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VANCOMYCIN MIC OF ENTEROCOCCUS SHOWING REDUCED ZONE OF INHIBITION BY DISK DIFFUSION METHOD

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Abstract: Enterococci are normal residents of the gastrointestinal and biliary tract and in lower number in the vagina and male urethra, occasionally in oropharyngeal secretions and on skin especially in perianal area. Enterococci have become one of the most common nosocomial pathogens. The incidence of nosocomial Enterococci infections has seen an increase in the last two decades and Enterococci are the second most common cause of nosocomial urinary tract and wound infections and the third most common cause of nosocomial bacteremias. Resistance to several commonly used antimicrobial agents is a remarkable characteristic of most of Enterococcal species. Isolation of Enterococci resistant to multiple antibiotics has become increasingly common in the hospital setting. The acquisition of Vancomycin resistance by Enterococci has seriously affected the treatment and infection control of these organisms. Identification at genus level was done by-Gram's Staining, Catalase andBile-Esculin Test. Detection of Vancomycin Resistant Enterococcus by Disk Diffusion Method, In this method Muller-Hinton Agar without NaCl was used. Antimicrobial susceptibility testing of the isolated strains of Enterococci to various antimicrobial discs was carried out according to Kirby-Bauer disc diffusion method. The Enterococci standard ATCC strain was used for quality control. The MIC value of 100 isolates varied from 0.5 - 32µg/ml. Among them 95 were VSE (MIC ≤ 4µg/ml), 4 were VIE (MIC 8-16 µg/ml) and 1 strain was VRE (MIC ≥ 32µg/ml). VRE strain was isolated from blood sample from a female patient. Thus prompt isolation, accurate identification and antibiotic susceptibility testing of Enterococci will help in the early identification of antibiotic resistant isolates, especially the Vancomycin resistant Enterococci and enable us to control their spread.

Keywords: Vancomycin, Enterococci, Nosocomial infections

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INTRODUCTION

Enterococci are Gram Positive Cocci that often occurs in pairs (diplococci) or in short chains and re difficult to distinguish from Streptococci on physical characteristics alone[1]. Enterococci are normal residents of the gastrointestinal and biliary tract and in lower number in the vagina and male urethra, occasionally in oropharyngeal secretions and on skin especially in perianal area[2]. Enterococci have become one of the most common nosocomial pathogens. Enterococci are traditionally regarded as low grade pathogens have emerged as an increasingly important cause of nosocomial infections in last decade[4].Enterococci are formidable pathogens because of their resistance to antimicrobial agents[3].

Resistance to several commonly used antimicrobial agents is a remarkable characteristic of most of Enterococcal species. Enterococci have both an intrinsic and acquired resistance to antibiotics making them important nosocomial pathogen. Isolation of Enterococci resistant to multiple antibiotics has become increasingly common in the hospital setting[4]. The intrinsic ruggedness of Enterococci also confers on unusual level of tolerance to several class of antibiotics[5]. Their resistant to penicillin's and cephalosporin's of several generations, the acquisition of high level resistance to amino glycosides and now due to emergence of Vancomycin resistance, these bacteria are often causing serious infections [6].

Since Vancomycin Resistant Enterococci (VRE) first reported in the 1980's, their geographic distribution and importance as nosocomial pathogens have continued to increase worldwide. The increasing incidence of Vancomycin resistance among the Enterococci have led to the selective isolation of Vancomycin Resistant Enterococci.[7]Vancomycin is the last line of defense against multiple drug resistant Enterococci, and for this reason the emergence of Vancomycin Resistant Enterococci (VRE) has caused great concern in the medical community.[8]

Thus the study was taken up to identify Vancomycin Intermediate Enterococci (VIE) and Vancomycin Resistant Enterococci (VRE) among the Enterococcus isolated from various clinical samples which showed reduced zone of susceptibility by disk diffusion method.

MATERIALS AND METHODS:

One hundred non-repetitive clinical isolates of Enterococcus that were consecutively isolated during the period of 4 months from December 2013 to March 2014 in Department of Microbiology, SVIMS, Tirupati were included in the study. Isolation was done by culture on Blood Agar and MacConkey Agar.

