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Potential interactions between vancomycin and meropenem in culture-negative periprosthetic joint infection: an in vitro study

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Abstract

Background Culture-negative periprosthetic joint infections (CN-PJIs) represent a critical subtype of PJI, and their high prevalence poses substantial challenges for treatment. CN-PJI is commonly managed utilizing antibiotic combinations, however, the interactions between these antibiotics have not been investigated. The aim of study was to investigate the synergistic and antagonistic effects of vancomycin (VAN) and meropenem (MEM), in an in vitro model of CN-PJI.

Methods Minimum Inhibitory Concentration (MIC), Minimum Bactericidal Concentration (MBC), Minimum Biofilm Inhibitory Concentration (MBIC), and Minimum Biofilm Eradication Concentration (MBEC) were determined for VAN and MEM. Fractional Inhibitory Concentration (FIC) and Fractional Biofilm Eradication Concentration (FBEC) indices were calculated to assess the synergistic or antagonistic effects of VAN in combination with MEM on *Staphylococcus aureus (S. aureus)*, *Staphylococcus epidermidis (S. epidermidis)*, and *Escherichia coli* (*E. coli)* incubated alone or in combination.

Results In the planktonic bacterial phase, MEM showed higher activity than VAN. Resistance increased when the bacteria were cultured together. The combination of VAN and MEM exhibited an indifferent effect against individual *Staphylococci* but an antagonistic effect against polymicrobial cultures. In biofilm, MEM demonstrated better antibiofilm activity than VAN, especially against *E. coli* biofilms. The combination of VAN and MEM showed an indifferent effect against *E. coli* and *S. epidermidis-E. coli* biofilms, but an antagonistic effect against *S. aureus*, *S. epidermidis*, *S. aureus-S. epidermidis*, and *S. aureus-E. coli* biofilms.

Conclusion This study provides valuable insights into the effectiveness of VAN and MEM combinations in treating CN-PJIs, highlighting the need for careful consideration when selecting antibiotic treatments for these infections.

Clinical trial number Not applicable.

Keywords Vancomycin, Meropenem, Culture-negative periprosthetic joint infection

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Introduction

Periprosthetic joint infection (PJI) is one of the most severe complications of arthroplasty. This condition can result in significant health issues, elevated mortality rates, and increased healthcare costs due to prolonged hospitalization [1]. The Musculoskeletal Infection Society (MSIS) working group presented diagnostic criteria for PJI in 2011 based on clinical findings, haematological tests, and preoperative cultures [2]. However, in practice, a substantial number of patients who are ultimately diagnosed with PJI have negative cultures pre- or intraoperatively [3], resulting in the clinical entity "culture-negative (CN)-PJI" [4]. Recent studies have reported a prevalence of CN-PJI as high as 42.1% [5], which poses a considerable challenge for the diagnosis and treatment of CN-PJI.

The use of next-generation sequencing (NGS) technology has resulted in a major breakthrough in the diagnosis of CN-PJI. The American Society for Microbiology has indicated that NGS has the potential to serve as a comprehensive diagnostic test, potentially transforming traditional clinical microbiology [6]. NGS has revealed a polymicrobial infection in 91.1% of CN-PJI cases, with a common set of species contributing to 82.4% of the polymicrobial profiles. *Escherichia coli (E. coli)*, *Staphylococcus epidermidis (S. epidermidis)*, *and Staphylococcus aureus* (*S. aureus*) ranked highest in terms of incidence and study-wide mean relative abundance, frequently emerging as the dominant microorganisms in polymicrobial infections [7]. Although NGS has increased the detection rate of CN-PJI, the appropriate and rational use of antimicrobials is the key to treating CN-PJI [4]. However, the choice of antibiotic regimen for CN-PJI is extremely difficult due to the lack of reliable data on the causative agent. Currently, vancomycin (VAN) in combination with a third-generation cephalosporin or carbapenem is considered to be the most common regimen for the treatment of CN-PJI [8]. This is the broadest spectrum combination covering gram-negative and gram-positive organisms, even including methicillin-resistant *Staphylococcus aureus* (MRSA) and methicillin-resistant *Staphylococcus epidermidis* (MRSE). Vancomycin is used as the primary antibiotic preoperatively, intraoperatively, and postoperatively because gram-positive organisms remain the most common pathogens [9]. In addition, carbapenem includes almost all gram-negative bacilli that cause PJI. This antibiotic regimen has been shown to be effective in the treatment of CN-PJI and to achieve similar rates of infection control as culture-positive PJI patients [3, 8]. However, some studies indicated that antibiotic combinations can exhibit synergistic or antagonistic effects, which significantly impact their overall efficacy [10, 11]. The specific interaction between VAN and carbapenem in the treatment of CN-PJI remains largely unexplored.

