

ORIGINAL RESEARCH

Prevalence and antibiogram of enterococcus faecalis isolated from various clinical samples in a tertiary care hospital in south Rajasthan with special reference to VRE

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Received: 09 June, 2023

Accepted: 11 July, 2023

ABSTRACT

Introduction: Enterococcus species are normal residents of the gastrointestinal, biliary tract, vagina and male urethra. Enterococcus faecalis has recently become the major pathogen exhibiting resistance to many antimicrobials which are commonly used with increased frequency to treat infections. The aim of this study was to determine prevalence and antimicrobial susceptibility pattern and Vancomycin resistant Enterococcus faecalis in patients attending a tertiary care hospital in southern Rajasthan. **Material and methods:** This study was conducted on 800 clinical samples in the Department of Microbiology, Pacific Medical College and Hospital Udaipur, Rajasthan, from January 2020 to January 2022. The samples included urine, blood, sputum, ascitic fluid, Pleural fluid, Pus, ET secretion collected aseptically from patients suffering from urinary tract infection (UTI), septicaemia, pyogenic infections and their culture and antibiotic sensitivity were performed as per standard recommendations of CLSI guidelines (Clinical and laboratory standard Institute) by disc diffusion method. **Results:** In our study, Out of total 800 samples, 109 samples were positive for the Enterococcus faecalis that form 13.62 % of all samples. Similarly 17 samples were positive for Enterococcus faecium which form 2.12% and 15 Enterococcus species were unidentified. Enterococcus faecalis isolates were 22.93%, 18%, 10%, 8%, 22%, 22%, 6%, 20 % of urinary, wound swab or pus, blood, ascitic fluid, sputum, pleural fluid and ET secretion samples respectively. Out of 109 of Enterococcus faecalis 29(26.60%) samples were Vancomycin resistant. **Conclusion:** Various studies have shown an increase in the rate of infection and antibiotic resistance in Enterococcus species. There is also a change in pattern of antibiotic resistance in Enterococcus species with an increased isolation rate of VRE. The outdoor patients have community acquired Enterococcus infection.

Key words: Vancomycin resistant Enterococcus faecalis, Cefoxitin, Clinical and laboratory standards Institute

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INTRODUCTION

The origin of Enterococcus species vary from environmental to animal and human resources in the natural flora and fauna. The main habitat of Enterococcus faecalis is the gastrointestinal tract of animals and human beings. Enterococci are part of normal flora of human intestine, biliary tract and lesser extent vagina and male urethra. Multidrug-resistant Enterococcus faecalis are among the most difficult microorganisms to treat in clinical settings because of resistance to all available antimicrobials and have very less antibiotic groups which can treat

infection. Enterococcus faecalis continues to be an important cause of multidrug resistant infection worldwide. This study was designed to isolate and antibiotic resistance profile of Enterococcus faecalis. Most of the samples were isolated from indoor patients rather than outdoor patients and man were more prominent to acquire infection of Enterococcus faecalis.

Enterococcus faecalis is well-known as nosocomial opportunistic pathogens¹. E. faecalis (80–90%) and E. faecium (5–10%) are the two most commonly isolated Enterococcal species from clinical samples in

hospital²⁻³. The most frequent infections caused by these organisms include urinary tract infections (UTIs) followed by intra-abdominal or intra-pelvic abscesses, endotracheal and blood stream infections⁴. Community-acquired infections due to *Enterococcus faecalis* are on the rise due to extensive use of broad spectrum antibiotics not used rationally which lead to spread antimicrobial resistance if not use rationale. However emergence of Vancomycin resistant *Enterococcus faecalis* (VRE) and their increasing prevalence worldwide has made it difficult to treat serious Enterococcal infections. Moreover, since Vancomycin-resistant Enterococci (VRE) from animal sources such as poultry and human foods of animal origin play avital role in human colonization and infection, significant level of VRE colonization may be found among persons not associated with the health care setting⁵. High-level aminoglycoside resistance (HLAR), β -lactamase production, and glycopeptides resistance including VRE have been reported among *Enterococcus faecalis*^{6,7}. Isolation of *Enterococcus faecalis* and other species from clinical samples is on the rise and poses a significant health concern, as they show intrinsic resistance to many group of antibiotics and may lead to a treatment failure⁸.

