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# Clinically Relevant Enterococcus Species and PCR Screening of Antibiotic Resistance and Virulence Factor Coding Genes

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**Abstract:** Enterococci are Gram positive cocci which are common residence of gastro intestinal tracts of humans and animals. But they are capable of causing severe infections, most often in hospitalised patients. Enterococci are important nosocomial pathogens and their intrinsic property of antibiotic resistance makes treatment difficult. Against this clinical significance, a study was conducted on the prevalence of vancomycin resistant enterococcal infections and their antibiotic sensitivity patterns at Sunrise Institute of Medical Sciences, a tertiary care hospital in Kochi, Kerala state. Various clinical specimens like blood, urine, abscess, vaginal swab etc. were microbiologically screened for the presence of antibiotic resistant Enterococci. The common phenotyping methods and genotyping protocols were used to identify the species for the study. The vancomycin resistant strains were typed genotypically by using 16SrDNA sequencing and the isolates were speciated to be *E. faecium*. A molecular screening of the isolates was also done for the presence of various virulence factor coding genes and for the genes which confer antibiotic resistance. The findings of the study revealed that 89.1 % isolated strains were multidrug resistant and a total of 4 antibiotic resistance genes were detected. Among the isolates the vancomycin resistance genes *vanA*, *vanB;* tetracycline resistance genes *tetA* and macrolide resistance gene *ermA* were screened. The presence of genes coding for various virulence factors were also detected among the isolates. However, we present an overview of antibiotic resistance pattern and virulence factors present in enterococci and we strongly believe that these results will surely help to design a new disinfection regime and antibiotic stewardship to control the incidence of infection.

**Keywords**: Enterococci, Multi drug resistance, Genotyping, Nosocomial infections, VRE

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## 1. INTRODUCTION

Enterococci are non- spore forming Gram- positive bacteria and is often seen in pairs as diplococci[1]. These facultative anaerobes colonize in the gastrointestinal tract of mammals, birds, reptiles and insects[2]. Enterococci make up less than 1% of the human adult gut microbiota[3]. They are also found in sand, fresh and marine water sediment and soil vegetations. Although enterococci have very low level of virulence[4,5], they have been associated with serious nosocomial infections recently. About 14% of nosocomial infections in USA between 2011 and 2014 were caused by enterococci[6]. Enterococci are resistant to Aminoglycoside, Cephalosporins, Clindamycin, Carbapenems, Glycopeptides, Lincosamides and Macrolide antimicrobial agents, which are the most routinely used drugs in hospitalized patients. Although cephalosporins was demonstrated to be a risk factor for increasing GI colonization by enterococci in hospitalized patient, [7]it is now found out that e*sp*, a gene associated with infection derived from outbreak strains, was present in 52% of *E. faecium* isolates and 40% of *E. faecalis* isolates causing bacteremia. [8]*Simenson et al*, studied the prevalence of resistance to Ampicillin, Gentamycin and Vancomycin in *E. faecalis* and *E. faecium* isolates from clinical specimens and use of antimicrobials in Nordic hospitals. And in his studies he detected a high proportion (31.4%) of *E. faecium* among blood culture isolates in which acquired Vancomycin resistance were not detected and amp resistance was not found in *E. faecalis* in contrast to 48.8% in *E. faecium* isolates. [9]*Jayaratne et al*., have used conventional PCR and reported various degrees of sensitivity and high degrees of specificity, thus providing encouraging results for the direct detection of VRE in these specimens. *Satake et al.,*[10] used methods by which they identified VRE within 8 hour of specimen submission by PCR of DNA extracted from clinical specimen. Previous Researchers[11] demonstrated the specificity of the PCR method in comparing the genetic organizations of Van A isolates from different sources and used purified DNA and found complete coincidence between genotype and phenotype for 30 VRE (17 Van A, 7 Van B and 6 Van C isolates). *Kariyamma et al.,*[12] have used multiplex PCR assay for simultaneous detection of glycopeptide resistance genotypes and for identification of clinically relevant Enterococci to the species level, which obtain definitive results within 48 hours. Infection control programs focus on identification of hospitalized patients infected with vancomycin-resistant enterococci (VRE) in order to minimize patient to patient transmission. Infections may arise from translocation of enterococcal cells from GI tract to other part of the host or to hospital environment. This is due to the opportunistic pathogenic nature of enterococcus. Infections are caused by endogenous or exogenous sources with a virulent lineage and have the ability to cause outbreaks and infections of epidemic proportions. Thus the present study was done to demonstrate the efficacy of molecular methods in screening of antibiotic resistance and also to identify the virulence factors associated with VRE infections. The screening of resistance genes and virulence factor genes will help to find out the prevention protocols which in turn will play a major role in infection control.

