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REVIEW

A Review Vancomycin Role in Gram Positive Biofilm-Associated Infections: Challenges and Emerging Solutions

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Abstract: Biofilm-associated infections pose a significant challenge in clinical settings due to their increased resistance to antibiotics and evasion of host immune responses. These infections are responsible for a large proportion of chronic and recurrent infections, leading to prolonged hospital stays, increased healthcare costs, and elevated morbidity and mortality rates. Vancomycin, a glycopeptide antibiotic, has long been a cornerstone in the treatment of infections caused by Gram-positive bacteria, particularly methicillin-resistant *Staphylococcus aureus* (MRSA). In addition, vancomycin-resistant Enterococcus (VRE) represents an important group of biofilm-forming pathogens, further complicating treatment strategies. However, its efficacy against biofilms remains a subject of ongoing research and debate. The ability of vancomycin to target biofilm-embedded bacteria is often hindered by multiple resistance mechanisms, including poor antibiotic penetration, metabolic adaptation of biofilm-associated cells, and the presence of persister cells. The aim of this review is to evaluate vancomycin's antibiofilm activity by examining its mechanism of action, pharmacokinetics, effectiveness, limitations, and potential strategies to enhance its therapeutic outcomes. Several novel approaches have been explored to augment vancomycin's antibiofilm activity, including combination therapies, adjuvant strategies, and nanotechnology-based drug delivery systems. Understanding these factors is crucial for optimizing therapeutic strategies and overcoming the persistent challenge of biofilm-related infections. This review synthesizes current evidence and highlights areas requiring further research to enhance vancomycin's efficacy against biofilm-associated infections.

Keywords: vancomycin resistance, biofilm-associated infections, combination therapy, drug delivery systems

Introduction

Bacterial biofilms are structured communities of microorganisms embedded within a self-produced extracellular polymeric substance (EPS) that confers protection against environmental stresses, antibiotics, and host immune responses.[1,2] Biofilm-associated infections are implicated in numerous clinical conditions, including catheter-related bloodstream infections, prosthetic joint infections, infective endocarditis, osteomyelitis, and chronic wounds.[3,4] The ability of bacteria

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to form biofilms significantly reduces the effectiveness of conventional antimicrobial treatments, necessitating the exploration of novel therapeutic approaches.[5,6]

Vancomycin, a glycopeptide antibiotic, is widely used for treating severe Gram-positive bacterial infections, including MRSA.[7,8] Its mechanism of action involves inhibiting bacterial cell wall synthesis by binding to the D-Ala-D-Ala terminus of peptidoglycan precursors, preventing transpeptidation and subsequent cell wall cross-linking.[9,10] Despite its efficacy against planktonic bacterial populations, vancomycin's ability to eradicate biofilm-embedded bacteria remains inconsistent due to several factors,[11] including limited diffusion through the EPS matrix, reduced bacterial metabolic activity within the biofilm.[12]

Several studies have assessed the antibiofilm activity of vancomycin, demonstrating variable efficacy depending on factors such as biofilm age, bacterial strain, antibiotic concentration, and treatment duration.[13,14] While high doses of vancomycin may improve penetration into biofilms,[15] complete eradication remains challenging due to the presence of persister cells—dormant bacterial cells that exhibit tolerance to antibiotics without undergoing genetic modifications.[16,17] Consequently, researchers have explored alternative approaches, such as combination therapies, biofilm-disrupting agents, and novel drug delivery systems, to increase vancomycin's effectiveness against biofilm-associated infections.[18,19] The aim of this review is to critically evaluate the role of vancomycin in Gram-positive biofilm-associated infections, highlighting its mechanisms of action, limitations in biofilm penetration, and emerging strategies to improve its therapeutic efficacy.

Mechanisms of Biofilm Resistance to Vancomycin

Biofilm-mediated resistance to vancomycin arises from multiple interconnected mechanisms, including, the EPS matrix, composed of polysaccharides, proteins, and extracellular DNA (eDNA), acts as a physical barrier that restricts the diffusion of vancomycin, leading to suboptimal antibiotic concentrations within deeper biofilm layers.[18,20,21] Furthermore, vancomycin's ability to penetrate biofilms is significantly hindered due to several factors, including its relatively large molecular size and poor diffusion through the protective extracellular polymeric substance (EPS) matrix. Moreover, Biofilms create a unique microenvironment in which bacteria adopt a sessile lifestyle, exhibit altered metabolic states, and express biofilm-specific resistance genes, making them inherently less susceptible to antibiotic treatment.[22,23] Therefore, bacteria within biofilms exhibit heterogeneity in metabolic activity.[24] Cells in deeper layers are often in a dormant or slow-growing state, reducing the efficacy of antibiotics that target actively dividing cells.[25] Also, a subpopulation of bacterial cells known as persister cells within the biofilm can enter a transient, non-replicative state, allowing them to withstand antibiotic exposure and later repopulate the biofilm once antibiotic pressure is removed.[26,27] On the other hand, Biofilm-forming bacteria can upregulate efflux pumps, actively extruding vancomycin and reducing its intracellular concentration.[28] Notably, the induction of biofilm-specific genes and the horizontal gene transfer of resistance elements as a result of close proximity of bacteria whin biofilms improve conjugation, leading to greater antibiotic tolerance and persistence.[29] (Table 1).

