Original Article

Increasing Antimicrobial Resistance Pattern in Enterococcus feacalis and Enterococcus feacium Isolated from Different Clinical Samples at Northwest General Hospital, KP, Pakistan

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Abstract

Objective: To determine Enterococcus species' frequency and antimicrobial resistance pattern isolated from different clinical samples.

Methodology: A cross-sectional study was conducted during the period of June and December 2023 in Northwest General Hospital Laboratory and Research Center. Patients referred by clinicians to medical laboratories for culture sensitivity tests were included after fulfilling inclusion-exclusion criteria. Different clinical samples, e.g. urine, blood, wound swabs, and semen, were collected aseptically after guiding the patients. All the samples were processed by inoculating on bacteriological media. Identification of the organisms was made on the basis of colonial characteristics, Gram staining, and biochemical tests. The antibiotic susceptibility profile of the isolated organisms was done according to the Kirby-Bauer disc diffusion method. For the comparison of categorical variables, the Fisher exact test was used. The statistical testing was carried out at the 2-tailed α level of 0.05

Results: Out of the total, 123 (31.9%) patient samples were culture-positive for Enterococcal species. Prevalence of Enterococcus feacalis and Enterococcus feacium was higher in urine and blood samples of patients belonging to age groups 31-40 years and >50 years. Enterococcus feacium showed higher level of resistance to all antibiotics than Enterococcus feacalis ($p \leq 0.0001$) with the exception of vancomycin ($p \leq 1.0$) and tigecycline ($p \leq 1.0$). Enterococcus feacalis showed the least resistance against nearly all antibiotics used in the study.

Conclusion: Overall prevalence of Enterococcal feacalis and Enterococcus feacium was higher in urine and blood samples. Overall prevalence of both enterococcal species was higher in patients age group >50years. Enterococcus feacium was found to be more resistant pathogen than Enterococcus feacalis.

Keywords: Antimicrobial Resistance; Enterococcus faecalis; Enterococcus feacium; Kirby-Bauer Disc Diffusion

Introduction

Enterococcus genus is a Gram-positive, catalase-negative, non-spore-forming facultative anaerobic bacteria that belongs to the Enterococcaceae family. Humans, animals, and the surrounding environment are frequently infested with these facultative anaerobic bacteria, which are often found in the gastrointestinal tract of humans and animals.[1] Enterococcus genus contains several species, such as Enterococcus hirae, Enterococcus mundtii, Enterococcus faecalis, and Enterococcus faecium.[2] Among these bacterial strains, Enterococcus faecalis is responsible for 85–90% infection, whereas Enterococcus faecium contributes 5–10% infection. Enterococcus spp. has become a leading cause of healthcare-associated infections ranging from endocarditis to UTIs.[3] It has been observed that Enterococcus species were the second or third common cause of current nosocomial urinary tract infections. Other infection like bacteremia, endocarditis, infection from catheter, wound and soft tissue infection, meningitis, respiratory infection, neonatal sepsis, intra-abdominal and pelvic infection were also caused by these bacterial strains.[4] High concentration of Enterococcal species in urine and their intrinsic and multidrug resistance pattern make them a challenging uropathogenic for clinicians.[5] Enterococcal species colonized normally in the gastrointestinal tract as a commensal flora, but their translocation from the gut often leads to bacteremia and increasing risk of serious infection. After being phagocytosed.[6] Multiple organ abscesses are often linked to a high death rate due to Enterococcal bacteremia, and metastatic abscesses can also develop in multiple organs. Enterococcus species are responsible for about 20% of native valve bacterial endocarditis and 6-7% of prosthetic valve endocarditis.[7] The rise in nosocomial infections and the evolution of antimicrobial resistance to various treatments have made Enterococcus species a global issue.[2] The reported death rates following Enterococcal bacteremia range from 19% to 48%.[8] According to estimates, vancomycin-resistant strains of Enterococcus faecium and Enterococcus faecalis were responsible for 100,000 to 250,000 incidents in 2019, and an additional 3.68 million deaths were indirectly linked to bacterial antimicrobial resistance.[9] Because Enterococcus species can acquire resistance mechanisms against a wide range of antimicrobial drugs, contributing to increased healthcare costs, morbidity, and mortality.[5] The causes behind increasing antimicrobial resistance patterns among Enterococcus species are multifaceted and influenced by various factors like indiscriminate use of broad-spectrum antibiotics, inadequate infection control measures in healthcare facilities, horizontal gene transfer among the bacterial species, and intrinsic ability of Enterococcus species regarding antimicrobial resistance acquisition and its development.[10-11] According to previous study conducted in this region, about 2.94% Enterococcus feacalis was responsible for urinary tract infection.[12] Unfortunately, emergence of these enterococcal species increasing day by day in this region. In 2022, another study reported a high prevalence of Enterococcus feacalis (12.8%) causing urinary tract infections among the patients belonging to this region.[13] There is no such data available regarding the provenance of Enterococcus species and its antimicrobial susceptibility profile in other clinical samples than urine in this region. To address this critical issue, a comprehensive research effort is imperative to understand the underlying antimicrobial resistance patterns in Enterococcal species. Therefore, the current study is designed to determine the frequency of Enterococcal species in different clinical samples and the selection of appropriate antimicrobial agents.