## 2. MATERIALS AND METHODS

### *2.1. Study Design And Area*

The study was conducted at Sunrise Institute of Medical Sciences (SIMS), Kerala, India between January 2018 and March 2019. This study was reviewed and approved by the institutional ethical committee of Sunrise Institute of Medical Sciences (SIMS/IEC/02/2022).

### *2.1 Sample Collection and Isolation of Enterococcus*

The clinical specimens were collected from the hospital wards, ICUs and surgical wards of Sunrise Institute of Medical Sciences Kerala, India. Clinical samples were collected as per the standard aseptic protocols. Non-clinical samples from hospital surfaces and materials were collected by sterile cotton swabs, and the collected specimens were inoculated into 10 ml tubes containing BHI broth and incubated at 37° C for 24 h. The enriched cultures were inoculated into Bile Esculinazide agar plates and incubated at 37°C. After 48 h incubation. observation was done, if more than half the medium is dark brown or black which indicated that, Enterococcus hydrolyzed esculin in the presence of bile. Colonies similar to *Enterococcus* species were further identified by gram staining and standard biochemical analysis. Further identification and antibiotic sensitivity patterns of the isolates were done with the help of Vitek 2 Copmact system GP identification and AST P626 cards.

### *2.2 Molecular Identification Studies*

Molecular characterization was conducted to confirm the identification of isolates which were resistant to vancomycin. The total Genomic DNA of the three selected strains were isolated by MagGenome Xpress DNA isolation kit, as per the manufacturer instruction (MagGenome, India). The isolated DNA were checked for its Quality and Quantity by UV spectroscopy through 260/280 nm wavelength absorption. Species identification was done by 16s rDNA gene sequencing using the universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492 R (5'-TACGGYTACCTTGTTACGACTT-3'). The 16s rDNA of each sample was amplified with standard protocol. PCR was done in step one plus real time PCR. The purified PCR products were sequenced using BigDye™ Terminator v3.1 Cycle Sequencing Kit as per the manufacturer instruction (Applied Biosystems, USA). Editing and contig assembly of the DNA sequences were performed using Ugene Ver 1.3. For additional verification, all the 16S rDNA gene sequences were compared with nucleotide sequences in the GenBank database (http://blast.ncbi.nlm.nih.gov/ Blast.cgi) using the Basic Local Alignment Search Tool (BLAST) search algorithm. All DNA sequences have been deposited in the GenBank database with the accession numbers. The tree was constructed with the sequence data matrix of the 16S rDNA gene. The sequences were aligned using Clustal W 1.8. The variable and incomplete sites at both the 5′ and 3′ ends of the 16S rDNA gene sequences were excluded from the alignment. Various regions were further aligned using MEGA- X. Regions that could not be aligned unambiguously were excluded from the analysis. Phylogenetic trees were inferred using the neighbor-joining (NJ) algorithm with the Kimura two-parameter model in MEGA –X. The strengths of the internal branches of the resultant trees were statistically evaluated by bootstrap analysis with 1000 bootstrap replications.

### *2.3 Determination Of Antibiotics Susceptibility (By Disc Diffusion Method)*

The antibiotic sensitivity of clinical isolates was assessed by disc diffusion method followed by CLSI guidelines[14] to confirm the antibiotic resistance pattern of clinical isolates. The disc diffusion assay was performed in brain heart infusion agar (BHI). The overnight cultures of all clinical isolates were sub-cultured in BHI broth until to reach the turbidity of 0.5 Mcfarland (1 x $10^8$ CFU/ml) standards. The sterile cotton swabs were used to uniformly spread of clinical isolates on the agar plates, the known antibiotics discs such as chloramphenicol, gentamicin, ciprofloxacin, ampicillin, penicillin, erythromycin, streptomycin, imipenem, vancomycin, clindamycin, bacitracin B, norfloxacin, tetracycline, carbenzillin and clarithromycin (Hi-Media, Mumbai, India) were placed over the swabbed plates and incubated at 37° C for 24 h. After incubation, the zone of inhibition was measured at mm scale[14].

### *2.4 Determination of Minimum Inhibitory Concentration Of Vancomycin*

MIC was determined by micro-broth dilution test using sterile 96-well microtitre plates. Antibiotic stock solution was prepared by dissolving vancomycin powder in sterile distilled water, and the concentration was adjusted to 512 µg/ml. A 1:10 dilution of 0.5 McFarland Standard was used; 50 µl each of antibiotic dilutions and organism suspension were mixed and incubated at 37°C for 24 hrs. The highest dilution which inhibited growth was considered MIC. MIC ≥32 µg/ml was considered to be indicative of resistant isolates[15].

### *2.5 Screening For Virulence Associated Genes*

Genes associated with virulence of Enterococcal isolates (responsible for functional characteristics, including secreted factors and cell surface determination cluster), was performed by PCR. The gene specific primers are listed in **Table 4**. *GelE*, *agg*, *esp*, and *hyl* genes were used in this study. Appropriate virulence genes were used as positive controls and sterile water used as negative controls in all tests[16].