Antibiofilm Activity of Vancomycin and Methods to Augment Its Efficacy

Vancomycin, despite being a cornerstone antibiotic for treating Gram-positive bacterial infections,[30] presents several limitations when used as a monotherapy, particularly against biofilm-associated infections.[31,32] One of the major challenges associated with vancomycin is its slow bactericidal activity, which can lead to prolonged treatment durations and increase the risk of persistent infections.[33] Additionally, the emergence of bacterial strains with decreased susceptibility to vancomycin, such as vancomycin-intermediate *Staphylococcus aureus* (VISA) and vancomycin-resistant *Enterococcus* (VRE), further complicates its clinical utility.[34,35] These strains exhibit adaptive resistance mechanisms, including thickened cell walls, alterations in cell envelope metabolism, and the upregulation of resistance genes, all of which contribute to reduced efficacy. Additionally, vancomycin's higher minimum inhibitory concentrations (MICs) against biofilm-embedded bacteria mean that even at therapeutic doses, the drug may fail to achieve sufficient intracellular concentrations to eradicate the infection completely.[36] This limitation often results in treatment failure, chronic infections, and enhanced recurrence rates.

Table 1 Mechanisms of Biofilm-Mediated Vancomycin Resistance

Notable Mechanisms	Reference No.
EPS matrix (polysaccharides, proteins, eDNA) acts as a physical barrier, restricting vancomycin diffusion	[18,20,21]
Large molecular size of vancomycin limits penetration through EPS	[18,20,21]
Biofilm microenvironment induces sessile lifestyle, altered metabolism, and biofilm-specific resistance genes	[22,23]
Heterogeneity in metabolic activity; deeper cells often dormant or slow-growing	[24,25]
Subpopulation enters transient, non-replicative state (persister cells) and repopulates after antibiotic removal	[26,27]
Upregulation of efflux pumps reduces intracellular vancomycin concentration	[28]
Biofilm-specific genes and horizontal gene transfer enhance tolerance and persistence	[29]

Prior research has evaluated vancomycin in comparison to other antibiotics for the eradication of biofilm. A study assessed the impact of cefepime, meropenem, piperacillin/tazobactam, and vancomycin on azole-resistant *Candida albicans* and *Candida tropicalis* in both planktonic and biofilm states. All tested antibiotics showed activity against Candida, reducing biofilm viability in both early and mature stages. Given their common use in hospital-acquired and catheter-related infections, these antibiotics may offer additional benefits in controlling *Candida* biofilm formation through antibiotic-lock therapy.[37] On the other hand, a different study examined the effects of vancomycin, daptomycin, and tigecycline on biofilm-associated *Staphylococcus* infections. Vancomycin failed to penetrate or kill bacteria within the biofilm. Daptomycin was bactericidal but did not inhibit or eradicate biofilm. Tigecycline effectively inhibited and eradicated biofilm while also killing bacteria within it. These findings suggest that tigecycline and daptomycin may be promising options for treating biofilm-associated infections.[38] Notably, other study tested vancomycin and linezolid against biofilms in an in vitro shunt model. While both antibiotics eradicated staphylococcal biofilms within two days, neither was effective against enterococcal biofilms. These findings support further clinical trials on linezolid as a potential non-surgical treatment option.[39] (Table 2).

To overcome these challenges and enhance the efficacy of vancomycin against biofilm-associated infections, several strategies have been explored. Combination therapy has emerged as a promising approach, wherein vancomycin is used alongside other antibiotics or biofilm-disrupting agents to achieve synergistic effects.[31] For instance, the combination of vancomycin with rifampin has displayed augmented penetration and improved bacterial eradication, it has been shown that a mouse model of prosthetic joint infection was utilized, in which *Staphylococcus aureus* was introduced into a knee joint containing a surgically implanted metallic device extending from the femur. This model was employed to assess whether the combination therapy of vancomycin and rifampin provides greater efficacy than vancomycin alone in treating such infections. Starting on postoperative day 7, vancomycin, with or without rifampin, was administered for

Table 2 Comparative Studies on Vancomycin Efficacy Against Biofilm-Associated Infections

Study Focus	Pathogen/Model	Main Outcomes	Reference
Limitations Of vancomycin monotherapy	Biofilm-associated Gram-positive bacteria	Slow bactericidal activity, VISA/VRE emergence, thickened cell walls, reduced efficacy against biofilm-embedded bacteria[	[30–36]
Comparative efficacy on *Staphylococcus* biofilm	Staphylococcus biofilm model	Vancomycin failed to kill biofilm bacteria; Daptomycin bactericidal but not biofilm-eradicating; Tigecycline both inhibited and eradicated biofilm	[38]
Shunt model biofilm eradication	*Staphylococcus* and *Enterococcus* biofilms	Both eradicated *Staphylococcus* biofilm within 2 days; ineffective against *Enterococcus* biofilm	[39]

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six weeks while retaining the implant. The study incorporated in vivo bioluminescence imaging, ex vivo CFU enumeration, X-ray imaging, and histological analysis to evaluate treatment outcomes. The findings demonstrated a significant therapeutic advantage when vancomycin was combined with rifampin compared to vancomycin monotherapy. Overall, these results indicate that this mouse model serves as a valuable preclinical system for evaluating antibiotic efficacy and combination therapies for prosthetic joint infections prior to advancing to more comprehensive human studies.[40]

