Original Article :

Antimicrobial Susceptibility of Enterococcus Sp to Linezolide: a 7-Year Retrospective Analysis at a Tertiary Care Hospital in Saudi Arabia

Syeda Nazia Kulsum[1] , Ali Faraz[1]

1. Assistant Professor of Microbiology, Department of Basic Medical Sciences,College of Medicine,Majmaah University, Al-Majmaah,Saudi Arabia.

Received on May 22, 2024, accepted for publication on Jan 12, 2025, dx.doi.org/10.5455/mjhs.2025.02.012

Corresponding author:

Syeda Nazia Kulsum, Assistant Professor of Microbiology, Department of Basic Medical Sciences, College Of Medicine, Majmaah University, Al-Majmaah,Saudi Arabia, Email id : naziakulsum9@gmail.com

OPEN ACCESS Distributed under Creative Commons CC BY-NC-ND 4.0

Abstract

Background and Aims:

To enhance antimicrobial and infection control strategies, it is crucial for researchers to analyze shifts in bacterial susceptibility patterns contributing to healthcare-associated infections (HAIs). This study aims to evaluate the susceptibility patterns of Enterococcus species isolated from patients at a tertiary care hospital in Saudi Arabia over a 7-year period, with a particular focus on linezolid.

Methods:

We conducted a retrospective analysis of antimicrobial susceptibility trends among Enterococcus species isolated from various clinical samples, including blood, urine, wound swabs, and other body fluids, collected between 2015 and 2021. The study utilized surveillance data from King Khalid Hospital in Al Majmaah, Saudi Arabia.

Result:

A total of 514 Enterococcus species isolates were obtained during the study period from various clinical specimens, including blood, urine, and wound swabs with 466 (90.66%) demonstrating susceptibility and 48 (9.33%) showing resistance to linezolid. There were no documented cases of mixed infections (involving more than one organism) in these samples. Each isolate was treated as a unique infection episode, and the analysis focused solely on Enterococcus species. No multiple isolates of Enterococcus species were found in the same sample or from the same patient during the study period. Each patient contributed only one isolate for analysis, ensuring no duplication in the data.

Conclusion:

Linezolid has demonstrated efficacy against Enterococcus species, exhibiting favorable clinical outcomes and a low resistance rate, thus representing a promising and cost-effective therapeutic option for treating Enterococcus-associated infections.

Keywords:

Enterococci, VRE, Linezolid, Antimicrobial Resistance, Healthcare Associated Infections

Introduction:

Initially considered as minor intestinal commensals, Enterococci, Gram-positive cocci, have undergone a transformation over the past two decades, emerging as significant pathogens. Owing to their intrinsic resistance to various commonly employed antibiotics, including Beta-lactams, aminoglycosides (when used alone), cephalosporins, co-trimoxazole, and clindamycin, treating enterococcal infections poses considerable challenges.[1] Vancomycin has traditionally served as the primary treatment for bloodstream infections (BSIs) caused by ampicillin-resistant enterococci; however, the prevalence of vancomycin-resistant enterococci (VRE) has risen significantly, accounting for approximately one-third of healthcare-associated enterococcal infections in the United States and over 20% in numerous European countries. Patients with VRE BSIs face mortality rates ranging from 20% to 46%, underscoring the severity of these infections. Notably, individuals with VRE-related BSIs exhibit a mortality risk 2.5 times higher compared to those infected with vancomycin-sensitive strains.[2]

In Saudi Arabia, Enterococcus species are increasingly recognized as significant pathogens contributing to healthcare-associated infections. Recent studies indicate a rising incidence of VRE infections within tertiary care hospitals in the region. For example, Almohammadi et al. (2019) reported that over 30% of Enterococcus isolates from clinical specimens in a tertiary hospital demonstrated vancomycin resistance, aligning with global trends of increased VRE prevalence.[3] These findings underscore the need for ongoing surveillance and evaluation of antimicrobial resistance patterns in Saudi healthcare settings. Local hospitals have primarily relied on vancomycin and linezolid for treating multidrug-resistant enterococcal infections; however, the emergence of resistance to these treatments is a growing concern.

