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ORIGINAL RESEARCH

Phenotypic Characterization Of Vancomycin-Resistant Enterococcus Isolates From Different Clinical Samples At A Rural Tertiary Care Hospital: A Significant And Relevant Research In The Field Of Microbiology

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Abstract
Vancomycin-resistant Enterococci (VREs) have increasingly become a major nosocomial pathogen worldwide**.** The increasing prevalence of vancomycin-resistant enterococci (VRE) in hospitals poses a serious threat to patients due to their multiple drug resistance. This study aimed to determine the prevalence of vancomycin resistance among the various Enterococcal species isolated from clinical samples,
Methods: A Total of 150 *enterococcus species* isolated from different clinical samples were subjected to vancomycin-resistant enterococcus (VRE) screening using conventional microbiological methods. Antibioticsusceptibility testingofall*enterococcal*isolatesand screening and confirmatory tests were performed to detect specific resistance mechanisms. Minimum inhibitory concentrations (MIC) of vancomycin were determined using the microdilution method.
Results: out of 150 enterococcus species isolated from different clinical samples were subjected to vancomycin resistance using conventional microbiological methods. The incidence rate of male VRE was 7/11 (63.6%), and female VRE was 4/11 (34.4%). Most VREs were from male patients of different age groups. Of these, 7 were *E. faecalis*& 4 were *E. faecium.*
Conclusion: Enterococci have emerged as a pathogen associated with severe nosocomial infections &*E. faecalis and E. faecium* cause most clinical infections.

Introduction

Enterococci is a Gram-positive bacterium, facultatively anaerobic, and widely distributed in nature and the digestive tracts of humans and animals. They are the second leading cause of healthcare-associated infection and are an important pathogen of urethral infection, soft tissue infection, sepsis, and meningitis (1,2). Among these are Enterococci, Enterococcus faecalis, and Enterococcus faecium, which are clinically significant. Enterococcus faecium is a nosocomial pathogen, meaning it is a type of bacteria that is resistant to most antibiotics and is typically acquired in a hospital setting; responsible for about 95% of human enterococcal infections and a leading cause of hospital-acquired and multidrug-resistant infection (1,3).

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The increasing prevalence of vancomycin-resistant enterococci (VRE) in hospitals poses a serious threat to patients due to their multiple drug resistance. This research is crucial as it sheds light on the potential reservoir of resistance genes and their ability to transfer to other bacterial strains, thereby influencing and potentially revolutionizing healthcare strategies. The implications of this research on healthcare strategies are profound and necessitate adaptation and change. (4)

Vancomycin-resistant enterococcal infections have become a significant concern within the healthcare community. Three distinct glycopeptide resistance phenotypes (Van A, Van B, Van C) exist, and they can be differentiated based on the level and inducibility of resistance to vancomycin and teicoplanin. The VanA type has acquired inducible resistance to both vancomycin and teicoplanin, while the VanB type has acquired inducible resistance to vancomycin but not to teicoplanin. (5)

The early detection of resistance profiles is a crucial step in preventing the spread of VRE in hospitals and the community. The present study aimed to determine the antimicrobial resistance profile and detect the prevalence of the vanA gene in Enterococci isolated from clinical samples. The study particularly emphasizes the phenotypic characterization of vancomycin resistance in enterococci. The findings of this research are of immense significance, providing a comprehensive understanding of the resistance profiles, particularly concerning vancomycin, and will be of immense value to the field.

Material & methods

This study was conducted at …….. Indore, MP. From....., 150 enterococcal strains were isolated from diverse clinical samples, including urine, blood, pus, pleural fluid, CSF, sputum, and ascitic fluid.

The colonies with typical enterococcal morphological characteristics were first identified based on Gram staining and standard biochemical tests, including arabinose utilization, growth in 6.5% NaCl, bile esculin degradation, and pyrrolidinyl β-naphthylamide (PYR) degradation (1)

Antibiotic susceptibility testing was conducted using the Modified Kirby Bauer disc diffusion method, following the guidelines outlined by the Clinical and Laboratory Standards Institute (6). Antibiotic discs (Himedia) with specified concentrations were employed for testing, including penicillin (10U/disc), ampicillin (10µg), high-level gentamicin (120µg), ciprofloxacin (5µg), vancomycin (30µg), and linezolid (30µg), nitrofurantoin (300 µg), Fosfomycin (), Tetracycline (), Minocycline (), doxycycline ().