In this study, the available NGS results of CN-PJI were utilized to construct monomicrobial and polymicrobial environments based on *S. aureus*, *S. epidermidis*, and *E. coli*, thereby accurately reflecting the in vitro environments of microorganisms commonly associated with CN-PJI. This research aims to provide insights into the efficacy of combining vancomycin and meropenem for CN-PJI treatment, potentially informing clinical decision-making and improving patient outcomes.

Methods

Bacterial strains and culture conditions

The bacterial strains utilized in the present study included *S. aureus* (ATCC 25923), *S. epidermidis* (ATCC 35984), and *E. coli* (ATCC 25922). These bacteria were suspended in Mueller–Hinton Broth II (MHB II; Biocorp, Warsaw, Poland) and incubated under aerobic conditions at 37 °C. After 24 h of incubation, the cultures were centrifuged (10 min, 2500 rpm), washed three times with phosphate-buffered saline (PBS; AppliChem, Darmstadt, Germany), and suspended in MHB II in the corresponding inocula.

Determination of minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC and MBC of vancomycin (VAN) and meropenem (MEM) were determined against *S. aureus*, *S. epidermidis*, and *E. coli* using the broth dilution method as recommended by the Clinical and Laboratory Standards Institute (CLSI) [11]. Suspensions of *S. aureus*, *S. epidermidis*, and *E. coli* were adjusted to a final concentration of 5×10^5 colony-forming units CFU/mL in MHB II. A volume of 100 µL of these bacterial suspensions was inoculated into the wells, either individually or in combination. The suspensions were then exposed to VAN and MEM solutions (0.015625-32 mg/L) in 100 µL of MHB II. The samples were incubated for 24 h under aerobic conditions at 37 °C. The MIC was defined as the lowest concentration of the antimicrobial agent that inhibited visible growth of the bacteria.

After incubating the bacteria with varying concentrations of the antibiotics, we meticulously transferred 100 µL from each well onto Tryptic Soy Agar (TSA) plates. Utilizing a sterile L-shaped spreader, we ensured the aliquots were evenly distributed across the agar surface, achieving uniform seeding. These plates were then incubated at 37 °C for a further 18–24 h to facilitate bacterial growth. Upon completion of the incubation period, we scrutinized the TSA plates for any signs of visible bacterial growth. The Minimum Bactericidal Concentration (MBC) was determined as the lowest concentration of the antimicrobial agent at which the agar plates exhibited no bacterial growth, signifying the eradication of 99.9% of the initial bacterial population. This critical

concentration reflects the minimal quantity of the antimicrobial agent necessary to achieve a bactericidal effect.

Fractional inhibitory concentration index (ΣFIC)

FIC was determined using the checkerboard assay. Suspensions of bacteria in MHB II, at an inoculum of 5×10^5 CFU/mL, were added to 96-well plates (Kartell, Noviglio, Italy) and exposed to various concentrations of VAN combined with MEM. Two-fold dilutions of the compounds were distributed across the wells (VAN: 0.03125-64 mg/L; MEM: 0.03125-64 mg/L). VAN was serially diluted along the plate, while the MEM was diluted from top to bottom. The samples were incubated under aerobic conditions at 37 ℃ for 24 h. The lowest concentration of the combined compounds that inhibited visible bacterial growth was considered the effective MIC for the combination.

