Original Research Article

# Charecterisation and resistance pattern of enterococcus species isolated from different clinical specimens in Rohilkhand region

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**Received: 24-05-2021 / Revised: 16-06-2021 / Accepted: 16-08-2021**

**Abstract**

**Objective:** The objective of this study was to isolate and characterize *Enterococcus* species on the basis of biochemical test, antibiotics susceptibility pattern, especially in regard to vancomycin resistance and high level amino glycoside resistance from various clinical specimens of patients attending the outdoor or admitted in Rohilkhand Medical College and Hospital. **Methods** This study includes 6572 different clinical specimens including urine, blood, pus, vaginal swab, throat swab, ascitic fluid, pleural fluid, cerebrospinal fluid and tissue pieces received for bacteriological culture in the department of microbiology. All these specimens were cultured and screened for growth of enterococci and suspected growths were isolated and identified. **Results** In this study, 100 *Enterococci* were isolated and identified. Out of 100 Enterococci isolated from various clinical specimen 56 were from urine, 33 from pus, 04 from vaginal swabs, 05 from blood cultures and 02 from body fluids (ascitic fluid). No Enterococci were isolated from catheter tip, CSF and pleural fluid. Seventy five isolates gave the biochemical tests and other characters similar to E. faecalis out of 100 isolates. Twenty isolates were identified as E. faecium, two were identified as E. avium, one each of E.durans, E. dispar and E. raffinosus. **Conclusion** Species identification is useful for epidemiological investigation of an outbreak and also for clinical decisions, particularly with regards to therapy as antimicrobial susceptibility differs in different species. This will not only help in initiation of effective therapy but also in the implementation of effective infection control measures.

**Keywords**: Epidemiological Investigation, Antimicrobial Susceptibility, Clinical Specimens Cerebrospinal Fluid, Bacteriological Culture Enterococci.



## Introduction

Enterococci are indigenous flora of intestinal tract, oral cavity and genitourinary tract of human and animals. They are important opportunistic pathogen especially in hospitalised patients[1,2]. Enterococci were traditionally regarded as low grade pathogens but have emerged as an increasingly important cause of nosocomial infections in the 1990s and the second most common opportunistic pathogen reported to the National Nosocomial Infection Surveillance (NNIS) system. The Centre for Disease Control and Prevention, in a survey on nosocomial infections, indicated that Enterococcus accounted for 13.9% infections, being next to Escherichia coli as a causative agent of hospital acquired urinary tract infections. Enterococci are capable of causing UTI, intra abdominal or pelvic wound infections, biliary tract infection and bacteremia. It also causes serious infections like endocarditis, meningitis and soft tissue infections[3,4].

In earlier Indian studies, *E. faecalis* has accounted for approximately 80-90% of clinical isolates, while *E.faecium* was isolated in the remaining 5-15% of cases[4,5].However, in recent times, a shifting spectrum of Enterococcal infections is being reported from different parts of the world with an increasing proportion being caused by *E. faecium*. This finding is important since E. faecium strains display a higher degree of drug resistance.*Enterococci* are intrinsically resistant to a wide range of antibiotics most notably beta-lactams and aminoglycosides, which are frequently used to treat infections with Gram positive cocci[9].

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In addition *Enterococci* have ability to acquire resistance to antimicrobial agents through transfer of plasmids, transposes, chromosomal exchange or mutations. Infection by *Enterococci* have traditionally been treated with cell wall active agents (eg- Penicillin and Ampicillin) in combination with an amino glycosides (streptomycin and Gentamycin).However emergence of high level resistance to amino glycosides (HLAR), β-Lactum antibiotics and MDR strains has led to failure of synergetic effect of combination therapy. The Vancomycin resistant *Enterococci* (VRE) mainly cause infection in acute care setting patients (like intensive care units), when the stay is increased for 15 days or more[12].Such a colonization pressure plays an important role in transmission of resistant organisms in hospitals. These patients transferred from such settings to the wards and other hospitals play the pivotal role in transmission of multidrug resistant *Enterococci*, so it requires strict enforce CDC guidance for infection detection, prevention, tracking, and reporting[13].The most common mode of multi-drug resistant organism transmission is via the hands of health care workers. Hands become transiently contaminated by contact with infected or colonized patients, or by contact with environmental surfaces in close proximity to the patient.Hence CDC has given same importance to multidrug resistant *Enterococci*, with that of Methicillin Resistant Staphylococcus aureus (MRSA) and Extended spectrum beta lactamases (ESBL) as a nosocomial pathogens.

