Research Article

Synergistic Effect of Daptomycin With Ceftaroline Against High-Level Gentamicin-Resistant Enterococcus Faecalis Isolates: A Checkerboard Synergy Study with Ai-Assisted Response Surface Analysis

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Abstract

The increasing prevalence of high-level gentamicin-resistant (HLGR) Enterococcus faecalis and the consequent loss of classical aminoglycoside–β-lactam synergistic therapy create a critical need to identify effective alternative combination treatments and improved analytical approaches to interpret antimicrobial interactions. HLGR is primarily mediated by the aac(6')-Ie-aph(2")-Ia aminoglycoside-modifying enzyme, which eliminates the traditional bactericidal synergy between aminoglycosides and cell-wall-active agents, thereby restricting treatment options to last-line antibiotics such as daptomycin and linezolid. In this context, the present study evaluated the in vitro synergistic interaction between daptomycin and ceftaroline against HLGR E. faecalis using checkerboard analysis with fractional inhibitory concentration index (FICI) interpretation, while integrating an AI-assisted analytical framework to improve interpretation of the concentration–interaction landscape. Clinical isolates were identified using VITEK-2, and the HLGR phenotype was screened with a 120 µg gentamicin disc, followed by PCR confirmation of the aac(6')-Ie-aph(2")-Ia resistance gene. Minimum inhibitory concentrations (MICs) were determined by broth microdilution in calcium-supplemented Mueller–Hinton broth according to CLSI 2024 guidelines, and checkerboard assays were conducted in 96-well microtitre plates across two-fold dilution matrices. Computational analyses included RBF-interpolated three-dimensional response surface modelling, FICI heatmap visualisation, and Random Forest classification with leave-one-out cross-validation (LOOCV). Individual MICs were ≥16 mg/L for daptomycin and ≥8 mg/L for ceftaroline, while combination testing produced a minimum FICI of 0.375 at 2 mg/L daptomycin + 2 mg/L ceftaroline and FICI = 0.500 at 4 mg/L + 2 mg/L, representing ≥4-fold MIC reductions without antagonism. The AI analysis identified a distinct synergy valley at low combined concentrations, with a Random Forest LOOCV accuracy of 78.6%, supporting the potential of this combination and highlighting the value of AI-assisted analysis in antimicrobial synergy studies.

Keywords: high-level gentamicin resistance; Enterococcus faecalis; daptomycin; ceftaroline; checkerboard assay; FICI; antimicrobial synergy

1. Introduction

A critical challenge in modern infectious disease management is the limited availability of effective therapeutic strategies against high-level gentamicin-resistant (HLGR) *Enterococcus faecalis*, which compromises traditional synergistic antibiotic regimens. Enterococcal infections continue to represent a substantial and growing burden in hospital settings worldwide. *Enterococcus faecalis* and *Enterococcus faecium* are the two predominant species of clinical relevance, collectively responsible for urinary tract infections, intra-abdominal sepsis, surgical-site infections, bacteraemia, and infective endocarditis (1,2). Among these, *E. faecalis* accounts for approximately 90–95% of clinical enterococcal isolates and demonstrates a marked propensity for endovascular infections, particularly infective endocarditis (3). As one of the leading causes of infective endocarditis worldwide, E. faecalis is associated with considerable morbidity and persistently high mortality despite advances in antimicrobial therapy (4,5). For decades, the cornerstone of treatment for severe enterococcal infections has been the synergistic combination of a cell-wall-active agent and an aminoglycoside, which provides bactericidal activity against otherwise tolerant organisms (4,5). However, the emergence and dissemination of high-level gentamicin resistance (HLGR), typically defined by gentamicin MIC values ≥500 µg/mL, have severely compromised this therapeutic strategy. HLGR is predominantly mediated by the bifunctional aminoglycoside-modifying enzyme AAC(6′)-APH(2′′), encoded by the *aac(6′)-Ie-aph(2′′)-Ia* gene, which abolishes the synergistic interaction between aminoglycosides and cell-wall-active agents, thereby limiting effective treatment options for serious enterococcal infections. Consequently, clinicians are increasingly required to rely on alternative

agents such as daptomycin and linezolid, although therapeutic failures, emerging resistance, and limited bactericidal activity continue to pose significant clinical challenges. Despite the recognised clinical importance of HLGR, systematic investigations evaluating novel synergistic antibiotic combinations against HLGR *E. faecalis* remain scarce. Therefore, the identification of effective combination therapies capable of restoring bactericidal activity represents an important unmet need in contemporary antimicrobial treatment strategies.

The epidemiology of HLGR *E. faecalis* is well documented but exhibits marked geographic heterogeneity. Hospital-based surveillance studies have reported prevalence rates ranging from approximately 20% to nearly 90%, reflecting differences in antimicrobial stewardship practices, infection-control measures, and local selective pressures (6–9). Arundathi et al. reported that 42% of clinical enterococcal isolates in their cohort were HLGR, with *E. faecalis* accounting for 72% of all isolates (10). Vigani et al. (11) reported that 45.5% (66/145) of *Enterococcus faecalis* bacteraemia episodes at a tertiary teaching hospital in Brazil were caused by HLGR isolates. Multivariate analysis identified older age, haematological malignancy, and prior vancomycin exposure as independent risk factors for HLGR bacteraemia, highlighting the clinical significance of aminoglycoside resistance in invasive enterococcal infections (11). The molecular basis of HLGR in the majority of clinical isolates is the bifunctional aminoglycoside-modifying enzyme encoded by the *aac(6')-Ie-aph(2")-Ia* gene, which acetylates and phosphorylates gentamicin, tobramycin, and netilmicin, preventing effective ribosomal binding (12,13). This gene is frequently borne on the Tn5281 transposon and is readily transferable via conjugation, facilitating its horizontal dissemination within hospital environments (12,13). Padmasini et al. (2014) demonstrated that the aac(6')-Ie-aph(2'')-Ia gene was the predominant genetic determinant associated with high-level gentamicin resistance among clinical enterococcal isolates, confirming its major role in mediating the HLGR phenotype and contributing to the loss of aminoglycoside–β-lactam synergistic activity (14). Given the mobile nature of the resistance determinant and observed epidemiological trends, HLGR enterococcal infections are expected to remain a persistent clinical challenge in tertiary care settings.