Linezolid, a synthetic antibiotic belonging to the oxazolidinone class, represents a novel approach in antimicrobial therapy. By obstructing the initiation of protein synthesis, linezolid exerts its bacteriostatic effect, distinguishing it from other antimicrobials. Its excellent absorption and high bioavailability enable swift transition to oral administration upon clinical stabilization.[4] Approved for treating certain gram-positive infections, including drug-resistant enterococcal strains, linezolid offers promise for therapy and improved survival rates among individuals infected with Enterococcus species. However, the emergence of Linezolid-resistant enterococcal strains worldwide presents a growing concern, primarily attributed to a point mutation in

the V domain of the 23S rRNA subunit, a constituent of ribosomes.[5]

Recognizing vancomycin-resistant Enterococcus faecium as a "high priority pathogen," the World Health Organization (WHO) emphasizes the urgent need for novel treatment modalities. E. faecium, categorized within the "ESKAPE" pathogen group along with other notorious pathogens, contributes significantly to nosocomial infections, demonstrating resistance to conventional antimicrobial agents.[6] Such resistance correlates with increased morbidity and mortality rates.[7,8] Formerly considered a last resort against multidrug-resistant microorganisms, vancomycin has faced challenges with the emergence of Vancomycin-Resistant Enterococci (VRE) among hospitalized patients undergoing prolonged antibiotic therapy.[9] Reports indicate vancomycin resistance in over 40% of E. faecium bloodstream isolates, with VRE prevalence varying by geographical region.[6] Consequently, the introduction of alternative treatment options such as linezolid has become imperative.

The escalating incidence of linezolid-resistant gram-positive bacteria in recent years, paralleling the widespread utilization of linezolid in clinical settings, underscores the heightened risk of linezolid resistance dissemination.

This study aims to evaluate the antimicrobial susceptibility patterns of Enterococcus species isolated from patients at King Khalid Hospital in Saudi Arabia over a 7-year period, focusing on the efficacy of linezolid. By analyzing these patterns, we aim to provide insights that can inform and optimize current infection control strategies and antimicrobial stewardship efforts in the region.

Methods:

A retrospective study was conducted spanning from January 2015 to December 2021, utilizing medical records and sample test reports obtained from patients at King Khalid Hospital in Al Majmaah, Saudi Arabia. Enterococcus species were isolated from various clinical specimens, including blood, urine, wound swabs, and other body fluids, collected from outpatient clinics and inpatient units by the microbiology laboratory. Information regarding Enterococcus species sensitivity and antibiotic resistance was extracted from laboratory reports.

A total of 514 patient medical files were comprehensively reviewed. Samples were collected in sterile containers and promptly processed upon receipt. Specimens were processed using standard microbiological procedures as outlined in "Clinical Microbiology Procedures Handbook" (Winn et al.)[10,11]. Enterococcus species were isolated using blood agar and bile esculin agar. The plates were aerobically incubated at

35–37°C for 24–48 hours

. Bacterial identification and antibiotic susceptibility patterns were determined using the fully automated MicroscanWalk-Away 96 Plus system (Siemens) in accordance with the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI), M100 Performance Standards for Antimicrobial Susceptibility Testing.[12,13] The methodologies, techniques, and processing protocols recommended by the manufacturer were strictly adhered to.

Results:

The sensitivity of Enterococcus species to linezolid is summarized in Table 1.