## 3. STATISTICAL ANALYSIS

All the experiments were performed in triplicate at least three times. The data were expressed as mean±sd. One-way ANOVA was performed using SPSS 20.0 software (SPSS, Chicago, IL, USA). Results with $*P < 0.05$ and $**P < 0.001$ were considered statistically significant.

## 4. RESULTS

### *4.1 Sample collection and Isolation of Enterococci*

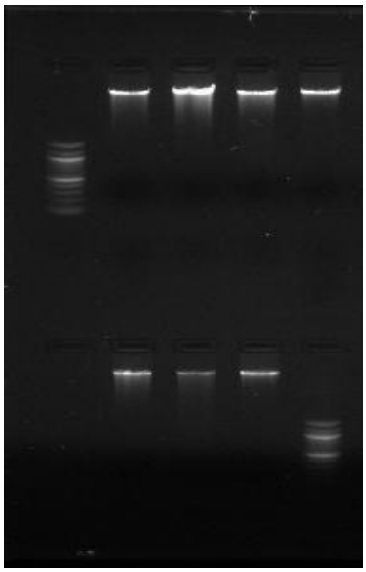
In this study, we collected 100 specimens in Sunrise Institute of Medical Sciences. Out of the samples, 93 enterococci were identified. The isolation rate of Enterococcus isolates was 93 % (93/100) in the present investigation. In the present study, four common species of Enterococci were isolated from 93 samples, *E. faecalis* (84.94%), *E. faecium* (12.90%), *E. raffinosus* (1.07%) and *E. avium* (1.07%). Of these, *E. faecalis* is a dominant species followed by *E. faecium*, *E. raffinosus* and *E. avium* (**Table 1**). The identification of isolates was performed with a fully automated identification system, Vitek 2 compact system (BioMerieux, Hazlewood, Mo.), with the help of Vitek GP identification cards and conventional biochemical tests[17].

**Table 1** Numbers of isolates of Enterococcal species from hospital wards

| Species | Isolates | Percentage of rate |
|---|---|---|
| *E. faecalis* | 79 | 84.94 % |
| *E. faecium* | 12 | 12.90 % |
| *E. raffinosus* | 1 | 1.07 % |
| *E. avium* | 1 | 1.07 |

### *4.2 Molecular identification*

The genomic DNA of the three selected isolates (JVA 1,2 & 3) were extracted and separated on agarose gel (**Figure 1**) and were extracted and subjected to PCR amplification. Consensus sequence of 16S rDNA gene was generated from forward and reverse sequence data using aligner software. The 16S rDNA gene sequence was used to carry out BLAST with the nrdatabase of NCBI genbank database. The sequences were submitted in GenBank database and the accession numbers are MK788123, MK788124, MK788125. Based on maximum identity score first ten sequences were selected and aligned using multiple alignment software program Clustal W. A distance matrix was generated using RDP database and the phylogenetic tree was constructed using MEGA X (**Figure 2**) and all the three strains were identified and confirmed as *Enterococcus faecium based* on sequencing and phylogenetic studies.



*The quality of the isolated DNA was analysed byusing 0.8% agaraose gel electrophoresis with ethidium bromide. Lane 1 & 10- 1Kb ladder, lane 2 to 9- isolated DNA of Entrococcus study strains.*

