



IDENTIFICATION OF ENTEROCOCCUS SPECIES ISOLATED FROM CLINICAL SPECIMENS AND DETECTION OF VANCOMYCIN RESISTANT ENTEROCOCCI.

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ABSTRACT

Although Enterococci have been considered of relatively low virulence, these organisms can cause serious infections like endocarditis, Urinary Tract Infection, wound infections particularly among hospitalized patients. *E. faecalis* had been the predominant Enterococcal species, accounting for 80 – 90% of all clinical isolates and *E. faecium* had accounted for 5 – 15%. The emergence of vancomycin resistant Enterococci (VRE) has alarmed the global infectious disease community for several reasons. First Enterococcal acquisition of vancomycin resistance leaves few options for disease management. Second conjugation experiments have confirmed vancomycin resistance gene transfer from Enterococci to *Staphylococcus aureus*. Vancomycin resistant Enterococci are those Enterococci that are resistant to vancomycin at a concentration greater than or equal to 32 mg/L. These Enterococci will contain genes Van A, Van B or Van D which confer resistance to vancomycin.our aim is to know incidence of Enterococcus infection and its antimicrobial sensitivity with reference to vancomycin resistant Enterococci. Total 7807 samples were received in the laboratory over a period of two years. The samples were processed and Enterococci were identified by standard biochemical test. Further characterization and speciation was carried out. Antimicrobial sensitivity pattern was detected and MIC of vancomycin resistant Enterococci was detected. Incidence of Enterococcal infection was 2% with *E. faecalis* was the commonest species isolated. Early detection of vancomycin resistant Enterococci and implementation of control measures will reduce the transmission of VRE.

KEY WORDS : Vancomycin resistant Enterococci ; Intrinsic resistance.



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INTRODUCTION

In 1980's based on genetic differences Enterococci were removed from the genus streptococcus and placed in their own genus Enterococcus⁽¹⁾. Although a dozen Enterococcus species have been identified only two are responsible for the majority of human infections. *E. faecalis* had been the predominant Enterococcal species, accounting for 80 – 90% of all clinical isolates and *E. faecium* had accounted for 5 – 15%. Other Enterococcus species are isolated less frequently and account for less than 5% of clinical isolates⁽¹⁾. Enterococci are considered a part of the normal flora of bowel, genital tract and anterior urethrae of humans⁽²⁾. Although Enterococci have been considered of relatively low virulence, these organisms can cause serious infections like endocarditis, Urinary Tract Infection, wound infections particularly among hospitalized patients⁽²⁾. The emergence of vancomycin resistant Enterococci (VRE) has alarmed the global infectious disease community for several reasons. First Enterococcal acquisition of vancomycin resistance leaves few options for disease management. Second conjugation experiments have confirmed vancomycin resistance gene transfer from Enterococci to *Staphylococcus aureus*⁽³⁾. One of the major reasons why these organisms survived in hospital environment is their intrinsic resistance to several commonly used antibiotics and their ability to acquire resistance to all currently available antibiotics either by mutation or transfer of genetic material through plasmids and transposons⁽¹⁾. Vancomycin resistant Enterococci are those Enterococci that are resistant to vancomycin at a concentration greater than or equal to 32 mg/L. These Enterococci will contain genes Van A, Van B or Van D which confer resistance to vancomycin.⁽⁴⁾

Objectives

- 1) To know the incidence of Enterococcus infection in a tertiary care hospital.
- 2) To determine of antimicrobial sensitivity patterns of Enterococcal isolates.
- 3) To know the extent of vancomycin resistant Enterococci in clinical isolates.

