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Increasing Trends of Linezolid Resistant *Enterococcus Species* in a Tertiary Care Hospital, Islamabad

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ABSTRACT

Objective: To conduct a five-year surveillance analysis of the epidemiological trends, demographic distribution and susceptibility patterns of linezolid-resistant *Enterococcus* (LRE), addressing a critical evidence gap in a region where no prior data is available.

Methodology: A retrospective, cross-sectional study was conducted from January 2020 to December 2024 at the Microbiology Laboratory, Shifa International Hospital, Islamabad. All non-duplicate *Enterococcus species* isolated from different specimens were included in the study. Demographic, specimen and susceptibility data were retrieved from the laboratory information system (LIS). *Enterococcus species* that were not susceptible to linezolid (including both intermediate and resistant isolates) were included in LRE. Data analysis was performed using SPSS software version 25.

Results: The overall rate of LRE was 1.7% (n=134/7936) increasing from 0.4 % (n=4/991) in 2020 to 2.1 % (n=34/1653) in 2024, exhibiting a significant upward trend (p value is < 0.001). Among the LRE 64.9% (87/134, 95% CI 56.8-73.0%) of the isolates were from the inpatient department (IPD). Blood cultures (49/134, 36.6%) yielded the highest count. Vancomycin and linezolid resistant *Enterococcus* (VLRE) accounted for 68.7% (92/134, 95% CI 60.8-76.5%). Antimicrobial susceptibility among both LRE (97.7%, 85/87) and VLRE (98.4%, 61/62) remained highest for tigecycline.

Conclusion: The study revealed an emerging trend of linezolid resistance among *Enterococcus* particularly in blood cultures and among inpatients, with limited therapeutic options, reflecting the clinical and nosocomial challenges of this resistance.

Keywords: Antimicrobial resistance, *Enterococcus* susceptibility, Linezolid resistant *Enterococcus,* Vancomycin resistant *Enterococcus.*

Authors' Contribution:
[1,2]Conception; Literature research; manuscript design and drafting; [3,4]Critical analysis and manuscript review; [5]Data analysis; Manuscript Editing.

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Introduction

Enterococcus is part of the normal flora of gut but may act as an opportunistic agent causing some diverse infections in humans particularly in hospital settings.[1] *Enterococcus* causes a variety of infections, mainly urinary tract, bloodstream, endocardial, intra-abdominal and prosthetic device infections. Over the last few decades, its clinical importance has markedly increased as it has emerged as a multi-drug resistant nosocomial pathogen from a harmless commensal.[2]

The emergence of the first case of vancomycin-resistant *Enterococcus* (VRE) in 1986 marked a significant milestone in antimicrobial resistance and has since become a major global healthcare concern.[3] Limited therapeutic options for the

infections caused by VRE is a serious concern in hospitals. In many low- and middle-income countries including Pakistan, treatment options are further restricted as other anti-enterococcal agents like daptomycin, are not easily available. Tigecycline also has activity against *Enterococcus*, but due to its low serum concentration, it is not considered as suitable treatment option for bloodstream infections. Consequently, linezolid remains one of the last resort therapeutic options against VRE.[4,5]

Linezolid was first launched in 2000 for clinical use following approval by FDA.[6] However, soon after its introduction, the linezolid resistance was reported from different regions of the world which is alarming for clinicians as this leaves very limited treatment options. This resistance occurs due to mutations in the 23S ribosomal RNA gene, and/or acquisition of transferable resistance genes such as the cfr, poxtA, and optrA genes.[7]

Linezolid resistance among *Enterococcu*s has been reported from different regions of the world. Treating these resistant organisms is challenging and also results in prolonged hospital stay, increased cost of healthcare and poor outcomes.

Despite the increasing global reports of LRE, data from Pakistan remain scarce, and no study has evaluated the long-term trend of linezolid resistance among *Enterococcus species* in the region. In our setting, more cases of linezolid resistance among *Enterococcus*, have been observed in recent years. To understand this trend, we analyzed data of five years' period for LRE. This study aimed to describe the increasing trend of LRE in our population, its demographic distribution, and susceptibility.