Table 1: Yearly Distribution of Enterococcus Species Sensitivity to Linezolid from 514 Clinical Isolates at King Khalid Hospital, 2015–2021:

YEAR	COUNT (n)	SENSITIVE (N)	PERCENTAGE SENSITIVE (%)
2021	74	68	91.89
2020	83	77	92.77
2019	88	73	82.95
2018	86	77	89.53
2017	40	37	92.00
2016	58	53	91.37
2015	85	81	95.29
TOTAL	514	466	90.66

The mean sensitivity of Enterococcus species to linezolid across the study period was calculated to be 91.54%. The standard deviation of sensitivity percentages was found to be 3.35%, indicating the variability of sensitivity values around the mean. Furthermore, the range of sensitivity percentages, representing the difference between the highest (95.29%) and lowest (82.95%) sensitivity observed, was determined to be 12.34%. These descriptive statistics are presented in table 2.

Table 2: Comprehensive Statistical Analysis Summary:

Statistic	Value
(%) Mean Sensitivity	91.54
Standard Deviation	3.35
Range	12.34%
Confidence Interval 95%	[94.02% ,89.06%]
(Multiple R (Correlation	0.511
(R-Square (Variance Explained	0.261
Adjusted R-Square	0.077
(Significance F (p-value	0.300

A %95 confidence interval was calculated to estimate the range within which the true mean sensitivity percentage of Enterococcus species to linezolid lies. This interval provides insight into the precision of our estimate and the variability of sensitivity percentages observed across the study period. A linear regression analysis was performed to quantify the relationship between time (represented by years) and sensitivity percentages. The regression coefficients were examined to determine the significance of the slope, indicating the presence or absence of a linear trend.

Regression Analysis Results:

Regression Statistics:

The linear regression analysis was conducted to assess the relationship between the years (representing time) and the sensitivity percentages of Enterococcus species to linezolid.

The multiple R-value of 0.511 indicates a moderate positive correlation between the years and sensitivity percentages. The R-squared value of 0.261 suggests that approximately

%26.1 of the variability in sensitivity percentages can be explained by the linear relationship with the years. The adjusted R-squared value of 0.077, adjusting for the number of predictors in the model, indicates that the linear model may not fully capture the variability in sensitivity percentages.

ANOVA: The analysis of variance (ANOVA) was performed to assess the overall significance of the regression model.

Coefficients:

The regression coefficients represent the estimated relationships between the years and sensitivity percentages. The coefficients and their significance are presented in Table 3.

Table 3: Coefficients:

Variable	Coefficient	Standard Error	t Stat	P-value	Lower 95%	Upper 95%
Intercept	2415.388	1954.158	1.236	0.284	-3010.223	7840.999
2021	-1.152	0.969	-1.190	0.300	-3.842	1.537

The coefficient for the year 2021 (-1.152) indicates the estimated change in sensitivity percentage for each unit increase in the year. However, it is not statistically significant (p = 0.300), suggesting that there may not be a significant linear trend in sensitivity percentages over the years. Overall, the results of the linear regression analysis suggest that there may not be a significant linear trend in sensitivity percentages of Enterococcus species to linezolid over the study period.

Interpretation:

The confidence interval suggests that we are 95% confident that the true mean sensitivity percentage of Enterococcus spe-

cies to linezolid falls within the calculated range [Lower bound 89.06%, Upper bound94.02%]]. Furthermore, the hypothesis test results indicate that there is not a significant linear trend in sensitivity percentages over time, providing insights into the temporal dynamics of susceptibility to linezolid among Enterococcus species.

Discussion:

Despite the fact that Enterococci were formerly thought to be low-virulent, they are now among the nosocomial pathogens that have surfaced as a substantial issue. Clinical infection is less prevalent than intestinal colonization with resistant Enterococcal strains. Patients who have been colonized are a possible source of organisms spreading to health care staff, the environment, and other patients.[14]

Over the 7-year period, 514 Enterococcus isolates were collected from various clinical specimens. The overall sensitivity to linezolid was 90.66%, with a slight variation across the years. The highest sensitivity was observed in 2015 (95.29%), while the lowest was in 2019 (82.95%). These variations may reflect changes in infection control practices or antimicrobial stewardship over time .And 48 (9.33%) exhibited resistance to linezolid. This relatively high resistance rate could be attributed to the increased use of linezolid in treating drug-resistant infections. Enterococcus species were primarily isolated from bloodstream infections and urinary tract infections, with a smaller proportion from wound infections and other body fluids. The emergence of linezolid resistance necessitates strict adherence to antimicrobial stewardship programs. Infection control measures, including isolation of VRE patients, proper hand hygiene, and environmental disinfection, play a crucial role in preventing the spread of resistant strains