**Fig1: DNA isolated from the bacterial culture of Enterococcus Species**

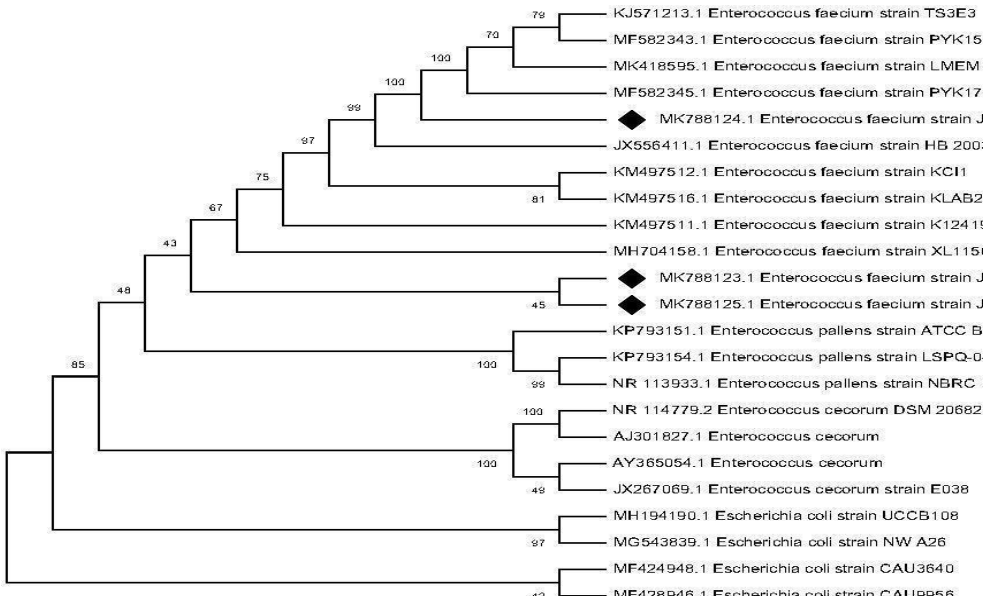



**Fig 2: Evolutionary relationships of taxa**

### *4.3 Antimicrobial resistance of Enterococcal isolates*

The present study showed that the most of the Enterococci isolates were multidrug resistant, ie., are resistant against conventional antibiotics groups such as aminoglycosides, glycopeptides, lincosamides, macrolides, β-Lactam, quinolones, polypeptides and others groups. (**Table 2 & 3 and Figure 3)**.

**Table 2** Antibiotic resistance profile of *Enterococcus species* by Kirby–Bauer disc diffusion method

| **Antibiotics** | ***E. faecium*** **N = 12** | ***E. faecalis*** **N = 79** | ***E. raffinosus*** **N = 1** | ***E. avium*** **N = 1** | **No. (%) isolate Total no.** |
|---|---|---|---|---|---|
| Gentamycin 120 | 10 | 70 | 0 | 0 | 86 % |
| Vancomycin | 12 | 75 | 0 | 0 | 93.5 % |
| Erythromycin | 11 | 68 | 0 | 0 | 84.9 % |
| Ampicillin | 12 | 79 | 1 | 1 | 100 % |
| Penicillin | 12 | 75 | 0 | 0 | 93.5 % |
| Ciprofloxacin | 10 | 70 | 0 | 0 | 86 % |
| Levofloxacin | 12 | 71 | 0 | 0 | 89.2 % |
| Tetracycline | 12 | 77 | 0 | 0 | 95.6 % |
| Linezolid | 10 | 69 | 0 | 0 | 84.9 % |

**Table 3** Antibiotic sensitivity Interpretive chart of *Enterococcus species* as per CLSI 2020

| Name Of Antibiotic Disc (Concentration) | Diameter of zone of inhibition(mm) | | |
|---|---|---|---|
| | Sensitive | Intermediate | Resistant |
| Ampicillin (10 µg) | >= 17 Mm | | <=16 Mm |
| Penicillin (10 Units) | >=15 Mm | | <=14 Mm |
| Linezolid (30 µg) | >=23 Mm | 22 – 21 Mm | <=20 Mm |
| Vancomycin (30 µg) | >= 17 Mm | 15 – 16 Mm | <=14 Mm |
| Gentamicin (120 µg) | | | |
| Ciprofloxacin (5 µg) | >=21 Mm | 16 -20 Mm | <=15 Mm |
| Levofloxacin (5 µg) | >= 17 Mm | 14 – 16 Mm | <=13 Mm |
| Nitrofurantoin (300 µg) | >= 17 Mm | 15 – 16 Mm | <=14 Mm |
| Tetracycline (30 µg) | >= 19 Mm | 15 – 18 Mm | <=14 Mm |
| Erythromycin (15 µg) | >=23 Mm | 14 – 22 Mm | <=13 Mm |
| Norfloxacin (10 µg) | >= 17 Mm | 13 -16 Mm | <=12 Mm |

**Figure 3:** Kirby-Bauer disc diffusion of the isolates

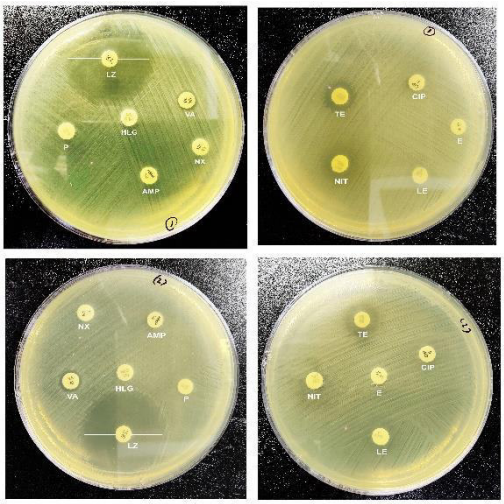



**Fig 3: LZ- Linezolid, VA- Vancomycin, NX- Norfloxacin, AMP- Ampicillin, P- Pencillin, HLG- Gentamycin 120, TE-Tetracycline, CIP- Ciprofloxacin, E- Erythromycin, LE- Levofloxacin, NIT-Nitrofurantoin**

### *4.4 Aminoglycoside resistance*

The present study found that Enterococci isolates were resistant against high level aminoglycosides. The high level aminoglycoside synergy was observed in 83.3 % of *E. faecium*, 88.6% of *E. faecalis* Notably, *E. raffinosus* and *E. avium* are susceptible to gentamicin.

