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Enhanced Anti-Biofilm Effects of Linezolid–Fosfomycin Combination against VRE *Enterococcus faecium*: An in Vitro Study

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ABSTRACT

We investigated the inhibitory potential of combining linezolid with fosfomycin against biofilms formed by vancomycin-resistant *Enterococcus faecium* (VREfm), aiming to provide a conceptual framework for managing infections associated with VREfm biofilms. This investigation used four clinical VREfm isolates (designated No. 2, No. 4, No. 5, and No. 6) sourced from the First Affiliated Hospital of Anhui Medical University. Synergy between linezolid and fosfomycin was evaluated through the checkerboard assay. Crystal violet staining quantified biofilm biomass suppression, whereas an Alamar Blue cell viability test measured metabolic function. The impact of drug exposure on the expression of genes implicated in biofilm development was probed using quantitative real-time polymerase chain reaction (RT-qPCR). Fractional inhibitory concentration index (FICI) values indicated that the linezolid-fosfomycin pairing acted synergistically against all VREfm isolates tested. Compared with linezolid alone, the dual-drug regimen markedly reduced both biomass accumulation and metabolic function in biofilms, particularly those at the mature stage. Data from RT-qPCR indicated that the combined therapy hindered biofilm development by repressing transcription of cylA, ebpA, and gelE during both early and late biofilm phases. Regarding mature biofilms specifically, the combination additionally downregulated asa1, atlA, and esp. Pairing linezolid with fosfomycin yielded a more robust suppression of VREfm biofilm formation than linezolid administered alone.

Keywords: Linezolid, Fosfomycin, Biofilm-formation genes, Vancomycin-resistant *Enterococcus faecium*

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Introduction

Enterococcus faecium has become a prominent opportunistic pathogen in healthcare environments, regularly implicated in urinary tract, intra-abdominal, and bloodstream infections [1]. Especially alarming is the growing difficulty in managing *E. faecium* infections due to the emergence of resistance to antimicrobial drugs, including vancomycin, which remains the cornerstone of Enterococcus treatment. Yet, its misuse has fueled a rising prevalence of vancomycin-resistant *E. faecium* (VREfm), leading to substantial mortality and therapeutic failure [2].

Given the escalating frequency of invasive VREfm illnesses, linezolid has emerged as a critical therapeutic option for VREfm infections. VREfm organisms generally retain susceptibility to linezolid. That said, studies have documented pronounced fluctuations in serum linezolid levels among severely ill individuals receiving the conventional regimen of 600 mg intravenously every 12 hours [3]. Such pharmacokinetic variability may predispose to resistance emergence and medication-related toxicity. Additionally, earlier exposure to linezolid was shown to constitute an independent predictor of drug-induced tolerance [4]. Data from the German National

Reference Center showed a rising proportion of linezolid-resistant VRE, from under 1% in 2008 to exceeding 9% by 2014 [5].

E. faecium is likewise among the microorganisms most adept at generating biofilms—a key contributor to disease pathogenesis [6]. A biofilm is a community of microbial cells permanently anchored to the surfaces of various medical devices, surrounded by extracellular polymeric substances (EPSs) comprising proteins, extracellular DNA, and polysaccharides [7]. Bacteria residing within biofilms can exhibit up to a thousandfold greater antibiotic resistance than free-floating forms and remain unresponsive to host immune defenses, thereby enabling prolonged survival and sustained infection even under intensive antibiotic therapy [8]. In addressing VREfm infections, especially those caused by biofilm-producing *E. faecium*, single-drug therapy frequently falls short and may provoke adverse effects owing to the necessity for extended treatment courses [9]. Consequently, discovering antibiotic pairings for VREfm infection management represents an urgent priority.

Therapeutic decisions should take into account unwanted side effects, drug penetration into target tissues, and possible pharmacological interactions. It has been demonstrated that escalating either the dosage or the duration of linezolid administration can provoke hematologic toxicity and promote the emergence of resistant organisms during the course of treatment [10]. Given the constraints of linezolid alone, resorting to multidrug regimens may be a viable alternative. Fosfomycin belongs to the older generation of antibiotics; it exerts broad-spectrum activity against Gram-positive and Gram-negative bacteria, including multidrug-resistant strains, by permanently disrupting the initial phase of peptidoglycan synthesis [11]. However, using fosfomycin as a standalone therapy can raise the MIC (minimum inhibitory concentration) of originally sensitive bacterial populations. Numerous investigations have revealed synergistic interactions between fosfomycin and a range of other antimicrobials when directed against Enterococcus species [12]. Our own laboratory has undertaken parallel investigations in this domain. We substantiated that the dual administration of linezolid and fosfomycin yielded a pronounced synergistic outcome against both vancomycin-sensitive and vancomycin-resistant Enterococci under in vitro conditions and within an in vivo context, employing time-kill curve methodology and a Galleria mellonella infection model, whilst averting the advent of resistance and diminishing the pathogenic potential of fosfomycin-susceptible and fosfomycin-resistant Enterococcus isolates [13, 14]. We also found that, with respect to VRE strains, neither linezolid nor fosfomycin alone could restrain the proliferation of resistant subpopulations, and that the acquisition of fosfomycin resistance incurred a trade-off in the form of attenuated VREfm virulence [15]. The co-administration of fosfomycin with either linezolid or tigecycline showed synergistic activity against VRE in time-kill analyses, achieving synergy in approximately 10% and 30% of cases, respectively [16]. When oritavancin was combined with fosfomycin, it enhanced antimicrobial susceptibility and produced synergism in 80% of the tested isolates, along with additivity in the remainder. Beyond that, the drug pairing showed either synergistic or additive effects in biofilm testing [17].

Fosfomycin retains efficacy against adherent populations of Enterococcus faecalis [18]. A high-level fosfomycin exposure (16×MIC) combined with daptomycin yielded considerably stronger antibiofilm activity relative to either agent alone and successfully eradicated adherent bacteria entrenched within established biofilms produced by linezolid-resistant E. faecalis isolates [19]. Linezolid has also been shown to inhibit biofilm formation by E. faecalis, and supplementing gentamicin significantly enhanced linezolid's biofilm-inhibitory capacity [20, 21]. That said, the question of whether linezolid combined with fosfomycin acts synergistically to suppress VREfm biofilms remains unresolved.

The process of biofilm generation unfolds across four distinct stages [22]. (1) Planktonic bacterial cells make initial contact with and affix to a surface, commencing biofilm development; (2) The attached organisms elaborate EPSs, fostering bacterial clustering alongside matrix deposition; (3) Biofilm architecture advances through multilayered growth, characterized by the emergence of micro-colonies interspersed with fluid-filled channels; (4) Biofilm structures achieve a state approximating maturity and initiate the dispersal of bacterial micro-colonies departing from the parent community to colonize distant sites, thereby propagating the infectious process. Recent scholarly work [23] has largely focused on the capacity of therapeutic agents to eliminate pre-existing mature biofilms. In contrast, comparatively little published research has examined how pharmacological interventions influence biofilms at immature stages. Given the developmental biology of biofilms, even successful eradication of mature biofilm communities does not preclude bacteria liberated during dispersal from relocating to unoccupied surfaces and re-initiating biofilm assembly. For this reason, achieving inhibition at the earliest stage of biofilm formation would significantly increase the likelihood of curing biofilm-related infections.