Similarly, pairing vancomycin with fosfomycin, has shown potential in overcoming biofilm-mediated resistance by attacking bacteria through multiple mechanisms. It has been demonstrated that the bactericidal effects of vancomycin and fosfomycin against MRSA in a rat biofilm model. The combination therapy showed synergistic killing in both in vitro and in vivo studies, reducing MRSA counts, inflammation markers, and biofilm structures compared to monotherapy. These findings suggest vancomycin and fosfomycin as a potential treatment for biofilm-associated MRSA infections.[41]

The effectiveness of vancomycin in addressing biofilm-associated infections is constrained by the physicochemical characteristics of biofilms, which impede antimicrobial penetration and diminish drug exposure to the bacterial cells contained within the matrix. Antibiotics such as gentamicin, fosfomycin, and rifampin are typically not used as monotherapy for Gram-positive infections. It may act as a synergistic agent by disrupting extracellular polymeric substances, enhancing penetration, providing antimicrobial action, targeting various bacterial populations, reducing resistance development, disrupting quorum sensing, and facilitating biofilm disruption and bacterial elimination.[42,43]

Another approach to improving vancomycin's activity involves the use of adjuvants that can enhance drug penetration or disrupt biofilm integrity. Agents such as DNase, dispersin B.[44] It has been shown that exposure to subinhibitory vancomycin levels strengthens biofilm persistence by restricting antibiotic penetration. Confocal microscopy with fluorescently labeled vancomycin revealed reduced drug diffusion in pre-exposed biofilms. Elevated extracellular DNA (eDNA) levels suggest a protective role against vancomycin. Supplementing with eDNA further increased vancomycin MIC and shielded biofilm cells.[45] Moreover, quorum sensing inhibitors have been investigated for their ability to break down the biofilm matrix and increase bacterial susceptibility to antibiotics. Quorum sensing inhibitors (QSI) have been explored as an antibiofilm strategy to increase bacterial sensitivity to antibiotics. This study evaluated the effects of QSI targeting *Pseudomonas aeruginosa, Burkholderia cepacia* complex, and *Staphylococcus aureus* in combination with conventional antibiotics. In vitro and in vivo models displayed that combining QSI with antibiotics including vancomycin enhanced bacterial killing and improved host survival in infected larvae and mice.[46]

Additionally, metal nanoparticles, liposomal formulations, and other nanotechnology-based drug delivery systems have been explored as a means of improving vancomycin's pharmacokinetics and enhancing its bioavailability at the site of infection. A study explores the cationic nanocarrier leciplex (LPX) for enhancing vancomycin hydrochloride (VAN) oral bioavailability by improving intestinal permeability. The optimized LPX formula demonstrated high entrapment efficiency (85.2%), stability, and a 2.3-fold increase in permeability. Pharmacokinetic studies showed a 2.99-fold rise in *Cmax* and 3.41-fold increase in AUC_{0-12}, confirming LPX's effectiveness.[47]

Notably, nanocarriers enhance vancomycin delivery by providing sustained release, targeted action, and reduced toxicity. It has been explored that the role in improving anti-MRSA activity and highlights simulation studies optimizing vancomycin-nanocarrier interactions. While nanocarrier-mediated delivery shows promise in enhancing efficacy and minimizing toxicity.[48] On the other hand, nanomedicine, particularly lipid-based nanosystems like liposomes, offers a promising strategy. Encapsulating antibiotics in liposomes enhances drug accumulation at infection sites, reduces toxicity, and protects against degradation. Additionally, liposomes can fuse with bacterial cells, potentially overcoming resistance and biofilm formation.[49,50] Studies have shown that liposomes encapsulating vancomycin enhance circulation time, resulting in substantially higher serum concentrations compared to the direct administration of free vancomycin.[51] Additionally, a liposomal formulation co-loaded with vancomycin achieved complete bone sterilization in an *S. aureus* osteomyelitis model, demonstrating its strong therapeutic potential for treating these severe infections.[52]

These novel drug delivery strategies may help circumvent the diffusion barrier posed by the EPS matrix and improve the overall effectiveness of vancomycin against biofilm-associated infections. A study investigated titanium alloy

implants modified with silver, titanium dioxide, and hydroxyapatite (HA) nanocoatings to enhance antibacterial properties. A dual-layered silver–HA coating effectively inhibited *Streptococcus sanguinis* growth and reduced biofilm formation significantly. In contrast, uncoated implants and titanium dioxide coatings showed no antibacterial effect. The nanocoatings remained highly stable in biological fluids, with minimal material loss. These findings suggest that silver–HA coatings can provide antibiofilm protection while maintaining HA's biocompatibility for improved osseointegration and bone healing.[53] Another study developed vancomycin-coated titanium implants using electrospinning nanotechnology to assess their antibacterial properties in vitro and in vivo. The coating demonstrated antibacterial efficacy without cytotoxicity in vitro. In vivo, X-ray, blood markers, and pathology confirmed its effectiveness against implant-associated infections. These findings suggest that vancomycin-coated titanium implants offer a promising strategy for infection prevention and treatment.[54] On the other hand, gold nanoparticles (GNPs) offer promising antibacterial potential due to their broad-spectrum activity and modifiability. It has been shown that pharmacokinetics of GNPs, vancomycin, and vancomycin-bound GNPs (VBGNP) after intravenous injection. The best-fit model, based on Akaike's information criterion, was a two-compartment model. VBGNPs exhibited enhanced plasma concentration and faster distribution. In vivo trials showed that 5 g/kg VBGNPs effectively reduced infection symptoms within seven days, whereas 15 g/kg vancomycin alone was insufficient. These findings highlight the potential of VBGNPs in treating MRSA-induced osteomyelitis.[55] Of note, Chitosan, known for its broad-spectrum antibacterial properties, was evaluated against VRSA and VREF in planktonic and sessile states. It effectively inhibited planktonic growth, reduced VREF by 6 log CFU in 30 minutes, and disrupted biofilms at 0.0125 mg/mL. These findings highlight chitosan's potential as a promising alternative for controlling vancomycin-resistant infections.[56] (Table 3).