Clean catch midstream urine was collected. The external genitals was thoroughly cleaned with soap and water and wiped with clean dry cloth. Middle part of the voided urine (about 5 ml) was collected in sterile wide mouthed screw capped container. Venipuncture site was prepared with tincture iodine and 70%alcohol. Blood (5-10) ml was drawn with sterile needle, syringe and transferred into the bottle containing 40 ml of brain heart infusion broth under aseptic precautions incubated at 37 ^{0}C. Pus or exudates was collected either by aspiration or using at least two sterile cotton swabs. After decontaminating the surface of the lesion, two swabs were taken from the depth of the wound or lesion. Care was taken not to touch the adjacent skin margins. Then the swabs were transported in sterile cotton plugged test tubes. Identification at genus level was done by-Gram's Staining, Catalase, Bile-Esculin Test.

Broth Dilution Method is used to detect the Minimum Inhibitory Concentration [MIC] of the antibiotic against Enterococci. Agar Dilution Method is a quantitative method for determining

the MIC of the antibiotics against Enterococci to be tested. Epsilometry strip method is an automated system for measuring the MIC of Enterococci. In the present study, the antibiotic sensitivity testing for Enterococcus is carried out by Agar Dilution Method. Antimicrobial susceptibility testing of the isolated strains of Enterococci to various antimicrobial discs was carried out according to Kirby-Bauer disc diffusion method. The Enterococci standard ATCC strain was used for quality control.

Before reading and interpretating the results, growth control and results with quality controls stains were checked. The minimum inhibitory concentration (MIC) was checked by examining the plates for the lowest concentration of Vancomycin that inhibited visible growth. For Vancomycin to Enterococci (1-128 µg / ml).[9] Sensitive ≤ 4 µg / ml, Intermediate 8-16 µg / ml, and Resistant ≥ 32 µg / ml.

RESULTS:

Out of 100 subjects, 55 (55%) patients were males and 45 (45%) patients were females.

Table 1: Sex distribution among the cases

Sex	Number	Percentage
Males	55	55%
Females	45	45%

Out of 100 samples, 72 were urine samples, 12 were blood samples, 9 were pus samples, 5 were catheter tip samples and 2 were body fluids as shown in the figure.

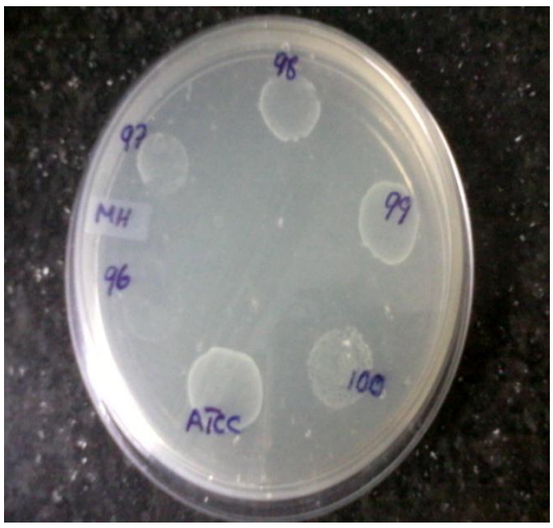
S.No	Sample	Number	Percent %
1.	Urine	72	72%
2.	Blood	12	12%
3.	Pus	9	9%
4.	Catheter tips	5	5%
5.	Body Fluids	2	2%

All the Enterococcal isolates were tested for Vancomycin susceptibility by disk diffusion method. The MIC of Vancomycin was determined using Agar Dilution Method. In the present study, all isolates exhibited reduced zone of inhibition by Disk Diffusion Method.

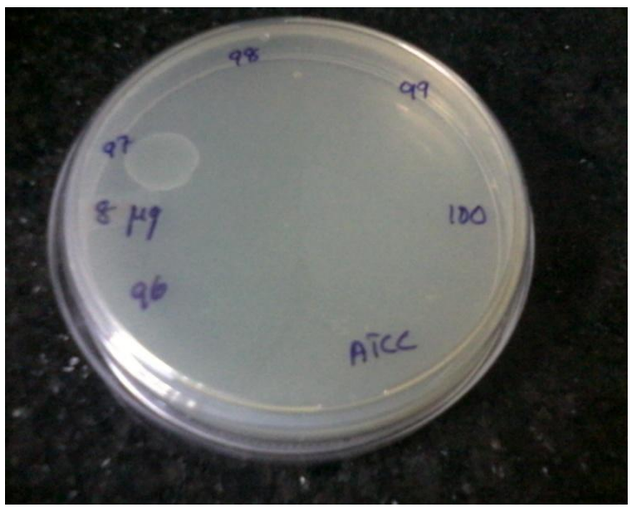
In the present study, out of 100 samples, 95 were VSE (MIC ≤ 4µg/ml), 4 were VIE (MIC 8-16 µg/ml) and 1 strain was VRE (MIC ≥ 32µg/ml). VRE strain was isolated from blood sample of female patient. Out of 4 VIE isolates 3 were from urine and 1 from catheter tip sample.