The fractional inhibitory concentration index (ΣFIC) was calculated according to the formula below:

$$\Sigma\mathrm{FIC} = \mathrm{FIC\ A} + \mathrm{FIC\ B}$$

FIC A = MIC of antimicrobial A in the combination/MIC of antimicrobial A alone.

FIC B = MIC of antimicrobial B in the combination/MIC of antimicrobial B alone.

The combination was classified as synergistic when the ΣFIC was ≤ 0.5. Indifference was indicated by a ΣFIC > 0.5 to ≤ 4 and antagonism was indicated when the ΣFIC was > 4 [11].

Determination of minimum biofilm inhibitory concentration (MBIC) and minimum biofilm eradication concentration (MBEC)

S.*aureus*, *S. epidermidis*, and *E. coli* bacterial suspensions were adjusted to initial inocula of 5×10^6 CFU/mL in MHB II and 100 μL of cell suspensions were seeded in the wells individually or in combination. Plates were incubated for 24 h at 37 ℃, and biofilms were allowed to form. Following incubation, wells were gently washed with PBS to remove planktonic bacteria. VAN (range: 1–8,192 mg/L) and MEM (range: 1–8,192 mg/L) was added to wells 100uL, and plates were incubated for 24 h at 37 °C. MBIC values were visually assessed, with no bacterial growth indicating the minimum inhibitory concentration [11–13]. The well with the established biofilm was used as a positive control, and the well with MHB II without antibiotic treatment was used as a negative control. The contents of the wells were then removed and the wells were rinsed with sterile PBS to remove residual antimicrobial agents. Fresh MHB II was added to each well and the plate was incubated at 37 ℃ for 24 h. The outer diameter (*OD*) of each well was measured at 595 nm using a microplate reader. MBEC was defined as the minimum antimicrobial concentration that inhibited bacterial regrowth of the treated biofilm compared with the bacterial-only control.

Fractional biofilm eradication concentration index (ΣFBEC)

ΣFBEC is a crucial parameter used to evaluate the overall efficacy of a drug in eliminating established biofilms. It integrates the effects of the drug across different time points and concentrations, providing a comprehensive perspective on the drug's mechanisms of action and effectiveness. Biofilms cultured on plate were exposed to combinations of VAN with MEM. Two-fold dilutions of compounds were distributed to the wells (VAN: 2–16,384 mg/L; MEM: 2–16,384 mg/L). VAN was serially diluted along the plate, while the MEM was diluted from top to bottom. The samples were incubated for 24 h at 37 ℃. The contents of the wells were then removed and the wells were rinsed with sterile PBS to remove residual antimicrobial agents. Fresh MHB II was added to each well and the plate was incubated at 37 ℃ for 24 h. The *OD* of each well was measured at 595 nm using a microplate reader. The presented results are the means of three results obtained on three different days.

The FBEC index (ΣFBEC) was calculated according to the formula below:

$$\Sigma\mathrm{FBEC} = \mathrm{FBEC\ A} + \mathrm{FBEC\ B}$$

FBEC A = MBEC of antimicrobial A in the combination/MBEC of antimicrobial A alone;

FBEC B = MBEC of antimicrobial B in the combination/MBEC of antimicrobial B alone.

The combination was classified as synergistic when the ΣFBEC was ≤ 0.5. Indifference was indicated by a ΣFBEC > 0.5 to ≤ 4 and antagonism was indicated when the ΣFBEC was > 4 [11].

