Emerging Genetic Mechanisms of Linezolid Resistance in Enterococci

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ABSTRACT

Background: The emergence of linezolid-resistant enterococci (LRE) represents a significant threat to public health, especially in hospital settings where vancomycin-resistant enterococci (VRE) already pose major treatment challenges. Linezolid, a synthetic oxazolidinone antibiotic, has been widely used as a last-resort treatment against multidrug-resistant Gram-positive infections. However, the increasing prevalence of resistance has been linked to various genetic determinants. These include chromosomal mutations in the 23S rRNA gene and ribosomal proteins, as well as the acquisition of mobile resistance genes such as *optrA*, *poxtA*, and *cfr*. The horizontal transfer of these genes—often carried on plasmids and transposons—facilitates the rapid spread of resistance across clinical, animal, and environmental isolates. This review provides a comprehensive overview of the emerging genetic mechanisms underlying linezolid resistance in *Enterococcus faecalis* and *Enterococcus faecium*, with a focus on molecular pathways, epidemiological trends, and implications for clinical treatment. Understanding these mechanisms is critical to developing effective surveillance systems and stewardship strategies aimed at curbing the dissemination of resistance in enterococcal populations.

Keywords: Enterococcus faecalis, Enterococcus faecium, linezolid resistance, *optrA*, *poxtA*, *cfr*, 23S rRNA mutations, antibiotic resistance genes, mobile genetic elements.

1. INTRODUCTION

Enterococci, particularly *Enterococcus faecalis* and *Enterococcus faecium*, have transitioned from being benign gut commensals to opportunistic pathogens of major clinical concern. These Gram-positive cocci are now recognized as leading causes of hospital-acquired infections, including bacteremia, urinary tract infections, surgical site infections, and infective endocarditis—especially among immunocompromised and critically ill patients [1,2]. The ability of enterococci to persist in harsh environments, form biofilms, and withstand disinfection procedures enhances their survival in healthcare settings, contributing to widespread nosocomial transmission [3].

Over the past two decades, the treatment of enterococcal infections has been severely complicated by the emergence of multidrug resistance. Vancomycin-resistant enterococci (VRE), once considered the

pinnacle of treatment difficulty, have now been joined by strains resistant to linezolid, a vital last-resort oxazolidinone antibiotic used against Gram-positive infections, including VRE [4,5]. Although linezolid was initially effective against these resistant pathogens, its extensive use has led to the selection and dissemination of resistant strains in both hospital and community environments [6].

Linezolid resistance in enterococci can arise through chromosomal mutations or acquisition of mobile resistance genes. The most common chromosomal mechanism involves mutations in the domain V of the 23S rRNA gene, particularly the G2576T substitution, which diminishes linezolid's ability to bind to the ribosomal RNA and inhibit protein synthesis [7]. On the other hand, plasmid-mediated genes such as *optrA*, *poxtA*, and *cfr* confer resistance through ribosomal protection or methylation, and they pose an even greater threat due to their potential for horizontal gene transfer across species and environments [8–10].

This review aims to provide an updated synthesis of the emerging genetic mechanisms of linezolid resistance in enterococci. Special attention is given to molecular pathways, the epidemiology of resistance genes, and the implications of mobile genetic elements in resistance dissemination. Understanding these mechanisms is essential for developing targeted diagnostic tools, guiding effective therapy, and implementing infection control and antimicrobial stewardship strategies.

Molecular Basis of Linezolid Action and Mechanisms of Resistance

Linezolid is a synthetic antibiotic from the oxazolidinone class, designed to inhibit bacterial protein synthesis by binding to the peptidyl transferase center of the 50S ribosomal subunit. Specifically, it targets domain V of the 23S rRNA, preventing the formation of the 70S initiation complex essential for translation. This unique mode of action made linezolid initially highly effective against multidrug-resistant Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) [11,12].

However, enterococci have rapidly evolved mechanisms to evade the inhibitory effects of linezolid. These mechanisms are generally categorized into two major groups: chromosomal mutations and acquisition of transferable resistance genes.