Motivated by these considerations, the present study set out to evaluate the dual regimen of linezolid plus fosfomycin directed against VREfm biofilms at both immature and mature phases, employing biofilm biomass quantification and metabolic activity profiling of bacteria residing within the biofilm matrix. The distinction between linezolid monotherapy and the linezolid-fosfomycin combination in modulating the expression of select biofilm-formation-linked genes was determined by RT-qPCR.

Materials and Methods

bacterial isolates

The entire collection of eight clinical VREfm isolates recovered from urine samples of patients at the First Affiliated Hospital of Anhui Medical University underwent preliminary screening for biofilm-forming proficiency. Based on these results, four clinical isolates (labeled No. 2, No. 4, No. 5, and No. 6) were selected as test organisms for the investigation. Confirmatory identification of all strains was performed using the automated VITEK-2 platform (BioMérieux, Marcy-l'étoile, France). Vancomycin-resistant Enterococcus ATCC 51299 functioned as the quality control reference organism. These bacterial strains constituted part of the institution's standard laboratory workflow. This activity received ethical clearance from the Ethics Committee of the First Affiliated Hospital of Anhui Medical University and adhered to the ethical standards delineated in the Declaration of Helsinki. Per prevailing national regulations within China, these isolates were procured within the framework of routine clinical patient care; accordingly, the requirement for informed consent was waived. None of the isolates incorporated into the study were collected with the specific intent of serving this research project.

Antimicrobials and media

Linezolid, fosfomycin, and vancomycin were sourced from the National Institute for Food and Drug Control of China (Beijing, China). Mueller–Hinton broth (MHB) (Oxoid, England) and MH agar (MHA) (Oxoid, England) were used as growth media for all experimental work, except that Brain Heart Infusion agar (BHIA, Oxoid, United Kingdom) was reserved solely for vancomycin susceptibility determinations. Furthermore, whenever fosfomycin was included in any medium, the preparation was supplemented with 25 mg/L glucose-6-phosphate (Sigma-Aldrich).

Assessment of in vitro susceptibility and determination of fractional inhibitory concentration index (FICI)

The agar dilution method was used to determine minimum inhibitory concentrations (MIC) for linezolid, fosfomycin, and vancomycin, in accordance with the recommendations of the Clinical and Laboratory Standards Institute [24]. To elaborate, plates of Mueller–Hinton agar and Brain Heart Infusion agar were formulated to incorporate successive two-fold decrements in the concentration of each respective antibiotic. Plates containing fosfomycin required the addition of glucose-6-phosphate to achieve a final concentration of 25 mg/L. Following this preparation, an inoculum delivering 5×10^5 colony-forming units (CFU) of bacterial cells was applied to the surface of each plate, after which incubation proceeded at 37°C over an 18–24 h window. The MIC endpoint was taken as the most dilute antibiotic preparation that completely suppressed colony emergence upon visual inspection. Following CLSI 2020 breakpoint guidance [24], the designation of resistant (R) was assigned when vancomycin MIC values met or surpassed 32 mg/L, linezolid MICs reached or exceeded 8 mg/L, and fosfomycin MICs attained or went beyond 256 mg/L. Quality assurance for the testing protocol relied upon vancomycin-resistant Enterococcus ATCC 51299 as the reference strain. Triplicate MIC assays were performed for each isolate.

Synergy between linezolid and fosfomycin across a range of concentrations was assessed using the checkerboard technique. The terminal concentrations tested for each drug extended from 1/16×MIC up to 2×MIC. Into each well of a 96-well plate, a bacterial suspension calibrated to 10^5 CFU/mL was dispensed. The prepared plates were then maintained under aerobic atmospheric conditions at 37°C for a full day.

Calculation of the fractional inhibitory concentration index (FICI) proceeded according to the relation: FICI = (MIC of agent A when paired / MIC of agent A when tested singly) + (MIC of agent B when paired / MIC of agent B when tested singly). Interpretation guidelines classified FICI readings of ≤0.5 as indicative of synergy, values falling between > 0.5 and 4 as reflecting no interaction, and any reading surpassing 4 as denoting antagonism [25].

In vitro determination of minimum biofilm eradication concentrations (MBECs)

The procedure for determining in vitro minimum biofilm eradication concentrations was based on the methodology previously cited [26]. Mature biofilm structures were generated by introducing bacterial suspensions of each strain—adjusted to a starting density of 5×10^6 CFU/mL in MHB—into microtiter wells and incubating them undisturbed at 37°C for 24 hours. Upon completion of biofilm maturation, the contents of the wells were aspirated, and the wells were subjected to three cycles of washing with PBS, after which MHB augmented with serially descending concentrations of either linezolid or fosfomycin—spanning a range from 64×MIC down to 2×MIC—was distributed into the wells. The plates were then incubated for a further 24 h. At the end of the antimicrobial challenge, wells were washed three times with PBS, and 0.01% Alamar Blue (Maokang Bio, China) in MHB was added. Alamar blue, which is alternatively termed resazurin, functions as a reporter dye whose reduction reflects the viability status of cells. Incubation continued for an additional 3 h at 37 °C, after which absorbance was measured at 570 nm and 600 nm using a microplate reader (Nanodrop, USA). The metabolic activity percentage (%MA) was derived through comparison with positive reference wells (bacteria maintained in plain MHB, equated to 100% activity) and negative reference wells (sterile MHB alone, equated to 0% activity), following the computational formula laid out in the product documentation:

The notation A_{570} signified the absorbance reading at 570 nm obtained from the antibiotic-exposed sample, whereas A_{600} indicated the corresponding reading at 600 nm from that same treated sample; P_{570} represented the 570 nm absorbance of the untreated control sample, and P_{600} corresponded to the 600 nm absorbance of the untreated control sample.

Data outputs were reported as $MBEC_{90}$ and $MBEC_{50}$ values, respectively defined as the minimal antimicrobial levels that suppressed bacterial metabolic function by at least 90±5% and 50±5%. The metabolic activity measurements used to assign MBEC values reflected the averaged outcome of three separate experimental runs performed on three distinct days.

Quantification of biofilm biomass

The consequences of exposing VREfm biofilm to linezolid alone versus linezolid in combination with fosfomycin were probed using the crystal violet staining technique, as described earlier [19]. To summarize, VREfm strains were seeded into 96-well polystyrene microtiter plates containing MHB fortified with the designated antimicrobial agent(s), and incubation was continued for 48 h to determine the biofilm-suppressive potential of the drug regimens. To contrast pharmacological effects on biofilms at early versus late developmental stages, the antimicrobial agents were introduced into the culture wells at one of two time points: either after a brief 2-hour attachment phase or after a full 24-hour biofilm establishment phase. After a total incubation duration of either 24 h or 48 h under static conditions—throughout which the antibiotic-laden medium was exchanged for fresh preparation every 24 hours—the overlying liquid was decanted, and each well was delicately rinsed with PBS on three successive occasions. The biofilm biomass remaining affixed to the well surfaces was subsequently stained with crystal violet, and the extent of staining was quantified by measuring absorbance at 590 nm on a microplate reader. Each experimental condition was run in triplicate technical replicates and repeated across three separate experimental sessions.

Determination of biofilm metabolic function

Resazurin-based viability detection served as the methodological cornerstone for gauging the metabolic integrity of biofilm-ensconced bacteria, a technique widely acknowledged for its consistency, replicability, and endorsement by expert bodies [27]. Upon termination of the previously specified 24 h or 48 h static growth period, the well contents were evacuated, and a triplicate PBS rinse cycle was performed with gentle agitation. Wells subsequently received an aliquot of MHB freshly prepared to contain 10% (v/v) Alamar blue chromogenic reagent and were returned to 37 °C for a 3-hour chromophore development step, as per the supplier's protocol (Maokang Bio, China). The computation of percent metabolic activity followed the arithmetic operations laid out in an earlier passage. For every treatment arm, three biological replicates were executed, staggered across separate calendar days.