Table 3 Investigated Strategies to Improve Vancomycin Efficacy Against Biofilm-Associated Infections

Strategy	Pathogen/Model	Main Findings	Ref.
Vancomycin + Rifampin	*S. aureus* prosthetic joint infection (mouse)	Combination improved penetration, bacterial eradication, and treatment outcome vs monotherapy	[40]
Vancomycin + Fosfomycin	MRSA biofilm (rat model)	Synergistic killing, reduced MRSA load, inflammation, and biofilm vs monotherapy	[41]
Adjuvants (DNase, dispersin B, eDNA)	*S. aureus* biofilm	DNase/dispersin B improved penetration; eDNA increased vancomycin MIC and biofilm persistence	[42,43]
Quorum Sensing Inhibitors + antibiotics	*P. aeruginosa, B. cepacia*, *S. aureus*	Enhanced killing and host survival in vitro and in vivo when combined with vancomycin	[44]
Nanocarriers (leciplex, liposomes, nanomedicine)	MRSA, osteomyelitis models	Improved bioavailability, serum levels, sustained release; liposomes achieved bone sterilization	[45–50]
Titanium implant nanocoatings (silver–HA, vancomycin-coated)	*S. sanguinis*, implant models	Inhibited biofilm growth, effective antibacterial activity, stable and biocompatible	[51,52]
Vancomycin-bound gold nanoparticles	MRSA osteomyelitis (rat)	Higher plasma concentration, faster distribution, superior infection control vs free vancomycin	[53]
Chitosan	VRSA, VREF (planktonic and biofilm)	Strong growth inhibition, biofilm disruption, effective at low concentrations	[54]

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Challenges

Several Key challenges Hinder the Effective Treatment of Biofilm-Associated Infections

Treating biofilm-associated infections presents numerous challenges, including difficulties in diagnosis due to the absence of specific markers, the complexity of microbial communities, and the limitations of conventional diagnostic methods for detecting biofilms.[57] Biofilm infections typically occur in vulnerable patients requiring medical devices and prosthetic implants, leading to chronic infections. The impacts of biofilm infections encompass persistence and recurrence, elevated morbidity and mortality rates, as well as increased healthcare costs.[58]

One major issue in studying biofilm is the standardization of biofilm models; inconsistencies in experimental models and assessment techniques contribute to variability in findings and impede cross-study comparisons.[59] Additionally, pharmacokinetic and pharmacodynamic considerations remain a concern, as the optimal vancomycin dosing regimen that maximizes antibiofilm activity while minimizing resistance development is not well established.[60,61] The emergence of vancomycin-resistant strains, including VISA and VRE, further complicates treatment efforts and highlights the urgent need for alternative therapies such as antimicrobial peptides, bacteriophage therapy, and novel adjuvants.[62,63] VRE is an emerging threat in healthcare settings. Analysis of 31 isolates from two hospitals in Italy revealed multidrug resistance, prevalent virulence genes, and dominant sequence type ST80.[64]

Lastly, the clinical translation of novel approaches remains limited; although many strategies show promise in laboratory settings, their effectiveness in clinical practice requires thorough validation through preclinical and clinical studies.[59,65,66] Figure 1 summarizes the challenging factors and the innovative method to improve vancomycin's biofilm eradication performance.

The primary challenges in standardizing in vitro and in vivo biofilm models for research and clinical relevance are multifaceted and encompass several levels. In vitro challenges arise from diverse model systems. Various biofilm models are available, including static, flow-cell, CDC biofilm reactor, and animal models, each replicating distinct facets of biofilm development. This diversity complicates the standardization and comparison of results. Variability in experimental conditions Variations in initial bacterial inoculum, growth media, surface materials, incubation durations, and environmental conditions (such as nutrient flow and shear forces) contribute to inconsistencies, impacting reproducibility and comparability.[43] Species and strain differences contribute to variability in biofilm formation among bacterial species and strains, which affects generalizability and underscores the need for standardized strains and conditions. Additionally,