Due to these factors, it has become important to study and discuss the spectrum of enterococcal infections along with their geographical distribution and antimicrobial resistance pattern. Species identification is useful for epidemiological investigation of an outbreak and also for clinical decisions, particularly with regards to therapy as anti-microbial susceptibility differs in different species. This will not only help in initiation of effective therapy but also in the implementation of effective infection control measures.

## Materials and methods

### Place of study

The present study was conducted in the department of microbiology Rohillkhand Medical College and Hospital.

**Duration of study**

December 2012 to December 2013.

**Sample size**

The study comprise of all clinical specimen like pus, blood, urine, body fluid, high vaginal swabs, swabs from surgical and non-surgical wound tissues, referred for bacterial culture and antibacterial sensitivity testing from patients of all age group and both sex who attended the O.P.D or were admitted in different wards of Rohilkhand medical college and hospital, Bareilly.

**Inclusion criteria**

Various samples like urine, pus ,blood, body fluids like pleural peritoneal fluid etc were screened for Enterococci for a period of 1year (Dec 2012 to Dec20013) and 100 isolates were taken for characterization. Detailed history of all the patients was recorded keeping in view of any significant part illness including, diabetes, chronic renal failure, malignancy, BPH, tuberculosis, any illness causing prolonged hospitalization.

**Exclusion criteria**

Samples without growth of *Enterococcus* species and also samples like stool, throat swab, sputum etc where they are present as normal commensal.

**Observation chart**

**Laboratory procedure**

**Part I (Identification and Specification of enterococci)**

(a) Culture of all specimens
(b) Identification of the enterococci species
(c) Characterization on the basis of biogrouping.

Following biochemical test were performed to identifying the isolated species in clinical materials.

- Growth in 6.5% NaCl
- Starch and bile aesculin hydrolysis
- Production of acetoin
- Pyruvate utilization
- Arginine decarboxylation
- Gelatin liquefaction
- Haemolysin production
- Tellurite reduction

**Part-II (Pattern of antibiotics resistance)**

(a) In vitro Antibiotic susceptibility enterococci isolates were carried out by disc diffusion method of Kirby-Bauer.
(b) High-level resistance to gentamicin and streptomycin by disc diffusion method and screen agar.
(c) MIC was determined by agar dilution method in strains resistant to vancomycin by disc diffusion method.
(d) Clinical correlation with regard to isolates of enterococci.

**Table 1: Distribution of enterococci isolated from various clinical specimens**

| Samples | Number of samples | No. of Enterococci |
|---|---|---|
| Urine | 2160 | 56 |
| Blood | 1280 | 5 |
| Pus | 1400 | 33 |
| Vaginal swab | 91 | 4 |
| Body fluids | 1641 | 2 |
| **Total** | 6572 | 100 |