Transcript profiling of biofilm determinants in VREfm through RT-qPCR

A panel of six genes with established roles in biofilm biogenesis was interrogated at the transcriptional level in clinical strains No. 5 and No. 6 using RT-qPCR, guided by protocols documented in prior publications [28]. To

capture effects on nascent biofilm structures, the VREfm organisms were dispersed into 100 mm × 20 mm nonpyrogenic polystyrene tissue culture dishes holding MHB augmented with linezolid at one-eighth of its MIC and fosfomycin at one-half of its MIC, with the incubation extending over a full day. To assess disruption of fully formed biofilms, parallel cultures were established in MHB fortified with linezolid at twice its MIC and fosfomycin at its MIC, and maintained for 48 h. Once the undisturbed incubation had concluded, total RNA was harvested from both the fluid-phase and surface-adherent bacterial fractions for subsequent RT-qPCR. Reaction mixtures were prepared with SYBR® Green Pro Taq HS Premix II (Rox Plus) (Agbio Co, Hunan, China), and thermal cycling with fluorescence acquisition was performed on a Roche LightCycler 96 platform (Switzerland). Transcript signals attributable to genes of interest were standardized to the concurrently amplified 16S rRNA internal control transcript, and fold-change values were derived using the comparative threshold cycle equation 2^(–ΔΔCT), where CT is the fractional cycle number at which amplification became detectable above background. **Table 1** provides a comprehensive list of oligonucleotide primer sets used in these RT-qPCR reactions. Triplicate measurements were obtained for each assay condition.

Table 1. PCR primers used for determining the expression levels of selected genes of vancomycin-resistant *E. faecium* isolates by RT-qPCR.

Target	Primer	Primer Sequence (5´-3´)	Reference
asa1	*asa1*-F	GCACGCTATTACGAACTATGA	[19]
	asa1-R	TAAGAAAGAACATCACCACGA	
atlA	*atlA*-F	AACAGCACCAACGGATTAC	[27]
	atlA-R	CATAGTCAGCATAGTTATTCATTG	
cylA	*cylA*-F	GGAGGATATGGTGACAAT	[19]
	cylA-R	TTACTTCTGGAGTTGCTAA	
esp	*esp*-F	AATTGATTCTTTAGCATCTGG	[28]
	esp-R	AGATTCATCTTTGATTCTTGG	
ebpA	*ebpA*-F	ATAATAACAACGCCATTCAA	[19]
	ebpA-R	ACATATCACCAGCATCTC	
gelE	*gelE*-F	TACACCATTATCCAGAACT	[19]
	gelE-R	CATCGCCATATTGAACTT	

Statistical framework

Dataset manipulation and statistical interrogation were performed in SPSS version 20.0 (SPSS, Inc., Chicago, IL, United States). Graphical output was rendered via GraphPad Prism, version 8.0 (GraphPad Software, Inc., San Diego, CA, United States). Single-factor analysis of variance (One-way ANOVA) served as the primary inferential test, with significance denoted as $P < 0.05$, $P < 0.01$, and $P < 0.001$, respectively.

Results and Discussion

Findings from MIC determinations and FICI ANALYSIS

The paired MIC readouts for linezolid and fosfomycin across the quartet of study isolates are cataloged in **Table 2.** Susceptibility to linezolid was uniform, all four strains yielding an MIC of 2 mg/L. Fosfomycin MICs, by contrast, ranged from 128 mg/L in three instances, with isolate No. 4 as the outlier, whose MIC reached 256 mg/L. It bears reiterating that vancomycin resistance was a universal trait among the strains under investigation.

Table 2. MICs and FICI of antimicrobial reagents against four strains.

Isolates	MIC in combination	MIC (mg/L)			
	FICI	LIN + FOS	VAN	FOS	LIN
ATCC51299	NE	NE	128	128	2
No.2	0.125	0.125+8	128	128	2
No.4	0.125	0.125+16	512	256	2
No.5	0.1875	0.125+16	512	128	2
No.6	0.125	0.125+8	512	128	2

Abbreviations: LIN = linezolid; FOS = fosfomycin; VAN = vancomycin; LIN+FOS = linezolid-fosfomycin combination; NE = not estimable.

The FICI indices, quantifying the combinatorial pharmacodynamic relationship between the two study drugs, are likewise entered in **Table 2.** All four VREfm strains yielded FICI values below the 0.5 cutoff, placing the linezolid-fosfomycin interaction firmly within the synergistic classification.

In vitro minimum biofilm eradication concentrations (MBECs)

The MBEC figures collated in **Table 3** underscore that VREfm populations dwelling within biofilm matrices exhibit dramatically elevated antimicrobial resistance compared with their free-living counterparts documented in **Table 2.** VREfm biofilms were more tolerant of fosfomycin than of linezolid. Fosfomycin $MBEC_{50}$ values distributed across a spectrum from 8×MIC up to 64×MIC among the four strains, while the corresponding span for linezolid $MBEC_{50}$ extended from 4×MIC to 32×MIC. A concordant pattern was observed in the $MBEC_{90}$ datasets. Biofilm cultures originating from strains No.4 and No.5 manifested particularly extreme drug tolerance, their $MBEC_{90}$ thresholds for both linezolid and fosfomycin verging on or exceeding 64×MIC. At the opposite end of the spectrum, the biofilm population generated by strain No.6 displayed the greatest sensitivity, with $MBEC_{90}$ values of 8×MIC for linezolid and 32×MIC for fosfomycin.

Table 3. $MBEC_{90}$ and $MBEC_{50}$ of antimicrobial agents against four strains.

Isolates	$MBEC_{50}$ (mg/L)		$MBEC_{90}$ (mg/L)	
	FOS	LIN	FOS	LIN
No. 2	1024	16	4096	64
No. 4	> 16.384	64	> 16.384	> 128
No. 5	8192	32	> 8192	128
No. 6	1024	8	4096	16

Abbreviations: LIN = linezolid; FOS = fosfomycin.

Analysis of biofilm biomass accumulation

Our opening comparative evaluation addressed whether the linezolid-plus-fosfomycin regimen surpassed linezolid monotherapy in limiting biofilm build-up during the incipient developmental window for each of the four VREfm isolates. At the juncture of drug addiction, the bacterial cells were transitioning from a planktonic state to surface anchorage and had begun elaborating EPSs to seed biofilm architecture. The results shown in **Figure 1** demonstrate that antibiotic levels below the MIC threshold can nonetheless disrupt biofilm progression. Pairing linezolid (at either 1/8×MIC or 1/4×MIC) with fosfomycin (1/4×MIC) consistently inhibited VREfm biofilm formation more robustly than linezolid alone, a pattern that held across the entire strain collection. A striking illustration emerged from strain No.5, where the co-application of linezolid at merely 1/4×MIC together with fosfomycin at 1/4×MIC outperformed even the higher-strength linezolid-alone condition of 1/2×MIC.