MIC for vancomycin was determined using the micro broth dilution method following the protocol and MIC breakpoints as per CLSI guidelines [6]. As part of quality control measures***, E. faecalis ATCC 29212*** and ***E. faecalis ATCC 51299*** were included as reference strains. These control strains ensure the accuracy and reliability of the MIC determinations, serving as benchmarks for the testing procedures. The process involves preparing a stock solution to determine vancomycin's minimum inhibitory concentration (MIC) against Enterococcus isolates using the agar dilution method.

Results

Out of 150 enterococcus isolates, ,68(45.3%) were *E. faecalis*, 52(34.6%) were *E. faecium*, and 30(20%) were other enterococcus species by phenotypic methods. The most common clinical samples from which enterococcus was isolated were pus 37(24.6%), ear swab 23(15.3%), urine 32(21.3%), Fluids & other 21(14%), Blood 19(12.6%) & sputum 18(12%). Where 11(7.3%) were VRE, and the highest number of VRE were isolated from pus & urine culture 3(27.2%) and 3(27.2%), respectively, followed by fluids & other 2(18.1%), blood 1(9.1%), ear swab 1(9.1%) and 1(9.1%) were from sputum samples.

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Table: Prevalence of VRE isolates from different clinical samples			
Clinical samples	**Number of Enterococcus (n=150)**	**VRE(n=11)**	**VRE %**
Pus	37(24.6%)	3	27.2%
Urine	32(21.3%)	3	27.2%
Blood	19(12.6%)	1	9.1%
Ear Swab	23(15.3%)	1	9.1%
Fluids & other	21(14%)	2	18.1%
Sputum	18(12%)	1	9.1
Total	**150**	**11**	**7.3%**

A total of 11(7.3%) isolates were found to be vancomycin-resistant using the broth microdilution technique as per CLSI guidelines. 7/68(10.2%) were vancomycin-resistant enterococcus faecalis, 4/52(7.7%) were vancomycin-resistant enterococcus faecium, and no VRE was isolated from other enterococcus species. All 11 isolates found vancomycin-resistant by the broth microdilution method showed MIC between ≥32µg/ml to ≥256µg/ml.

Table 2: Minimum Inhibitory Concentration (MIC) values of Vancomycin against the VRE strains.								
VRE isolates n= 11	**Vancomycin MIC values (µg/ml)**							**Total**
	Intermediate (8-16 µg/ml)		**Resistant (≥32µg/ml)**					
	8 µg/ml	**16 µg/ml**	**32 µg/ml**	**64 µg/ml**	**128 µg/ml**	**256 µg/ml**	**512 µg/ml**	
E. faecalis			3	2	1	1		7
E. faecium			2		2			4
Total			5	2	3	1		11

The sensitivity pattern of *E. faecalis and E. faecium* shows that 100% were sensitive to linezolid. Where *E. faecalis* shows that 67(98.5%) were sensitive to ampicillin, followed by 56(82.3%) were to gentamycin (HLG), **61 (89.7%)** were to vancomycin, 19(27.9%) were to ciprofloxacin, 15(22.1%) were to penicillin. In the case of *E. faecium***38(92.3%)** were sensitive to vancomycin followed by 42(82.7%) were to gentamycin (HLG), 14(26.9%) were to ciprofloxacin, 9(17.3%) were to penicillin.