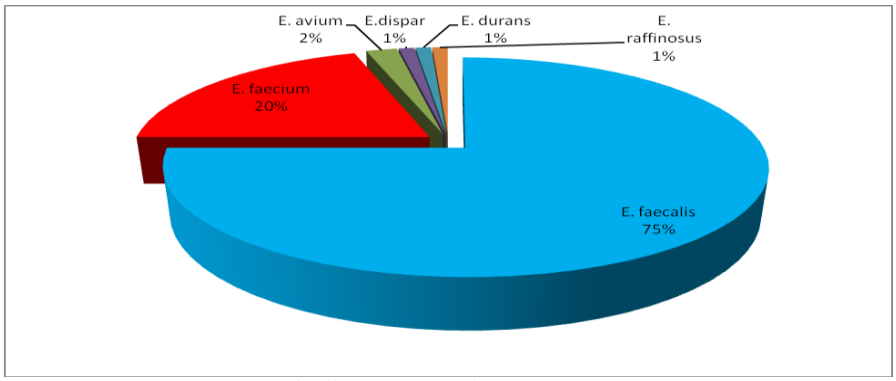


**Fig 1: Percentage of different species of *enterococci* isolated in this study**

**Table 2: Biochemical characterization of enterococcus species isolated ***

| No. of Isolates (n=100) | MAN | SOR | ARG | ARA | SBL | RAF | TEL | MOT | PIG | SUC | PYU | Species |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 75 | + | - | + | - | + | - | + | - | - | + | + | E. faecalis |
| 20 | + | - | + | + | V | V | - | - | - | + | - | E. faecium |
| 2 | + | + | - | + | + | - | - | - | - | + | + | E. avium |
| 1 | - | - | + | - | - | + | - | - | - | + | + | E. dispar |
| 1 | - | - | + | - | - | - | - | - | - | - | - | E. durans |
| 1 | + | + | - | + | + | + | - | - | - | + | + | E. raffinosus |

**Table 3: Species distribution of *enterococci* in various clinical specimens**

| Sample | Total No. of *Enterococci* | E. faecalis | E. faecium | E. dispar | E. durans | E. avium | E. raffinosus |
|---|---|---|---|---|---|---|---|
| Urine | 56 | 42 | 13 | - | - | 1 | - |
| Blood | 5 | 4 | 1 | - | - | - | - |
| Pus | 33 | 28 | 2 | 1 | 1 | 1 | - |
| Vaginal Swab | 4 | 1 | 2 | - | - | - | 1 |
| Other Samples(body fluids | 2 | 0 | 2 | - | - | - | - |
| Total | 100 | 75 | 20 | 1 | 1 | 2 | 1 |

**Table 4: Number of *enterococcus* isolated from clinical specimens of inpatients and outpatients.**

| Specimen | Species | No. of Isolates | IPD (n=2184) | OPD (n=1010) |
|---|---|---|---|---|
| Urine | E. faecalis | 42 | 20 | 22 |
| | E. faecium | 13 | 11 | 2 |
| | Others-(E.avium) | 1 | 0 | 1 |
| Blood | E. faecalis | 4 | 4 | 0 |
| | E. faecium | 1 | 1 | 0 |
| | Others | 0 | 0 | 0 |
| Others(pus, Vaginal swab, body fluids) | E. faecalis | 29 | 19 | 10 |
| | E. faecium | 6 | 5 | 1 |
| | Others-(E.avium, E.dispar, E.durans, E.raffinosus) | 4 | 3 | 1 |
| | **Total** | **100** | **63** | **37** |

**Table 5: Antibiotic sensitivity pattern of urinary isolates**

| Antibiotic | E. faecalis (n=42) | | | E. faecium (n=13) | | | Other spp. (n=1) | | |
|---|---|---|---|---|---|---|---|---|---|
| | S | I | R | S | I | R | S | I | R |
| **A** | 12 | 2 | 28 | 4 | - | 9 | 1 | - | - |
| **T** | 8 | 4 | 30 | 3 | - | 10 | - | - | 1 |
| **HSG** | 22 | 1 | 19 | 6 | 2 | 5 | 1 | - | - |
| **S** | 23 | 1 | 18 | 9 | 2 | 2 | - | - | 1 |
| **Nx** | 4 | 3 | 35 | 2 | 1 | 10 | - | - | 1 |
| **Va** | 41 | - | 1 | 13 | - | - | 1 | - | - |
| **Te** | 28 | 3 | 11 | 6 | 1 | 6 | 1 | - | - |
| **Lz** | 42 | - | - | 13 | - | - | 1 | - | - |
| **Pm** | 18 | - | 24 | 3 | - | 10 | - | - | 1 |
| **Nt** | 32 | 3 | 7 | 10 | 1 | 2 | 1 | - | - |