AIMS AND OBJECTIVE

- To determine prevalence of *Enterococcus faecalis* species in patients.
- To detect antimicrobial resistance pattern of *Enterococcus faecalis* isolated from various clinical samples.
- To detect Vancomycin resistant *Enterococcus faecalis* in clinical samples.

MATERIAL AND METHODS:

1. Design of the Study:

- This prospective study was carried out in the Dement of Microbiology at Pacific Medical College and Hospital in Udaipur, Rajasthan, from January 2020 to January 2022.
- In the laboratory, 800 samples were processed to identify *Enterococcus faecalis*, of which 109 were isolated and identified from patients. Similarly 17 samples were positive for *Enterococcus faecium* which form 2.12% and 15 *Enterococcus* species were unidentified samples.
- The isolated *Enterococcus faecalis* were then processed for antimicrobial resistance pattern and VRE.

2. Time required for research:

- The period of the research was determined by the Pacific Medical University.
- The study began on January 2020, after receiving ethical permission from the Institutional Ethical

Committee (IEC), and it lasted till January, 2022.

3. Criteria for Inclusion: All patient samples which were received in department of Microbiology in Pacific Medical College and Hospital Udaipur.

4. Criteria for Exclusion:

- Normal flora samples, such as stool samples, were omitted from the research.

- Incomplete request form.

5. Collection of Samples:

- Samples were collected in sterile containers using adequate aseptic procedures and according to the particular requirements for each sample type as per standard guidelines.

- Urine, pus, swabs, blood, skin scraping, secretions, and catheter tips were all used in the investigation.

- Stool samples were omitted since *Enterococcus faecalis* is a natural flora of the colon.

- A total of 800 *Enterococcus* samples were analyzed for the isolation of *Enterococcus faecalis* and to determine their antimicrobial susceptibility with Vancomycin resistance pattern.

6. Bacterial Culture:

- A laminar air flow cabinet was used to process samples under sterile conditions.
- Subcultures were carried out on Nutrient agar, Blood agar and MacConkey agar.

7. Biochemical Analysis:

The following criteria were used to identify *Enterococcus* species:

- Bacterial colony, morphological properties on various culture medium.
- Gram staining is used to identify gram-positive cocci in pairs and short chains.
- Test for Bile-esculin susceptibility.
- PYR test.
- Sugar fermentation test.

RESULT AND OBSERVATION

In our study we included clinical samples i.e. Urine, ET, Blood, Pus, Sputum, Ascitic fluid and Pleural fluid from both indoor and outdoor patients. These samples were streaked aseptically on Nutrient agar, MacConkey agar and Blood agar plates. Plates were incubated at 37°C for 24 hours. Following overnight incubation of the samples, colonies of *Enterococcus faecalis* were seen as grey colonies of about 0.5-1mm in diameter with gamma haemolysis on blood agar and smooth surface and convex margins, creamy or whitish color on nutrient agar. Tiny, small pink colonies developed on MacConkey agar after 24 hours of incubation.

Table 1: Number of Isolated Enterococcus species from different types of clinical samples

