



Research Article

STUDY ON VIRULENCE FACTORS & ANTIMICROBIAL RESISTANCE AMONG *ENTEROCOCCI* SP., ISOLATED FROM VARIOUS CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL, KANCHIPURAM, INDIA

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Abstract- *Enterococcus* previously considered as a normal commensal of gut, is developing fast as a pathogen causing serious and life threatening nosocomial infections. This study was undertaken to determine the prevalence factors & their association with antimicrobial resistance. A total of 56 clinical samples were isolated from a tertiary care hospital. All these samples were identified as per standard conventional methods and detected for the production of virulence factors such as Haemolysin, Gelatinase, & Biofilm production. Antimicrobial susceptibility testing was carried out by disc diffusion method according to the CLSI guidelines. Among 56 isolates, *E. faecalis* were 43(76.7%), & *E. faecium* were 13(23.3%). The highest percentage of antibiotic resistance was seen in Erythromycin (83.7% in *E. faecalis* and 84.7% in *E. faecium*) followed by Ciprofloxacin (76.7% in *E. faecalis* and 77% in *E. faecium*), Tetracycline (74.4% in *E. faecalis* and 46% in *E. faecium*) and Ampicillin (32.5% in *E. faecalis* and 84.7% in *E. faecium*). In this study, overall 30.2% of *E. faecalis* & 23.1% of *E. faecium* showed biofilm production, haemolysin production were 46.5% of *E. faecalis* and 15.3% of *E. faecium*, followed by Gelatinase production were 21% of *E. faecalis* and 15.3% of *E. faecium*. Though the prevalence of vancomycin resistant *Enterococci* (VRE) is comparatively high in our study, hence regular monitoring of vancomycin resistance is very crucial for early finding, treatment, application of preventive and control measures.

Keywords- *Enterococci*, *Haemolysin*, *Gelatinase production*

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Introduction

Enterococcus previously considered as a normal commensal of gut, is developing fast as a pathogen causing serious and life-threatening nosocomial infections. This is attributed to attainment of multi drug resistance and virulence factors of the organisms. They can be opportunistic agents of infections in elder patients and immunocompromised patients who received antimicrobial drugs and immunosuppressive therapy, circulatory failure or clinically aggressive procedures. *Enterococci* are the most common aerobic and facultative anaerobic, gram positive cocci found in the intestinal as normal flora of humans and other animals [1]. For many years *Enterococcus* spp were supposed to be harmless to humans and was considered not important therapeutically. They are emerging nosocomial pathogens due to the increasing antibiotic pressure, high degree of resistance to aminoglycosides, erythromycin, tetracycline and more recently vancomycin [2]. The characteristic of *Enterococci* that makes them such difficult pathogens is their inherent resistance to a number of antimicrobial agents. *Enterococci* exhibit low levels of intrinsic resistance to penicillin's, cephalosporins, carbapenems, carbacephems, aminoglycosides and lincosamides. They also have acquired genes to resist the action of glycopeptides such as vancomycin and teicoplanin [3]. Major cause of intrinsic resistance is believed due to commonly used antibiotics and their ability to acquire resistance to all currently available antibiotics, either by mutation or by receipt of foreign genetic material through the transfer of plasmids and transposons [4]. With this background, the following study was undertaken to determine the prevalence of *Enterococcal* species, isolated from clinical samples along with their antimicrobial susceptibility patterns, virulence factors.

Materials & Methods

This retrospective study was carried on to 56 consecutive isolates of *Enterococci* isolated from different clinical samples (exudates, urine, blood and body fluids) of Hospitalized and Out Patient Department of Meenakshi medical college, Enathur, Kancheepuram. This study was approved by the Institute Ethical Committee. All the samples were identified by standard culture methods, biochemical tests and antibiotic susceptibility test by using Kirby-Bauer disc diffusion, followed by Detection of Virulence Factors such as Haemolysin production, Gelatinase production, & Biofilm production.

Identification of *Enterococci* Sp

Collection, processing, isolation and speciation of *Enterococcus* species from different clinical specimens were carried out as per standard conventional bacteriologic methods. All gram-positive cocci that are catalase negative are confirmed as *Enterococcus* genus with growth on and blackening of bile-esculin agar, growth in the presence of 6.5% sodium chloride (salt tolerance test) and heat tolerance test. Further *Enterococcus* species were identified by potassium tellurite reduction, arginine dihydrolase test and sugar fermentation test [5].