**Table 6: Antibiotic sensitivity pattern of enterococci isolated from blood**

| Antibiotic | E. faecalis (n=4) | | | E. faecium (n=1) | | | Other spp. (n=0) | | |
|---|---|---|---|---|---|---|---|---|---|
| | S | MS | R | S | MS | R | S | MS | R |
| **A** | 1 | - | 3 | - | - | 1 | - | - | - |
| **T** | 2 | - | 2 | - | - | 1 | - | - | - |
| **G** | 2 | - | 2 | 1 | - | - | - | - | - |
| **Sm** | 2 | - | 2 | 1 | - | - | - | - | - |
| **Cp** | 2 | - | 2 | - | - | 1 | - | - | - |
| **E** | 3 | - | 1 | - | - | 1 | - | - | - |
| **Va** | 3 | - | 1 | 1 | - | - | - | - | - |
| **Te** | 4 | - | - | 1 | - | - | - | - | - |
| **Lz** | 4 | - | - | 1 | - | - | - | - | - |
| **Pm** | 2 | - | 2 | - | - | 1 | - | - | - |

**Table 7: Antibiotic sensitivity pattern of enterococci isolated from pus, vaginal swabs and other body fluids**

| Antibiotic | E. faecalis (n=29) | | | E. faecium (n=6) | | | Other spp. (n=4) | | |
|---|---|---|---|---|---|---|---|---|---|
| | S | MS | R | S | MS | R | S | MS | R |
| **A** | 10 | 1 | 18 | 1 | - | 5 | 2 | - | 2 |
| **T** | 4 | - | 25 | 1 | - | 5 | 1 | - | 3 |
| **G** | 12 | 2 | 15 | 2 | - | 4 | 0 | - | 4 |
| **Sm** | 14 | - | 15 | 4 | - | 2 | 2 | 1 | 1 |
| **Cp** | 9 | - | 20 | 4 | - | 2 | - | - | 4 |
| **E** | 4 | - | 25 | 1 | - | 5 | - | - | 4 |
| **Va** | 28 | - | 1 | 5 | - | 1 | 4 | - | - |
| **Te** | 27 | 1 | 1 | 6 | - | - | 4 | - | - |
| **Lz** | 29 | - | - | 6 | - | - | 4 | - | - |
| **Pm** | 7 | 3 | 19 | 1 | - | 5 | 2 | - | 2 |

**Results**

This study includes 6572 different clinical specimens including urine, blood, pus, vaginal swab, throat swab, ascitic fluid, pleural fluid, cerebrospinal fluid and tissue pieces received for bacteriological culture in the department of microbiology, Rohilkhand Medical College and Hospital during the period of December-2012 to December-2013.All these specimens were cultured and screened for growth of E*nterococci* and suspected growths were isolated and identified. In this study, 100 *Enterococci* were isolated and identified. Out of 100 *Enterococci* isolated from various clinical specimen 56 were from urine, 33 from pus, 04 from vaginal swabs, 05 from blood cultures and 02 from body fluids (ascitic fluid). No *Enterococci* were isolated from catheter tip, CSF and pleural fluid. 75 isolates gave the biochemical tests and other characters similar to E. faecalis out of 100 isolates. Twenty isolates were identified as E. faecium, two were identified as E. avium, one each of E.durans, E. dispar and E. raffinosus.