1. Chromosomal Mutations

The most well-characterized mechanism of linezolid resistance in enterococci is point mutations in the domain V region of the 23S rRNA gene. Among these, the G2576T mutation (Escherichia coli numbering) is the most commonly reported and has been associated with high-level resistance [13]. Since enterococci possess multiple copies of the rRNA operon (typically 4–6), the degree of resistance often correlates with the number of mutated alleles [14].

In addition to 23S rRNA mutations, alterations in ribosomal proteins L3 and L4 (encoded by *rplC* and *rplD*) have been implicated in resistance. These proteins are located near the linezolid binding site and may indirectly affect the antibiotic's binding affinity by altering ribosome conformation [15].

2. Plasmid-Mediated Resistance Genes

Plasmid-borne genes present a greater epidemiological concern due to their ability to spread horizontally between bacterial species.

- **optrA**: Encodes an ATP-binding cassette F (ABC-F) protein that mediates ribosomal protection, preventing linezolid from binding to its ribosomal target. It also confers resistance to phenicols, suggesting its role in multidrug resistance contexts [16].
- **poxtA**: Similar to *optrA*, this gene encodes another ABC-F ribosomal protection protein. It confers low-level resistance to linezolid and phenicols and is often found co-located with other resistance determinants [17].
- **cfr**: Encodes a methyltransferase that modifies adenine at position A2503 in the 23S rRNA, thereby reducing binding affinity of linezolid and several other antibiotics including phenicols, lincosamides, pleuromutilins, and streptogramin A (the PhLOPSA phenotype) [18].

These genes are often carried on transposons, integrative conjugative elements, or plasmids, making them highly mobile and a significant threat for cross-species dissemination. Notably, the detection of *optrA* and *poxtA* in both clinical and animal-derived isolates highlights the potential role of the food chain and agricultural use of antimicrobials in the propagation of resistance [19].

Understanding these genetic pathways is crucial not only for accurate diagnosis and surveillance but also for the development of targeted therapeutic approaches and preventive measures.

Epidemiology and Global Dissemination of Linezolid Resistance Genes

The global dissemination of linezolid-resistant *Enterococcus* species, especially *E. faecalis* and *E. faecium*, reflects a concerning trend in both hospital and community settings. Although initially rare, resistance rates have been increasing due to both clonal spread and horizontal gene transfer, particularly of plasmid-mediated resistance genes such as *optrA*, *poxtA*, and *cfr* [20].

1. Clinical Settings and Healthcare Environments

Hospitals remain the primary hotspots for the emergence and spread of linezolid-resistant *Enterococcus* (LRE) strains. Intensive care units (ICUs), transplant wards, and long-term care facilities are particularly affected due to the frequent use of broad-spectrum antibiotics and the presence of immunocompromised patients. Several studies have documented outbreaks of LRE associated with epidemic clones carrying *optrA* or *cfr*, particularly in regions with high linezolid consumption [21].

Clonal outbreaks of *E. faecium* ST117 and ST480, harboring *poxtA* or *optrA*, have been reported in European countries such as Ireland, Denmark, and Spain, as well as in parts of Asia and South America [22]. These clones often exhibit multidrug-resistant profiles, further limiting therapeutic options and increasing the burden on healthcare systems.

2. Zoonotic and Environmental Reservoirs

A striking aspect of linezolid resistance is its detection in non-clinical environments. Multiple surveillance studies have identified *optrA*, *poxtA*, and *cfr* genes in enterococcal isolates from food-producing animals, retail meat products, animal manure, and water sources [23]. This has raised serious concerns about the role of the One Health interface in the spread of antimicrobial resistance.

In particular, *optrA* has been frequently found in *Enterococcus* species isolated from poultry, swine, and cattle in regions such as China, Korea, and the Middle East. These findings suggest that agricultural use of antibiotics, even those unrelated to linezolid, may co-select for resistance determinants due to genetic linkage on mobile elements [24].

3. Mobile Genetic Elements and Global Spread

The mobility of resistance genes is facilitated by insertion sequences, transposons, and conjugative plasmids, which allow for rapid dissemination across bacterial species and environmental boundaries. For instance, IS1216 and Tn6674 have been implicated in the mobilization and recombination of *optrA*-carrying plasmids [25].