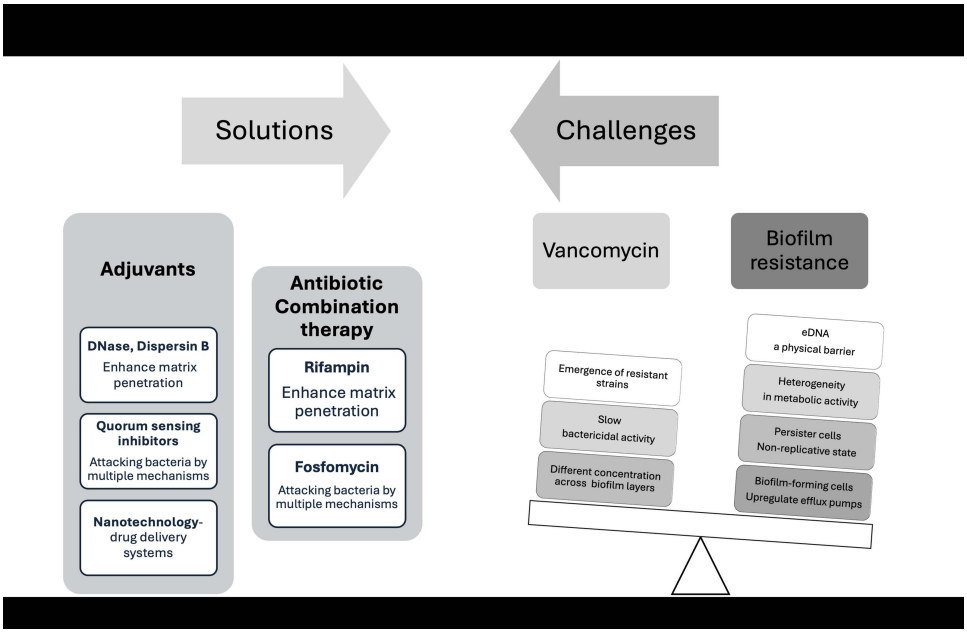

Figure 1 Summary of the factors hinders vancomycin efficacy against biofilm and the novel method to improve vancomycin's biofilm eradication performance.

the lack of consensus on readouts is evident, as different studies employ various metrics (eg, biomass staining, viable counts, structural imaging), complicating the standardization of evaluation protocols.Reproducibility and scalability are critical challenges, as ensuring models are reproducible across laboratories and scalable for high-throughput testing is complex.[67]

The in vivo model may encounter challenges, including limited clinical correlation. Numerous models fail to adequately replicate the complexity of human infections, including immune responses, tissue interactions, and multi-species biofilms, thereby restricting their translatability. The limitations of in vivo models include ethical, practical, and cost constraints that restrict the standardization of animal models. Additionally, physiological differences between animals and humans impede direct clinical relevance. Complex models that more accurately replicate in vivo conditions often exhibit lower reproducibility and require greater resources, while simpler models may lack clinical relevance; thus, achieving a balance between these factors is challenging.[68] The precise replication of the microenvironment of human tissues, encompassing immune responses and fluid dynamics, presents significant challenges, thereby constraining the clinical relevance of experimental models. Ultimately The complexity of interactions among multiple microbial species in natural infections is significant, and standardized multi-species biofilm models remain in the development phase.[43,68]

Discussion

This study provides a comprehensive synthesis of current strategies to enhance vancomycin's efficacy against biofilm-associated infections, integrating combination therapies, adjuvants, and nanotechnology-based approaches. Additionally, it emphasizes the clinical relevance of emerging resistance mechanisms, including VRE and VISA, highlighting potential therapeutic avenues and guiding future research for improved management of biofilm-mediated infections. Biofilm-associated infections remain a major clinical challenge due to their enhanced resistance to antibiotics and evasion of host immune responses. Vancomycin, while a cornerstone for treating Gram-positive infections such as MRSA, shows limited efficacy against biofilm-embedded bacteria due to multiple resistance mechanisms, including restricted penetration, metabolic heterogeneity, persister cells, and efflux pumps. Strategies to overcome these barriers—such as combination therapy (vancomycin with rifampin or fosfomycin), biofilm-disrupting adjuvants (DNase, dispersin B, quorum sensing inhibitors), and advanced nanotechnology-based delivery systems (liposomes, gold nanoparticles, coated implants)—have displayed enhanced antibiofilm activity in preclinical models.[40–54]

Despite these advances, significant challenges remain. Standardization of biofilm models and assessment methods is lacking, complicating comparison across studies.[55] Optimizing vancomycin pharmacokinetics and dosing for biofilm infections remains unresolved,[56,57] and the emergence of vancomycin-resistant strains such as VISA and VRE further limits clinical options.[58–60] Moreover, while many innovative strategies show promise in vitro or in animal models, their translation into clinical practice requires further validation.[55,61,62]

This study highlights these persistent challenges and provides a comprehensive synthesis of current approaches to enhance vancomycin efficacy against biofilms. By systematically evaluating combination therapies, adjuvants, and nanotechnology-based delivery methods, our work underscores potential therapeutic avenues and emphasizes the need for integrated strategies to improve outcomes in patients with biofilm-associated infections.

Conclusion

Vancomycin remains a cornerstone in treating Gram-positive infections but shows limited effectiveness against biofilms. Emerging strategies—such as combination therapies, biofilm-disrupting agents, and nanotechnology-based delivery systems—offer potential to enhance its activity. Future research should focus on optimizing therapeutic protocols, standardizing biofilm evaluation methods, and developing innovative approaches to overcome resistance, thereby improving patient outcomes in biofilm-associated infections.

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically

reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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References