Methodology

A retrospective, cross-sectional study was conducted over the period of five years from 1st January 2020 to 31st December 2024 in Microbiology laboratory, Shifa International Hospital. Islamabad.

All non-duplicate *Enterococcus species* (other than *Enterococcus casseliflavus* and *Enterococcus gallinarum*) isolated during this time span from all culture samples (including blood, urine, wound, pus, tissue, catheter tips and others) received in the laboratory were included in the study. *Enterococcus casseliflavus* and *Enterococcus gallinarum* were excluded from the study as being intrinsic resistant to vancomycin. Any duplicate *Enterococcus* isolate from the same patient was excluded.

Patient demographic details including age, gender and hospital setting, as well as clinical sample types, were extracted from laboratory information system (LIS). Patient ages were grouped as 0-17, 18-29, 30-39, 40-49, 50- 59, 60-69 and ≥70 years. The susceptibility of *Enterococcus species* was also retrieved from the LIS.

Identification of *Enterococcus species* was carried out using conventional biochemical tests (including Gram staining, catalase test, bile esculin hydrolysis, and Lancefield grouping) and VITEK 2 compact system (bioMérieux, France), following the manufacturer's instructions.

Antimicrobial susceptibility testing was performed by the Kirby-Bauer disk diffusion method on Mueller-Hinton agar and VITEK 2 Gram-positive susceptibility card and interpreted according to latest Clinical and Laboratory Standards Institute (CLSI) M100.[8] European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints were used where CLSI breakpoints were not available like tigecycline susceptibility.[9]

A panel of antibiotics commonly used against *Enterococcus* was tested i-e ampicillin, vancomycin, clarithromycin, chloramphenicol, doxycycline/minocycline, tigecycline, linezolid, ciprofloxacin (urine samples only), and nitrofurantoin (urine samples only). Clarithromycin and chloramphenicol susceptibility testing was not performed for urine isolates, in accordance with CLSI recommendations. Tigecycline susceptibility was also not performed in urine cultures as it is not approved for urinary tract infections. In our study, all *Enterococcus Species* that were not susceptible to linezolid (i-e. intermediate or resistant as response

rate may be lower or not achieved by usual therapeutic concentration, with normal dosage respectively) were included as LRE.

Data analysis was performed using SPSS software version 25. Patient age was summarized as mean, median, and interquartile range (IQR). Yearly trend, gender, age groups, hospital setting, sample type, and antimicrobial susceptibility were reported as percentages with 95% confidence interval (95% CI*)*. The Chi-square test was used to assess the yearly trends of LRE, and Fisher's exact test was used to compare tigecycline susceptibility between LRE and VLRE cases. A p-value ≤0.05 was considered statistically significant.

The study was approved by Institute Ethical and Research Committee (IRB. 353-25) on 21-08-2025.

Results

During this five years' study period, total 8019 *Enterococcus* were isolated. Eighty-three isolates were *Enterococcus casseliflavus/ Enterococcus gallinarum* and excluded. Out of remaining 7936 *Enterococcus,* 134 (1.7%, 95%CI 1.4-2.0%) were LRE. Co-resistance to vancomycin was observed in 92 cases (68.7%, 95%CI 60.8-76.5%). LRE cases increased from 0.4 % to 2.1 % from 2020 to 2024 exhibiting significant upward trend (p value is <0.001). Out of 134 LRE, 88% cases were resistant while 12% showed intermediate susceptibility (Table I).

Demographic analysis revealed 74 cases (55.2%, 95% CI 46.8-63.6%) of LRE were isolated from samples of male patients while 60 case (44.8%, 95% CI 36.4-53.2%) from female patients. Significantly higher number of cases i-e 87 (64.9%, 95% CI 56.8-73.0%) cases were from inpatient department (IPD) while 47 (35.1%, 95% CI 27.0-43.2%) were from outpatient department (OPD) (p value 0.0001). Age distribution showed highest number of LRE cases in 60-70 years (n=29) and 50-59 years (n=29) age group, followed by 70 years plus (n=26), 40-49 years (n=20), 18-29 years (n=14 years). Lowest frequency was in pediatric patients i-e 0-17 years (n=8) and 30-39 years (n=8) age group. Mean and median age was 50±21 years and 53 years respectively with IQR 36-67 years.