In a study done in Nepal, highest frequency of susceptibility was observed for linezolid (97.8%).[15] In study done in other places like Ethiopia, Bangladesh, India, Al BAHA Saudi Arabia all Enterococcus spp. isolates, were sensitive to linezolid, reported that none of the Enterococci isolates were resistant to linezolid .[14,16,17,18] In contrast the study done in a hospital in Nigeria the susceptibility was 57.8% to linezolid (30µg}.[19]

In a study done in China E. faecalis clinical isolates from the patients with UTI in this hospital setting, the frequency of linezolid or tedizolid-resistant/intermediate isolates were 22.61 and 13.04%, respectively, In present study Enterococcus sp(9.33%) were resistant to linezolide.20 In Australia resistance pattern was E. faecalis was 11% and E.faecium was 4 % respectively.[21] In Qassim region among uropathogens E. faecalis was resistant to linezolid 16.7%.[22] Present study shows En-

terococcus spp still has good sensitivity to linezolid. The microbial culture and sensitivity of the isolates from samples should be carried out as a routine before starting the antimicrobial therapy. Current knowledge of the antibiotic sensitivity/resistance patterns of Enterococcus spp at a particular geographical region is a guiding factor for choosing an appropriate empirical antimicrobial treatment.

Beyond the immediate clinical implications, the emergence of drug-resistant Enterococcus strains also highlights the critical need for ongoing research into innovative antimicrobial therapies. With the continual evolution of resistance mechanisms, staying ahead of the curve in drug development is imperative. Encouragingly, recent advancements in the field of antimicrobial research have yielded promising candidates, including novel antibiotics and combination therapies that exhibit efficacy against resistant Enterococcus strains. Moreover, these innovations not only provide hope for more effective treatment but also underscore the importance of maintaining a multifaceted approach to combating antimicrobial resistance. By fostering collaboration between researchers, healthcare providers, and policymakers, we can collectively address this ever-evolving challenge and strive for a future where infections caused by drug-resistant Enterococcus species become increasingly manageable.

Conclusion:

In this context, the findings from present study and the wealth of research from various geographical regions serve as invaluable building blocks for a comprehensive understanding of Enterococcus resistance patterns. They not only inform local treatment strategies but also contribute to the global effort to combat antimicrobial resistance. As we move forward, the collective knowledge gained from these studies will continue to shape the landscape of empirical antimicrobial treatment, emphasizing the importance of precision medicine in an era where personalized therapeutic approaches are increasingly pivotal in the fight against drug-resistant pathogens.

References:

1. Yadav G, Thakuria B, Madan M, Agwan V, Pandey A. Linezolid and vancomycin-resistant enterococci: a therapeutic problem. J Clin Diagn Res. 2017;11(8):GC07-GC11.
2. Balli EP, Venetis CA, Miyakis S. Linezolid versus daptomycin for treatment of vancomycin-resistant enterococcal bacteremia: a systematic review and meta-analysis. Antimicrob Agents Chemother. 2014;58(2):734-9.
3. Almohammadi AA, Alqahtani MA, Alshehri MS, Alharbi SM, Almutairi

MM. Antimicrobial resistance patterns of Enterococcus species isolated from clinical specimens in a tertiary hospital in Saudi Arabia. Ann Saudi Med. 2019;39(5):340-5.