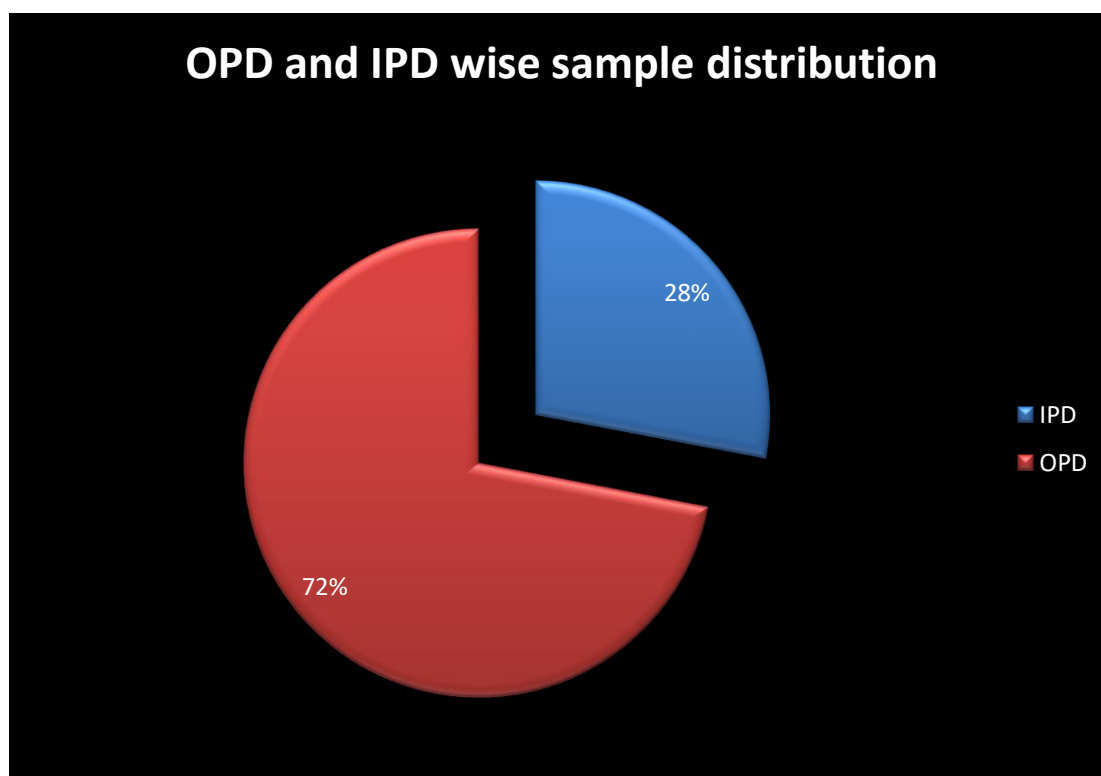
S. No.	Type of Clinical Sample	Number of Isolated Enterococcus faecalis(n)	Percentage (%)
1	Urine	25	22.93
2	ET	20	18.34
3	Blood	10	9.17
4	Sputum	22	20.18
5	Pus	18	16.51
6	Ascitic fluid	8	7.33
7	Pleural fluid	6	5.50
	Total samples	109	100%

In our research study total of 800 clinical samples were processed, 109 Enterococcus faecalis were isolated. Similarly 17 samples were positive for Enterococcus faecium which form 2.12% and 15 Enterococcus species were unidentified of all samples during further biochemical estimation. On their specific characteristic features, from various clinical samples, 25 samples of Enterococcus faecalis were isolated from urine culture, 20 were isolated from ET culture, 10 were isolated from blood culture by automated and conventional methods, 22 were isolated from sputum, 18 were isolated from pus

culture, 8 Enterococcus faecalis were isolated from ascitic fluid culture and 6 Enterococcus faecalis were isolated from pleural fluid (table:1).