Results

In vitro antibacterial activity

MICs and MBCs against *S. aureus*, *S. epidermidis*, and *E. coli* were determined for VAN in comparison with MEM. Except for *E. coli*, *staphylococci* (ATCC 25923, ATCC 35984) MIC values for VAN were 1 mg/L, while the MBC values were consistently 16 mg/L. In contrast, MEM demonstrated high activity (MIC < 1 mg/L) against both *staphylococci* and *E. coli*, corresponding MBC values also less than 1 mg/L. Resistance increased markedly when the polymicrobial communities were cultured. The MIC of VAN against *S. aureus-S. epidermidis*, *S. aureus-E. coli*, and *S. epidermidis-E. coli* was recorded at 4 mg/L, with MBC range of 8–32 mg/L. Moreover, the MIC of MEM against polymicrobial community culture was 0.0625 mg/L, and the MBC values varied from 0.125 to 2 mg/L (Table 1).

Table 1 Minimum inhibitory concentration (MIC; mg/L) and minimum bacterial concentration (MBC; mg/L) values of Vancomycin (VAN) compared to meropenem (MEM) against *S. aureus*, *S. epidermidis* and *E. coli* individually or in combination

Species	MIC (mg/L)		MBC (mg/L)	
	VAN	MEM	VAN	MEM
S. aureus	1	0.125	16	0.25
S. epidermidis	1	0.125	16	0.25
E. coli	/	0.015625	/	0.015625
S. aureus-S. epidermidis	4	0.0625	8	2
S. aureus-E. coli	4	0.0625	32	1
S. epidermidis-E. coli	4	0.0625	8	0.125

S. aureus, Staphylococcus aureus; S. epidermidis, Staphylococcus epidermidis; E. coli, Escherichia coli

Table 2 Fractional inhibitory concentration index (ΣFIC) for all tested combinations between Vancomycin (VAN) and meropenem (MEM) against *S. aureus*, *S. epidermidis* and *E. coli* individually or in combination

Species	ΣFIC	Interaction
S. aureus	2.25	indifference
S. epidermidis	2.25	indifference
E. coli	-	-
S. aureus-S. epidermidis	4.06	antagonism
S. aureus-E. coli	4.06	antagonism
S. epidermidis-E. coli	8.06	antagonism

S. aureus, Staphylococcus aureus; S. epidermidis, Staphylococcus epidermidis; E. coli, Escherichia coli

In vitro evaluation of the combined effects of VAN and MEM

The effect of the combination of VAN and MEM was evaluated using the checkerboard microdilution method. FIC values obtained indicated an indifferent effect for the combination of VAN and MEM when assessed against *S. aureus* and *S. epidermidis*. Notably, this combination exhibited an antagonistic effect on *S. aureus-S. epidermidis*, *S. aureus-E. coli*, and *S. epidermidis-E. coli.* The most striking antagonism was observed in the combination of *S. epidermidis and E. coli* (Table 2).

In vitro antibiofilm activity

When planktonic bacteria accumulate and form biofilms, higher concentrations of antibiotics are required for their removal. MEM exhibited high activity against biofilms formed by *S. aureus* and *S. epidermidis*, with an MBIC of 8 mg/L and MBEC of 32 mg/L, which was 256 times the MIC. In contrast, *E. coli* forms biofilms that demonstrated elevated levels of resistance to antibiotics, with an MBIC of up to 16 mg/L and an MBEC of up to 128 mg/L, representing 8,192 times the MIC. VAN was less effective against biofilms than MEM, whereas it showed considerable activity against *staphylococci*, and its efficacy remained relatively limited. The MBIC for VAN ranged from 32 to 64 mg/L and the MBEC ranged from 256 to 512 mg/L, which corresponded to 256 to 512 times the MIC. Notably, MEM demonstrated enhanced activity when co-cultured with the bacteria. Combinations of *S. aureus-S. epidermidis*, *S. aureus-E. coli* and *S. epidermidis-E. coli*, the MBIC was 1 mg/L, while the MBEC ranged from 8 to 64 mg/L, representing 128 to 1,024 times the MIC range. In contrast, VAN exhibited reduced activity against the same bacterial combinations, with MBIC ranging from 128 to 512 mg/L and MBEC reaching as high as 4,096 mg/L, which corresponded to 1,024 times the MIC (Table 3).