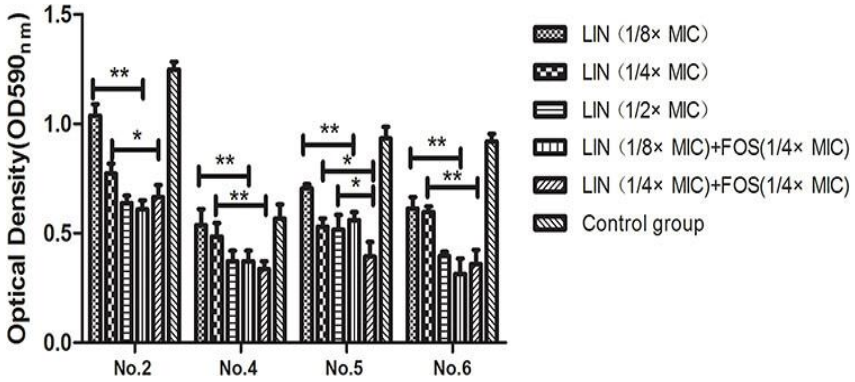


Figure 1. Sub-minimum inhibitory concentrations (1/8, 1/4, or 1/2×MIC) of linezolid, combined with 1/4 MIC of fosfomycin, inhibited VREfm biofilm formation at the initial stage. Notes: Data represent the average of three independent experiments (mean ± SD). $P < 0.05$, $P < 0.01$. Abbreviations: LIN = linezolid; FOS = fosfomycin; LIN+FOS = linezolid-fosfomycin combination.

A complementary line of inquiry explored whether linezolid deployed at escalated doses (2, 4, or 8×MIC) together with fosfomycin (1×MIC) possessed the wherewithal to dislodge mature, pre-established VREfm biofilms beyond what linezolid could accomplish when given as a standalone agent. The evidence presented in **Figure 2** attests to

the substantial biofilm-removal capacity of the dual-therapy approach. Tellingly, even the strongest linezolid-only treatment (8×MIC) proved less effective at biofilm clearance than the combined regimen.

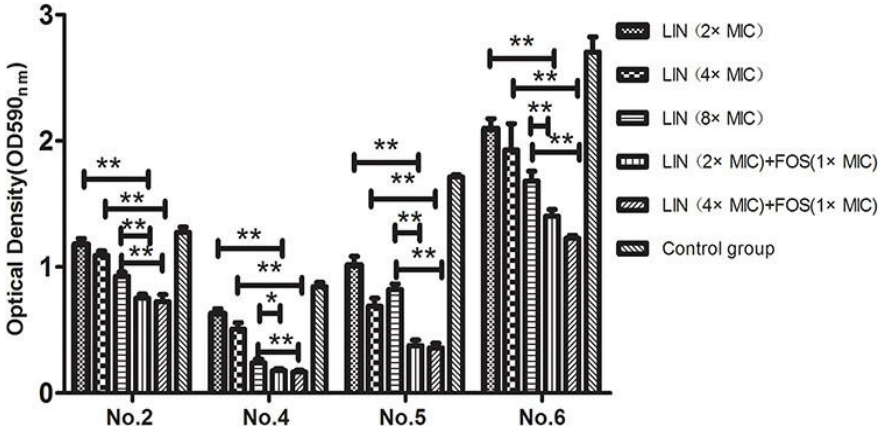


Figure 2. High concentrations (2, 4, or 8×MIC) of linezolid, combined with 1×MIC fosfomycin, inhibited VREfm biofilm formation at the mature stage. Notes: Data represent the average of three independent experiments (mean ± SD). $P < 0.05$, $P < 0.01$. Abbreviations: LIN = linezolid; FOS = fosfomycin; LIN+FOS = linezolid-fosfomycin combination.

Measurement of biofilm metabolic performance

During the earliest phase of biofilm development, linezolid at the middle and upper dosing strata (1/4 and 1/2×MIC) depressed the metabolic output of biofilm-embedded organisms by a margin exceeding 50% uniformly across the four test strains. As the data in **Figure 3** make evident, fosfomycin co-administration markedly strengthened the metabolic suppression conferred by linezolid at its lower and mid-range concentrations (1/8 and 1/4×MIC). Focusing on strain No.6, the degree of metabolic dampening elicited by linezolid at the low and moderate strengths (1/8 and 1/4×MIC) when accompanied by fosfomycin (1/4×MIC) outstripped that induced by linezolid at the high strength (1/2×MIC) applied by itself.

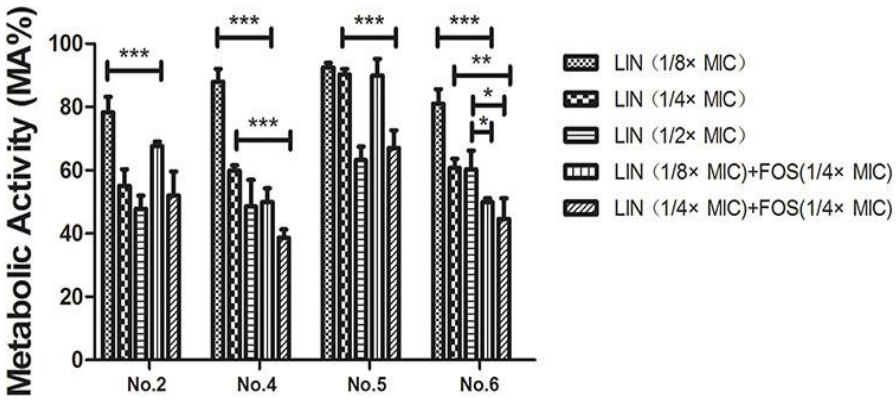


Figure 3. Sub-minimum inhibitory concentrations (1/8, 1/4, or 1/2×MIC) of linezolid, combined with 1/4 MIC of fosfomycin, inhibited cells in VREfm-biofilm at the initial stage. Notes: Data represent the average of three independent experiments (mean ± SD). $P < 0.05$, $P < 0.01$, $P < 0.001$. Abbreviations: LIN = linezolid; FOS = fosfomycin; LIN+FOS = linezolid-fosfomycin combination.

When the focus shifted to mature biofilm assemblies, both antibiotics demonstrated considerably blunted metabolic interference, a trend plainly visible in **Figure 4**. For three of the four strains—No.2, No.4, and No.5—the percentage reduction in metabolic activity within mature biofilms never crossed the 50% threshold, regardless of whether linezolid was administered singly or paired with fosfomycin. The addition of fosfomycin at 1×MIC did, nonetheless, augment linezolid's performance in every isolate tested, with the amplification being particularly pronounced in strains No.4 and No.5. A noteworthy result was that the intermediate linezolid dosage (4×MIC) combined with fosfomycin (1×MIC) exerted metabolic restraint superior to what was accomplished by the maximum linezolid concentration (8×MIC) delivered independently.