### *4.5 Glycopeptides resistance*

Vancomycin is an important antibiotic agent in glycopeptide group. 100 % of *E. faecium* isolates were resistant to vancomycin followed by 94.9 % of *E. faecalis*. None of the *E. raffinosus and E. avium* isolates was resistant to vancomycin treatment.

### *4.6 Lincosamides and Macrolides resistance*

86% of *E faecalis*, 91.6% of *E. faecium*. *E. raffinosus* and *E. avium* were susceptible to erythromycin. At the same time, 87.3% of *E. faecalis*, 100% of *E. faecium*. And *E. raffinosus* and E. *avium* were susceptible to clarithromycin.

### β-Lactam and Quinolones resistance

100% of *E. faecalis*, 100% of *E. faecium*, 100% of E. roffinosus and 100% of *E. avium* isolates were resistant to ampicillin. The present study found that Enterococci isolates were resistant to quinolones group of antibiotics such as ciprofloxacin. The ciprofloxacin resistance was observed in 88.6 % of *E. faecalis*, 83.3 % of *E. faecium* and 100 % of *E. raffinosus* and 100 % of *E. avium*. And, 88 % of *E. faecalis*, 85 % of *E. faecium* were resistant to levofloxacin.

Other antibiotics such as tetracycline, linezolid were also tested for susceptibility. The results showed that 80% of *E. faecalis*, 80% *E. faecium*, were resistant to tetracycline and note that *E. raffinosus* and *E. avium* were susceptible to tetracycline. And, 87.3 % of *E. faecalis*, 83.3% of *E. faecium* were sensitive to linezolid.

### *4.7 Detection of vancomycin resistant strains*

The *E. faecium* and *E. faecalis* isolates were resistant to vancomycin, remaining isolates *E. raffinosus* and *E. avium* were susceptible to vancomycin. 12isolates of *E. faecium* and 75 isolates of *E. faecalis* were resistance to vancomycin (**Table4**)

.

| Table 4 Detection of vancomycicn resistant enterococcai species | | | | | |
|---|---|---|---|---|---|
| **Species** | **10 µg/ml** | **20 µg/ml** | **30 µg/ml** | **40 µg/ml** | **50 < µg/ml** |
| *E. faecium* | - | 2 | 5 | 6 | 6 |
| *E. faecalis* | - | 2 | 3 | 29 | 21 |

### *4.8 Physiological test*

The results showed the presence of different enzymatic virulence activity such as hemolysis, gelatinase, DNase and lipase activity. The maximum level of hemolysis activity was exhibited by *E. faecium* (71%). Gelatinase enzymatic activity was exhibited by *E. faecium* (68%) followed by *E. faecalis* (32%). And DNase activity was observed, 58% of *E. faecium* strains exhibited DNase activity followed by *E. faecalis* 17. 6 %. Also, lipase activity was exhibited by only *E. faecium* strains (43.7%).

### *4.9 Screening of antimicrobial resistance genes*

The study has confirmed the carriage of different antimicrobial resistance genes. Among the isolates the vancomycin resistance genes *vanA* and *vanB* were detected in 75.4% and 74.5% of the strains. Similarly, the tetracycline resistance genes *tetA* were detected in 46.2% of the strains. Further, erythromycicn resistance gene *ermA* was detected in 41.5% of the strains. (**Table 5 & 6).**

| **Table 5:** Primers used for detection of Antibiotic resistance genes. | | |
|---|---|---|
| **Genes** | **Oligonucleotide sequences (5'-3')** | **Length (bp)** |
| *tetA* | GGCACCGAATGCGTATGAT<br>AAGCGAGCGGGTTGAGAG | 480 |
| *vanA* | GCGCGGTCCACTTGTAGATA<br>TGAGCAACCCCCAAACAGTA | 314 |
| *vanB* | AGACATTCCGGTCGAGGAAC<br>GCTGTCAATTAGTGCGGGAA | 220 |
| *ermA* | TCTAAAAAGCATGTAAAAGAA<br>CTTCGATAGTTTATTAATATTAGT | 645 |
| 16S rDNA | AGAGTTTGATCMTGGCTCAG<br>TACGGYTACCTTGTTACGACTT | 1506 |

| **Table 6** Prevalence and distribution of antimicrobial resistance genes among the Enterococcus isolates. | | | | | | |
|---|---|---|---|---|---|---|
| **Antibiotics Resistance genes** | ***Antibiotic* Resistance Phenotype[a]** | ***E. faecium* N = 12** | ***E. faecalis* N = 79** | ***E. raffinosus* N = 1** | ***E. avium* N = 1** | **No. (%) isolate Total no.** |
| *vanA* | VAN | 10 | 59 | 0 | 0 | 74.1 % |
| *vanB* | VAN | 9 | 59 | 0 | 0 | 73.1 % |
| *tetA* | TET | 5 | 40 | 0 | 0 | 48.3 % |
| *ermA* | ERY | 4 | 40 | 0 | 0 | 47.3 % |

