ISSN: 2581 – 3633
PubMed - National Library of Medicine - ID: 101745081

International Journal of Medical Science and Advanced Clinical Research (IJMACR)

Available Online at: www.ijmacr.com

Volume – 6, Issue – 1, Janaury - 2023, Page No. : 166 - 173

A study of vancomycin and linezolid sensitivity pattern in enterococcus species from various clinical samples from a tertiary care hospital, Jaipur, Rajasthan

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How to citation this article: Dr. Anita Lamba, Dr. Susheel Kumar singh, Dr. Mamta Lamba, Dr. Rajni Sharma, Dr. Akriti Aggarwal, "A study of vancomycin and linezolid sensitivity pattern in enterococcus species from various clinical samples from a tertiary care hospital, Jaipur, Rajasthan", IJMACR- January - 2023, Volume – 6, Issue - 1, P. No. 166 – 173.

Type of Publication: Original Research Article

Conflicts of Interest: Nil

Abstract

Introduction: Enterococci are recognized as opportunistic pathogens, as well as commensals in both humans and animals. They have emerged as a major cause of difficult-to-treat nosocomial and community acquired infections, as the organism have intrinsic and acquired resistance to many antibiotics.

Objectives: To isolate and identify clinically relevant Enterococcus species from all the clinical samples and also to study their antibiotic susceptibility pattern and to evaluate occurrence of vancomycin and linezolid resistance in Enterococcus species.

Material and method: A laboratory based observational descriptive study was carried out for a period of one year from May 2020 to April 2021 at the Department of Microbiology, SMS medical college, Jaipur. A total of 210 isolates of Enterococcus species obtained from various clinical samples. Identification was done with standard biochemical methods and Antibiotic susceptibility testing was done on Muller Hilton agar plate by Kirby Bauer disc diffusion method. All methodology was followed as per Clinical and Laboratory Standards Institute (CLSI) 2019 guidelines.

Results: Out of 210 isolates of Enterococcus studied, maximum no of isolates obtained from Urine (153, 72.85%); followed by blood (32, 15.2%), pus (16, 7.61%) and peritoneal fluid (6, 2.85%). Sensitivity towards High level Gentamicin (HLAR) and Ampicillin was seen in 124(59%) & 54(26%) Enterococcal isolates

respectively. Overall Vancomycin sensitivity was seen in 184 (88%) isolates. Linezolid still showed good sensitivity, with 205 (98%) isolates found to be sensitive.

Conclusion: Isolation of Enterococcus species resistant to most of the higher antibiotics like vancomycin, linezolid and teicoplanin with high prevalence of High-Level Aminoglycoside Resistance (HLAR), is a major concern as such isolates have associated with many hospital acquired infections and have limited or no therapeutic option.

Keywords: CLSI, HLAR

Introduction

Enterococci are Gram positive oval cocci, arranged in pairs or short chains. They produce smooth, grey colonies, either non-hemolytic or alpha-hemolytic. They are widely distributed in nature and around 35 species have been identified till now. They are part of normal flora of human intestine, oral cavity, female genital tract and male urethra [1]. Over last two decades, Enterococci have emerged as an important agent of human disease mainly Hospital Acquired Infections, because of their resistance to antimicrobial agents to which other Streptococci are generally susceptible [2].

According to various studies Enterococci are the second most common cause of urinary tract infections (UTI) and third most common cause of Bacteremia from hospital acquired infections [3,4].

Intrinsic resistance to a variety of antibiotics like Cephalosporins, clindamycin, aminoglycosides (except high level aminoglycoside resistance), & cotrimoxazole are found in Enterococci. (CLSI2022) is of major concern. Acquired resistance to Vancomycin (VRE, Vancomycin-resistant Enterococci) is increasing now a days, which account for more than 30% of Enterococcal infections. More than 90% of VRE isolates are E. faecium, while E. faecalis accounts for remaining 5% to 10% only [5]. Acquired resistance to vancomycin is mediated by the van gene clusters, which alter the drug target from D-alanyl-D-alanine to D-alanyl-D-lactate [6]. VRE are of important concern as Vancomycin is an antibiotic of choice to treat fatal infections caused by drug resistant Enterococcus spp[7].

Earlier the mainstay of treatment of enterococcal infections over the years was penicillin with gentamycin due to synergistic action. Because of the development genetically acquired resistance to gentamicin, vancomycin became the agent of choice. But in past few years because of development of vancomycin resistance, linezolid became the last resort antibiotic. Presently many circulating strains are reported to have acquired resistance to most of the available therapeutic options including vancomycin and linezolid which are thought to be antibiotics of last resort in serious Enterococcal infection.