Antimicrobial susceptibility testing for *Enterococcus* sp.

Antimicrobial susceptibility testing was performed on Mueller Hinton agar as per CLSI guidelines. The following antibiotics were tested- Vancomycin (30µg), Ciprofloxacin (30 µg), High level Gentamicin (120 µg), High level streptomycin (300µg), Erythromycin (15µg), Tetracycline (30 µg), Ampicillin (10 µg), as per CLSI guidelines.

Detection of Virulence Factors

Haemolysin production

Hemolysin production will be detected by inoculating *Enterococci* on to freshly prepared beef heart infusion agar supplemented with 5% human blood. Plates were incubated at 37°C and observed after 24 and 48 hours. A clear zone of β hemolysis around the bacterial colonies indicated the production of hemolysin [6].

Gelatinase production

Gelatinase production will be detected by gelatin stab method which employs nutrient gelatin deep tubes that containing 12% gelatin and heavy inoculum from a pure culture of *Enterococci* is stabbed into the media. The gelatin media is incubated for 48hrs and then placed into the refrigerator for approximately 30mins. If the gelatin is still intact the media will solidify in the refrigerator and a negative test result is recorded. If the organism has produced sufficient gelatinase, the tube will remain liquid and will not solidify in the refrigerator; this has been recorded as a positive result [7].

Biofilm production

Colonies of *Enterococci* which had grown overnight on blood agar were inoculated in trypticase soy broth (Hi-media laboratories, Mumbai, India) with 0.5% glucose and incubated at 37°C overnight. This overnight growth was diluted 1:100 in the TSB with glucose. The micro titre plates were incubated aerobically at 37°C for 48 hours. At the end of 48 hours, the culture was discarded from the wells. The wells were gently washed three times with distilled water. The plates were then inverted and air dried at room temperature and finally the adherent biofilms are stained with 1% crystal violet for 20 minutes. The plates were then washed 5 times. The absorbance of the biofilm on the bottom surface of each well was determined at 490 nm with an ELISA microplate reader [8].

Result

Among 56 isolates, *E. faecalis* were 43(76.7%), & *E. faecium* were 13(23.3%), shown in [Table-1]. The highest percentage of antibiotic resistance was seen in Erythromycin (85.3% in *E. faecalis* and 90.9% in *E. faecium*) followed by Ciprofloxacin (73.1% in *E. faecalis* and 81.8% in *E. faecium*), Tetracycline (73.1% in *E. faecalis* and 45.4% in *E. faecium*) and Ampicillin (24.3% in *E. faecalis* and 72.7% in *E. faecium*) were shown in [Table-2]. In this study, overall 31.71% of *E. faecalis* & 21.27% of *E. faecium* showed biofilm production, haemolysin production were 48.7% of *E. faecalis* and 18% of *E. faecium*, followed by Gelatinase production were 22% of *E. faecalis* and 18% of *E. faecium* were shown in [Table-3][Fig-1-3].

Table-1 Distribution of *Enterococcus* sp.

Enterococcus Sp.,	No. of isolates	Percentage (%)
<i>E. faecalis</i>	43	76.7
<i>E. faecium</i>	13	23.3
Total	56	100

Table-2 Antibiotic Susceptibility Pattern of *Enterococcal* sp.

Antibiotics	<i>E. faecalis</i> 43(76.7%)				<i>E. faecium</i> 13(21.3%)			
	Sensitive		Resistant		Sensitive		Resistant	
	No	%	No	%	No	%	No	%
Erythromycin	7	16.2	36	83.7	2	15.3	11	84.7
Ciprofloxacin	10	23.4	33	76.7	3	23	10	77
Tetracycline	11	25.6	32	74.4	7	54	6	46
Ampicillin	29	67.4	14	32.5	2	15.3	11	84.7
High level Gentamicin	20	46.5	23	53.5	6	46	7	54
High level streptomycin	26	60.4	17	39.5	5	38.4	8	61.5
Vancomycin	38	88.3	5	11.6	11	84.6	2	15.3

Table-3 Virulence Characteristics of *Enterococcus* sp.