Prevalence of Enterococcus faecalis in OPD and IPD samples

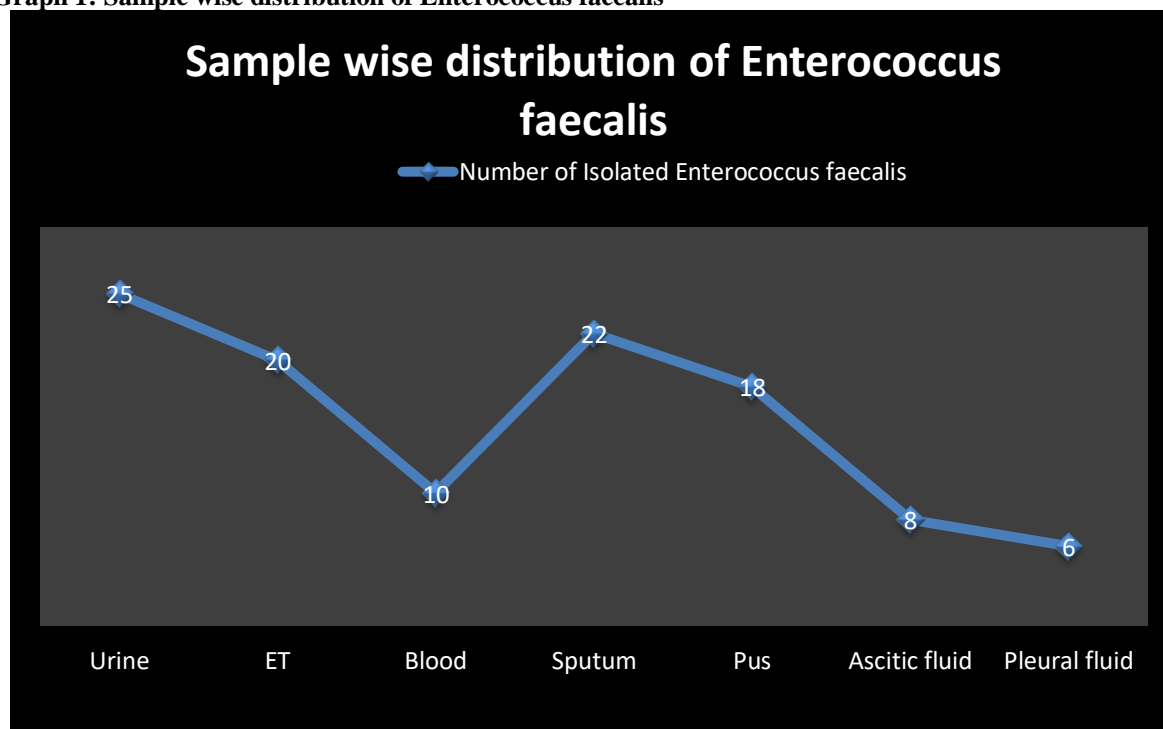
During the study period 109 Enterococcus faecalis were recovered from different clinical samples. The majority of the specimens were from inpatients department accounting 78 samples form 71.55% and 31 (28.45%) from outpatient department samples.



Studies clearly revealed that maximum numbers of Enterococcus faecalis were observed from urine culture whereas minimum numbers of Enterococcus faecalis were found in pleural fluid samples.

As per our observation Enterococcus faecalis isolated from various samples showed lowest resistance against Linezolid 03 (2.75%) followed by Tigecycline (3.66%), Teicoplanin (5.50%), Daptomycin (9.17%)

and Rifampicin (11%). Vancomycin showed 26.60% resistance. Ampicillin and Tetracycline showed resistance i.e. 41% and 66%. Erythromycin and Trimethoprim sulfomethoxazole showed 26.66% resistance. Both antibiotics Benzyl penicillin and Clindamycin showed 33.33% resistance. Similarly Levofloxacin and Gentamycin showed 55.04% and 46.66% resistant.

Graph 1: Sample wise distribution of Enterococcus faecalis

According to our study maximum resistance was shown by Cefoxitin and minimum resistance was shown by Linezolid followed by Tigecycline, Teicoplanin, Daptomycin and Rifampicin.

Table 2: Antimicrobial susceptibility test for Enterococcus faecalis in various samples (N=109)

Antibiotics	Disc Con. (µg)	Resistant Strains (n)	Percent of resistant Strains (%)	Sensitive Strains (n)	Percent Sensitive strains (%)
Ampicillin	30µg	45	41.28	64	58.71
Vancomycin	30µg	42	26.60	67	73.39
Teicoplanin	30µg	06	5.50	103	94.50
Daptomycin	50µg	10	9.17	99	90.82
Erythromycin	15µg	65	59.63	44	40.36
Tetracycline	30µg	72	66.05	37	33.94
Levofloxacin	5µg	60	55.04	49	44.95
Ciprofloxacin	5µg	62	56.88	47	43.11
Gentamicin	10µg	48	44.03	61	55.96
Clindamycin	2µg	42	38.53	67	61.46
Cefoxitin	30µg	92	84.40	17	15.59
Benzyl penicillin	30 µg	36	33.02	73	66.97
Trimethoprim Sulfomethoxazole	1.25µg/23.75µg	34	31.19	75	68.80
Tigecycline	15µg	04	3.66	105	96.33
Rifampicin	5µg	12	11.00	97	89.00
Linezolid	30 µg	03	2.75	106	97.24