MATERIALS AND METHODS

The study was conducted in the Department of Microbiology of NKP Salve Institute of Medical Sciences and Research Centre, Nagpur. This prospective study was conducted over a period of February 2011 to December 2012. A total of 7807 samples were collected from both out-patient and in-patient of all clinical departments. They comprised of urine, blood, pus, wound swabs, body fluids, high vaginal swabs and catheter tips. All specimens are subjected to gram staining first. Then they were inoculated into Blood agar, MacConkeys agar and incubated at 37°C for 18 – 24 hours. If growth was observed, colony morphology and gram stain morphology were studied. The gram positive and gram negative organisms were identified by battery of tests as per protocol. The gram negative organisms and other gram positive organisms were excluded and only Enterococci were included. Enterococci were identified on the basis of colony characteristics, gram staining, salt tolerance test, bile esculin hydrolysis, catalase, arginine decarboxylase, VP and PYR test and mannitol fermentation test. They were further characterized upto species level by using standard test like motility, pigment production and sugar fermentation tests like arabinose, lactose, raffinose, sucrose.^(5,6) Antimicrobial susceptibility pattern was done in Muller-Hinton agar by Kirby-Bauer disc diffusion method according to CLSI guidelines.⁽⁷⁾ All strains were tested for susceptibility using penicillin (10ug), ampicillin (10ug), Vancomycin (30ug), gentamicin, norfloxacin, tetracycline, erythromycin, nitrofurantoin, linezolid etc. Resistance to vancomycin was screened by using vancomycin screen agar.⁽⁴⁾ The minimum inhibitory concentration was determined by using the standard agar dilution method for vancomycin.⁽⁸⁾ Control organism for susceptibility testing of Enterococci used were : Enterococcus faecium ATCC 51299 – Resistant to vancomycin (MIC-32 mg/l) Enterococcus faecalis ATCC 29212 – Sensitive to vancomycin

RESULTS

A total of 7807 samples were collected from both out and in-patients. Among these 4149 (53.14%) were culture positive. Out of total culture positive, 80 (2%) Enterococcus species were isolated from various clinical samples. Enterococci was isolated more from urine sample (56.25%). Table no. 1 shows distribution of Enterococci in different types of samples. 80% of Enterococci were isolated from hospitalized patients and 20% from outdoor patients. Table no. 2 shows distribution of Enterococci species isolated. Enterococcus faecalis was the commonest species isolated (78.75%) followed by Enterococcus faecium (16.25%) and others 5% which includes E. avium, E. raffinosus and E. cassaliflavus. Table no. 3 shows speciation of Enterococcus

species in different samples. 60 (95.23%) of Enterococcus faecalis were sensitive to vancomycin. 12 (92.30%) of Enterococcus faecium were sensitive to vancomycin. In contrast E. avium, E. raffinosus and E. cassaliflavus were 100% sensitive to vancomycin and other drugs. Antibiotic Sensitivity pattern of individual species is shown in Table no. 4. 3 (4.76%) strains of Enterococcus faecalis and 1 (7.69%) of Enterococcus faecium were resistant to vancomycin detected by vancomycin screen agar. Vancomycin inhibitory concentration level among isolates were shown in Table no. 5. 3 (6.66%) isolates of Enterococcus faecalis showed MIC of > 256 mg/ml and 1 (7.69%) isolates of Enterococcus faecium showed MIC of > 512 mg/ml by agar dilution method which were considered as resistant.

Table No. 1
Isolation of Enterococci in various clinical specimens.

Sr. No.	Sample	Number of Enterococci
1	Urine	45
2	Blood culture	10
3	Pus	9
4	Body fluid	7
5	Catheter tip	4
6	Stool	2
7	Wound swab	2
8	Vaginal swab	1
	Total	80

Table No. 2
Distribution of Enterococcal species isolated.

Sr. No.	Organism	Number of species
1	E. faecalis	63
2	E. faecium	13
3	E. raffinosus	2
4	E. avium	1
5	E. cassaliflavus	1
	Total	80

Table No. 3
Specification of Enterococci in various samples.

Organism	Urine	Blood culture	Pus	Body fluid	Catheter tip	Stool	Wound swab	Vaginal swab
E. faecalis (n=63)	38	7	6	5	3	1	2	1
E. faecium (n=13)	6	2	2	1	1	1	-	-
E. raffinosus (n=2)	1	1	1	-	-	-	-	-
E. avium (n=1)	-	1	-	-	-	-	-	-
E. cassaliflavus (n=1)	-	-	-	1	-	-	-	-
Total	45	10	9	7	4	2	2	1

Table No. 4
Antibiotic sensitivity pattern of various Enterococcal species.

Drug	Organisms				
	E.faecalis (n=63)	E.faecium (n=13)	E.raffinosis (n=2)	E.avium (n=1)	E.cassaliflavus (n=1)
Erythromycin (15 ug)	88 %	76.92 %	100 %	100 %	100 %
Nitrofurantoin (300 ug)	100 %	100 %	100 %	100 %	100 %
Vancomycin (30 ug)	93.33 %	92.30 %	100 %	100 %	100 %
Tetracyclin (30 ug)	97.77 %	100 %	100 %	100 %	100 %
Ciprofloxacin (10 ug)	66.66 %	76.92 %	100 %	100 %	100 %
Gentamicin (120 ug)	71.11 %	76.92 %	100 %	100 %	100 %
Ampicillin (10 ug)	22.22 %	23.07 %	100 %	100 %	100 %
Penicillin (10 ug)	44.44 %	38.46 %	100 %	100 %	100 %
Linezolid (30 ug)	95.55 %	84.61 %	100 %	100 %	100 %

Table No. 5
MIC for vancomycin resistant Enterococci by agar dilution method.