1. Thi MTT, Wibowo D, Rehm BHA. Pseudomonas aeruginosa biofilms. *Int J Mol Sci*. 2020;21(22):1–25. doi:10.3390/ijms21228671
2. Viganor L, Howe O, McCarron P, McCann M, Devereux M. The antibacterial activity of metal complexes containing 1,10- phenanthroline: potential as alternative therapeutics in the era of antibiotic resistance. *Curr Top Med Chem*. 2016;17(11):1280–1302. doi:10.2174/1568026616666161003143333
3. Ryder MA. Catheter-related infections: it's all about biofilm. *Topics Adv Practice Nurs*. 2005;5(3).
4. Khardori N, Yassien M. Biofilrns in device-related infections. *J Indus Microbiol Biotechnol*. 1995;15(3):141–147. doi:10.1007/BF01569817
5. Mirghani R, Saba T, Khaliq H, et al. Biofilms: formation, drug resistance and alternatives to conventional approaches. *AIMS Microbiol*. 2022;8(3):240–278. doi:10.3934/microbiol.2022019
6. Gonzales PR, Pesesky MW, Bouley R, et al. Synergistic, collaterally sensitive β-lactam combinations suppress resistance in MRSA. *Nat Chem Biol*. 2015;11(11):855–861. doi:10.1038/nchembio.1911.Synergistic
7. Leclercq R, Derlot E, Duval J, Courvalin P. Plasmid-mediated resistance to vancomycin and teicoplanin in enterococcus faecium. *N Engl J Med*. 1988;319(3):157–161. doi:10.1056/nejm198807213190307
8. Haddadin AS, Fappiano SA, Lipsett PA. Methicillin resistant Staphylococcus aureus (MRSA) in the intensive care unit. *Postgrad. Med. J*. 2002;78(921):385–392. doi:10.1136/pmj.78.921.385
9. Kardos G, Laczkó L, Kaszab E, et al. Phylogenetic analysis of the genes in d-ala-d-lactate synthesizing glycopeptide resistance operons: the different origins of functional and regulatory genes. *Antibiotics*. 2024;13(7):1–18. doi:10.3390/antibiotics13070573
10. De Rosa M, Verdino A, Soriente A, Marabotti A. The odd couple(S): an overview of beta-lactam antibiotics bearing more than one pharmacophoric group. *Int J Mol Sci*. 2021;22(2):1–21. doi:10.3390/ijms22020617
11. Song T, Duperthuy M, Wai SN. Sub-optimal treatment of bacterial biofilms. *Antibiotics*. 2016;5(2):1–18. doi:10.3390/antibiotics5020023
12. Pinto RM, Soares FA, Reis S, Nunes C, Van Dijck P. Innovative strategies toward the disassembly of the EPS matrix in bacterial biofilms. *Front Microbiol*. 2020;11(May):1–20. doi:10.3389/fmicb.2020.00952
13. Li J, Xie S, Ahmed S, et al. Antimicrobial activity and resistance: influencing factors. *Front Pharmacol*. 2017;Vol. 8:1–11. doi:10.3389/fphar.2017.00364
14. Slobodníková L, Fialová S, Rendeková K, Kováč J, Mučaji P. Antibiofilm activity of plant polyphenols. *Molecules*. 2016;21(12):1–15. doi:10.3390/molecules21121717
15. Jefferson KK, Goldmann DA, Pier GB. Use of confocal microscopy to analyze the rate of vancomycin penetration through Staphylococcus aureus biofilms. *Antimicrob. Agents Chemother*. 2005;49(6):2467–2473. doi:10.1128/AAC.49.6.2467-2473.2005
16. Fisher RA, Gollan B, Helaine S. Persistent bacterial infections and persister cells. *Nat Rev Microbiol*. 2017;15(8):453–464. doi:10.1038/nrmicro.2017.42
17. Chowdhury N, Wood TL, Martínez-Vázquez M, García-Contreras R, Wood TK. DNA-crosslinker cisplatin eradicates bacterial persister cells. *Biotechnol. Bioeng*. 2016;113(9):1984–1992. doi:10.1002/bit.25963
18. Singh R. Revolutionizing antimicrobial therapies through biofilm-targeted nanomedicine. *Environ. Microbiol. Rep*. 2021;78–97.
19. Nadar S, Khan T, Patching SG, Omri A. Development of antibiofilm therapeutics strategies to overcome antimicrobial drug resistance. *Microorganisms*. 2022;10(2):1–28. doi:10.3390/microorganisms10020303
20. Campoccia D, Montanaro L, Arciola CR. Extracellular DNA (eDNA). a major ubiquitous element of the bacterial biofilm architecture. *Int J Mol Sci*. 2021;22(16):9100. doi:10.3390/ijms22169100
21. Flemming HC, Neu TR, Wozniak DJ. The EPS matrix: the 'house of biofilm cells,'. *J Bacteriol*. 2007;189(22):7945–7947. doi:10.1128/JB.00858-07
22. Kollef MH. Limitations of vancomycin in the management of resistant staphylococcal infections. *Clinl Infect Dis*. 2007;45:213–221. doi:10.1086/519470
23. Kluin OS, Busscher HJ, Neut D, van der Mei HC. Poly(trimethylene carbonate) as a carrier for rifampicin and vancomycin to target therapy-recalcitrant staphylococcal biofilms. *J Orthop Res*. 2016;34(10):1828–1837. doi:10.1002/jor.23194
24. Stewart PS, Franklin MJ. Physiological heterogeneity in biofilms. *Nat Rev Microbiol*. 2008;6(3):199–210. doi:10.1038/nrmicro1838
25. Stojowska-Swędrzyńska K, Kuczyńska-Wiśnik D, Laskowska E. New strategies to kill metabolically-dormant cells directly bypassing the need for active cellular processes. *Antibiotics*. 2023;12(6):1044. doi:10.3390/antibiotics12061044
26. Marro FC, Laurent F, Josse J, Blocker AJ. Methods to monitor bacterial growth and replicative rates at the single-cell level. *FEMS Microbiol Rev*. 2022;46(6):1–30. doi:10.1093/femsre/fuac030
27. Kester JC, Fortune SM. Persisters and beyond: mechanisms of phenotypic drug resistance and drug tolerance in bacteria. *Crit. Rev. Biochem. Mol. Biol*. 2014;49(2):91–101. doi:10.3109/10409238.2013.869543
28. Alav I, Sutton JM, Rahman KM. Role of bacterial efflux pumps in biofilm formation. *J Antimicrob Chemother*. 2018;73(8):2003–2020. doi:10.1093/jac/dky042