4. Ament PW, Jamshed N, Horne JP. Linezolid: its role in the treatment of gram-positive, drug-resistant bacterial infections. Am Fam Physician. 2002;65(4):663-70.
5. Krawczyk B, Wysocka M, Kotłowski R, Bronk M, Michalik M, Samet A. Linezolid-resistant Enterococcus faecium strains isolated from one hospital in Poland: commensals or hospital-adapted pathogens? PLoS One. 2020;15(5):e0233504.
6. Dadashi M, Sharifian P, Bostanshirin N, Hajikhani B, Bostanghadiri N, Khosravi-Dehaghi N, et al. Global prevalence of daptomycin, tigecycline, and linezolid-resistant Enterococcus faecalis and Enterococcus faecium strains: a systematic review and meta-analysis. Front Med. 2021;8:720647.
7. O'Neil J. Tackling drug-resistant infections globally: final report and recommendations. 2016. Available from: https://amrreview.org/sites/default/files/160518_Final%20paper_with%20cover.pdf.
8. Shrivastava SR, Shrivastava PS, Ramasamy J. WHO releases global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics. J Med Soc. 2018;32:76.
9. Sarathy MV, Balaji S, Rao TJM. Enterococcal infections and drug resistance mechanisms. In: Model organisms for microbial pathogenesis, biofilm formation and antimicrobial drug discovery. Singapore: Springer; 2020. p. 131-58.
10. Winn W, Allen S, Janda W, et al. Koneman's color atlas and textbook of diagnostic microbiology. 6th ed. Philadelphia: Lippincott Williams & Wilkins; 2006.
11. Murray PR, Baron EJ, Jorgensen JH, et al. Manual of clinical microbiology. 9th ed. Washington, DC: ASM Press; 2007.
12. Clinical and Laboratory Standards Institute (CLSI). Performance standards for antimicrobial susceptibility testing. 33rd ed. CLSI supplement M100; 2023.
13. Jorgensen JH, Ferraro MJ. Antimicrobial susceptibility testing: a review of general principles and contemporary practices. Clin Infect Dis. 2009;49(11):1749-55.
14. Ferede ZT, Tullu KD, Derese SG, et al. Prevalence and antimicrobial susceptibility pattern of Enterococcus species isolated from different clinical samples at Black Lion Specialized Teach-

ing Hospital, Addis Ababa, Ethiopia. BMC Res Notes. 2018;11:793.

15. Karna A, Baral R, Khanal B, et al. Characterization of clinical isolates of enterococci with special reference to glycopeptide susceptibility at a tertiary care center of Eastern Nepal. Int J Microbiol. 2019;2019:7936156.
16. Islam TAB, Shamsuzzaman SM. Isolation and species identification of enterococci from clinical specimens with their antimicrobial susceptibility pattern in a tertiary care hospital, Bangladesh. J Coast Life Med. 2015;3(10):787-90.
17. Bhatt P, Patel A, Sahni AK, et al. Emergence of multidrug-resistant enterococci at a tertiary care centre. Med J Armed Forces India. 2015;71(2):139-44.
18. Alzahrani MA, Ali MS, Anwar S. Bacteria causing urinary tract infections and its antibiotic susceptibility pattern at a tertiary hospital in Al-Baha region, Saudi Arabia: a retrospective study. J Pharm Bioallied Sci. 2020;12(4):449-56.
19. Prevalence and antibiotics susceptibility profile of Enterococcus spp. isolated from some hospitals in Abuja, Nigeria. Afr J Clin Exp Microbiol. 2017;18(3):154.
20. Ma X, Zhang F, Bai B, et al. Linezolid resistance in Enterococcus faecalis associated with urinary tract infections of patients in tertiary hospitals in China: resistance mechanisms, virulence, and risk factors. Front Public Health. 2021;9:570650.
21. Coombs GW, Pearson JC, Daley DA, et al. Molecular epidemiology of enterococcal bacteremia in Australia. J Clin Microbiol. 2014;52(3):897-905.
22. The antibiotic susceptibility patterns of uropathogens isolated in Qassim, Saudi Arabia. Microbiology Department, College of Medical Laboratory Sciences, University of Khartoum; 2023.