All 04 isolates of E. faecalis were sensitive to teicoplanin and linezolid Out of 04 isolates of E. faecalis, 03 were resistant to ampicillin and 01 sensitive to it. Two were resistant and 02 were sensitive to each of tetracycline, high strength gentamicin,high strength streptomycin and ciprofloxacin. One was resistant and 03 were sensitive to erythromycin and vancomycin. Two were resistant and 02 sensitive to pristinomycin. Out of 75 E. faecalis strains, 42 were isolated from urine, 04 from blood, 28 from pus, 01 from vaginal swabs and none isolated from other samples. Out of 20 E.

faecium strains, 13 were isolated from urine, 01 from blood, 02 from pus, 02 from vaginal swab and 02 from other samples. One E. avium was isolated from pus and 01 from urine. One each of E.dispar and E.durans were isolated from pus and 01 E.raffinosus isolated from high vaginal swab.

One E. faecium was isolated from blood and was resistant to ampicillin, tetracycline, ciprofloxacin and erythromycin. It was sensitive to high strength gentamicin, streptomycin, vancomycin, teicoplanin linezolid and pristinomycin. Out of the 100 isolates, 63 were isolated from the hospitalized patients and only 37 isolates were from the outdoor patients.56 isolates were from urine samples containing 42 E. faecalis, 13 E. faecium and 01 other species. Out of the 42 E. faecalis isolates 20 were from admitted patients and 22 from outdoor patients. From urine samples out of 13 E. faecium isolates, 11 were from admitted and 02 from outdoor patients. 01 E.avium species was isolated from outdoor patient. Five isolates were from blood samples in which 04 were from E.faecalis and 01 from E. faecium. 39 isolates were from pus, vaginal swab, body fluids containing 29 E. faecalis, 06 E. faecium. Out of the 29 E. faecalis isolates 19 were from admitted patients and 10 from outdoor patients. Out of 06 E. faecium isolates from pus, vaginal swabs, body fluids samples, 05 were from admitted and 01 from outdoor patients. One each species of E.avium, E.dispar, E.durans and E.raffinosus were isolated in which 03 were from admitted and one from outdoor patient.

In 42 E. faecalis isolates, 28 were resistant to ampicillin and 30 to tetracycline.Thirty five isolates were resistant to norfloxacin. Eighteen were resistant to high strength streptomycin, 19 were resistant to high strength gentamicin. One isolates were resistant to vancomycin, 11 were resistant to teicoplanin. Twenty four were resistant to pristinomycin and 07 to nitrofurantoin. All were sensitive to linezolid.

In 13 E. faecium isolates, 09 were resistant to Ampicillin, 10 were resistant to tetracycline.Five isolates were resistant to high strength gentamicin and 02 were resistant to streptomycin (high level). Ten were resistant to Norfloxacin. One isolates were resistant to vancomycin. 06 isolate were resistant to teicoplanin. All were sensitive to linezolid. One E. avium isolates, was resistant to tetracycline, norfloxacin and pristinomycin. It was resistant to high strength streptomysin were as sensitive to high strength gentamycin. It was sensitive to vancomycin, teicoplanin, linezolid and nitrofurantoin.

Out of 29 E. faecalis strains 18 were resistant, 10 were sensitive and 01 was intermediate sensitive to ampicillin. Twenty five isolates were resistant and 04 sensitive to tetracycline. Fifteen isolates were resistant, 12 sensitive and 02 intermediate sensitive to gentamicin. Fifteen isolates were resistant and 14 were sensitive to high strength streptomycin. Twenty isolates were resistant and 09 sensitive to ciprofloxacin and twenty five were resistant and 04 sensitive to erythromycin. One isolate was resistant, 01 intermediate sensitive and 15 sensitive to vancomycin, teicoplanin and linezolid. Nine isolates were resistant, 03 intermediate sensitive and 05 sensitive to pristinomycin .Out of 6 E. faecium, 05 isolates were resistant and 01 sensitive to ampicillin, tetracycline, erythromycin and pristinomycin. Two isolates were resistant and 04 sensitive to high strength gentamicin, high strength streptomycin and ciprofloxacin. All 06 were sensitive to vancomycin, teicoplanin and linezolid. Among other 04 species, all were resistant to streptomycin, erythromycin and ciprofloxacin and were sensitive to vancomycin, teicoplanin and linezolid. 02 isolates were resistant and 02 sensitive to ampicillin and Pristinomycin.