29. Olsen I. Biofilm-specific antibiotic tolerance and resistance. *Eur. J. Clin. Microbiol. Infect. Dis*. 2015;34(5):877–886. doi:10.1007/s10096-015-2323-z
30. Vazquez-Guillamet C, Kollef MH. Treatment of gram - positive infections in critically ill patients. *BMC Infect Dis*. 2014;14(1):1–8. doi:10.1186/1471-2334-14-92
31. Seah J, Lye DC, Ng TM, Krishnan P, Choudhury S, Teng CB. Vancomycin monotherapy vs. combination therapy for the treatment of persistent methicillin-resistant Staphylococcus aureus bacteremia. *Virulence*. 2013;4(8):734–739. doi:10.4161/viru.26909
32. Randolph AG, Xu R, Novak T, et al. Vancomycin monotherapy may be insufficient to treat methicillin-resistant staphylococcus aureus coinfection in children with influenza-related critical illness. *Clinl Infect Dis*. 2019;68(3):365–372. doi:10.1093/cid/ciy495
33. Lee AS, de Lencastre H, Garau J, et al. Methicillin-resistant Staphylococcus aureus. *Nat Rev Dis Primers*. 2018;4(1):1–23. doi:10.1038/nrdp.2018.33
34. Turner NA, Sharma-Kuinkel BK, Maskarinec SA, et al. Methicillin-resistant Staphylococcus aureus: an overview of basic and clinical research. *Nat Rev Microbiol*. 2019;17(4):203–218. doi:10.1038/s41579-018-0147-4
35. Prematunge C, MacDougall C, Johnstone J, et al. VRE and VSE bacteremia outcomes in the era of effective VRE therapy: a systematic review and meta-analysis. *Infect Control Hosp Epidemiol*. 2016;37(1):26–35. doi:10.1017/ice.2015.228
36. Tang YC, J H, Chen CC, et al. In vitro efficacy of antimicrobial agents against high-inoculum or biofilm-embedded meticillin-resistant Staphylococcus aureus with vancomycin minimal inhibitory concentrations equal to 2 µg/mL (VA2-MRSA). *Int J Antimicrob Agents*. 2011;38(1):46–51. doi:10.1016/j.ijantimicag.2011.02.013
37. Sidrim JJC, Teixeira CEC, Cordeiro RA, et al. β-Lactam antibiotics and vancomycin inhibit the growth of planktonic and biofilm Candida spp.: an additional benefit of antibiotic-lock therapy? *Int J Antimicrob Agents*. 2015;45(4):420–423. doi:10.1016/j.ijantimicag.2014.12.012
38. Ozturk S, Gunay B, Ertugrul N, M B, Sakarya. Effects of vancomycin, daptomycin, and tigecycline on coagulase-negative staphylococcus biofilm and bacterial viability within biofilm: an in vitro biofilm model. *Can. J. Microbiol*. 2016;62(9):735–743. doi:10.1139/cjm-2015-0855
39. Bayston R, Ullas G, Ashraf W. Action of linezolid or vancomycin on biofilms in ventriculoperitoneal shunts in Vitro. *Antimicrob. Agents Chemother*. 2012;56(6):2842–2845. doi:10.1128/AAC.06326-11
40. Niska JA, Shahbazian JH, Ramos RI, Francis KP, Bernthal NM, Miller LS. Vancomycin-rifampin combination therapy has enhanced efficacy against an experimental staphylococcus aureus prosthetic joint infection. *Antimicrob. Agents Chemother*. 2013;57(10):5080–5086. doi:10.1128/AAC.00702-13
41. Shi J, Mao N-F, Wang L, et al. Efficacy of combined vancomycin and fosfomycin against methicillin-resistant Staphylococcus aureus in biofilms in vivo. *PLoS One*. 2014;9(12):1–14. doi:10.1371/journal.pone.0113133
42. Borges NH, Suss PH, Ortis GB, Dantas LR, Tuon FF. Synergistic activity of vancomycin and gentamicin against staphylococcus aureus biofilms on polyurethane surface. *Microorganisms*. 2025;13(5):1119. doi:10.3390/microorganisms13051119
43. Roberts AEL, Kragh KN, Bjarnsholt T, Diggle SP. The limitations of in vitro experimentation in understanding biofilms and chronic infection. *J Mol Biol*. 2015;427(23):3646–3661. doi:10.1016/j.jmb.2015.09.002
44. Kaplan JB, LoVetri K, Cardona ST, et al. Recombinant human DNase i decreases biofilm and increases antimicrobial susceptibility in staphylococci. *Journal of Antibiotics*. 2012;65(2):73–77. doi:10.1038/ja.2011.113
45. Doroshenko N, Tseng BS, Howlin RP, et al. Extracellular DNA impedes the transport of vancomycin in Staphylococcus epidermidis biofilms preexposed to subinhibitory concentrations of vancomycin. *Antimicrob. Agents Chemother*. 2014;58(12):7273–7282. doi:10.1128/AAC.03132-14
46. Brackman G, Cos P, Maes L, Nelis HJ, Coenye T. Quorum sensing inhibitors increase the susceptibility of bacterial biofilms to antibiotics in vitro and in vivo. *Antimicrob. Agents Chemother*. 2011;55(6):2655–2661. doi:10.1128/AAC.00045-11
47. Abdellatif MM, Ahmed SM, El-Nabarawi MA, Teaima M. Oral bioavailability enhancement of vancomycin hydrochloride with cationic nanocarrier (leciplex): optimization, in vitro, ex vivo, and in vivo studies. *Scientia Pharmaceutica*. 2023;91(1). doi:10.3390/scipharm91010001
48. Nyandoro VO, Ismail EA, Tageldin A, et al. Potential of nanocarrier-mediated delivery of vancomycin for MRSA infections. *Expert Opin Drug Delivery*. 2025;22(3):347–366. doi:10.1080/17425247.2025.2459756
49. Gomez ZG, Hosseinidoust A. Liposomes for antibiotic encapsulation and delivery. *ACS Infect. Dis*. 2020;6(5):896–908. doi:10.1021/acsinfecdis.9b00357
50. Ferreira M, Ogren M, Dias JNR, et al. Liposomes as antibiotic delivery systems: a promising nanotechnological strategy against antimicrobial resistance. *Molecules*. 2021;26(7):2047. doi:10.3390/molecules26072047
51. Muppidi K, Wang J, Betageri G, Pumerantz AS. PEGylated liposome encapsulation increases the lung tissue concentration of vancomycin. *Antimicrob. Agents Chemother*. 2011;55(10):4537–4542. doi:10.1128/AAC.00713-11
52. Kadry MABAA, Al-Suwayeh SA, Abd-Allah ARA, Bayomi MA. Treatment of experimental osteomyelitis by liposomal antibiotics. *J Antimicrob Chemother*. 2004;54(6):352. doi:10.1093/jac/dkh465
53. Besinis A, Hadi SD, Le HR, Tredwin C, Handy RD. Antibacterial activity and biofilm inhibition by surface modified titanium alloy medical implants following application of silver, titanium dioxide and hydroxyapatite nanocoatings. *Nanotoxicology*. 2017;11(3):327–338. doi:10.1080/17435390.2017.1299890
54. Zhang L, Yan J, Yin Z, et al. Electrospun vancomycin-loaded coating on titanium implants for the prevention of implant-associated infections. *Int J Nanomed*. 2014;9(1):3027–3036. doi:10.2147/IJN.S63991
55. Shaker OMS, Ibrahim AF. Pharmacokinetic of gold nanoparticle-vancomycin delivery in osteomyelitis induced by mrsa in rabbits. *Biochem Cell Archiv*. 2022;22(1):1–14.
56. Costa ME, M E, Silva S, et al. Investigation of chitosan's antibacterial activity against vancomycin resistant microorganisms and their biofilms. *Carbohydr. Polym*. 2017;174(1):1–23. doi:10.1016/j.carbpol.2017.06.087
57. Xu Y, Dhaouadi Y, Stoodley P, Ren D. Sensing the unreachable: challenges and opportunities in biofilm detection. *Curr Opin Biotechnol*. 2020;64:79–84. doi:10.1016/j.copbio.2019.10.009
58. Mendhe S, Badge A, Ugemuge S, Chandi D. Impact of biofilms on chronic infections and medical challenges. *Cureus*. 2023. doi:10.7759/cureus.48204
59. Magana M, Sereti C, Ioannidis A, et al. Options and limitations in clinical investigation of bacterial biofilms. *Clin Microbiol Rev*. 2018;31(3):1–49. doi:10.1128/CMR.00084-16