Table I: Yearly trend of linezolid resistant *Enterococcus*

Year	*Enterococcus species* isolated	Linezolid Intermediate	Linezolid Resistant	Total LRE n (%)
2020	991	2	2	4 (0.4)
2021	1772	0	12	12 (0.7)
2022	1818	8	41	49 (2.7)
2023	1702	2	33	35 (2.1)
2024	1653	4	30	34 (2.1)
total	7936	16	118	134 (1.7)

Table II: Antimicrobial Susceptibility Profile of LRE

Antibiotics	Number tested*	Susceptible n (%)
Ampicillin	132	26 (19.7)
Vancomycin	134	42 (31.3)
Doxycycline	126	52 (41.3)
Tigecycline	87	85 (97.7)
Minocycline	28	12(42.9)
Clarithromycin	100	5 (5)
Chloramphenicol	81	27 (33.3)
Nitrofurantoin	33	28 (84.8)
Ciprofloxacin	32	7 (21.9)

***** The total number tested varied due to some agents not tested for specific sample type or susceptibility data was not available for some isolates due to retrospective nature of study.

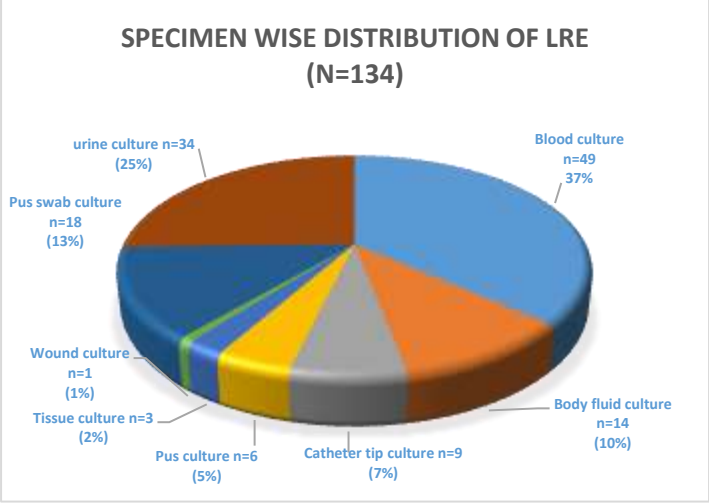


Figure 1: Specimen Wise Distribution of LRE

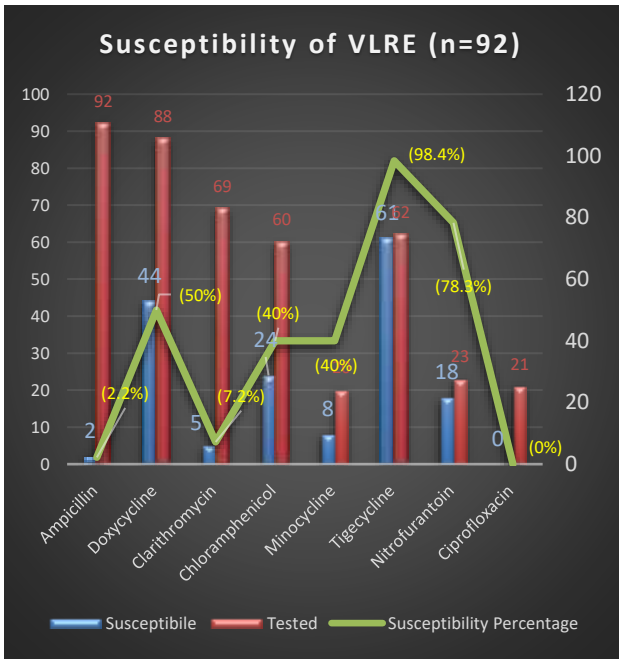


Figure 2: Antimicrobial Susceptibility of VLRE

Discussion

Enterococcus, being intrinsically resistant to many antibiotics and continuously developing resistance to anti-enterococcal antibiotics, is one of the most serious threats to antibiotic resistance control efforts. In this five-year analysis, the overall rate of LRE over five years was 1.7%. Though the rate was modest but its increasing trend is concerning as it gradually increased from 0.4 % (4 cases in 2020) to 2.1% (34 cases in 2024). LRE has been reported globally with variable prevalence. A systematic review by Guan et al in 2024 highlighted the global increase in antimicrobial resistance including linezolid.[10] In Europe Mališová et al reported 8% linezolid resistance among *Enterococcus* over the period of 10 years.[11] A five-year surveillance by Bai B et al. documented an increase in resistance from 1.89 % to 7.04 % from 2011 to 2015 in Chinese hospital setting.[12]

In Pakistan a study was conducted in Karachi in 2024 to investigate the genome sequencing of 13 LRE isolated over a period of two years. It highlighted presence of multiple resistance mechanisms among LRE, reflecting emerging resistance challenge. Nasir SA et al. recommended development of national guidelines for judicious use of linezolid.[5]

In April 2025 Rauschenberge et al concluded that extended hospital stay increases the likelihood of linezolid resistance and recommended that infection control practices should emphasize on antimicrobial stewardship as well, given that linezolid resistance was rarely associated with transmission events.[13] Inpatient predominance in our study supports this association. The higher rate in the IPD setting and blood cultures may reflect prolonged antibiotic exposure, longer hospital stay, invasive procedures like use of central line, and can increase risk of nosocomial transmission.[14]