Virulence Factors	<i>E. faecalis</i>	<i>E. faecium</i>
Biofilm Producers	13(30.2%)	3(23.1%)
Haemolysin Producers	20(46.5%)	2(15.3%)
Gelatinase Producers	9(21%)	2(15.3%)

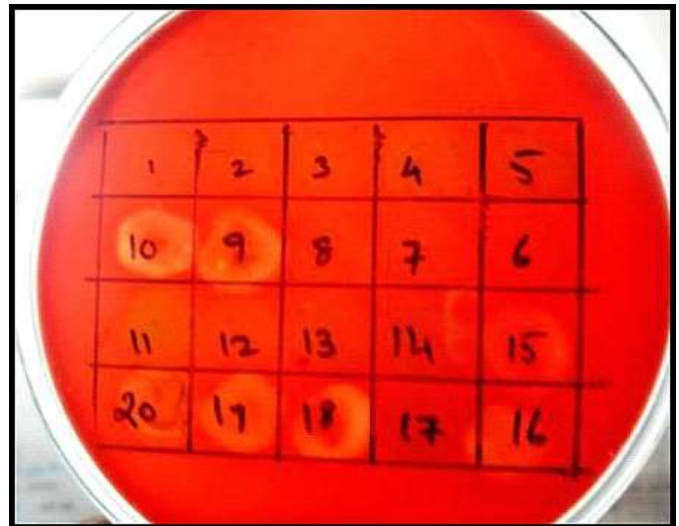


Fig-1 Hemolysin production

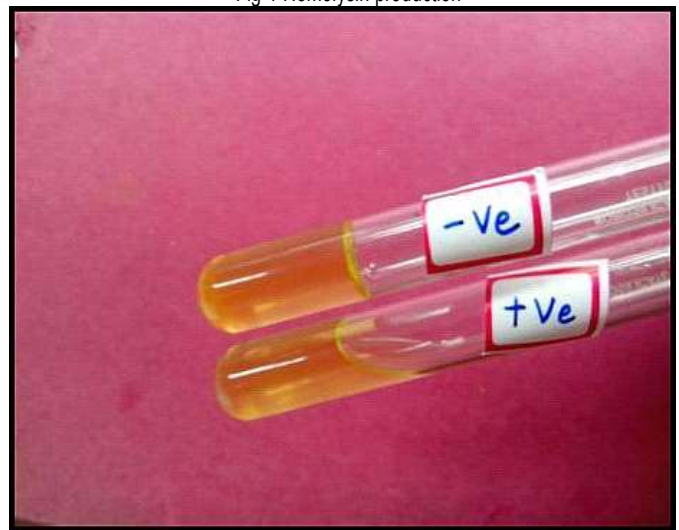


Fig-2 Gelatinase production

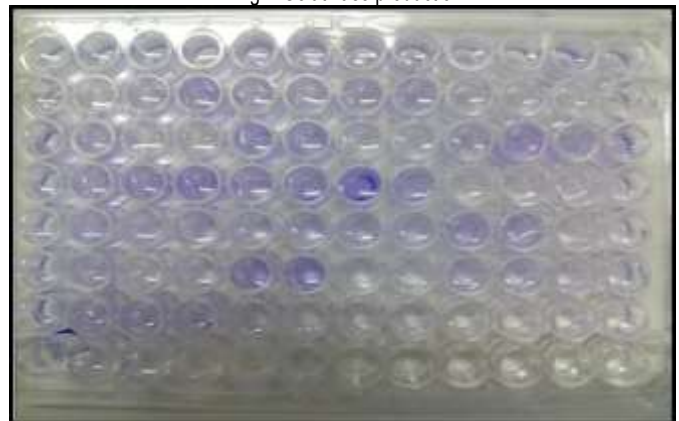


Fig-3 Biofilm formation by Microtitre plate method

Discussion

Enterococcal infections and its antibiotic resistance are one among the most important global health problems causing significant morbidity in the general population. In our study, we have got 76.7% of *E. faecalis* and 23.3% of *E. faecium* which is close to study of Vinod Kumar *et al* 2011[18]. Several other studies done in India and abroad reports that 80 to 90% of *Enterococci* are *E. faecalis* and 10 to 20% of *Enterococci* are *E. faecium*. This finding is of potential concern *E. faecium* is more commonly associated with Vancomycin resistance than other *Enterococci* [18]. Antibiotic resistance in *Enterococci* is either intrinsic or acquired.