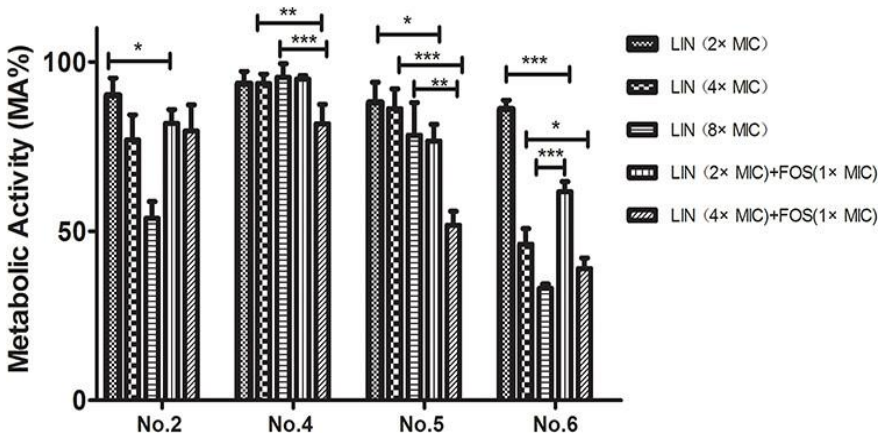
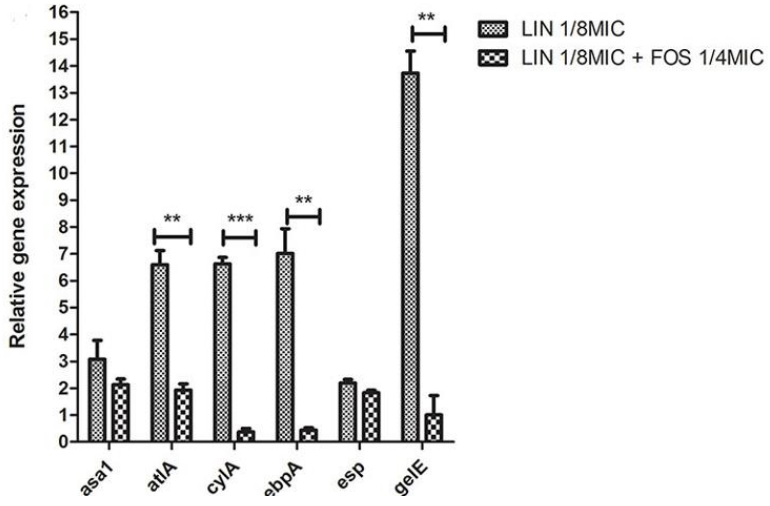


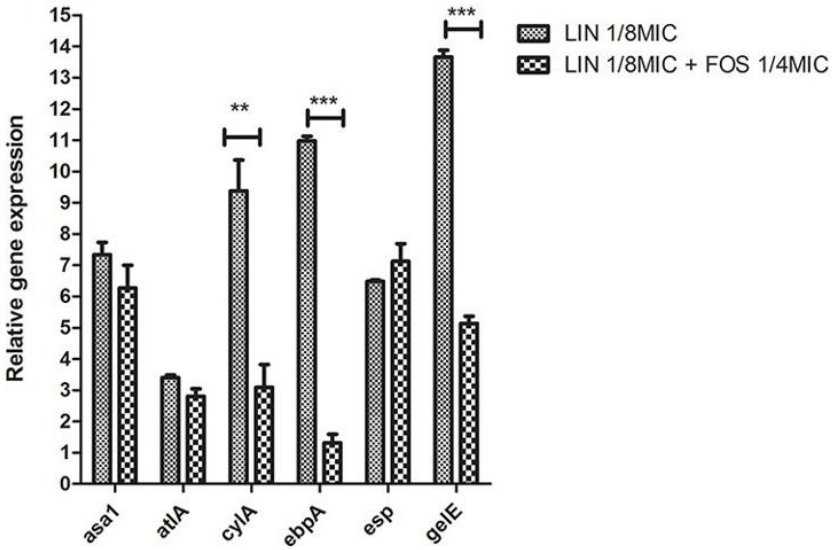
Figure 4. High concentrations (2, 4, or 8×MIC) of linezolid, combined with 1×MIC of fosfomycin, inhibited cells in the mature VREfm biofilm. Notes: Data represent the average of three independent experiments (mean ± SD). $P < 0.05$, $P < 0.01$, $P < 0.001$. Abbreviations: LIN = linezolid; FOS = fosfomycin; LIN+FOS = linezolid-fosfomycin combination.

Quantitative comparison of biofilm gene transcript abundance

As the final experimental component, strains No. 5 and No. 6 were singled out for RT-qPCR-based quantification of messenger RNA levels for six biofilm-implicated genetic elements. As conveyed by **Figure 5,** fosfomycin treatment left the expression of asa1 and esp unaltered in either strain, and similarly produced no change in atlA transcript levels within strain No.6. By contrast, statistically meaningful decreases in cylA, ebpA, and gelE transcript abundance were recorded when the bacterial populations were challenged for 24 h with the combination of linezolid at 1/8×MIC and fosfomycin at 1/4×MIC, a condition designed to intercept biofilm genesis at its formative stage.



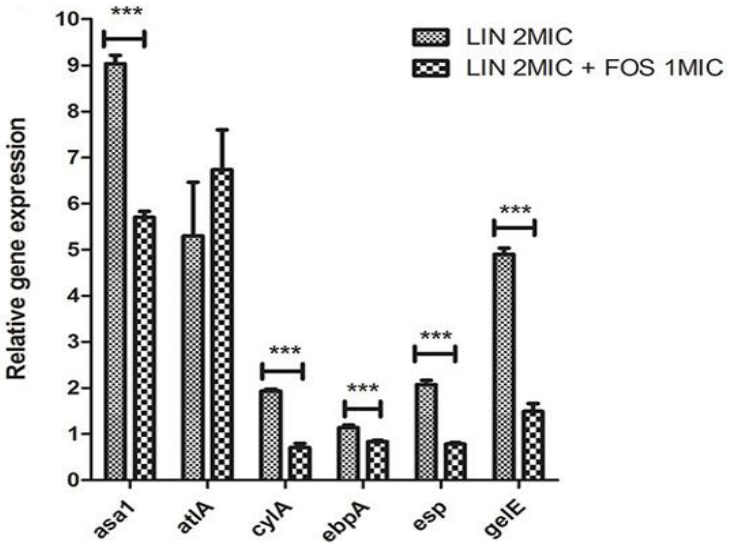
a)



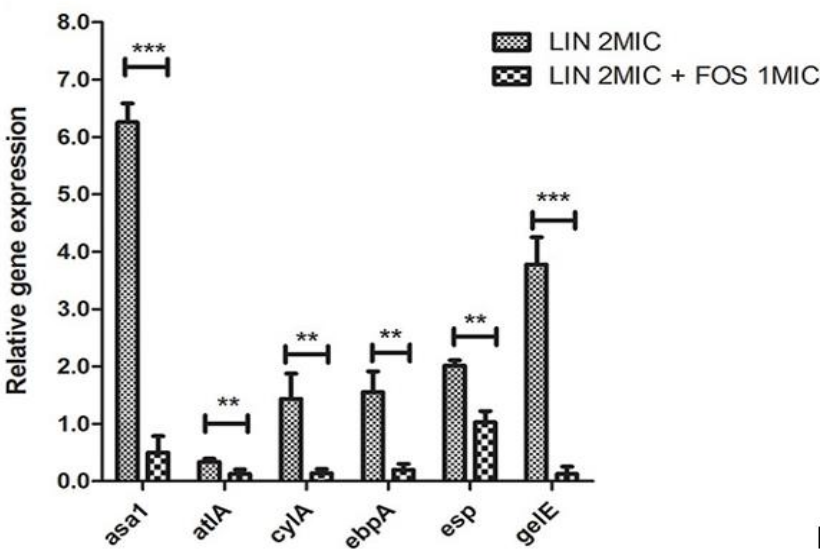
b)

Figure 5. Relative gene expression of biofilm formation-related genes of isolates No.5 and No.6 with linezolid alone or linezolid combined with fosfomycin treatment in the initial stage. Notes: Data represent the average of three independent experiments (mean ± SD). $P < 0.01$, $P < 0.001$; No.5 strain (a); No.6 strain (b). Abbreviations: LIN = linezolid; FOS= fosfomycin; LIN+FOS = linezolid-fosfomycin combination.