The present study is aimed at detecting the antimicrobial resistance pattern among Enterococcus isolates obtained from various clinical specimens, with a special emphasis on vancomycin and linezolid resistance and to discuss therapeutic option on these multidrug resistant Enterococcal isolates.

Material and methods

A total 210 Enterococcal isolates from various clinical samples (like urine, blood, pus, peritoneal fluids and others.) were included in the study. Clinical samples were collected from patients suspected of bacterial infection visiting the IPD/OPD of SMS Hospital, Jaipur, Rajasthan from May 2020 to April 2021. All the samples were processed in department of Microbiology using standard laboratory protocol. The approval from

the Institutional Research and Ethical committee was obtained before conducting the study.

Only samples yielding growth of *Enterococcus* spp were included. The suspected *Enterococcal* isolates grown on the primary plating media such as Blood agar and MacConkey agar, after incubation at 37°C for 24 hours, were selected for further identification. Preliminary tests for identification such as Gram stain, catalase test, bile esculin test were performed on the selected isolates. Catalase negative, Gram Positive cocci in pairs and short chains with bile esculin positive isolates were selected and processed further. After the identification of Enterococci, antimicrobial susceptibility testing was done by Kirby-Bauer disc diffusion method on Muller Hinton agar as per CLSI guidelines 2019.

The antibiotics tested were as follows

For urinary isolates: Ampicillin (10 µg), Ciprofloxacin (5µg), High level Gentamicin (120µg), Tetracyc line (30 µg), Vancomycin (30µg), Linezolid (30µg), Nitro furanto in (30µg) and Fosfomycin (200U).

For isolates from other sites like pus, wound, blood & others–Ampicillin (10µg), Erythromycin (15µg), Doxycycline (30µg), High level Gentamicin (120µg), Vancomycin (30µg), Teicoplanin (30µg), Linezolid (30 µg) and Ciprofloxacin (5 µ) g.

Anti-biotic disc were procured from HI media laboratories Pvt. Ltd. Mumbai, India.

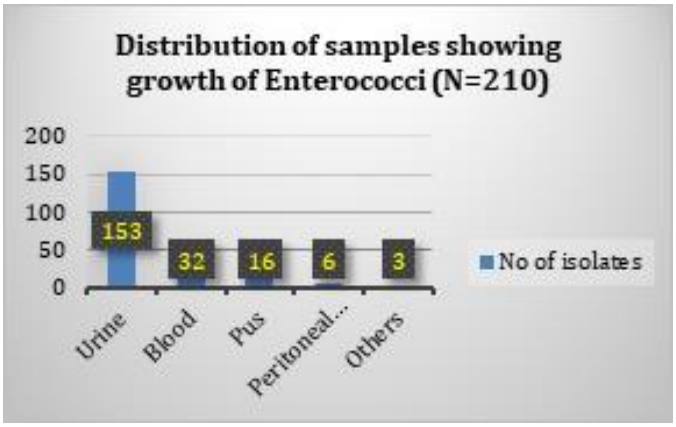
Results

A total of 210 Enterococcal isolates were studied from various clinical samples, like urine, blood, pus, peritoneal fluid and others. Majority of the Enterococcal isolates were from urine specimens 153 (73%), followed by blood (15%), pus (8%) peritoneal fluid (3%), and others (2%). Enterococci were isolated predominantly from IPD patients (83%) than from OPD patients (17%). Maximum number of isolates were from female patients (121, 58%) as compared to male patients (89, 42%). The age distribution ranged from 1 day to 86 years with maximum number of isolates were in the age group of 30 to 39 years (44, 21%). 27 (13%) isolates were from the age group 0-10 years, out of which ,10 (5%) isolates were from neonates (0 to 28 days) and 7(3%) from infants (28 days to one year-old) which is considered to be alarming.

Table 1: Distribution of samples showing growth of Enterococci (n=210)

S.No.	Type of samples	No of isolates	% of isolates
1	Urine	153	73%
2	Blood	32	15%
3	Pus	16	8%
4	Peritoneal fluid	6	3%
5	Others	3	2%
6	Total	210	100%

Fig 1: Distribution of samples showing growth of Enter ococci (n=210)



The antibiotic sensitivity results were interpreted by measuring the zone of inhibition of growth around each disc as per CLSI guidelines 2019.

Majority of Enterococcal isolates showed least sensitivity for Ciprofloxacin (9%), and Ampicillin

(26%). Sensitivity towards High level Gentamicin and Ampicillin was seen in 124 (59%) & 54 (26%) Enterococcal isolates respectively.