Methodology

The current study was cross-sectional and was conducted between June 2023 and December 2023 at Northwest General Hospital Laboratory & Research Center Peshawar. Ethical approval for this research work was taken from the Institutional Research Board (IRB&EC/2023-HIS/058) of Northwest General Hospital and Research Center. A total of 385 patients referred by clinicians for culture sensitivity tests were analyzed after fulfilling inclusion and exclusion criteria. Patients were advised that urine culture, blood culture, wound swab culture, body fluids culture, and semen culture tests were included. Patients aged <10 years were excluded. Moreover, contaminated growth and mixed growth on blood agar media (different colonial characteristics) were excluded.

After proper guidance, the patient's urine, blood, wound swab, body fluids, and semen samples were collected aseptically in a sterile container provided by laboratory staff. Blood samples were collected according to standard microbiological protocols to avoid skin flora contamination. All the samples were processed in the Microbiology section of Northwest General Hospital Laboratory and Research Centre following standard microbiological aseptic technique. All the samples, which comprised of urine, blood, wound/pus, body fluids, and semen, were inoculated on bacteriological media such as blood agar and cysteine lactose electrolyte deficient (CLED) agar by using Laminar Flow Hood to avoid contamination and incubated at 37° for 24 hours.

After incubation, identification of the organisms was done on the basis of colonial characteristics on blood agar. Enterococcal species producing non-hemolytic small colonies on blood agar.

Enterococcal species are Gram-positive cocci that look like coccobacilli or ovals, either in single, pairs, or chains.

Biochemical tests such as catalase and bile esculin test was used for further confirmation. Enterococcal species are catalase negative. Bile esculin is a presumptive test comprised of glycosidic coumarin derivative, Enterococcal bacteria hydrolyzing esculin in the presence of bile salt and producing a black diffusible complex which turns the medium to brow black color. Differentiation between Enterococcus faecalis and Enterococcus faecium was done by using ampicillin disc. Enterococcus feacalis is sensitive to ampicillin, producing a clear zone of inhibition around the disc, while Enterococcus feacium showed resistance, and no zone of inhibition was seen around the ampicillin disc.[14]

Antibiotic susceptibility profile of the isolated organisms was done according to Kirby-Bauer disc diffusion method. A bacterial suspension was made by taking isolated colonies from fresh overnight culture plate turbidity equal to 0.5Mcfarland standard solution. A bacterial lawn was prepared by spreading bacterial suspension on a blood agar plate. Antibiotic discs obtained from (Oxoid PVT) were placed at a distance of 25 mm apart from each other on bacterial lawn. The antimicrobials used were erythromycin (ERY), ampicillin (AMP), nitrofurantoin (F), vancomycin (V), ciprofloxacin (CIP), gentamicin (CN), rifampicin (RIF), fosfomycin (FOS), linezolid (LZD) and tigecycline (TGC). Interpretation of the result was don on the basis of zone of inhibition around each antimicrobial disc. According to Clinical Laboratory Standard Institute. Sensitivity zone for ampicillin (>17mm), erythromycin (>23mm), nitrofurantoin (>17mm), vancomycin (>17mm), ciprofloxacin (>21mm), gentamycin (>10mm), rifampicin (>20mm), fosfomycin (>16mm), linezolid (>23mm) and tigcyclin (>19mm) (15).