Among these LRE, 68.6% isolates were resistant to both vancomycin and Linezolid. This situation is challenging, as these antibiotics have a critical role in managing severe enterococcal infections.[15] Our findings align with global trends, highlighting co-resistance of LRE to multiple antimicrobials, severely limiting therapeutic options. Similarly, Rani V et al., in 2024 concluded that increase in LRE is associated with increased use of antibiotics and regular surveillence is required to monitor it.[16] Yadav et al addressed multidrug resistance in enterococcus in a study carried out in India and raised concern of limited therapeutic options.[17]

Tigecycline (97.7%) showed high in vitro activity against these resistant organisms, offering a possible salvage option. Dadashi et al also demonstrated good susceptibility of tigecycline against enterococcal infections worldwide; however, its use is restricted in bloodstream infections due to pharmacokinetic constraints.[18]

Studies have linked the emergence of linezolid resistance to chromosomal mutations in the 23S rRNA gene and transferable resistance genes such as optrA, poxtA, and cfr. Wang et al. identified optrA as the most common cause resistance gene in LRE.[19] Similarly, Rani et at., in a study conducted at a tertiary care hospital India, linked 83% of LRE with optrA gene.[20] The detection of such resistance

elements raises concern about risk of horizontal gene transfer and dissemination in hospital environments.[21] This emphasizes the need for continuous molecular surveillance of such resistant organisms.

Limitations: This study has its limitations. First the data collection was collected from a single center. Secondly molecular characterization of resistance mechanisms was not performed, which would have provided deeper insights into the underlying mechanisms of linezolid resistance. Third, clinical outcomes and treatment history was not available. Despite these limitations, our results provide valuable baseline data on the emerging problem of linezolid resistance among *Enterococcus* and underline the urgent need for multicenter surveillance and molecular epidemiological studies.

Conclusion

The study revealed an emerging trend of linezolid resistance among *Enterococcus*. Most resistant cases were recovered from blood cultures and predominantly from inpatients, with limited therapeutic options, reflecting the clinical and nosocomial challenges posed by this resistance. Tigecycline exhibited good susceptibility against LRE and can be used for complicated skin and soft tissue infections and intra-abdominal infections, but has limitations in the treatment of bacteremia. These findings highlight the urgent need for stringent antimicrobial stewardship, strict infection control measures, and availability of more treatment options. Continuous monitoring and surveillance throughout the country is crucial to determine the true burden and prevent further dissemination of linezolid-resistant *Enterococcus*.

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