Recent genomic epidemiology studies using whole-genome sequencing (WGS) have shown the polyclonal nature of LRE outbreaks, supporting the theory that resistance dissemination is driven not only by clonal expansion but also by independent acquisition events across diverse strains and geographies [26].

4. Surveillance and Reporting Challenges

Despite the growing threat, global surveillance for linezolid resistance remains patchy and often limited to high-income countries. Many low- and middle-income regions lack robust laboratory capacity to detect and report these resistance genes, especially those carried on plasmids. This underreporting creates a blind spot in our understanding of the true global burden and hampers coordinated control efforts [27].

The integration of LRE monitoring into national and international antimicrobial resistance (AMR) surveillance programs, including those supported by the WHO Global Antimicrobial Resistance Surveillance System (GLASS), is crucial for timely response and containment.

Clinical Implications and Treatment Challenges of Linezolid-Resistant Enterococci

The emergence of linezolid-resistant *Enterococcus* (LRE) species presents serious clinical challenges, particularly in patients with limited treatment options due to multidrug resistance. Linezolid has long served as a reliable agent against vancomycin-resistant enterococci (VRE), and its compromise threatens the management of severe Gram-positive infections in both inpatient and outpatient settings [28].

1. Therapeutic Limitations

Linezolid-resistant strains are frequently co-resistant to multiple other antibiotic classes, including aminoglycosides, macrolides, and glycopeptides. This resistance narrows treatment options

significantly, especially in cases of bloodstream infections, endocarditis, or deep-seated infections requiring bactericidal agents. While daptomycin and tigecycline remain options, their efficacy is variable and may be limited by pharmacokinetic constraints or emerging resistance [29].

For central nervous system (CNS) infections or endovascular foci, linezolid was previously favored due to its good tissue penetration. The loss of its efficacy in resistant strains presents additional therapeutic dilemmas, often necessitating off-label or combination therapies with uncertain outcomes [30].

2. Diagnostic and Empiric Therapy Challenges

The accurate detection of linezolid resistance is crucial for guiding therapy but is not always routinely performed in many microbiology laboratories, especially in resource-limited settings. Standard susceptibility tests may not detect low-level resistance mediated by certain *optrA* or *poxtA* variants. Moreover, empiric therapy based on prior resistance patterns may lead to treatment failure when resistance emerges unnoticed [31].

Delayed initiation of appropriate therapy has been linked to increased morbidity, length of hospital stay, and healthcare costs. In critically ill patients, this delay may also be associated with higher mortality [32].

3. Risk Factors and Clinical Outcomes

Linezolid resistance is more frequently observed in patients with prior exposure to linezolid, prolonged hospitalization, intensive care unit admission, and those with immunosuppressive conditions. Patients colonized with LRE are at increased risk of subsequent infections, particularly if invasive procedures are required [33].

Clinical outcomes in LRE infections are often poorer than those with susceptible isolates. Studies have demonstrated higher relapse rates, prolonged bacteremia, and increased incidence of complications such as endocarditis or persistent urinary tract infections [34].

4. Infection Control Considerations

From an infection control perspective, LRE strains pose a heightened risk of nosocomial transmission. Colonized or infected patients can serve as reservoirs for transmission within healthcare settings, particularly in the absence of strict hygiene protocols. The capacity of *Enterococcus* to survive on surfaces for extended periods and its frequent involvement in biofilms exacerbate this risk [35].

Infection control measures—such as patient cohorting, contact precautions, environmental decontamination, and staff education—are essential to limit spread. Molecular typing of outbreak strains may also help trace transmission pathways and inform targeted interventions.

Prevention and Control Strategies for Linezolid-Resistant Enterococci

Preventing the spread of linezolid-resistant *Enterococcus* (LRE) in healthcare and community settings is a critical priority due to the organism's ability to persist in the environment, form resilient biofilms,

and transmit resistance genes horizontally. Effective control demands a multifaceted approach, combining infection prevention practices, robust surveillance, and antimicrobial stewardship.