For comparison of categorical variables, the Fisher exact test was used. The statistical testing was carried out at the 2-tailed α level of 0.05. The data was analyzed using GraphPad Prism, version 8.0.2 (GraphPad Software, Inc., USA)

Results

In the current study, a total of 385 clinical samples were collected from male and female patients. Out of the total, 123(31.9%) samples comprised of males (59) and females (64) were culture-positive for Enterococcal species. The overall prevalence of Enterococcus feacalis and Enterococcus feacium in both gender was 61(49.6%) and 62(50.4%), respectively, shown in Table 1. The frequency of Enterococcus faecalis was higher in females (87%) than in males (60%) in urine samples, while the frequency of Enterococcus faecium was higher in males (69%) compared to female patients (57%). In the blood sample, the frequency of Enterococcus feacium was found to be higher in males (27.5%) and females (37%) than in Enterococcus feacalis. Frequency of Enterococcus feacalis and Enterococcus faecium in other clinical samples e.g body fluid, wound swab and semen was relatively lower as shown in table 2. Overall distributions of Enterococcal species in different age group was shown in table 3. Frequency of Enterococcus faecalis was found higher in urine 6(60%) followed by blood 2(20%) and wound 2(20%).in patients [10-20 years], While Frequency of Enterococcus faecium in blood and urine was 2(66.6%) and 1(33.3%) respectively in the same age group. Frequency of Enterococcus faecalis in urine sample was 10(100%) in patient [21-30 years]. Frequency of Enterococcus faecalis in urine, wound swabs and semen sample was 4(50%), 2(25%) and 2(25%) respectively in patient [31-40years], while prevalence of Enterococcus faecium in urine, blood and wound samples was 6(46.2%), 6(46.2%) and 1(17.6%) respectively in the same age group. Enterococcus faecalis frequency was higher in urine sample 10(100%) then Enterococcus faecium 2(33.3%) in patients [41-50 years]. The frequency of Enterococcus faecalis and Enterococcus faecium in the urine samples was 15(78.9%) and 29(67.4%), respectively, in patients. While frequency of Enterococcus faecium in blood, body fluids and wound samples was 10(23.3%), 3(7.0%) and 1(2.3%) respectively in the same age group.

Enterococcus feacium showed higher level resistance to all antibiotics used in the study than Enterococcus feacalis ($p \le 0.0001$) with exception of Vancomycin ($p \le 1.0$) and Tigecycline ($p \le 1.0$). The least resistance was observed in Enterococcus feacium against tigecycline (0%), fosfomycin (5%), linezolid (8%), and vancomycin (15%), as shown in Table 4. While urinary tract infection caused by Enterococcus faecalis showed the least resistance to ampicillin (0%), tigecycline (0%), linezolid (0%), vancomycin (16%), nitrofurantoin (22%), and fosfomycin (24%). Enterococcus feacalis isolated from urine, wound swab, body fluid, and semen samples showed higher resistance levels to ciprofloxacin, which is 87%, 100%, 100%, and 100%, respectively. Tigecycline, aminoglycosides, rifampicin, and linezolid, were the drugs that showed the least resistance and were considered the drugs of choice against Enterococcus facial. Among Enterococcus feacalis and Enterococcus feacium isolates, some strains were resistant to vanco-

Table 1. Overall prevalence of Enterococcus faecalis and Enterococcus faecium

Bacterial Species	E. faecalis	E. faecium	Total
Gender	n(%)	n(%)	n(%)
Male	30(24.4)	29(23.5)	59(100)
Female	31(25.2)	33(26.3)	64(100)
Total	61(49.6)	62(50.4)	123(100)

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Table 2. Gender-wise Prevalence of Enterococcus faecalis and Enterococcus faecium isolated from various clinical samples

Organisms	Gender (n)	Urine n(%)	Blood n(%)	Fluids n(%)	Wound /Pus n(%)	Semen n(%)
E. faecalis	Male (30)	18(60)	1(3)	2(7)	5(17)	4(13.3)
	Female (31)	27(87)	2(6.5)	-	2(6.5)	-
E. faecium	Male (29)	20(69)	8(27.5)	1(3.5)	-	-
	Female (33)	19(57)	12(37)	-	2(6)	-

Table 3. Prevalence of Enterococcus faecalis and Enterococcus faecium in patients age groups