Table 3 Minimum biofilm inhibitory concentration (MBIC; mg/L) and minimum biofilm eradication concentration (MBEC; mg/L) values of Vancomycin (VAN) compared to meropenem (MEM) against *S. aureus*, *S. epidermidis* and *E. coli* individually or in combination

Species	MBIC (mg/L)		MBEC (mg/L)	
	VAN	MEM	VAN	MEM
S. aureus	32	8	512	32
S. epidermidis	64	8	256	32
E. coli	128	16	8,192	128
S. aureus-S. epidermidis	128	1	4,096	64
S. aureus-E. coli	512	1	4,096	64
S. epidermidis-E. coli	512	1	4,096	8

S. aureus, Staphylococcus aureus; S. epidermidis, Staphylococcus epidermidis; E. coli, Escherichia coli

Activity of VAN applied in combination with MEM against biofilms

To quantify the apparent interactions observed and enable comparisons with previous and future studies, the FBEC index was calculated for VAN when used in the MEM combination. FBEC is a modification of the FIC index, which is used to detect synergism or antagonism between two antibiotics. The FBEC values indicated an indifferent effect of the combination of VAN and MEM when assessed against *E. coli* and *S. epidermidis-E. coli*. Notably, this combination exhibited an antagonistic effect on *S. aureus*, *S. epidermidis*, *S. aureus-S. epidermidis*, *and S. aureus-E. coli.* The most striking antagonism was observed in *S. epidermidis and S. aureus-E. coli* (Table 4).

Discussion

This study investigated the interactions between VAN and MEM in an in vitro model of CN-PJI. This study utilized NGS results of CN-PJI to construct monomicrobial and polymicrobial environments based on *S. aureus*, *S. epidermidis*, and *E. coli*. The results showed that the combination of VAN and MEM exhibited an indifferent effect against *S. aureus* and *S. epidermidis* but an antagonistic effect on polymicrobial communities. MEM demonstrated higher activity against biofilms than VAN. The combination of VAN and MEM showed indifferent

Table 4 Fractional biofilm eradication concentration index (ΣFBEC) for all tested combinations between Vancomycin (VAN) and meropenem (MEM) against *S. aureus*, *S. epidermidis* and *E. coli* individually or in combination

Species	ΣFBEC	Interaction
S. aureus	4.25	antagonism
S. epidermidis	9.00	antagonism
E. coli	0.51	indifference
S. aureus-S. epidermidis	4.06	antagonism
S. aureus-E. coli	8.13	antagonism
S. epidermidis-E. coli	2.00	indifference

S. aureus, Staphylococcus aureus; S. epidermidis, Staphylococcus epidermidis; E. coli, Escherichia coli

effects against *E. coli* and *S. epidermidis-E. coli* biofilms, but an antagonistic effect on *S. aureus*, *S. epidermidis*, *S. aureus-S. epidermidis*, and *S. aureus-E. coli* biofilms. These findings may inform future clinical guidelines and contribute to more effective treatment strategies for bacterial infections, ultimately improving patient outcomes in healthcare settings.