**Statistical analysis**

Data was compiled using MS excel 2007 and analysis was done with the help of Epi-Info 7 software. Frequency and percentage were calculated & statistical test (Chi Square) was applied wherever applicable; $p<0.05$ was taken as statistically significant.

**Discussion**

*Enterococci* are gram positive cocci presenting as harmless commensals to multifaceted deadly pathogens. The most frequent infection caused by them is urinary tract infections followed by wound infection and blood infections. Although about a dozen of *Enterococcus* species have been identified, only two are responsible for the majority of human infection i.e *Enterococcus faecalis* and *Enterococcus faecium*. Although less frequently or even rarely, several of the other enterococcal species, including E. avium, E. cecorum, E. dispar, E. durans, E. gallinarum, E. gilvus, E. hirae, E. mundtii, E. pallens, E.feacalis variant strains, have also been isolated from human sources[3]

Infection rate of *Enterococcus* in Indian population varies in different geographic areas. In this study, 100 *Enterococci* were isolated from 6572 bacteriological specimen accounting the infection rate to be 1.52 percent. S. Sreeja et al[14] from Bangalore found an infection rate of 2.3 percent in their study over 5555 clinical samples while Jyotsna Agarwal et al. in 2009 from Lucknow found an isolation rate to be 1.46 percent[15]. Rupali S Shinde et al .in 2012, from Mumbai found an isolation rate of 5.5 percent during the study period of one year, 54 isolates of *Enterococci* were collected from 980 culture-positive samples[16]. Suddhanshu Bhardwaj et al. in 2013 from Bhopal found an infection rate of 1.66 percent. They isolated 150 *Enterococci* species from 9024 clinical samples like urine, blood, pus, CSF, stool, asceptically collected fluids and aspirates from patients[17].

The maximum number of *Enterococcus* strains isolated in this study was from urine specimen (56%) followed by pus (33%) and blood (5%). Only 6% of *Enterococcus* strains were isolated from body fluids which include vaginal swab (4%), ascitic fluid (2%). No Enterococcus was isolated from pleural fluid and CSF. Among these patients with Enterococcal urinary tract infection, 25% were from OPD and 31% from patient admitted in different wards. Catheterization, genitourinary tract surgeries, prolong use of third generation cephalosporin were amongst the greatest risk factors found. The number of *Enterococcus* isolated from pus was 33% and most of these isolated strains were from orthopaedics and surgery wards. *Enterococcus* isolated from blood was 5 %. Most of these isolated strains were from paediatrics ward and NICU. S. Sreeja et al. isolated 31% *Enterococcus* species from urine specimen out of 128 *Enterococcus* but the maximum number of isolates were from pus (43 %).[14] Baragundi Mahesh et al. in 2010 found 50 (41.6%) enterococcal isolates from urine out of total 120 Enterococcus followed by blood, pus and body fluids.[18] Gosh Reena et al. in 2011, from Kolkata isolated 46.49% *Enterococus* from urine. A total of 73 *Enterococcus* strains were isolated from urine and the species were E.faecalis 52, E.faecium 7, E.casseliflavus 2, E.dispar 8 and 4 E.durans[19]. Preeti Srivastava et.al in 2013 from north India isolated 70 % *Enterococcus* from urine and E. faecalis 92% constituted the predominant isolate followed by E.faecium 8%.[20]