Patients age Group	Organisms	n	Urine n(%)	Blood n(%)	Body fluids n(%)	Wound/Pus n(%)	Semen n (%)
10-20 years	E. faecalis	10	6(60%)	2(20%)	-	2(20%)	-
	E. faecium	3	1(33.3%)	2(66.6%)	-	-	-
21-30 years	E. faecalis	10	10(100%)	-	-	-	-
	E. faecium	01	1(100%)	-	-	-	-
31-40 years	E. faecalis	08	4(50%)	-	-	2(25%)	2(25%)
	E. faecium	13	6(46.2%)	6(46.2%)	-	1(7.6%)	-
41-50 years	E. faecalis	10	10(100%)	-	-	-	-
	E. faecium	06	2(33.3%)	2(33.3%)	-	-	2(33.3%)
>50 years	E. faecalis	19	15(78.9)	1(5.2)	-	3(15.9)	-
	E. faecium	43	29(67.4)	10(23.3)	3(7.0)	1(2.3)	-

Table 4. Percentage of antimicrobial resistance of Enterococcus faecalis and Enterococcus faecium isolated from different clinical

Patients age Group	Organisms	n	AMP n(%)	N n(%)	V n(%)	CIP n(%)	CN n(%)	RIF n(%)	FOS n(%)	LZD n(%)	TGC n(%)	ERY n(%)
Urine	E. faecalis	45	0(0)	10(22)	7(16)	39(87)	14(31)	19(42)	11(24)	0(0)	0(0)	!
	E. faecium	39	39(100)	30(77)	6(15)	38(98)	39(100)	39(100)	2(5)	3(8)	0(0)	!
Blood	E. faecalis	3	0(0)	!	0(0)	!	1(33)	1(33)	!	0(0)	0(0)	3(100)
	E. faecium	20	20(100)	!	8(40)	20(100)	0(0)	4(20)	!	5(25)	0(0)	20(100)
Wound	E. faecalis	7	0(0)	!	0(0)	7(100)	3(43)	3(43)	!	0(0)	0(0)	7(100)
	E. faecium	2	2(100)	!	0(0)	2(100)	2(100)	0(0)	!	0(0)	0(0)	2(100)
Body fluids	E. faecalis	2	0(0)	!	0(0)	2(100)	0(0)	0(0)	!	0(0)	0(0)	0(0)
	E. faecium	1	1(100)	!	0(0)	1(100)	1(100)	1(100)	!	0(0)	0(0)	1(100)
Semen	E. faecalis	4	0(0)	!	0(0)	4(100)	4(100)	0(0)	!	0(0)	0(0)	4(100)
	p-value		<0.00001	<0.00001	1.000	0.005	<0.00001	<0.00001	0.0002	0.0068	1.00	1.00

!: not tested, AMP: ampicillin, N: nitrofurantoin, V: vancomycin, CIP: ciprofloxacin, CN: Gentamycin, RIF: Rifampicin, FOS: Fosfomycin, LZD: Linezolid, TGC: tigecycline, ERY: erythromycin, p-value (Result of chi-square Fisher Exact test), The result is significant at p- value <0.05.

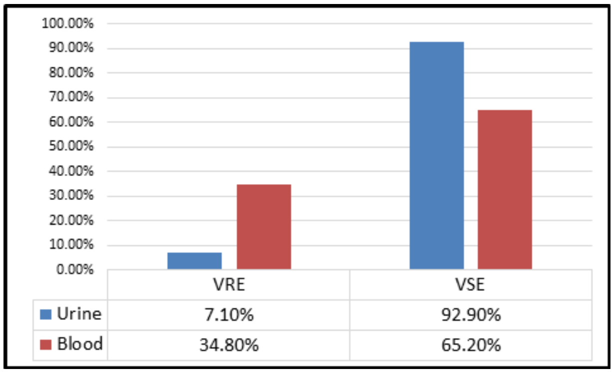

Figure 1: Percentage of vancomycin-resistant enterococci and vancomycin-sensitive enterococci in urine and blood samples

mycin. The prevalence of vancomycin-resistant strain was higher in blood samples (34.8%) than in urine samples (7.1%) shown in Figure 1.