1. Infection Prevention and Control (IPC) in Healthcare Settings

In hospital environments, LRE can spread rapidly among high-risk patient populations, especially in intensive care units and transplant wards. Standard precautions, including strict hand hygiene, use of personal protective equipment (PPE), and patient isolation, are foundational in reducing transmission [36].

Environmental cleaning plays a vital role, as *Enterococcus* species can survive for prolonged periods on inanimate surfaces. High-touch areas and reusable medical equipment should be disinfected with agents proven effective against resistant Gram-positive organisms. Biofilm removal strategies are particularly important in managing devices such as catheters and endotracheal tubes [37].

2. Screening and Early Detection

Active surveillance through rectal swabs or molecular detection techniques (e.g., PCR assays targeting *optrA*, *poxtA*, or *cfr*) can identify colonized individuals and facilitate early intervention. Screening is especially recommended for high-risk patients, including those with a history of prolonged antibiotic exposure, previous VRE colonization, or recent intensive care admission [38].

Early identification of colonized or infected patients allows for implementation of isolation protocols and helps reduce hospital-wide outbreaks. Additionally, routine monitoring of resistance trends enables real-time updates to empiric therapy guidelines and infection control strategies.

3. Antimicrobial Stewardship

Linezolid resistance is frequently associated with inappropriate or prolonged use of the drug. Stewardship programs that promote appropriate antibiotic selection, dose optimization, and duration limitation are essential to preserving the efficacy of last-resort antimicrobials [39].

Incorporating rapid diagnostics and susceptibility testing into treatment decisions ensures that linezolid is reserved for infections where it is truly indicated. De-escalation strategies based on microbiological results and real-time feedback to prescribers have shown success in reducing unnecessary linezolid use [40].

4. One Health and Environmental Surveillance

Since resistance genes like *optrA* and *poxtA* have been detected in both human and animal isolates, One Health approaches are vital. Monitoring food sources, agricultural antibiotic use, and wastewater contamination helps track zoonotic and environmental pathways of resistance dissemination.

International cooperation is necessary to develop harmonized surveillance frameworks, particularly in regions with unregulated antibiotic usage in livestock. Public health policies must address both clinical and non-clinical drivers of resistance to prevent further spread.

Conclusion

The rise of linezolid-resistant *Enterococcus* species underscores the dynamic and evolving nature of antimicrobial resistance in modern healthcare. What was once a reliable last-line antibiotic is now compromised by both chromosomal mutations and the global dissemination of mobile resistance genes such as *optrA*, *poxtA*, and *cfr*. These genetic mechanisms not only threaten current therapeutic options but also highlight the remarkable adaptability of *Enterococcus* spp. in response to antimicrobial pressure.

As these resistant strains become more prevalent in hospitals, communities, animals, and the environment, a coordinated, multidisciplinary response is urgently needed. Effective strategies include enhanced infection prevention and control, judicious antibiotic stewardship, and investment in rapid diagnostics and novel antimicrobials. Equally important is the integration of One Health principles, acknowledging that resistance is not confined to human medicine but is intricately linked to veterinary and environmental sectors.

Understanding and addressing the genetic mechanisms of linezolid resistance in *Enterococci* is essential to safeguarding the efficacy of this vital antimicrobial class and preventing further erosion of our therapeutic arsenal.

REFERENCES

1. Bai B, Hu K, Zeng J, et al. Linezolid consumption facilitates the development of linezolid resistance in Enterococcus faecalis in a tertiary-care hospital: a 5-year surveillance study. Microb Drug Resist. 2019;25(6):791-798.
2. Mendes RE, Deshpande LM, Jones RN. Linezolid update: stable in vitro activity following more than a decade of clinical use and summary of associated resistance mechanisms. Drug Resist Updat. 2014;17(1-2):1-12.
3. Brenciani A, Morroni G, Schwarz S, Giovanetti E. Oxazolidinones: mechanisms of resistance and mobile genetic elements involved. J Antimicrob Chemother. 2022;77(10):2596-2621.
4. Antonelli A, D'Andrea MM, Brenciani A, et al. Characterization of poxtA, a novel phenicol–oxazolidinone–tetracycline resistance gene from an MRSA of clinical origin. J Antimicrob Chemother. 2018;73(7):1763-1769.
5. Sadowy E. Linezolid resistance genes and genetic elements enhancing their dissemination in enterococci and streptococci. Plasmid. 2018;99:89-98.
6. Liu BG, Yuan XL, He DD, et al. Research progress on the oxazolidinone drug linezolid resistance. Eur Rev Med Pharmacol Sci. 2020;24(18).
7. Ruiz-Ripa L, Feßler AT, Hanke D, et al. Mechanisms of linezolid resistance among enterococci of clinical origin in Spain—detection of optrA- and cfr(D)-carrying E. faecalis. Microorganisms. 2020;8(8):1155.