Most commonly isolated *Enterococcus* species in India are E. faecalis followed by E. faecium. In this study, E.faecalis were isolated in 75% of total specimens and E.faecium in 20% of the specimens. Very few unusual species of *Enterococci* (non faecalis, non faecium) were identified which included E.avium (2%), E.durans (1%), E.raffinosus (1%), E.dispar (1%). In few studies from central India more number of unusual species of *Enterococcus* was isolated. This could be due to the geographical variation in distribution of the Enterococcal species in different areas. Mendiratta et al in 2008 from Maharashtra recovered 85.3% E.faecalis, 14.7% E.faecium from various clinical specimens.[21]VA Rahangdale et al in the same year from Nagpur recovered 123 enterococcal strains of which 64.23% were E. faecalis, 32.52% E. faecium, 2.44% E. gallinarum and 0.81% E. raffinosus[22]. However Revati Sharma et al in 2013from Mumbai observed a shift in spectrum with Enterococcus faecalis (50%) followed by Enterococcus faecium (30%). She also reported 27 cases (20%) of unusual species of *Enterococci* which included; E.avium (7%), E.raffinosus (4%), E.casseliflavus (4%), E.durans(4%), E.gallinarum (1%)[21]

Chaudhary et al in 2007 from PGIMS, Rohtak, India isolated 260 enterococcal strains from various clinical specimens in which E.faecalis were 72.3%, E.faecium 17.3%, E.raffinosus, E.durans 2.3%, E.casseliflavus 1.92% and E.dispar 1.53%. Polymicrobial

infection was noted in 17% of isolates.[78] We also found co-infection of Escherichia coli and Enterococcus species (colony count of both $>10^5$CFU/ml) in 5 patients of urinary tract infection. Diabetes and prostatitis and were found to be the existing co-morbid condition in these patients[24]. Preeti srivastava et al. in 2013 from Jaipur also isolated 100 enterococcus species from various clinical samples. E.fecalis (92%) constituted the predominant isolate.

Enterococci have emerged as an increasingly important cause of nosocomial infection. A major reason why these organisms survive in hospital environment is the intrinsic resistance to several commonly used antibiotics and perhaps more importantly, their ability to acquire resistance to several commonly used antibiotics either by mutation or by receipt of plasmids and transposons. Enterococci with high level amino glycosides resistance (HLAR), β-Lactamase production and glycopeptides resistance including Vancomycin Resistant Enterococci (VRE) have emerged, posing a therapeutic challenge to physicians due to ease of acquiring and transferring antimicrobial drug resistance.Most of the isolates in this study were sensitive to Linezoid(100%), Vancomycin(96%) and Teicoplanin(86%). Norfloxacin, Ampicillin and Tetracycline were mostly found to be resistant. The sensitivity pattern in our study showed that Enterococci are becoming increasing resistant to traditional antibiotic therapy. Some commonly available antibiotics like Norfloxacin and Nitrofurantoin known to have good anti-enterococcal activity and used in urinary tract infection patients were also resistant in around 50% of patients. Chaudhary et al in 2007 from PGIMS, Rohtak, India, isolated 260 Enterococcal Strains from various clinical specimens and observed the maximum susceptibility with Vancomycin 98%, followed by Teicoplanin 88%, Linezolid 79%[24]. S. Sreeja et al in 2012, observed in their study that 47% isolates were resistant to Penicillin, 45% to Ampicillin, 50% to Ciprofloxacin and 47% to High strength Gentamicin[14]. Preeti srivastava et al. in 2013 also isolated 100 Enterococcus species from various clinical samples and all were found to be susceptible to Linezoid and Vancomycin with least sensitive to ciprofloxacin and tetracycline. In their study Linezolid showed (100%) sensitivity followed by Vancomycin (91.5%), Nitrofuration (88.5%), Norfloxacin (77 %), Gentamicin (60%), Cefoperazone (54%), Pepracillin (46%) and Ciprofloxacin (42%)[20].S.Shafiyabi et.al in 2013,found resistance patterns of the isolates were 100%, 90%, 85%,72.5%, 60% and 65% to Cephalexin, Gentamicin, Cotrimoxazole, Erythromycin and Penicillin and Tetracycline respectively. Two isolates (5%) showed Vancomycin resistance, one from urine and one from wound discharge each, both belonging to E. faecalis. All the isolates were sensitive to Linezolid.[25]