Discussion

Enterococcal species recently emerged as an opportunistic pathogen primarily associated with hospital settings, particularly long-term hospitalized patients.[16] Recently, infections caused by Enterococcal species have great clinical concern due to the acquisition of resistance against various antimicrobial agents.[17] In the current study, the frequency of both Enterococcal species was found to be higher in urine (42.4%) and blood samples (32.8%). These findings are relatively higher than the previous study conducted in Iraq,[18] where the frequency of Enterococcus species in urine and blood samples was 33.9% and 5.6%, respectively. This variation may be due to the small sample size in the later study and differences in the geographical distribution of the patients. According to previous data.[19] Enterococcal species become the second most common uropathogenic after Escherichia coli, responsible for urinary tract infection. These findings supported the present evidence, where the frequency of both Enterococcal species was higher in urine samples. Enterococcal species causing urinary tract infections, bacteremia, and endocarditis have drawn special attention in recent years as multidrug-resistant bacteria responsible for nosocomial infections.[20] In the present study, Enterococcus feacium was the most prevalent pathogen isolated from blood samples, which increases the susceptibility of individuals to developing endocarditis. Among Enterococcal species, particularly Enterococcus faecium and Enterococcus faecalis, are the third most common cause of nosocomial infections in clinical settings after Pseudomonas aeruginosa and Staphylococcus aureus.[21] In the present study, we observed that both Enterococcal species were accountable for urinary tract infection, bacteremia, and wound infection. The prevalence of Enterococcus feacalis was higher than Enterococcus feacium in female patients compared to males. These findings showed similarities with previous study.[22]

Among Enterococcal species, Enterococcus faecalis was seen to be a more prevalent uropathogenic responsible for urinary tract infection in the age group > 50 years. This higher frequency may be due to weak immune systems, hormonal changes in females after forty years of age, and any comorbidity or chronic underlying diseases contributing to risk factors that make individuals susceptible. These findings showed an agreement with the previous data.[23] The emergence of multi-drug resistant Enterococci in clinical settings has become a challenge for clinicians regarding their management and treatment.[24] Over the past 20 years, Enterococci have emerged as a prominent source of hospital-acquired infections. The adhesion, colonization, immune response evasion, pathogenicity, and severity of the infection are all influenced by a number of virulence factors.[25] Enterococci have effective genetic exchange systems that allow the genes encoding virulence determinants to be passed from susceptible strains to resistant ones.[26] For the last few years, Enterococcus faecium has shown resistance against cell wall inhibitors and nucleic acid inhibitors, e.g., ampicillin, imipenem, and fluoroquinolones, and limited therapeutic options for treating urinary tract infections.[27] This evidence supports our current findings, where Enterococcus faecium showed resistance to these antimicrobial agents. Only fosfomycin, linezolid and vancomycin were found to be effective against Enterococcal feacium. Self-prescription sub-optimal doses, irrational use of antibiotics, and quackery are the common factors contributing to high antibiotic resistance patterns in this region. On the other hand, all Enterococcus faecalis strains isolated from various clinical samples showed sensitivity against nearly all antibiotics used in the current study except ciprofloxacin. These findings were also supported by another previous data.[14] Based on the current antimicrobial susceptibility profile, phenotypic differentiation between Enterococcus faecalis and Enterococcus faecium should be done with the use of an ampicillin disc. Enterococcus feacalis is found sensitive against ampicillin while Enterococcus feacium was found resistant.

This hike in resistance mechanisms of Enterococcus faecium made it a major clinical concern throughout the world. Antimicrobial resistance is a persistent issue, with vancomycin-resistant enterococci being particularly significant. In 2017, World Health Organization (WHO)[28] classified vancomycin-resistant Enterococcus faecium as a high-priority pathogen that necessitates further research and development of novel antibiotics. In the present study, vancomycin resistance Enterococcus feacium was more prevalent in blood samples than urine, which showed similarity with previous data.[22] Further study required keeping large sample sizes to address the emergence of vancomycin resistance enterococci in clinical settings. Moreover, work is needed

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to understand and explore the underlying mechanism of antimicrobial drug resistance.

Conclusion:

Overall prevalence of Enterococcal feacalis and Enterococcus feacium was higher in urine and blood samples. Overall prevalence of both enterococcal species was higher in patients age group >50years. Enterococcus feacium was found to be more resistant pathogen than Enterococcus feacalis.

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