Table: Antibiotic sensitive pattern of *E. faecalis* & *E. faecium*				
Antibiotics	***E. faecalis (n=68)***		***E. faecium (n=52)***	
	Sensitive	**Resistant**	**Sensitive**	**Resistant**
Ampicillin	67(98.5%)	1(1.5%)	17(32.7%)	35(67.3%)
Penicillin	15(22.1%)	53(77.9%)	9(17.3%)	43(82.6%)
Vancomycin	**61 (89.7%)**	**7(10.3%)**	**38(92.3%)**	**4(7.7%)**
Linezolid	100 (100%)	0%	100%	0%
Ciprofloxacin	19(27.9%)	49(72.1%)	14(26.9%)	38(73.1%)
Gentamycin (HLG)	**56(82.3%)**	**9(17.64%)**	**42(82.7%)**	**9(17.3%)**
Antibiotics	**Antibiotics sensitive and resistant pattern of *E. faecalis* & *E. faecium* (only for urine isolates) N=9**			
	E. faecalis (n=15)		***E. faecium (n=11)***	
Tetracycline (U)	12(80%)	3(20%)	8(72.7%)	3(27.3%)
Doxycycline (U)	12(80%)	3(20%)	9(81.8%)	2(18.2%)
Minocycline (U)	12(80%)	3(20%)	9(81.8%)	2(18.2%)

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Fosfomycin (U)	14(93.3%)	1(6.7%)	10(91.9%)	1(9.1%)
Nitrofurantoin (U)	13(86.7%)	2(13.3%)	10(91.9%)	1(9.1%)

Discussion

Enterococci have become increasingly prevalent as a significant causative agent of nosocomial infections inside hospital settings and opportunistic infections in persons with impaired immune systems [7-9]. It is widely acknowledged that these microorganisms have the potential to induce a range of severe and potentially fatal infections, including endocarditis, bloodstream infections, wound infections, and urinary tract infections [7-9]. Therefore, it is crucial to identify them early and implement appropriate treatment according to the antimicrobial susceptibility pattern. The rise of VRE has exacerbated the situation, resulting in limited choices for antibiotics to treat this multi-drug-resistant organism.

In the present study, 11(7.3%) isolates were found vancomycin-resistant by broth microdilution. All isolates showed resistance to vancomycin, with MIC ranges between ≥32μg/ml and≥256μg/ml.

The first reported VRE isolate in India was documented in New Delhi by Mathur et al. in 1999. [10]. In another study by Taneja et al., eight (5.55%) VRE were detected by the E-test and agar dilution method.[11]

Similarly, Fernandes and Dhanashree, in a study of 150 enterococcal isolates, reported a prevalence of vancomycin resistance of 8.6%.[12]. Shafiyabi et al. also reported a prevalence of 5% of vancomycin resistance among enterococci isolates in a tertiary care centre in South India.[13]. Vancomycin resistance is low in India compared to Western countries, although its rising rate is concerning. [14]

Our study aims to evaluate the frequency of vancomycin resistance in the clinical isolates of Enterococci obtained from patients in this region. Approximately 150 confirmed cases of Enterococcal isolates were obtained from various clinical samples, encompassing urine, blood, and pus.

In our study, out of 150 enterococcus isolates, the maximum isolates were from pus 37(24.6%), ear swab 23(15.3%), urine 32(21.3%), Fluids & other 21(14%), Blood 19(12.6%) & sputum 18(12%). Where 11(7.3%) were VRE, and the highest number of VRE were isolated from pus & urine culture 3(27.2%) and 3(27.2%), respectively, followed by fluids & other 2(18.1%), blood 1(9.1%), ear swab 1(9.1%) and 1(9.1%) were from sputum samples.

A study conducted by Akhter S [15] observed a VRE isolation rate of 4.65% in *E. faecalis*. Their investigation [16] reported a VRE isolation rate of 1.4%, all of which were E. faecium. This rate is lower than the rate observed in our study.

Vancomycin resistance in our isolates is a stark reminder of the importance of strictly enforcing antibiotic policies and robust infection control measures. It underscores the need for vigilant measures to prevent the emergence and spread of multi-drug-resistant bacteria, highlighting the significance of judicious antibiotic use and stringent infection control practices in healthcare settings.