On studying the species wise antibiotic sensitivity pattern, we found that amongst E. faecalis isolates one of the most commonly used antibiotic Norfloxacin , Ciprofloxacin (quinolones group) was sensitive in only 12% and 9% of isolates respectively. Similarly, other traditional antibiotics like Ampicillin, Tetracycline, Erythromycin were sensitive in only 34.67%, 24% and 9.33% isolates respectively. Resistance of E.faecalis to High strength Gentamycin and high strength streptomycin was also 52% and 53.33% indicating a failure of combination therapy of amino glycosides and cell wall inhibitors. E.faecium were found to slightly more resistant than E.faecalis especially to drugs like Ciprofloxacin, Erythromycin, and Pristinomycin. The resistance to high strength streptomycin in E.feacium was found to be 80% while that of high strength gentamycin was only 55%. S. Sreeja et al also observed around 50% *Enterococcus* to be resistant to Ampicillin, Penicillin, Ciprofloxacin and high strength Gentamycin. They also found E.faecium to be more resistant to the available antibodies as compared to E.faecalis.[14] Revati Sharma et al observed sensitivity to Teicoplanin in 85.5% but sensitivity to Linezolid only in 86.9% against E.faecalis. E.faecium and observed 12% resistance against Linezolid in E.faecalis and E.faecium.

For long, Enterococci have been treated by combining aminoglycosides with β-lactams which overcomes the intrinsic resistance exhibited by Enterococci and a synergistic effect is usually achieved since the intracellular penetration of aminoglycosides is facilitated by cell wall active agent. However, Enterococci have acquired HLR to Gentamycin that has resulted in resistance to synergism between gentamycin and penicillin. In this study 49% and 41% were found resistant to HSG and HSS by disc diffusion method. High level Gentamycin resistance was found to be slightly higher than high level Streptomycin resistance with no significant difference between E.faecalis and E.faecium (P>0.05). Mendiratta et al in 2008, in his study found 46% of the enterococci to be resistant to HLR Gentamicin and combined HLGR and HLSR was significantly ($p<0.05$) higher in E. faecium (59.1%) than E. faecalis (7.8%).[21] Sanal C. Fernandes et.al in 2013, found frequency of HLGR among the enterococcal isolates to be 53 per cent with no significant difference seen between E. faecalis and E. faecium isolates. High level streptomycin resistance was found in 49.3 per cent of the isolates in their study, with E. faecium (59%) showing greater resistance as compared to E. faecalis (49%),[26] Saroj Golia et al., 2014, found HLGR to be more common in E. faecium isolates (68%) compared to E. faecalis (56%) strains. HLSR was also found to be more in E.faecium (48%) than in E.faecalis (40%)[27]

**Conclusion**

Species identification is useful for epidemiological investigation of an outbreak and also for clinical decisions, particularly with regards to therapy as antimicrobial susceptibility differs in different species. This will not only help in initiation of effective therapy but also in the implementation of effective infection control measures.

**What this study add to existing knowledge**

At last we will also stress here again that the in-discriminate use of antibiotics without doing antibiotic susceptibility testing, prolong and inappropriate use of antibiotics lead to emergence of resistance. A good antibiotic policy should be laid down between clinician and microbiologist in all tertiary care hospitals and a strict antibiotic regime should be applied by clinicians. As only limited drugs are available for the treatment of multidrug resistance Enterococcus, irrational use of antibiotics should be avoided. A periodic surveillance is also must to monitor resistance patterns in various clinical isolates for planning effective treatment strategies.

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**Conflict of Interest: Nil**
**Source of support: Nil**