SPECIATION OF ENTEROCOCCI ISOLATED FROM CLINICAL SPECIMENS AND DETERMINATION OF HLG AND VANCOMYCIN RESISTANCE

Saleem Mohamedali¹, Nagma Rafi², Kaviraj Mahadevan³, Gopal Rangasamy⁴

¹Professor, Department of Microbiology, Sri Manakula Vinayagar Medical College and Hospital, Puducherry.

²Postgraduate Student, Department of Microbiology, Sri Manakula Vinayagar Medical College and Hospital, Puducherry.

³Research Associate, Central Research Lab, Sri Manakula Vinayagar Medical College and Hospital, Puducherry.

⁴Professor and HOD, Department of Microbiology, Sri Manakula Vinayagar Medical College and Hospital, Puducherry.

ABSTRACT

BACKGROUND

Enterococci constitute a significant proportion of the normal flora of the oropharynx, gastrointestinal and genitourinary tract. The incidence of Enterococcal infections is on the rise over the past few years. Enterococci are resistant to a number of drugs including β -lactamase glycopeptides and aminoglycosides limiting the options for treatment.

Aims and Objectives- The aim of the study is to speciate the enterococci isolated from clinical specimens and to determine the prevalence of High Level Gentamycin and Vancomycin resistance among the isolates.

MATERIALS AND METHODS

In the present study, a total of 50 strains of Enterococci were isolated from various clinical samples collected over a period of 6 months. Speciation of the isolates and their resistance to HLG and Vancomycin were determined by disc diffusion and E-test.

RESULTS

The maximum no. of isolates were from female and urine constituted the most common sample with highest yield. *E. faecalis* was the most common species. HLGR Enterococci was formed in 13/50 strain, only one strain 1/50 of enterococci was vancomycin resistant.

CONCLUSION

HLG and vancomycin resistance among enterococci should be monitored to prevent the transfer of resistance genes to other bacteria. It also helps to improve therapy and control nosocomial spread of enterococci.

KEYWORDS

Enterococci, High Level Gentamicin, Vancomycin Resistance.

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BACKGROUND

Enterococci constitute a significant proportion of the normal flora of the oropharynx, gastrointestinal tract, perianal skin and vagina.¹ They are the common contaminants of open wounds and bed sores.² *E. faecalis* and *E. faecium* are the two most common species associated with urinary tract, intra-abdominal, pelvic, biliary tract, respiratory tract infections and neonatal sepsis.³ The incidence of Enterococcal infections has increased in the recent years making it the second most common nosocomial pathogen. *E. faecalis* is the predominant strain among enterococci, although, in recent times the rate of infection caused by *E. faecium* has also increased remarkably.⁴ Enterococci are resistant to a number of antimicrobial agents including β -lactam antibiotics, glycopeptides and aminoglycosides.⁵ The emergence of

multidrug resistant strains with high level aminoglycoside resistance (HLAR), β -lactamase production and glycopeptide resistance including vancomycin resistant enterococci (VRE) results in therapeutic failure, posing a serious threat to the life of patients.⁶

The genome of enterococci encodes aminoglycoside modifying enzymes (AME). These enzymes possess the ability to reduce the synergetic effect of aminoglycoside antibiotics on the cell wall of bacteria.⁷ The presence of genes *aac(6')-Ie-aph(2'')-Ia* mediates HLR to gentamicin. Identifying HLGR Enterococcus and modifying antibiotic preferences is of most concern in regard to treatment and infection control.⁸

Vancomycin is often used in the treatment of enterococcal infections. Vancomycin is a glycopeptide antibiotic which acts by blocking the biosynthesis of bacterial cell wall. Vancomycin resistant enterococci phenotypes evolve as a result of changes in the peptidoglycan precursors which are the major constituents of bacterial cell wall. These changes reduce the affinity to glycopeptide antibiotics (Vancomycin). Thereby, the potential target is made unavailable, resulting in the emergence of vancomycin resistant enterococci (VRE).⁹ Identification of enterococcal species and their antibiotic resistance pattern with respect to HLGR and VRE were taken to concern in this pilot study.

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Corresponding Author:

Dr. Saleem Mohamedali,

Professor,

Department of Microbiology,

Sri Manakula Vinayagar Medical College
and Hospital, Puducherry.

E-mail: pondysaleem@gmail.com

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MATERIALS AND METHODS

The study was conducted in the Department of Microbiology, Sri Manakula Vinayagar Medical College and Hospital, Puducherry, India. This retrospective study was conducted over a period of six months from July 2016 to December 2016 during which 50 strains of Enterococci were isolated from various clinical samples. The strains originated from urine, pus, blood, high vaginal swab (HVS), drain tube (DT), throat swab, sputum and body fluid. The genus was confirmed by Gram stain, catalase test, bile aesculin hydrolysis. Speciation was done based on Facklam conventional method & fermentation of mannitol, raffinose, sorbitol and arabinose (Figure 1).

Antimicrobial susceptibilities were determined by Kirby-Bauer disc diffusion method with high level gentamicin (120 µg) and vancomycin (30 µg). Standard procedures were followed and results were interpreted according to CLSI guidelines.

Epsilometer (E) test was done in VRE [Resistant in disc diffusion method] strains using a vancomycin strip of concentration gradient 0.016 to 256 µg/mL and the MICs were recorded.