***[a] VAN – Vancomycin, TET – Tetracyclin, ERY – Erythromycin***

### *4.10 Occurrence of virulence genes in Enterococci*

The occurrence of a group of virulence genes detected in all isolates and the primers used for the detection are shown in **Table 7** and **Table 8** and the gel photos of PCR are showed in **figure 5**. In particular *GelE*, 114 (53%), *agg*, 189 (89%), *hyl*, 143 (67%) and *esp*, 110 (51. 8%) were confirmed. Only three *E. faecium* isolates possessed all the tested virulence genes, while 7 *E. raffinosus* and 2 *E. avium* strains carried none of the tested virulence genes. It indicated that *E. faecium* had a potential for higher virulence and *E. raffinosus* and *E. avium* were comparatively less virulent. It was brought out that there was a correlation between biofilm formation and the presence of the *ace* gene in *E. faecium* ($P < 0.001$). The possession of *GelE*, genes were mainly found in *E. faecium* ($P < 0.005$) and these genes had a statistical significance with the formation of biofilms among *E. faecium*.

| **Table 7** Primers used for detection of virulence genes. | | |
|---|---|---|
| **Genes** | **Oligonucleotide sequences (5' -3')** | **Length (bp)** |
| *gel*E | ACCCCGTATCATTGGTTT<br>ACGCATTGCTTTTCCATC | 419 |
| *agg* | CACGTAATTCTTGCCCACCA<br>AAACGGCAAGACAAGTAAATA | 520 |
| *esp* | CGGTCATACCGACGACCAAA<br>TGTCACATCGCCATCGACTT | 745 |
| *hyl* | ACAGAAGAGCTGCAGGAAATG<br>GACTGACGTCCAAGTTTCCAA | 276 |

**Table 8** Prevalence and distribution of virulence genes among Enterococcus sp .

| Virulence genes | *E. faecium* (N = 12) | *E. faecalis* (N = 79) | *E. raffinosus* (N = 1) | *E. avium* (N = 1) | No. (%) isolate Total no. |
|---|---|---|---|---|---|
| *cylA* | 4 | 7 | 0 | 0 | 11 (11.8 %) |
| *gelE* | 8 | 5 | 0 | 0 | 13 (13.9 %) |
| *agg* | 4 | 12 | 0 | 0 | 16 (17.2 %) |
| *hyl* | 6 | 4 | 0 | 0 | 10 (10.7 %) |

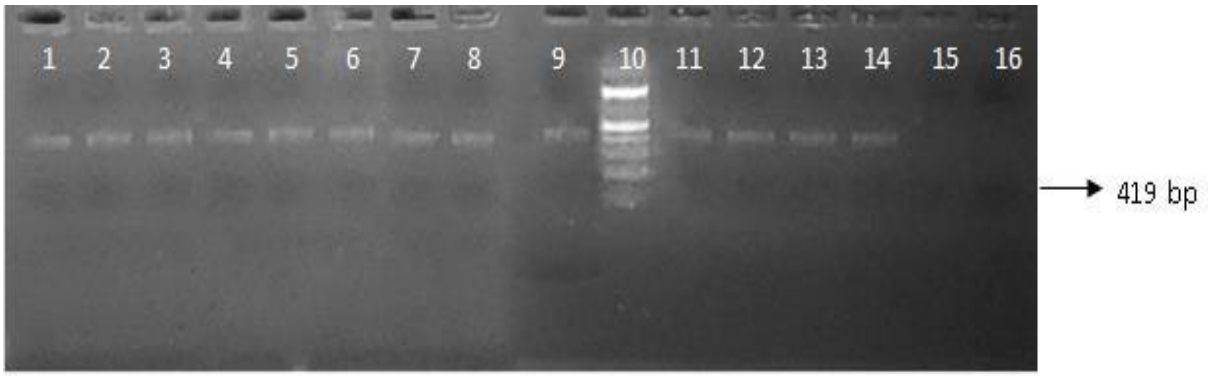



**Fig 4(a)**

Representative gel of presence of virulence factor gelE in the isolates were screened with the gene specify primers presences of 419 bp amplicon confirms the presences of virulence gene. Lane 10- 100 bp ladder except lane 16 and 15 all other isolates shows positive for gelE gene