The transcriptional data about mature biofilms, assembled in **Figure 6,** indicate that all six gene targets underwent significant downregulation, the lone exception being atlA in strain No.5. Transcripts originating from asa1 and gelE were notably suppressed across both test strains following a 48-hour exposure to linezolid at 2×MIC concurrently with fosfomycin at 1×MIC, a regimen aimed at eradicating established biofilm communities.



a)



b)

Figure 6. Relative gene expression of biofilm formation-related genes of isolates No.5 and No.6 with linezolid alone or linezolid combined with fosfomycin treatment in the mature stage. Notes: Data represent the average of three independent experiments (mean ± SD). $P < 0.01$, $P < 0.001$; No.5 strain (a); No.6 strain (b). Abbreviations: LIN = linezolid; FOS = fosfomycin; LIN+FOS = linezolid-fosfomycin combination.

Medical concern surrounding biofilm-associated infections has escalated considerably, encompassing pathologies related to indwelling devices, recalcitrant infections in the absence of prosthetic material, and even the functional failure of biomedical devices. Bacterial populations of enterococci favor an aggregated, community-based mode of existence and are implicated in roughly one-quarter of catheter-associated urinary tract infections. These microbes are routinely cultured from wound specimens and are increasingly identified in cases of infective endocarditis; critically, all the aforementioned infectious conditions involve biofilms [29]. Given the inherent nature of biofilm structures, enterococcal infections rooted in biofilms are exceptionally refractory to clearance and simultaneously serve as foci for bacterial seeding and as genetic reservoirs for antimicrobial resistance traits [30].

The presence of multidrug-resistant strains substantially magnifies the therapeutic complexity of biofilm-mediated infections. Serving as a principal treatment option for VRE infections, linezolid has been demonstrated across numerous studies to inhibit biofilm formation by enterococci, whether deployed as single-agent therapy or in combination with rifampicin or gentamicin [21, 31]. Fosfomycin also possesses biofilm-attenuating activity against various bacterial genera, including Enterococcus species [32]. In the current investigation, we demonstrated that linezolid and fosfomycin exhibit synergistic antibacterial activity against VRE, corroborating prior findings from our research collective [14]. Further evidence validating that fosfomycin can interact with linezolid in either a synergistic or additive fashion has emerged from a time-kill assay examining 32 VREfm urinary tract isolates [33]. It is worth emphasizing that the overwhelming majority of those strains were sourced from urine samples. A separate clinical inquiry conducted at a medical center in Thailand, involving 26 patients with VRE, identified synergistic activity between fosfomycin and linezolid [34]. The bacterial strains evaluated in that study originated from multiple anatomical compartments, including the circulatory system, the urinary apparatus, and the gastrointestinal tract. On the whole, the published literature evaluating the therapeutic value of combining fosfomycin with linezolid against VRE has relied on urinary tract clinical specimens, highlighting a gap that warrants further investigation using Enterococcus isolates derived from more diverse infection sites.

Findings from biofilm biomass staining assays indicated that fosfomycin markedly enhanced linezolid-mediated biofilm suppression against VRE, with this effect especially notable at the mature biofilm stage. As illustrated in **Figure 2**, the biofilm cultivated over 24 hours showed a near doubling in the combined-treatment cohort compared with the linezolid-exclusive cohort. This phenomenon was particularly apparent for isolates No. 4 and No. 5. Of considerable significance, the optical density readings at 590 nm obtained from wells receiving the dual-drug regimen were lower than those from the linezolid-only arm, even at the highest concentration (8×MIC). This

outcome carries meaningful translational implications. This suggests that supplementing therapy with fosfomycin might obviate the need for maximally dosed linezolid when addressing VRE infections—an important consideration, given that linezolid at elevated doses predisposes patients to adverse sequelae, such as lactic acidosis [35]. Looking at the early attachment window, the degree of biofilm restriction conferred by pharmacological intervention fell short of that observed against mature biofilms; the co-therapy and linezolid-only arms reduced biofilm biomass by approximately 50% and 30%, respectively.

Regarding metabolic function determinations, linezolid demonstrated bactericidal activity against organisms residing within biofilms, and the presence of fosfomycin further enhanced this activity. The magnitude of killing varied on an isolate-by-isolate basis. In strain No. 6, the combination approach consistently outperformed monotherapy in achieving bacterial eradication, regardless of whether the biofilm was incipient or fully developed. The scenario with strain No.2 diverged somewhat: enhanced bacterial killing relative to linezolid alone was confined to the lower linezolid dosing condition (1/8×MIC against early biofilm or 2×MIC against mature biofilm) when partnered with fosfomycin, whereas at the 1/4×MIC, 1/2×MIC, 4×MIC, and 8×MIC linezolid tiers, no meaningful distinction emerged between monotherapy and the combination **(Figures 3 and 4).** This partial discordance with the biofilm suppression data suggests that therapeutic agents may, in addition to outright bacterial killing, operate through other mechanisms to diminish biofilm accumulation. The RT-qPCR outputs lent weight to this supposition. A considerable number of genetic loci govern enterococcal biofilm biogenesis. Six such determinants were singled out for transcriptional analysis within the RT-qPCR component of this work. The gelE coding sequence, contained within the gelE-sprE transcriptional unit, dictates production of gelatinase. This enzyme plays a critical role early in biofilm establishment by proteolytically cleaving the collagen-anchoring adhesin Ace [36]. By degrading Ace, gelatinase facilitates bacterial surface attachment, promoting further spread and niche colonization. Gelatinase also triggers the mobilization of N-acetylglucosaminidase (AtlA), an essential autolytic enzyme that initiates lysis of a bacterial subfraction (autolysis) and liberates extracellular DNA (eDNA). eDNA serves a glue-like function critical for both adherence and architectural maintenance within biofilms [37]. The data presented in **Figure 5** reveal that linezolid combined with fosfomycin curbed gelatinase elaboration—and thereby hindered bacterial substratum adhesion—by transcriptionally downregulating gelE during the biofilm initiation phase. After 24 hours of undisturbed incubation, the biofilm approaches structural completion, and diminished gelatinase output can correspondingly curtail eDNA discharge through dampened autolytic activity. This resultant loss of biofilm cohesion and anchoring strength renders the matrix more vulnerable to disruption **(Figure 6).** Concurrently, the linezolid-fosfomycin regimen suppressed cylA transcript levels, further limiting biofilm expansion. The cylA gene, which contributes to toxin structure and biological function, is part of the PL promoter that governs the cytolysin operon, which is situated alongside cylLL, cylLS, cylM, cylB, and cylI [38]. The cytolysin exotoxin can perforate and lyse target cell membranes, an event indispensable for eDNA genesis.

Upon biofilm maturation, the linezolid-fosfomycin pairing additionally curbed generation of aggregation substance (AS), a filamentous surface glycoprotein encoded by the asa1 locus **(Figure 6).** The dual-antibiotic regimen depressed asa1 transcript abundance further than linezolid alone. Surface-displayed AS promotes intercellular aggregation and heightened antibiotic tolerance, properties conducive to biofilm development [39]. This molecular observation aligns with our phenotypic finding of diminished biofilm biomass and reduced MIC values within the combination therapy arm.