RESULTS

In the present pilot study, a total of 50 strains of Enterococci were isolated from different clinical samples. Most (50%) of the strains were isolated from the urine of female patients (Table 1). The sum of 50 strains comprised of 10 species as identified by Facklam conventional method & fermentation of mannitol, raffinose, sorbitol and arabinose (Table 2). *E. faecalis* (30%) was the most common isolate followed by *E. faecium* (26%). HLG resistance was detected in 13 strains and vancomycin resistance was found among 5 strains by disk diffusion method. 2 strains of *E. faecium* and two of *E. canis* which were vancomycin resistant by disc diffusion method has shown sensitivity in E-test (Figure 2). One strain of *E. malodoratus* was found to be resistant to vancomycin in disc diffusion and also in E-test (Table 4).

Sample	Male	Female
Urine	6	25
Pus	5	4
Blood	0	1
High Vaginal Swab	0	1
Drain Tube	1	1
Urethral Tube	1	1
Throat Swab	1	0
Ascitic Fluid	1	0
Sputum	2	0
Total	17	33

Table 1. Gender wise Distribution of the Isolates among Various Clinical Samples

Species	N (%)
<i>E. faecalis</i>	15(30)
<i>E. faecium</i>	13(26)
<i>E. durans</i>	3(6)
<i>E. casseliflavus</i>	1(2)
<i>E. avium</i>	5(10)
<i>E. dispar</i>	3(6)
<i>E. canis</i>	2(4)
<i>E. malodoratus</i>	5(10)
<i>E. haemoperoxidus</i>	1(2)

<i>E. cecorum</i>	2(4)
Total	50 (100)

Table 2. Speciation of Enterococcal Isolates

Species	HLGR	VRE
<i>E. faecalis</i>	5(27%)	-
<i>E. faecium</i>	4(22%)	2(40%)
<i>E. casseliflavus</i>	1(6%)	-
<i>E. avium</i>	1(6%)	-
<i>E. canis</i>	2(11%)	2(40%)
<i>E. malodoratus</i>	-	1(20%)

Table 3. Distribution of HLGR and VRE by Disc Diffusion Method

VRE, N = 5	E- Test Sensitive [MIC- µg/mL]	E- Test Resistant [MIC- µg/mL]
<i>E. faecium</i> (N = 2)	2 [1.5, 1.4]	-
<i>E. malodoratus</i> (N = 1)	-	1 [>32]
<i>E. canis</i> (N = 2)	2 [1.2, 1.5]	-

Table 4. E test against VRE Determined by Disc Diffusion Method

Figure 1. Speciation by Fermentation of Mannitol, Raffinose, Sorbitol, Arabinose

Figure 1. E- Test Showing MIC of a VRE Strain (By Disc Diffusion Method) against False VRE

DISCUSSION

Enterococci were initially considered as rare infectious pathogens but have emerged as an important opportunistic pathogen among hospitalised patients of late.¹⁰ These organisms are resistant to most of the antimicrobial agents in use, making it difficult to treat.^{11,12}

The maximum yield of Enterococci was from urine sample (31/50) which is similar to the studies by Suresh et al, Uma et al.^{13,14} A high rate of isolation of *E. faecalis* similar to our study has been reported by Kishk et al, Kanthishree et al, Swati et al.^{3,15,16}

High Level Gentamicin Resistance (HLGR)

In the present study, HLGR was found among 6% of *E. casseliflavus*, *E. avium* and 11% of *E. canis* strains. None of the other five species showed HLGR. The only strain of *E. malodoratus* which was resistant to vancomycin was susceptible to HLG. HLGR was exhibited by 5 of *E. faecalis* and 4 of *E. faecium* which is concordant with the previous studies.^{3,17} Synergistic effect of aminoglycosides act as the best choice to eradicate Enterococcal infection, but the evolution of HLAR especially HLGR strains became more challenging in infection control.¹⁸

VRE

The emergence of VRE is a major problem in management of hospital acquired infections. Vancomycin serves as the first line of defence against HLAR Enterococci, VRE makes the antibiotic treatment ineffective and poses a challenge to the clinicians.¹⁹ Transfer of VR genes to VS enterococci and other bacteria limits the use of vancomycin in control of nosocomial infections. Hence, performing antibiotic sensitivity test is important for therapeutic purpose. In testing the sensitivity to vancomycin, E-test is effective in the determination of false resistant strains in a dosage dependent manner. Only one strain of *E. malodoratus* was found to be resistant to vancomycin both by disc diffusion and E test. In our study, it shows that prevalence of VRE is low in our area (2%). A higher rate of resistance (10%) to vancomycin was detected based on disc diffusion done, giving a higher false positive rate of VRE. It is therefore necessary to confirm all VRE isolates by confirmatory test using MIC agar test or E test. Most of the studies have used only disc diffusion for detecting VRE and hence not comparable.²⁰

The health care industry is in need to discover potential agents to fight against VRE. Quinupristin/Dalfopristin and Linezolid are the recently developed antibiotics which are the candidates of choice for VRE. In vitro susceptibility tests have shown promising results and their therapeutic application is in clinical trials.²¹

CONCLUSION

The distribution of Enterococci species in nosocomial infections and the rate of HLGR and VRE at our hospital is similar in trends with the other recent reports across the country. Susceptibility test should be done for HLG before administration and gentamicin can be used along with cell wall active antibiotics like vancomycin, ampicillin that have synergistic effect against enterococci. E- Test is efficient and essential to avoid false identification of VRE. Multifaceted infection control practices can reduce the transmission of HLGR and VRE genes across bacteria and aid in the prevention of hospital-acquired infections (HAIs).

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