Ampicillin showed 41.28% resistance, Levofloxacin, Ciprofloxacin, and Gentamicin showed 54.04%, 56.88%, and 44.03% resistance respectively. Similarly Clindamycin, Benzyl penicillin and Trimethoprim sulfomethoxazole showed 38.53%, 33.02% and 31.19% resistance against E. faecalis obtained from urine samples.

Detection of Vancomycin Resistant Enterococcus faecalis

After that and by a large distance, the most effective and least effective antibiotics were Linezolid and Cefoxitin respectively against Vancomycin resistant Enterococcus faecalis. The most effective antibiotics were Linezolid (100%) and Tigecycline (96.55%) of all VRE. Highest resistant to all VREs were showed by Cefoxitin (100%) against the samples. Then after the cefoxitin, tetracycline showed 24 (82.75%).

Table 3: Antimicrobial susceptibility test for Vancomycin resistant Enterococcus faecalis (VRE) in various samples (N=29)

Antibiotics	DiscCon. (µg)	Resistant Strains (n)	Percent of resistant Strains (%)	Sensitive Strains (n)	Percent Sensitive strains (%)
Ampicillin	30µg	18	62.60	11	37.93
Vancomycin	30µg	29	100	0	00
Teicoplanin	30µg	1	3.44	28	96.55
Daptomycin	50µg	1	3.44	28	96.55
Erythromycin	15µg	19	65.51	10	34.48
Tetracycline	30µg	24	82.75	5	17.24
Levofloxacin	5µg	8	27.58	21	72.41
Ciprofloxacin	5µg	19	65.51	10	34.48
Gentamicin	10µg	20	68.96	9	31.03
Clindamycin	2µg	17	58.62	12	41.37
Cefoxitin	30µg	29	100	0	00
Benzyl penicillin	30 µg	18	62.02	11	37.39
Trimethoprim Sulfomethoxazole	1.25µg/2 3.75µg	20	68.96	9	31.03
Tigecycline	15µg	1	3.44	28	96.55
Rifampicin	5µg	2	6.44	27	93.10
Linezolid	30 µg	00	00	29	100

According to the findings age and sex had no effect on resistance to any antibiotic. Using generalized linear models with logistic regression, we found no effect of age, sex or their combination on resistance to antibiotics. VRE samples were showed maximum susceptibility against Linezolid that was 100% followed by Teicoplanin, Daptomycin, Tigecycline which was 96.55% for each. Maximum resistant VRE samples against Vancomycin and cefoxitin that was 100% and followed by Tetracycline (82.75%) and Gentamicin (68.96%).

Table 4: Specimen-wise distribution of isolated Enterococcus faecalis, Vancomycin resistant Enterococcus faecalis & their percentage

S. No.	Type of Clinical Sample	No. of clinical Sample	Total No of E. faecalis isolates (n)	Vancomycin resistant E. faecalis (n)	Percentage Resistance species (%)
1	Urine	150	25	8	27.58
2	Blood	15	10	4	13.79
3	Sputum	30	22	6	20.68
4	Ascitic fluid	10	08	3	10.34
5	Pleural fluid	15	06	2	7.24
6	Pus	08	05	1	3.44
7	ET	80	20	5	17.24
	Total Samples	800	109	29 (26.60%)	100

DISCUSSION

In our study Out of total 800 samples, 109 samples were positive for the Enterococcus faecalis that form 13.62% of all samples. Similarly 37 samples were positive for Enterococcus faecium which form 4.62% of all samples. Enterococcus faecalis isolates for 22.93%, 18%, 10%, 8%, 22%, 22%, 6%, 20 % of urinary, wound swab or pus, blood, ascitic fluid, sputum, pleural fluid and ET secretion. Total 49 samples were positive for other unidentified species of Enterococcus that forms 18% of samples. Other species rather than Enterococcus species and Enterococcus faecium are difficult to identify and have similarities in conventional biochemical reaction but they can identify conveniently by automated culture methods.