60. Álvarez R, Cortés LEL, Molina J, Cisneros JM, Pachón J. Optimizing the clinical use of vancomycin. *Antimicrob. Agents Chemother*. 2016;60 (5):2601–2609. doi:10.1128/AAC.03147-14
61. Elbarbry F. Vancomycin dosing and monitoring: critical evaluation of the current practice. *Eur J Drug Metab Pharmacokinet*. 2018;43(3):259–268. doi:10.1007/s13318-017-0456-4
62. Perl TM. The threat of vancomycin resistance. *Am J Med*. 1999;106(5 A):26–37. doi:10.1016/S0002-9343(98)00354-4
63. Baëtz B, Boudrioua A, Hartke A, Giraud C. Alternatives to fight vancomycin-resistant staphylococci and enterococci. *Antibiotics*. 2021;10(9):1116. doi:10.3390/antibiotics10091116
64. Lombardi A, Ripabelli G, Sammarco ML, Tamburro M. Enterococcus faecium as an emerging pathogen: molecular epidemiology and antimicrobial resistance in clinical strains. *Pathogens*. 2025;14(5):483. doi:10.3390/pathogens14050483
65. Svenson S. Clinical translation of nanomedicines. *Curr. Opin. Solid State Mater. Sci*. 2012;16(6):287–294. doi:10.1016/j.cossms.2012.10.001
66. Zheng C, Li M, Ding J. Challenges and opportunities of nanomedicines in clinical translation. *BIO Integration*. 2021;2(2):57–60. doi:10.15212/bioi-2021-0016
67. Pang L, Lin H, Yang F, Deng D. Editorial: mechanisms of biofilm development and antibiofilm strategies. *Front Microbiol*. 2023;14. doi:10.3389/fmicb.2023.1190611.
68. Khan J, Tarar SM, Gul I, Nawaz U, Arshad M. Challenges of antibiotic resistance biofilms and potential combating strategies: a review. *3 Biotech*. 2021;11(4):169. doi:10.1007/s13205-021-02707-w