MIC value	No of samples	Percentage
≤ 4µg/ml	95	95%
8-16 µg/ml	4	4%
≥ 32µg/ml	1	1%

Growth in Muller Hinton Agar without Vancomycin:



Growth in Muller Hinton Agar with Vancomycin:



DISCUSSION:

The present study was undertaken considering the paucity of data on Vancomycin resistant Enterococci (VRE) due to the fact that Enterococci are the second leading cause of hospital acquired infection and the third leading cause of bacteraemia. Vancomycin resistance can be difficult to detect in clinical microbiology laboratory. Disk diffusion sensitivity testing by standard 30µg Vancomycin frequently misclassifies intermediately susceptible isolates as fully susceptible. Presently MIC determinations by broth or agar dilution or by E test are the gold standard for determining Vancomycin susceptibility, but these methods are not suitable for routine use in the diagnostic laboratories.

In the present study a total of 100 Enterococcus isolates were investigated for the period of 4 months from December 2013 to March 2014. The strains were collected from various clinical specimens including urine, blood, pus, catheters, and body fluids, from the patients of different inpatient and outpatient departments of SVIMS hospital. Among the 100 Enterococci isolates, 72 samples were urine, 12samples were blood, 9 were pus samples, 5samples were catheters tips and 2 were body fluid samples.

The MIC value of 100 isolates varied from 0.5 - 32µg/ml. Among them 95 were VSE (MIC ≤ 4µg/ml), 4 were VIE (MIC 8-16 µg/ml) and 1 strain was VRE (MIC ≥ 32µg/ml). VRE strain was isolated from blood sample from a female patient.

In Taneja Net al study which was done on 2000-2001 showed that 5.5% resistant isolates of *Enterococcus sps* [10]. In another study (Devi SP et al) it was proved that 20% a high percentage of vancomycin resistant isolates of Enterococcus were isolated. But a low-level resistance (11.6%) of *Enterococcus* isolates was reported in the study of Ranghdale VA et al in 2007.

The experience of Prakash et al (2005) shows the issue is more complex. There is an impression that control on Vancomycin prescriptions keeps the VRE rates low. Finding the 1% VRE in the present study are corroborative with the Mathur P et al publication that was done on 2003 in Indian J Med Res 118, July 2003:25-28.[11] Wide spread use of Vancomycin to treat infections caused by Enterococci and other Gram positive cocci has lead to the emergence of Vancomycin resistance.The alarmingly increasing trend of VRE infection calls for the implementation of an effective infection control policy which would help in containing this bioterror, if not completely eradicating it.

CONCLUSION:

Though Enterococci have long been recognized as low virulence bacteria occurring as commensals in the human intestine, currently their role in causing infections is better defined. The use of broad-spectrum antibiotic agents as a substitute for precise diagnostics or to enhance the likelihood of therapeutic success increases the rate of selection of multi drug resistant bacteria.

VRE have become common in many hospitals throughout the world and, once established, show to be very difficult to eradicate. The increasing incidence of multi drug resistant among clinical isolates can be a therapeutic challenge. Therefore infection control measures are of prime importance in preventing the establishment of these pathogens and controlling the spread. Control measures aimed at reducing the incidence of VRE colonization and infection in healthcare settings should include: hand washing with an antiseptic or a waterless antiseptic agent, routine screening for Vancomycin resistance among clinical isolates, rectal surveillance cultures contact isolation for patients with VRE and antimicrobial stewardship.

All the clinical microbiology laboratories should routinely test the MIC of Vancomycin for multidrug resistant Enterococci for appropriate treatment of patients and also for implementation of infection control measure to prevent the spread of resistance. Thus prompt isolation, accurate identification and antibiotic susceptibility testing of Enterococci will help in the early identification of antibiotic resistant isolates, especially the Vancomycin resistant Enterococci and enable us to control their spread.

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