The esp genetic determinant, which codes for the enterococcal surface protein (Esp) displayed on the bacterial exterior, resides within a 153-kb pathogenicity island, and its transcriptional activity markedly elevates cell surface hydrophobicity and the capacity to adhere to underlying substrates [40]. Published accounts indicate that the esp gene of E. faecalis plays an important role in biofilm formation, though it is not absolutely required for this process [41]. That said, there exists little dispute that Esp potentiates biofilm development [42]. Evidence further indicates that Esp substantially augments primary attachment to polystyrene and polyvinyl chloride plastics used in urine collection receptacles [43], and Esp transcription was shown to enhance in vitro adhesion to murine bladder and kidney epithelial cells [44]. Consequently, the diminished ESP transcriptional output observed under combination treatment could prove clinically advantageous by lowering the incidence of VRE-related urinary tract infections. The endocarditis- and biofilm-associated pili (Ebp), expressed from the cotranscribed ebpABC operon, are assembled from three constituent proteins—EbpA, EbpB, and EbpC—and contribute to both biofilm establishment and endocarditis pathogenesis. The terminal adhesin subunit EbpA facilitates binding to host-derived fibrinogen and collagen and is implicated in urinary tract infections, catheter-related urinary tract infections, and endocarditis [45]. Our data revealed a pronounced decline in ebpA transcript levels in the dual-

drug treatment group, particularly during the nascent phase of biofilm formation **(Figure 5).** A plausible explanation for this observation may lie in the fact that ebpA through srtC are transcribed as a polycistronic mRNA, and disruption mutants lacking functional ebpA exhibit severely diminished transcription of the downstream ebpB and ebpC genes. Additionally, phase-contrast microscopic examination of mutant strains deficient in ebpA, ebpB, ebpC, and srtC revealed a significant departure from wild-type behavior, specifically at the initial attachment step of biofilm formation, indicating that these genetic elements are operant during the early cell-to-surface interplay phase of the multi-stage biofilm developmental program [46].

Several constraints of the present study warrant acknowledgment. To begin with, the test panel comprised only four clinical isolates, all recovered from patients' urinary specimens. This limited strain set may fall short of capturing the full diversity of VREfm. To achieve broader applicability and enhanced representativeness, future work should incorporate a larger collection of isolates drawn from varied anatomical infection sites and spanning a wider range of MIC values. Secondly, the entire experimental work was conducted in vitro and therefore could not account for the influence of the host immunological response. Consequently, additional investigations employing animal models appear necessary to more fully evaluate the contribution of immune system factors. A further limitation is that, while our RT-qPCR analysis provided a quantitative assessment of six biofilm-associated gene transcripts, it did not encompass the remaining biofilm-related gene repertoire nor other potentially confounding variables.

Conclusion

Taken together, the combination of linezolid and fosfomycin exerted synergistic activity against VREfm. The combined regimen suppressed biofilm biomass across all four VREfm isolates studied and diminished the metabolic function of viable organisms within the biofilm matrix. The RT-qPCR findings revealed that the linezolid-fosfomycin combination led to significant downregulation of cylA, ebpA, and gelE, each of which plays a key role in driving biofilm genesis. In the context of mature biofilm, the dual-antibiotic treatment additionally repressed the expression of asa1, atlA, and esp. These collective observations suggest that linezolid combined with fosfomycin may constitute a feasible therapeutic strategy for managing infections caused by VREfm biofilms.

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References

1. Gao W, Howden BP, Stinear TP. Evolution of virulence in *Enterococcus faecium*, a hospital-adapted opportunistic pathogen. Curr Opin Microbiol. 2018;41:76-82.
2. Joshi S, Shallal A, Zervos M. Vancomycin-resistant enterococci: epidemiology, infection prevention, and control. Infect Dis Clin North Am. 2021;35(4):953-68.
3. Zoller M, Maier B, Hornuss C, Neugebauer C, Dobbeler G, Nagel D, et al. Variability of linezolid concentrations after standard dosing in critically ill patients: a prospective observational study. Crit Care. 2014;18(4):R148.
4. Smith TT, Tamma PD, Do TB, Dzintars K, Zhao Y, Cosgrove SE, et al. Prolonged linezolid use is associated with the development of linezolid-resistant *Enterococcus faecium*. Diagn Microbiol Infect Dis. 2018;91(2):161-3.
5. Klare I, Fleige C, Geringer U, Thürmer A, Bender J, Mutters NT, et al. Increased frequency of linezolid resistance among clinical *Enterococcus faecium* isolates from German hospital patients. J Glob Antimicrob Resist. 2015;3(2):128-31.
6. Azeredo J, Sutherland IW. The use of phages for the removal of infectious biofilms. Curr Pharm Biotechnol. 2008;9(4):261-6.

7. Blackledge MS, Worthington RJ, Melander C. Biologically inspired strategies for combating bacterial biofilms. Curr Opin Pharmacol. 2013;13(5):699-706.
8. Rogers SA, Huigens RW III, Cavanagh J, Melander C. Synergistic effects between conventional antibiotics and 2-aminoimidazole-derived antibiofilm agents. Antimicrob Agents Chemother. 2010;54(5):2112-8.
9. Goić-Barišić I, Radić M, Novak A, Rubić Z, Boban M, Tonkić M. Vancomycin-resistant *Enterococcus faecium* colonization and *Clostridioides difficile* infection in a hematologic patient. Acta Clin Croat. 2020;59(3):523-8.
10. Ye JJ, Shie SS, Cheng CW, Yang CC, Huang PY, Wu TS, et al. Clinical characteristics and treatment outcomes of vancomycin-resistant *Enterococcus faecium* bacteremia. J Microbiol Immunol Infect. 2018;51(6):705-16.
11. Dijkmans AC, Zacarías NVO, Burggraaf J, Mouton JW, Wilms EB, van Nieuwkoop C, et al. Fosfomycin: pharmacological, clinical and future perspectives. Antibiotics (Basel). 2017;6(4):24.
12. Tong J, Jiang Y, Xu H, Liu Y, Zhao X, Zhang J, et al. In vitro antimicrobial activity of fosfomycin, rifampin, vancomycin, daptomycin alone and in combination against vancomycin-resistant enterococci. Drug Des Devel Ther. 2021;15:3049-55.
13. Qi C, Xu S, Wu M, Zhu S, Yang Y, Cui J, et al. Pharmacodynamics of linezolid-plus-fosfomycin against vancomycin-susceptible and -resistant enterococci in vitro and in vivo in a *Galleria mellonella* larval infection model. Infect Drug Resist. 2019;12:3497-505.
14. Li Y, Peng Y, Zhang N, Wang J, Zhang J, Xu H, et al. Assessing the emergence of resistance in vitro and in vivo: linezolid combined with fosfomycin against fosfomycin-sensitive and -resistant *Enterococcus*. Infect Drug Resist. 2022;15:4995-5010.
15. Wang S, Liu H, Mao J, Jiang Y, Tong J, Xu H, et al. Pharmacodynamics of linezolid plus fosfomycin against vancomycin-resistant *Enterococcus faecium* in a hollow fiber infection model. Front Microbiol. 2021;12:779885.
16. Tang HJ, Chen CC, Zhang CC, Su BA, Li CM, Weng TC, et al. In vitro efficacy of fosfomycin-based combinations against clinical vancomycin-resistant *Enterococcus* isolates. Diagn Microbiol Infect Dis. 2013;77(3):254-7.
17. Lagatolla C, Mehat JW, La Ragione RM, Luzzati R, Di Bella S. In vitro and in vivo studies of oritavancin and fosfomycin synergism against vancomycin-resistant *Enterococcus faecium*. Antibiotics (Basel). 2022;11(10):1334.
18. Oliva A, Furustrand Tafin U, Maiolo EM, Jeddari S, Bétrisey B, Trampuz A. Activities of fosfomycin and rifampin on planktonic and adherent *Enterococcus faecalis* strains in an experimental foreign-body infection model. Antimicrob Agents Chemother. 2014;58(3):1284-93.
19. Zheng JX, Sun X, Lin ZW, Qi GB, Ji X, Yan YJ, et al. In vitro activities of daptomycin combined with fosfomycin or rifampin on planktonic and adherent linezolid-resistant isolates of *Enterococcus faecalis*. J Med Microbiol. 2019;68(3):493-502.
20. Zheng J, Chen Z, Lin Z, Sun X, Lin WH, Yan Y, et al. Radezolid is more effective than linezolid against planktonic cells and inhibits *Enterococcus faecalis* biofilm formation. Front Microbiol. 2020;11:196.
21. Luther MK, Arvanitis M, Mylonakis E, LaPlante KL. Activity of daptomycin or linezolid in combination with rifampin or gentamicin against biofilm-forming *Enterococcus faecalis* or *E. faecium* in an in vitro pharmacodynamic model using simulated endocardial vegetations and an in vivo survival assay using *Galleria mellonella* larvae. Antimicrob Agents Chemother. 2014;58(8):4612-20.
22. Davies D. Understanding biofilm resistance to antibacterial agents. Nat Rev Drug Discov. 2003;2(2):114-22.
23. Del Pozo JL. Biofilm-related disease. Expert Rev Anti Infect Ther. 2018;16(1):51-65.
24. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 30th informational supplement. CLSI document M100-S30. Wayne (PA): Clinical and Laboratory Standards Institute; 2020.
25. Davis H, Brown R, Ashcraft D, Pankey G. In vitro synergy with fosfomycin plus doxycycline against linezolid- and vancomycin-resistant *Enterococcus faecium*. J Glob Antimicrob Resist. 2020;22:78-83.
26. Paduszynska MA, Greber KE, Paduszynski W, Sawicki W, Kamysz W. Activity of temporin A and short lipopeptides combined with gentamicin against biofilm formed by *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Antibiotics (Basel). 2020;9(9):566.