Intrinsic traits are due to resistance to semisynthetic Penicillinase resistant penicillin's, cephalosporin, low level of aminoglycosides and low level of clindamycin, whereas acquired resistance includes resistance to chloramphenicol, erythromycin, High level aminoglycosides, penicillin, fluoroquinolones & vancomycin. Antibiotics resistance among *Enterococci* is a global problem. In our study the highest antibiotic resistance was seen against Erythromycin 83.7% to *E. faecalis* and 84.7% to *E. faecium* which correlates with, Fernandes, *et al*, (2013) [11], have reported highest resistance to Erythromycin 81% to *E. faecalis* and 90.1% to *E. faecium* [9-11].

In our study 32.5% of *E. faecalis* and 84.7% of *E. faecium* were resistant to Ampicillin. Many studies have also reported that *E. faecium* is more resistant than *E. faecalis* [12]. Many studies have also reported that *E. faecium* is more resistant than *E. faecalis* [12-14] also reported a high percentage of ampicillin resistance in their study (100% to *E. faecium* and 58% to *E. faecalis*). Salem-Bekhit *et al* (2012) [9], also reported ampicillin resistance of 15.7% in *E. faecalis* and 70.4% in *E. faecium*. In our study 76.7% of *E. faecalis* are resistant to ciprofloxacin and 77% of *E. faecium* are resistant to ciprofloxacin also reported by Sarika Jain *et al* 2011[11].

In our study we have isolated 15.3% *E. faecium* and 11.6% *E. faecalis* showing higher resistance to vancomycin. Similar findings were also reported by Karmarkar *et al.*, (2004) [14] who also reported resistance among *E. faecium*. Our study showed around HLG resistance of 53.5% to *E. faecalis* and 54% to *E. faecium* and HLS resistance of 39.5% to *E. faecalis* and 61.5% to *E. faecium*. In our study resistance to High level aminoglycoside resistance was seen higher in *E. faecium* than in *E. faecalis*, similar findings were observed by Prakash *et al* (2005) [19] who also reported significantly higher resistance to High level gentamycin and High-level Streptomycin by *E. faecium* than in *E. faecalis*. Similar result was also observed by Mendiratta *et al* (2008) [16]. High HLGR in *E. faecalis* and HLSR in *E. faecium* observed has also been reported [14-16] as also vice versa [12]. In our study 46.5% of *E. faecalis* produced hemolysin which was in agreement previous studies and 18.1% of *E. faecium* produced hemolysin which is in correlation with Timur *et al* 2006[16]. In our study 21% of *E. faecalis* and 15.3% of *E. faecium* produced gelatinase which were in concordance with Timur *et al* 2006 in which 12.7% of *E. faecalis* and 39% of *E. faecium* produced gelatinase [15-19].

Conclusion

Enterococcus has obviously occurred as a medically significant organism, causing outbreaks of many nosocomial infections.

Application of research: Though the prevalence of vancomycin resistant *Enterococci* (VRE) is comparatively high in our study, yet regular monitoring of vancomycin resistance is very crucial for early finding, treatment, application of preventive and control measures.

Research Category: Medical Microbiology

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University: MAHER University, Chennai, 600078, Tamil Nadu

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Author Contributions: All authors equally contributed

Author statement: All authors read, reviewed, agreed and approved the final manuscript. Note-All authors agreed that- Written informed consent was obtained from all participants prior to publish / enrolment

Study area / Sample Collection: Hospitalized and Out Patient Department of Meenakshi medical college, Enathur, Kancheepuram

Conflict of Interest: None declared

Ethical approval: Ethical approval taken from Meenakshi Medical College Hospital & Research Institute, Kanchipuram, 631552, MAHER University, Chennai, 600078, Tamil Nadu, India.

Ethical Committee Approval Number: Nil

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