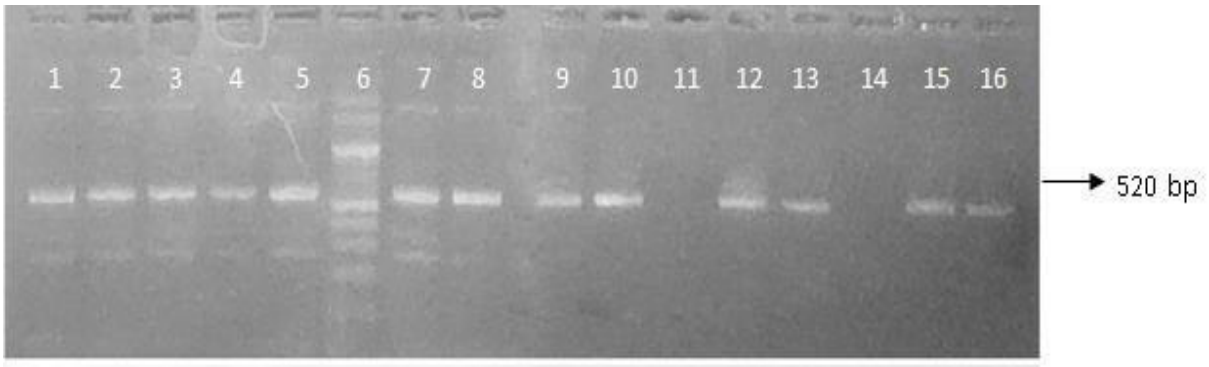



**Fig 4(b)**

Representative gel of presence of virulence factor agg in the isolates were screened with the gene specify primers presences of 520 bp amplicon confirms the presences of virulence gene. Lane 6- 100 bp ladder except lane 11 and 14 all other isolates shows positive for agg gene

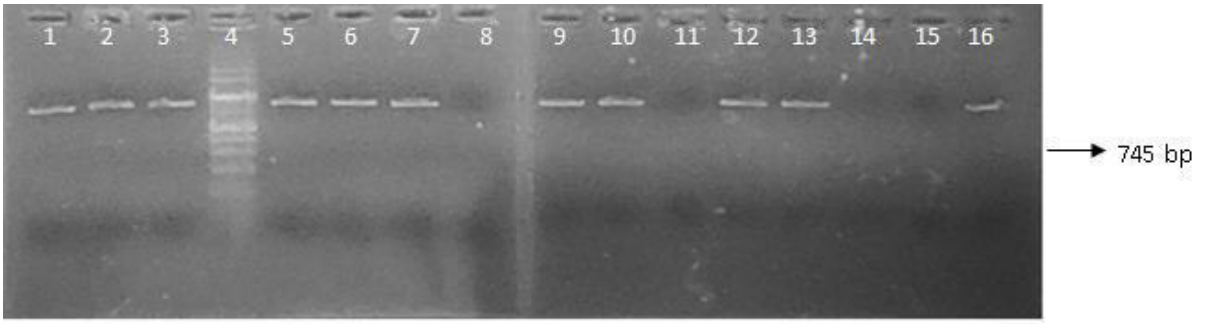



**Fig 4(c)**

Representative gel of presence of virulence factor esp in the isolates were screened with the gene specify primers presences of 745 bp amplicon confirms the presences of virulence gene. Lane 4- 100 bp ladder except lane 8, 11, 14 and 15 all other isolates shows positive for esp gene

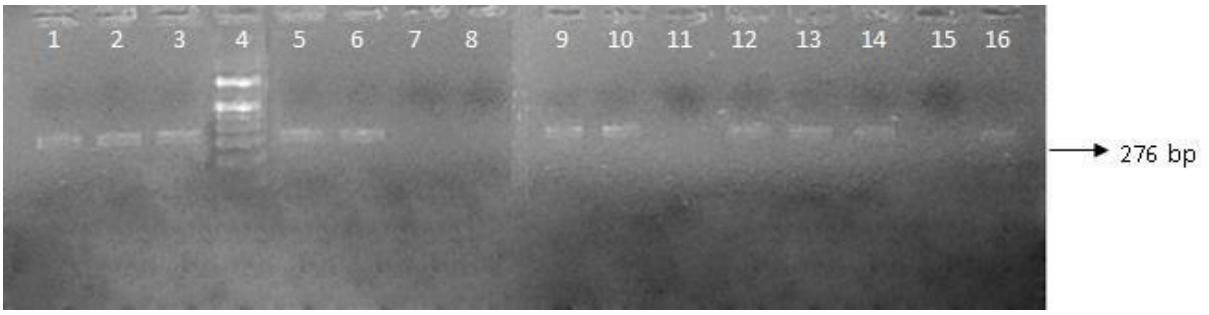



**Fig 4(d)**

Representative gel of presence of virulence factor hyl in the isolates were screened with the gene specify primers presences of 276 bp amplicon confirms the presences of virulence gene. Lane 4- 100 bp ladder except lane 7, 8, 11 and 15 all other isolates shows positive for hyl gene