8. Egan SA, Shore AC, O'Connell B, et al. Linezolid resistance in Enterococcus faecium and Enterococcus faecalis from hospitalized patients in Ireland: high prevalence of the MDR genes optrA and poxtA. J Antimicrob Chemother. 2020;75(7):1704-1711.
9. Misiakou MA, Hertz FB, Schønning K, et al. Emergence of linezolid-resistant Enterococcus faecium in a tertiary hospital in Copenhagen. Microb Genom. 2023;9(7):001055.
10. Sassi M, Guérin F, Lesec L, et al. Genetic characterization of a VanG-type vancomycin-resistant Enterococcus faecium clinical isolate. J Antimicrob Chemother. 2018;73(4):852-855.
11. He T, Shen Y, Schwarz S, et al. Genetic environment of the transferable oxazolidinone/phenicol resistance gene optrA in Enterococcus faecalis isolates of human and animal origin. J Antimicrob Chemother. 2016;71(6):1466-1473.
12. Crowe-McAuliffe C, Murina V, Turnbull KJ, et al. Structural basis for PoxtA-mediated resistance to phenicol and oxazolidinone antibiotics. Nat Commun. 2022;13(1):1860.
13. Schwarz S, Zhang W, Du XD, et al. Mobile oxazolidinone resistance genes in Gram-positive and Gram-negative bacteria. Clin Microbiol Rev. 2021;34(3):e00088-20.
14. Sharkey LK, O'Neill AJ. Antibiotic resistance ABC-F proteins: bringing target protection into the limelight. ACS Infect Dis. 2018;4(3):239-246.
15. Vester B. The cfr and cfr-like multiple resistance genes. Res Microbiol. 2018;169(2):61-66.
16. Wilson DN, Hauryliuk V, Atkinson GC, O'Neill AJ. Target protection as a key antibiotic resistance mechanism. Nat Rev Microbiol. 2020;18(11):637-648.
17. Zhang X, Jiang C, Zhou C. Diagnosis of Enterococcus faecalis meningitis associated with long-term cerebrospinal fluid rhinorrhoea using metagenomics next-generation sequencing: a case report. BMC Infect Dis. 2021;21:1-4.
18. Wada Y, Harun A, Yean CY, Zaidah AR. A Nanoplex PCR assay for the simultaneous detection of vancomycin- and linezolid-resistant genes in Enterococcus. Diagnostics (Basel). 2023;13(4):722.
19. Torres C, Alonso CA, Ruiz-Ripa L, et al. Antimicrobial resistance in Enterococcus spp. of animal origin. In: Antimicrobial Resistance in Bacteria from Livestock and Companion Animals. 2018:185-227.
20. Ma X, Zhang F, Bai B, et al. Linezolid resistance in Enterococcus faecalis associated with urinary tract infections of patients in a tertiary hospital in China: resistance mechanisms, virulence, and risk factors. Front Public Health. 2021;9:570650.
21. Chen H, Du Y, Xia Q, Li Y, Song S, Huang X. Role of linezolid combination therapy for serious infections: review of the current evidence. *Eur J Clin Microbiol Infect Dis.* 2020;39:1043–1052.
22. Fernandes GFS, Scarim CB, Kim SH, Wu J, Castagnolo D. Oxazolidinones as versatile scaffolds in medicinal chemistry. *RSC Med Chem.* 2023;14(5):823–847.
23. Jin B, Wang T, Chen JY, et al. Synthesis and biological evaluation of 3-(pyridine-3-yl)-2-oxazolidinone derivatives as antibacterial agents. *Front Chem.* 2022;10:949813.
24. Leach KL, Brickner SJ, Noe MC, Miller PF. Linezolid, the first oxazolidinone antibacterial agent. *Ann N Y Acad Sci.* 2011;1222(1):49–54.
25. Buckley ME, Ndukwe AR, Nair PC, et al. Comparative assessment of docking programs for docking and virtual screening of ribosomal oxazolidinone antibacterial agents. *Antibiotics.* 2023;12(3):463.
26. Gatti M, Raschi E, De Ponti F. Serotonin syndrome by drug interactions with linezolid: clues from pharmacovigilance-pharmacokinetic/pharmacodynamic analysis. *Eur J Clin Pharmacol.* 2021;77:233–239.
27. Shu L, Huo BN, Yin NG, et al. Clinical drug interactions between linezolid and other antibiotics: for adverse drug event monitoring. *Pharmacol Res Perspect.* 2024;12(4):e1236.