A synergy mechanism between Ampicillin and High-level Gentamicin which was found in 39(19%) isolates. Sensitivity for Teicoplanin, Doxycycline and Erythromycin, which were tested in samples other than urine were found to be 88%, 68% and 11% respectively. In urine samples, Nitrofurantoin sensitivity was seen in 112 (73%) isolates, while majority of isolates showed good sensitivity for Fosfomycin (97%).

Overall Vancomycin sensitivity was seen in 184 (88%) isolates. Linezolid still showed good sensitivity, with 205 (98%) isolates found to be sensitive.

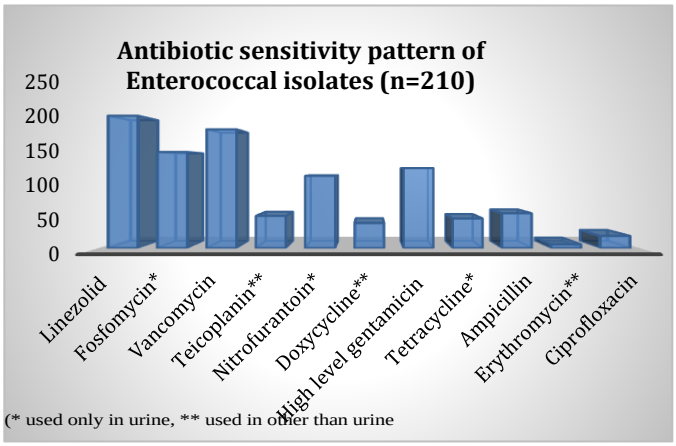
Linezolid resistance was seen in 5 (2%) Enterococcal isolates, which were isolated from urine samples, however, these isolates were sensitive to Tetracycline (4/5, 80%), Nitrofurantoin (3/5, 60%), and Fosfomycin (5/5,100%).

Table 2: Antibiotic sensitivity pattern of Enterococcal species by Kirby-Bauer disc diffusion method (N=210)

S. No	Name of antibiotic	Total no of isolates	% Sensitive
1	Linezolid	205	(98%)
2	Fosfomycin*	149	(97%)
3	Vancomycin	184	(88%)
4	Teicoplanin**	50	(88%)
5	Nitrofurantoin*	112	(73%)
6	Doxycycline**	39	(68%)
7	High level gentamicin	124	(59%)
8	Tetracycline*	46	(30%)
9	Ampicillin	54	(26%)
10	Erythromycin**	6	(11%)
11	Ciprofloxacin	19	(9%)

(* used only in urine, ** used in other than urine)

Fig 2: Antibiotic sensitivity pattern of Enterococcal species by Kirby- Bauer disc diffusion method (N=210)



Discussion

A total of 210 Enterococcal isolates were recovered from various clinical samples like urine, blood, pus and peritoneal fluid and others. In our study, majority of the Enterococcal isolates were from urine specimens 153 (73%), followed by blood (15%), pus (8%) peritoneal fluid (3%), and others (2%). Similar finding of higher rate of Enterococci isolated from urine samples have been reported by Dr Binita Bhuyan et al,[8] (81.4%) and by Chakraborty A et al,[9] 66%. The second most common sample in this study was blood (32, 15%) as also reported by Dr Binita Bhuyan et al,[8]10.3%, by David P. Kateete et al,[10] 18 % and Zelalem Tena Ferede et al,[11] 40% in their study. The majority of the specimens were from inpatients (83%) than from outpatients (35%) which is in correlation with the findings of Dr Binita Bhuyan et al [8],who have reported 139(96%) of the 145 Enterococcus isolates were from hospitalized patients and 5(4%) specimens from outpatients. Similar finding also reported by Garima Yadav et al [12], from IPD patients (189, 94.5%) than from OPD patients (11, 5.5%) and also by Amelework

Yilema et al [13] Of which, in-patients and out patients accounted for 6.8% (20/24) and 4.4% (4/24), respectively. The reason for high rate of isolation of Enterococci from females (58%) in our study might be due to the proximity of urethra to the perineal area due to shorter length of urethra in females leading to higher rate of UTI in females, which was similar to Garima Yadav et al,[12] (142, 71%) from female as compared to male patients (58, 29%).Majority of the isolates in our study were obtained from the age group of 30 to 39 year 44(21%) which is similar to other studies as reported by Adhikari RP et al, [14] 13 (21.7%) . The frequency of High-Level Gentamicin resistance was found in 86 (41%) Enterococcal isolates, which was also observed by, Adhikari RP et al,[14] 55%, Atreyi Chakraborty et al [9], 45.75% and Puneet Bhatt et al,[15] 53% which is in close resemblance to our study. HLGR in Enterococci is of great concern these days because this results in failure of synergistic bactericidal effect of beta-lactam and Amino glycosides therapy against Enterococcal infections. Resistance to high concentration of amin glycosides in Enterococci is due to the production of aminoglycosides modifying enzymes and gene mutation of antibiotic target. A synergy mechanism was seen between Ampicillin and High-Level Gentamicin which was seen in around 39(19%) isolates in our study, similar findings were observed by Atreyi Chakraborty et al,[9] 54.25%.