In the planktonic bacterial phase, VAN exhibited bactericidal activity against *Staphylococcus spp*. At lower concentrations, VAN demonstrated bacteriostatic effects with an MIC of 1 mg/L. Conversely, at higher concentrations, VAN displayed bactericidal activity with an MBC of 16 mg/L. This dose-dependent activity underscores the importance of appropriate dosing in clinical settings to achieve desired therapeutic effects. MEM was highly effective against all tested bacteria, including both gram-positive (*Staphylococcus spp*.) and gram-negative (*E. coli*). Both the MIC and MBC values for MEM were determined to be less than 1 mg/L, indicating potent antibacterial activity at relatively low concentrations. This broad-spectrum activity renders MEM a valuable option for treating infections caused by various bacterial species. Furthermore, we investigated the antibacterial activities of VAN and MEM against polymicrobial infections. The effectiveness of VAN decreased significantly in polymicrobial infections, with the MIC increasing to 4 mg/L and MBC values ranging from 8 to 32 mg/L. This reduction in efficacy suggests that the presence of polymicrobial organisms may enhance the resistance to VAN. In contrast, MEM maintained a relatively high activity in polymicrobial organisms, with an MIC of 0.0625 mg/L and MBC values ranging from 0.125 to 2 mg/L. The sustained effectiveness of MEM in polymicrobial infections underscores its potential utility in treating such conditions. However, Li et al. [14] suggested that patients with polymicrobial PJI should be a broad spectrum of antibiotics or a combination of multiple antibiotics to ensure adequate coverage against various microorganisms. Consequently, this study further explored the efficacy of VAN in combination with MEM in cases of polymicrobial infections. Notably, antagonistic effects were observed when VAN and MEM were administered together, with the most pronounced antagonism occurring in combinations of *S. epidermidis* and *E. coli*. This finding challenges the prevalent practice of combining antibiotics to enhance therapeutic efficacy, and underscores the complexity of antibiotic interactions in polymicrobial infections. These results highlight the importance of antibiotic stewardship in healthcare settings. Appropriate selection, dosing, and combination of antibiotics are essential to maximize therapeutic efficacy while minimizing the risk of developing antibiotic resistance. Furthermore, this study emphasizes the necessity for a nuanced and tailored approach for treating polymicrobial infections, considering the intricate interactions between different bacterial species and antibiotics.

As CN-PJI progress, bacteria form biofilms. Biofilm formation poses a considerable challenge for the treatment of infections. In this study, MEM demonstrated high activity against gram-positive bacteria, with an MBIC of 8 mg/L and MBEC of 32 mg/L. These relatively low concentrations indicate MEM's effectiveness against *staphylococcal* biofilms. However, MEM exhibited reduced efficacy against *E. coli* biofilms compared with *staphylococci*, with an MBIC of 16 mg/L and a markedly higher MBEC of 128 mg/L, suggesting that *E. coli* biofilms are more resistant to MEM treatment. In contrast, VAN was less effective than MEM against the tested bacterial species. For *staphylococcal* biofilms, VAN required higher concentrations (MBIC: 32–64 mg/L, MBEC: 256–512 mg/L) compared to MEM. This reduced effectiveness may be attributed to VAN's limited permeability to *staphylococcal* biofilms, as reported by Dall et al. [10]. The elevated concentrations of VAN required to inhibit and eradicate *staphylococcal* biofilms indicate that achieving therapeutic levels in clinical settings may pose challenges, potentially leading to treatment failure or necessitating prolonged therapy. Interestingly, in polymicrobial biofilms, MEM exhibited enhanced activity with decreased MBIC (1 mg/L) and MBEC (8–64 mg/L) values. This unexpected finding suggests that MEM may have synergistic effects in polymicrobial infections, possibly due to interactions between different bacterial species or alterations in biofilm structure. The enhanced activity of MEM in these scenarios could render it a valuable option for treating complex polymicrobial infections associated with CN-PJI. Conversely, VAN activity was further diminished in polymicrobial infections, with increased MBIC (128–512 mg/L) and MBEC (up to 4,096 mg/L) values. This substantial reduction in efficacy underscores the challenges associated with using VAN for polymicrobial infections, as extremely high concentrations are required to inhibit and eradicate biofilms. However, in clinical practice, joint infections are often treated with high-dose intraventricular (IV) antibiotics. He et al. [15]