28. Di Franco S, Alfieri A, Pace MC, et al. Blood stream infections from MDR bacteria. *Life (Basel).* 2021;11(6):575.
29. Dadashi M, Sharifian P, Bostanshirin N, et al. The global prevalence of daptomycin, tigecycline, and linezolid-resistant *Enterococcus faecalis* and *Enterococcus faecium* strains from human clinical samples: a systematic review and meta-analysis. *Front Med (Lausanne).* 2021;8:720647.
30. Zarzecka U, Zakrzewski AJ, Chajęcka-Wierzchowska W, Zadernowska A. Linezolid-resistant *Enterococcus* spp. isolates from foods of animal origin—the genetic basis of acquired resistance. *Foods.* 2022;11(7):975.
31. Schwarz S, Zhang W, Du XD, et al. Mobile oxazolidinone resistance genes in Gram-positive and Gram-negative bacteria. *Clin Microbiol Rev.* 2021;34(3):10.1128/CMR.00088-20.
32. Brenciani A, Morroni G, Schwarz S, Giovanetti E. Oxazolidinones: mechanisms of resistance and mobile genetic elements involved. *J Antimicrob Chemother.* 2022;77(10):2596–2621.
33. Antonelli A, D'Andrea MM, Brenciani A, et al. Characterization of *poxtA*, a novel phenicol–oxazolidinone–tetracycline resistance gene from an MRSA of clinical origin. *J Antimicrob Chemother.* 2018;73(7):1763–1769.
34. Wilson DN, Hauryliuk V, Atkinson GC, O'Neill AJ. Target protection as a key antibiotic resistance mechanism. *Nat Rev Microbiol.* 2020;18(11):637–648.
35. Crowe-McAuliffe C, Murina V, Turnbull KJ, et al. Structural basis for PoxtA-mediated resistance to phenicol and oxazolidinone antibiotics. *Nat Commun.* 2022;13(1):1860.
36. Fostier CR, Monlezun L, Ousalem F, Singh S, Hunt JF, Boël G. ABC-F translation factors: from antibiotic resistance to immune response. *FEBS Lett.* 2021;595(6):675–706.
37. He T, Shen Y, Schwarz S, et al. Genetic environment of the transferable oxazolidinone/phenicol resistance gene *optrA* in *Enterococcus faecalis* isolates of human and animal origin. *J Antimicrob Chemother.* 2016;71(6):1466–1473.
38. Auckland C, Teare L, Cooke F, et al. Linezolid-resistant enterococci: report of the first isolates in the United Kingdom. *J Antimicrob Chemother.* 2002;50(5):743–746.
39. Ruggero KA, Schroeder LK, Schreckenberger PC, Mankin AS, Quinn JP. Nosocomial superinfections due to linezolid-resistant *Enterococcus faecalis*: evidence for a gene dosage effect on linezolid MICs. *Diagn Microbiol Infect Dis.* 2003;47(3):511–513.
40. Diaz L, Kiratisin P, Mendes RE, Panesso D, Singh KV, Arias CA. Transferable plasmid-mediated resistance to linezolid due to *cfr* in a human clinical isolate of *Enterococcus faecalis*. *Antimicrob Agents Chemother.* 2012;56(7):3917–3922.