Enterococcus faecalis is the most important cause of hospital acquired infections in patients who have weakened immune system, wide spread use of antimicrobial agents responsible for the development of resistance to antimicrobial agents. Enterococcus faecalis has become increasingly important because of their ability to cause serious infections such as endocarditis, bacteremia, pus forming abscess, intra-abdominal and urinary tract infections. Enterococcus faecalis is responsible for increasing resistance to different antimicrobials which include β -lactam antibiotics, aminoglycosides and most importantly glycopeptides like Vancomycin. There is a limited evidence of Teicoplanin resistance in comparison to Vancomycin. Serious Enterococcus faecalis infections are often refractory to treatment with a high mortality rate in healthcare setup.

S.N.	Studies	Year	Vancomycin resistant Enterococcus Faecalis
1.	Arif et. al.	April 2019	30%
2.	Yangzom et. al	August 2019	13.5%
3.	Mohanty et. al	July 2005	0%
4.	Khandelwal et. al.	July 2020	14.10%
5.	Vaghela et. al	December 2018	49%
6.	Yadav et. al.	May 2022	26.66%

In contrary to Vaghela et.al. in their study according to Enterococcus faecalis isolation distribution shows 49% that were found to be 26.66% in our study. This is comparable with the study carried out by **Khandelwal et. al.**(2020) (14.10%) Enterococcus faecalis isolates.

Present study in comparison for E. faecalis isolates shows similarity with **Sachan et. al.** in which out of 283 enterococci isolated predominant species were Enterococcus faecalis (82.33%). Vancomycin resistance were observed among 6.01% of Enterococcal isolates, respectively but in our study it VRE was 26.66% out of all samples.

Present study shows similarity with **Rana et. al** Out of 100 Enterococci, 70 Enterococcus faecalis, 21 E. faecium and 09 other Enterococcus species were isolated. The results showed that majority of Enterococci were isolated from male patients (59%), from urine samples (59%) and from medicine dement (36%). AST showed overall high resistance to Penicillin (98%) Ampicillin (86%), Gentamicin (85%), Ciprofloxacin (60%), Vancomycin (12%) (VRE), Minimum resistance was observed with Linezolid (3%) that is similar to our study which shows 2.75% resistant in out of total samples.

Shekhawat et. al Out of 100 isolates of Enterococci, 90% (90) were E.faecalis, 1% (1) was E. faecium and 9% (9) others. The maximum number of Enterococcus isolates obtained from urine 76% (76) followed by pus 14% (14), blood 6% (6) and tracheal swab 4% (4) that is similar to ur findings but antimicrobial resistant pattern showed some contrary results. The sensitivity pattern of the isolates showed an increased resistance to antibiotics like Amoxicillin (66%), Tetracycline (64%), high Gentamycin (63%) and Ciprofloxacin (60%). All the isolates were sensitive to Linezolid (100%), 88% to Vancomycin, 59% to Nitrofurantoin and 44% to amoxicillin. In our study linezolid was not susceptible for all samples.

In a study conducted by **Arif et. al** April 2019 shows 30 % Vancomycin resistant Enterococcus faecalis isolates which is similar to our study in which Vancomycin resistant Enterococcus faecalis isolates are 26% of total number.

CONCLUSION

Prevention of Enterococcus faecalis infections in general and VRE in particular needs active surveillance cultures and aggressive implementation of infection control measures in healthcare setup. This study demonstrates the increased prevalence of multidrug resistant Enterococcus faecalis, thus

responsible for a serious therapeutic threat in upcoming era. This situation warrants the implementation of efficient infection control and prevention program and regular surveillance of antimicrobial resistance of Enterococcus faecalis in order to establish a rational antibiotic policy for the better management of Enterococcus faecalis infections.

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