mentioned that conventional IV antibiotic administration without inducing systemic toxicity usually only achieves 2–3 times the joint MIC, making it difficult to reach the MBEC level at the site of joint infection, leading to CN-PJI treatment failure. The inability to deliver adequate antibiotic concentrations to the affected area through systemic administration is a major hurdle in the effective treatment of these infections. Intra-articular infusion has emerged as a promising alternative for overcoming the limitations of systemic antibiotic administration. This approach delivers high antibiotic concentrations directly to the affected area, bypassing the constraints of systemic administration. Ji et al. [16] and Whiteside et al. [17] studies also demonstrated that in the single-stage management of chronically infected total hip arthroplasties, intra-articular infusion achieved 89.2–95% infection-free rates over a midterm follow-up period of approximately 4.8 to 5.3 years. These outcomes demonstrate that local antibiotic infusion solutions offer a promising avenue for improving outcomes, with the potential to revolutionize the management of these challenging infections and improve patient recovery and quality of life. In addition, surprisingly, the combination of VAN and MEM exhibited antagonistic effects, particularly on *S. epidermidis* biofilms and *S. aureus-E. coli* co-cultures. This finding suggests that concurrent use of these antibiotics may diminish their overall efficacy against certain polymicrobial infections. These results underscore the complexity of managing biofilm-associated infections, particularly those involving polymicrobial infections. The differential effectiveness of MEM and VAN against various bacterial biofilms and their combinations highlights the necessity of careful consideration when selecting antibiotic treatments for polymicrobial infections. Clinicians should consider factors such as the likely causative organisms, potential synergistic or antagonistic effects of antibiotic combinations, and ability to achieve therapeutic concentrations at the infection site.

This study has several limitations that should be considered when interpreting the results. While the bacterial strains used were among the most common identified through reference NGS results, their designation as reference strains ensures consistency and reproducibility under experimental conditions; however, it may not fully capture the variability and resistance mechanisms present in clinical isolates. This limitation affects the generalizability of our findings to real-world scenarios, where bacterial populations exhibit significant heterogeneity in terms of virulence factors, biofilm formation, and antibiotic susceptibility. Additionally, the in vitro nature of the study may not accurately replicate the complex biological environment of a human joint, potentially limiting the applicability of the findings in clinical settings. The interactions between VAN and MEM were assessed under controlled laboratory conditions, which may not account for the variability in drug interactions that can occur in vivo due to patient-specific pharmacokinetics and pharmacodynamics. Furthermore, we have not yet explored the specific mechanisms behind the antagonistic interactions between VAN and MEM. To enhance the relevance of our study, we will also consider including more recent studies that discuss alternative treatment approaches for CN-PJI. Therefore, future research should include in vivo studies and clinical trials to validate these findings and further investigate the implications of antibiotic interactions in the treatment of CN-PJI.

Conclusion

This study examined the interactions between VAN and MEM in an in vitro model of CN-PJI, revealing significant insights into antibiotic efficacy against bacterial pathogens. The combination showed indifferent effects against *S. aureus* and *S. epidermidis*, but exhibited antagonistic effects on polymicrobial communities, suggesting that concurrent use may not be advisable. Furthermore, MEM demonstrated superior activity against biofilms compared to VAN, indicating its potential as a more effective treatment for biofilm-related infections. These findings highlight the necessity for clinicians to reassess current treatment protocols involving VAN and MEM, and to consider alternative antibiotic combinations that may enhance therapeutic efficacy. Additionally, the study underscores the importance of evidence-based antibiotic selection and the risks of empirical combination therapy, which could inform future clinical guidelines and lead to more effective strategies for managing bacterial infections, ultimately improving patient outcomes in healthcare settings.

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Author contributions

Liqin Yao: Writing - original draft, Methodology, Data curation. Youcai Ma: Writing - original draft, Methodology. Rui Liu: Writing - original draft, Methodology. Yicheng Li: Investigation, Methodology. Xuebin Sun: Methodology, Data curation. Tuerhongjiang Wahafu: Methodology, Data curation. Li Cao: Writing - review & editing, Supervision. Wenbo Mu: Writing - review & editing, Supervision, Funding acquisition.

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Data availability
All data generated or analyzed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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