27. Pettit RK, Weber CA, Pettit GR. Application of a high-throughput Alamar Blue biofilm susceptibility assay to *Staphylococcus aureus* biofilms. Ann Clin Microbiol Antimicrob. 2009;8(1):28.
28. Dubrac S, Boneca IG, Poupel O, Msadek T. New insights into the WalK/WalR (YycG/YycF) essential signal transduction pathway reveal a major role in controlling cell wall metabolism and biofilm formation in *Staphylococcus aureus*. J Bacteriol. 2007;189(22):8257-69.
29. Ch'ng JH, Chong KKL, Lam LN, Wong JJ, Kline KA. Biofilm-associated infection by enterococci. Nat Rev Microbiol. 2019;17(2):82-94.
30. Zheng JX, Wu Y, Lin ZW, Pu ZY, Yao WM, Chen Z, et al. Characteristics of and virulence factors associated with biofilm formation in clinical *Enterococcus faecalis* isolates in China. Front Microbiol. 2017;8:2388.
31. Holmberg A, Mörgelin M, Rasmussen M. Effectiveness of ciprofloxacin or linezolid in combination with rifampicin against *Enterococcus faecalis* in biofilms. J Antimicrob Chemother. 2012;67(2):433-9.
32. Bari AK, Belalekar TS, Poojary A, Rohra S. Combination drug strategies for biofilm eradication using synthetic and natural agents in KAPE pathogens. Front Cell Infect Microbiol. 2023;13:1155699.
33. Descourouez JL, Jorgenson MR, Wergin JE, Lanspa MJ, Ashbee HR, Pavia AT, et al. Fosfomycin synergy in vitro with amoxicillin, daptomycin, and linezolid against vancomycin-resistant *Enterococcus faecium* from renal transplant patients with infected urinary stents. Antimicrob Agents Chemother. 2013;57(3):1518-20.
34. Hemapanpairoa J, Changpradub D, Thunyaharn S, Santimaleeworagun W. Vancomycin-resistant enterococcal infection in a Thai university hospital: clinical characteristics, treatment outcomes, and synergistic effect. Infect Drug Resist. 2019;12:2049-57.
35. Mao Y, Dai D, Jin H, Wang Y. The risk factors of linezolid-induced lactic acidosis: a case report and review. Medicine (Baltimore). 2018;97(36):e12114.
36. Dundar H, Brede DA, La Ragione RM, El-Gendy AO, Diep DB, Nes IF. The fsr quorum-sensing system and cognate gelatinase orchestrate the expression and processing of proprotein EF_1097 into the mature antimicrobial peptide enterocin O16. J Bacteriol. 2015;197(13):2112-21.
37. Thomas VC, Hiromasa Y, Harms N, Thurlow L, Tomich J, Hancock LE. A fratricidal mechanism is responsible for eDNA release and contributes to biofilm development of *Enterococcus faecalis*. Mol Microbiol. 2009;72(4):1022-36.
38. Van Tyne D, Martin MJ, Gilmore MS. Structure, function, and biology of the *Enterococcus faecalis* cytolysin. Toxins (Basel). 2013;5(5):895-911.
39. Süssmuth SD, Muscholl-Silberhorn A, Wirth R, Susa M, Marre R, Rozdzinski E. Aggregation substance promotes adherence, phagocytosis, and intracellular survival of *Enterococcus faecalis* within human macrophages and suppresses respiratory burst. Infect Immun. 2000;68(9):4900-6.
40. Tendolkar PM, Baghdayan AS, Shankar N. Putative surface proteins encoded within a novel transferable locus confer a high-biofilm phenotype to *Enterococcus faecalis*. J Bacteriol. 2006;188(6):2063-72.
41. Kristich CJ, Li YH, Cvitkovitch DG, Dunny GM. Esp-independent biofilm formation by *Enterococcus faecalis*. J Bacteriol. 2004;186(1):154-63.
42. Tendolkar PM, Baghdayan AS, Gilmore MS, Shankar N. Enterococcal surface protein, Esp, enhances biofilm formation by *Enterococcus faecalis*. Infect Immun. 2004;72(10):6032-9.
43. Toledo-Arana A, Valle J, Solano C, Arrizubieta MJ, Cucarella C, Lamata M, et al. The enterococcal surface protein, Esp, is involved in *Enterococcus faecalis* biofilm formation. Appl Environ Microbiol. 2001;67(10):4538-45.
44. Leendertse M, Heikens E, Wijnands LM, van Luit-Asbroek M, Teske GJ, Roelofs JJ, et al. Enterococcal surface protein transiently aggravates *Enterococcus faecium*-induced urinary tract infection in mice. J Infect Dis. 2009;200(7):1162-5.
45. Nielsen HV, Flores-Mireles AL, Kau AL, Kline KA, Pinkner JS, Neiers F, et al. Pilin and sortase residues critical for endocarditis- and biofilm-associated pilus biogenesis in *Enterococcus faecalis*. J Bacteriol. 2013;195(19):4484-95.
46. Nallapareddy SR, Singh KV, Sillanpää J, Garsin DA, Höök M, Erlandsen SL, et al. Endocarditis and biofilm-associated pili of *Enterococcus faecalis*. J Clin Invest. 2006;116(10):2799-808.