## 5. DISCUSSION

Enterococci are an exclusive pathogen for humans/animals, the rapid increasing of enterococci resistance against antibiotics will leads to increased mortality and morbidity rate in clinical settings[1]. The present study investigated the prevalence of enterococci and antimicrobial resistance patterns of enterococci isolated from the hospital wards including ICU wards that have high risk for enterococci colonization. Multidrug resistant *E. faecium* has caused invasive infections, not only for *E. faecium*, *E. faecalis* also joined together to caused nosocomial infections[1]. But the available data exhibit that *E. faecium* caused a large number of nosocomial infections compared than *E. faecalis*[18]. The isolates obtained from the present study *E. faecium* (12.90%) followed by *E. faecalis* (84.94%) while *E. raffinosus* and *E. avium* observed by (1.07%) and (1.07%) respectively (**Table. 4**). The obtained species distribution was comparable to the distribution of enterococcal species in other studies[19]. The present study showed that *E. faecalis* is predominant enterococci species followed by *E. faecium*, which is in contrast with a previous study from the Egypt recorded that *E. faecium* as a dominant species followed by *E. faecalis*[19]. A total of 15 antibiotics groups were used to detect the enterococci antimicrobials susceptibility. The enterococci isolates have the ability of resistance against penicillin and ampicillin. Besides, *E. faecium* is less susceptible than *E. faecalis* against β-lactam antibiotics, behind the reason is enterococci cell wall are low affinity to penicillin binding proteins[20]. The present study also exhibits a similar result, *E. faecalis* resistance rate is lower than the *E. faecium*. The result was compared with previous study[21] also documented that *E. faecalis* lower resistance than the *E. faecium* against ampicillin. Although *E. faecium* and *E. faecalis* isolates were resistant to penicillin which is similar level of resistance reported from the previous study[22]. In this study, the antibiotics resistance phenotype and their respective resistance genes were detected among the Emterococcus isolates. Tetracycline resistance genes like *tetA* were confirmed among the isolates (**Table. 6)**. The erythromycin resistance genes *ermA* were identified among the erythromycin resistant enterococci. The present findings were in accordance with the previous studies[23, 16] interestingly. The oxazolidinones group of antibiotics such as tedizolid and linezolid were reported to be very effective in treating of Gram positive multidrug resistance bacterial infections. Noteworthy, these virulence genes were noted to be generally linked with mobile genetic elements of horizontal transmission[24,25] and the study suggest that, regular monitoring of oxazolidinone resistant enterococci is required for prevent and control the spread of resistant genes to the humans/animals and environments. Similarly, quinolones resistance genes such as *qnrA*, *qnrB*, *qnrS* and *qepA* were significantly present in the quinolones (ciprofloxacin and norfloxacin) resistant enterococci isolates. Furthermore, virulence genes were detected which are responsible for biofilm formation, hemolysis and gelatinase activity. We found that *esp* gene present in the *E. faecium* and *E. faecalis* isolates. An *esp* gene is responsible to regulate the biofilm formation. The presence of *esp* could be a possible reason for enterococci isolates biofilm formation. And *ace*, genes also involved in enterococci biofilm development, in particular, *E. faecium* and *E. faecalis* biofilm development[26]. The present study found that *esp* gene expression in biofilm forming enterococci isolates. Notably, *esp* highly present in *E. faecium* compared than *E. faecalis* (**Table. 8 and Figure 4).** Hemolysis (hyl) and protease gelatinase (GelE) plays an important role in enterococcal pathogenesis[27]. These genes were found in higher level in *E. faecium* isolates.[28]

## 6. CONCLUSION

The findings of the study revealed that 89.1 % isolated strains were multidrug resistant and a total of 15 antibiotic resistance genes were detected. In particular, the commonality of antibiotic resistance and possession of various virulence factors among the enterococcal isolates is a serious cause of concern. The present screening also indicated that a hospital wards would be the common reservoir of enterococcus pathogens. This study strongly suggested that appropriate sterilization of article/places is required with prepared surveillance additives covering the place so as to contain any further spread. The development of antibiotic resistance genes and virulence genes in enterococci from hospital wards including ICU wards is a serious global concern because they can able to transfer to humans via while used those things. Finally, the present investigates that hospital wards are reservoirs of antimicrobial resistant enterococci with possible virulence genes. Therefore, we suggest sterilization and surveillance programs to control the enterococci appearance in environments.

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## 8. CONFLICTS OF INTEREST

Conflict of interest declared of none.

## 9. AUTHOR CONTRIBUTION

Mr. Jijo G Vaghese conceived and planned the experiments. Mr. Jijo G Vaghese and Ms. Fiji E carried out the experiments with the support from Dr. B. Anandharaj All authors discussed the results and contributed to the final manuscript. Dr. B. Anandharaj supervised the entire project.

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