Conclusion

Enterococci have emerged as a pathogen associated with severe nosocomial infections recently. Urinary tract infections are the most frequent infections caused by enterococci, followed by wound infections and bloodstream infections. E. faecalis and E. faecium cause most clinical infections. For β-lactam- and aminoglycosides-resistant, Gram-positive bacteria, vancomycin is generally used. Treating severe infections caused by vancomycin-resistant enterococci has emerged as one of the leading clinical challenges for physicians because of limited therapeutic options. In this study, **11** isolates were vancomycin resistant. These isolates are highly resistant to vancomycin using the broth microdilution method.

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References

1. Chopjitt P, Boueroy P et al. Genomic characterization of vancomycin-resistant *Enterococcus faecium* clonal complex 17 isolated from urine in tertiary hospital in Northeastern Thailand. DOI 10.3389/fmicb.2023.1278835.
2. Sun, H. L., Liu, C., Zhang, J. et al. (2019). Molecular characterization of vancomycin-resistant enterococci isolated from a hospital in Beijing, China. J. Microbiol. Immunol. Infect. 52, 433–442. doi: 10.1016/J.JMII.2018.12.008.
3. Ahmed, M. O., and Baptiste, K. E. (2018). Vancomycin-resistant enterococci: a review of antimicrobial resistance mechanisms and perspectives of human and animal health. Microb. Drug Resist. 24, 590–606. doi: 10.1089/MDR.2017.0147.
4. Hong-Li Sun, Chang Liu et al. Molecular characterization of vancomycin-resistant enterococci isolated from a hospital in Beijing, China. Journal of Microbiology, Immunology and Infection (2019)52,433e442.
5. Jaiswal S, Kumar A, Saxena S et al. Phenotypic and Genotypic Characterization Of Vancomycin-Resistant Enterococcus Isolates From Clinical Samples At A Rural Tertiary Care Hospital. DOI: 10.53555/jptcp.v31i1.4118.
6. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing, CLSI supplement M100S, 33rh ed. Wayne, Pennsylvania: CLSI; 2023.
7. Qamar MU, Rizwan M, et al. A retrospective study of antimicrobial susceptibility and clinical characteristics of multidrug-resistant polymicrobial infections in Pakistan 2019–2021. Future Microbiology. 2023 Nov;18(17):1265-77
8. Ma J, Song X, Li M, Yu Z, et al. Global spread of carbapenem-resistant Enterobacteriaceae: Epidemiological features, resistance mechanisms, detection and therapy. Microbiological Research. 2023 Jan 1; 266:127249
9. Tiwari A, Krolicka A, et al. Antibiotic resistance monitoring in wastewater in the Nordic countries: A systematic review. Environmental Research. 2024 Apr 1; 246:118052
10. Mathur P, Chaudhary R, Dhawan B, et al. Vancomycin-resistant Enterococcus bacteraemia in a lymphoma patient. Indian J Med Microbiol. 1999; 17:194-5.
11. Taneja N, Rani P, Emmanuel R, Sharma M. Significance of vancomycin-resistant enterococci from urinary specimens at a tertiary care centre in Northern India. Indian J Med Res 2004; 119:72-4.
12. Fernandes SC, Dhanashree B. Drug resistance and virulence determinants in clinical isolates of Enterococcus species. Ind J Med Res 2013; 137:981-5.
13. Shafiyabi S, Mariraj J, Sumathi S, Shanmugam, Krishna S. Emergence of vancomycin-resistant enterococci in a tertiary care hospital in South India. Int J Pharm Biomed Res 2013; 4:111-3.
14. Deshpande VR, Karmarkar MG, Mehta PR. Prevalence of multidrug-resistant enterococci in a tertiary care hospital in Mumbai, India. J Infect Dev Ctries 2013; 7:155-8.
15. Akhter S, Asna ZH, Rahman MM. Prevalence and antimicrobial susceptibility of enterococcus species isolated from clinical specimens. Mymensingh Medical Journal: MMJ. 2011 Oct 1;20(4):694-9.
16. Kirdar S, Sener AG, Arslan U, et al. Molecular epidemiology of vancomycin-resistant Enterococcus faecium strains isolated from haematological malignancy patients in a research hospital in Turkey. Journal of medical microbiology. 2010 Jun;59(6):660-4.