In urine samples, Nitrofurantoin resistance was seen in 41(27%) isolates and Fosfomycin resistance was seen in only 4(3%) isolates in our study, similar finding also recovered by Dr Binita Bhuyan et al,[8] where 17% resistance was observed for Nitrofurantoin and also by Aasish Karna et al,[16] where 18% resistance was found for Nitrofurantoin. Vancomycin resistance was observed in 26(12%) isolates in the present study, quite lower than the findings of VRE have been reported in studies by Chimanjita Phukan et al,[17] 24%, Amelework Yilema et al, [13] 41.7% and Garima Yadav et al,7% [12] which may be due to irrational use of these antibiotics in different geographical areas.

Emergence of resistance against the antibiotics like Vancomycin and Linezolid which were considered as last resort antibiotics for multidrug resistant enterococcl infections till now, is a matter of concern which emphasizes the need to explore other therapeutic options in such conditions and using them more rationally. Linezolid an oxazolidinone, introduced only in year 2000, exerts antibacterial activity by inhibiting the formation of the 70S initiation complex becomes the drug of choice for many VRE infections. Linezolid resistance in *Enterococcus* was reported from India in 2013 [18,19] and since then there has been increase in incidence of reporting Linezolid resistant VRE [17,18,20,21].

Table 3: The rates of VRE and Linezolid resistance reported from similar studies are shown in the table below.

S. No.	Author	Place and year	Vre %	Linezolid resistance
1	Puneet bhatt et al [15]	Pune, India (2015)	14%	2%
2	Atreyi chakraborty et al [9]	West Bengal, India (2015)	0%	0%
3	Chimanjita phukan et al [17]	North east, India (2016)	24%	4.5%
4	Garima	Meerut, India	7%	2%

	Yadav et al [12]	(2017)		
5	Present study	Jaipur, India (2021)	12%	2%

parental and oral formulations and the oral formulation is almost 100% bioavailable [22]. In this study, Linezolid resistance was 2%; similar results were seen in other studies as shown in [Table 3] [17,18,20,21] Mutation in the genes encoding 23S rRNA, an important part of drug binding site of ribosome is the most common mechanism of Linezolid resistance and this selection for mutated genes in rRNA was originally demonstrated in Staphylococci and have subsequently identified in Enterococci as well, and it is associated with longer duration of therapy.

This calls for proper use and de-escalation whenever Linezolid is intended for use. The other mechanism of Linezolid resistance transferable plasmid-mediated resistance to Linezolid due to cfr gene is a major threat due to its potential to spread across species [23].

Newer therapeutic option like Quinupristin/ Dalfopristin and Daptomycin has greatly increased the therapeutic option for the treatment of serious VRE infections.

Quinupristin/ Dalfopristin, a combination of Sreptogramin A (Dalfopristin) and strep to gramin B (Quinupristin) and Daptomycin a lipopeptide, anti-microbial agent is effective for VRE.

Conclusion

Enterococci are emerging as an important pathogen causing variety of hospital acquired infections and also cause community acquired infections contributing significantly to patients morbidity and mortality. The emergence of vancomycin resistant Enterococci and Linezolid resistance worsens the problem further because of the multidrug resistance exhibited by these agents leaving fewer therapeutic options for the clinicians in treating the serious life threatening VRE infections. In our study we isolated a total of 210 Enterococcal isolates from various clinical samples. Of these 26 isolates were identified as Vancomycin Resistant Enterococci with a prevalence rate of about 12% and around 5 (2%) isolates were found Linezolid resistant. They showed resistance to multiple antibiotics like Ampicillin, Doxycycline and exhibited higher rate of High-level Gentamicin resistance. The emergence of 2% Linezolid resistant VRE from the hospital is a matter of concern as till now they are considered to be last resort for treatment in patients infected with VRE. Very few reports of Linezolid resistant VRE are reported from India. The approved drug in such case Quinupristin/ Dalfopristin is inherently resistant to most of the Enterococcal species. This calls for the need of strict enforcement of antibiotic policies, coupled with greater adherence to infection control measures to prevent emergence and spread of antibiotic resistant bacteria.

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