Organisms	MIC
E. faecalis (n=3)	> 256 ug/ml
E. faecium (n=1)	> 512 ug/ml

DISCUSSION

Enterococcus is one of the common cause of nosocomial infection with increasing frequency of antimicrobial resistance to most of the currently used antibiotics. Due to this Enterococcus have emerged as one of the leading challenges for physicians and surgeons in the treatment of infections. The present study include phenotypic characterization and resistant pattern of Enterococci to vancomycin. Enterococci were isolated from various clinical specimens with the prevalence rate of 2%. Where as in Acharya et al prevalence rate of Enterococci was 0.57% and Jada s et al showed (10.77%) prevalence rate which indicates variation in prevalence rate.^(9,10) In the present study, 80% of Enterococci were isolated from hospitalised patients and 20% from out door patients. Similar Findings were shown in Acharya et al where (72%) Enterococci were isolated from hospitalized patient and (28%) from out-patients. This might be because longer hospitalized stay and immunocompromised conditions are known risk factors for nosocomial infections.⁽⁹⁾ In the present study, urine was the most common sample from which Enterococci were isolated (56.25%). Similar results were found in Jada et al, among different samples Enterococci was isolated more from urine samples (59%).⁽¹⁰⁾

P.J. Desai et al showed frequent and higher isolation of Enterococci from urine sample followed by pus.⁽¹¹⁾ Prakash VP et al showed that the most common source of Enterococcal isolation were the urine, blood, pus etc.⁽¹²⁾ In the present study, among the 80 isolated Enterococci, Enterococcus faecalis was the predominant isolate (78.75%) followed by Enterococcus faecium (16.25%) and others (5%). These findings matches with the findings of Chaudhari et al where Enterococcus faecalis isolated were (72.3%) and Enterococcus faecium (17.3%) out of 260 Enterococci.⁽¹³⁾ Udo and colleagues isolated (85.3%) of Enterococcus faecalis and (7.7%) of Enterococcus faecium out of 415 Enterococci.⁽¹⁴⁾ Ghoshal and colleagues isolated 685 Enterococci out of them 67% were Enterococcus faecalis and 33% were Enterococcus faecium. Species identification of isolates enabled us to acces species specific antimicrobial resistance characteristics.⁽¹⁵⁾ Among Enterococci, Enterococcus faecium has emerged as an important nosocomial pathogen during the past decade. It is a cause of significant mortality and morbidity in hospitalized patients. Notorious for resistance to multiple antimicrobial agent, the aquisition of vancomycin resistance has made this organism a formidable pathogen. Erythromycin, Ciprofloxacin and Tetracycline

sensitivity was (88.88%),(66.66%)and (97.77%) in *E. faecalis* and (76.92%),(76.92%) and (100%) in *E. faecium* respectively, which is very high. Penicillin and Gentamicin showed sensitivity of (44.44%) and (71.11%) in *E. faecalis* and (38.46%) and (76.92%) in *E. faecium* respectively. All strains showed 100% sensitivity to Linezolid. Other Enterococci like *E. avium*, *E. Cassaliflavus*, *E. raffinosus* showed 100% sensitivity to all drugs. 5% of Enterococci strains showed vancomycin resistance by screening and macrobroth dilution methods. . In the present study vancomycin resistant *E. faecium* (7.69%) was the predominant pathogen with MIC > 512 ug/ml. Out of 3 vancomycin resistant strains of *E. faecalis*, 2(5.26%) were isolated from urine, and 1(50%) from wound swab. Single strain of vancomycin resistant *E. faecium* was isolated from pus(50%).This might be because

of Vancomycin usage has increased in hospitals following the emergence of methicillin resistant staphylococcus aureus. The emergence of VRE is also due to inappropriate use of cephalosporins as well as poor infection control measures. Chaudhari et al isolated 5 vancomycin resistant Enterococci, out of which two were *E. faecalis* and three *E. faecium* out of 260 Enterococci.⁽¹³⁾ Jada and colleagues isolated 1 VRE out of 100 Enterococci.⁽¹⁰⁾

CONCLUSION

Early detection and prompt reporting of vancomycin resistance in Enterococci and implementation of appropriate infection control measures will prevent person to person transmission of VRE in hospital premises.

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