Daptomycin is a cyclic lipopeptide antimicrobial that exerts bactericidal activity through calcium-dependent insertion into the cytoplasmic membrane, causing membrane depolarisation, disruption of the transmembrane potential, and inhibition of macromolecular synthesis (15–17). Emerging evidence indicates that daptomycin functions through a dual-targeting strategy (18). By forming Ca^{2+}-dependent complexes with phosphatidylglycerol and lipid II, it disrupts peptidoglycan biosynthesis, while simultaneously inducing membrane depolarisation through a mechanistically independent pathway. This multifaceted mode of action is believed to underlie its sustained clinical effectiveness against multidrug-resistant Gram-positive pathogens and the comparatively low frequency of high-level resistance (18). Although daptomycin is widely employed as a salvage agent in HLGR infections, monotherapy carries the risk of selecting for daptomycin non-susceptible variants, and bactericidal efficacy in difficult-to-treat enterococcal infections is not consistently achieved. Ceftaroline fosamil is a fifth-generation cephalosporin with high affinity for penicillin-binding protein 5 (PBP5) — the low-affinity PBP that mediates β-lactam tolerance in enterococci — as well as for PBPs 1–4, enabling broad disruption of peptidoglycan cross-linking (19–21). The structural compromise imposed by ceftaroline reduces cell-wall rigidity and alters the net anionic surface charge of the bacterial envelope, lowering the electrostatic barrier to daptomycin membrane insertion and thereby potentiating its bactericidal activity (22,23). In a comprehensive in vitro assessment of vancomycin-resistant Enterococcus faecalis and Enterococcus faecium, Smith et al. reported that ceftaroline was the most effective β-lactam partner for daptomycin, achieving the largest reductions in daptomycin MICs and the strongest synergistic killing effects. Enhanced daptomycin binding following ceftaroline exposure was identified as a key mechanism underlying this synergy (24). Sakoulas et al. showed that ceftaroline restores daptomycin activity against daptomycin-nonsusceptible *E. faecium* by increasing daptomycin membrane binding and enhancing susceptibility to the host defence peptide LL-37, highlighting the role of ceftaroline-induced cell surface remodelling in promoting both antibiotic and innate immune killing (25). Clinical support for this synergistic interaction was subsequently provided by Sakoulas et al. (26) who successfully treated recurrent left-sided infective endocarditis caused by high-level gentamicin-resistant Enterococcus faecalis using a daptomycin–ceftaroline combination. The favourable clinical outcome highlighted the potential of this regimen as a salvage therapeutic strategy for refractory enterococcal endovascular infections and encouraged further investigation of β-lactam-enhanced daptomycin therapy.

Despite accumulating in vitro and clinical evidence, FICI-based checkerboard data specifically against HLGR *E. faecalis* with genotypically confirmed resistance remain limited. Furthermore, conventional tabular FICI reporting may underrepresent the spatial extent of synergistic interactions across the concentration matrix. Computational methods such as interpolated three-dimensional response surfaces, heatmap visualisation, and supervised machine learning

classifiers offer a means to render these interactions in a more informative and reproducible format, as has been applied in oncological drug combination research. The present study was therefore designed to: (i) confirm HLGR phenotype and *aac(6')-Ie-aph(2")-Ia* genotype in clinical *E. faecalis* isolates; (ii) determine individual MICs by broth microdilution per CLSI 2024 guidelines; (iii) evaluate daptomycin–ceftaroline synergy by standardised checkerboard assay with FICI calculation; and (iv) apply an AI-assisted workflow comprising three-dimensional response surface modelling, FICI heatmap, and Random Forest classification with leave-one-out cross-validation to enhance interpretability of the pharmacodynamic interaction landscape.

2. Materials and Methods

2.1 Bacterial Isolates

Clinical *E. faecalis* isolates were recovered from blood culture, urine, wound pus, and exudate specimens from inpatients at SRM Medical College Hospital and Research Centre, Chennai, India. Primary identification was performed using the VITEK 2 compact automated identification system (bioMérieux, France), supplemented by standard biochemical tests including aesculin hydrolysis, bile aesculin agar, growth in 6.5% NaCl broth, and the pyrrolidonyl-β-naphthylamide (PYR) reaction. All isolates were cryopreserved in 20% glycerol at −70°C. *E. faecalis* ATCC 29212 served as the quality control strain throughout all antimicrobial testing.

2.2 HLGR Phenotypic Screening

HLGR screening was performed by disc diffusion using a 120 µg high-content gentamicin disc (Hi-Media, India) on Mueller–Hinton agar, incubated at 37°C for 18–24 hours. Isolates exhibiting no zone of inhibition or a zone diameter below the interpretive breakpoint were designated as HLGR, consistent with CLSI 2024 criteria (CLSI M100™ Performance Standards for Antimicrobial Susceptibility Testing, 2024; Ledina et al., 2026). Susceptibility to vancomycin, linezolid, teicoplanin, and ampicillin was also assessed concurrently.

2.3 Molecular Confirmation of Resistance Genotype

Genomic DNA was extracted from HLGR isolates using a commercial extraction kit. PCR amplification targeting the *aac(6')-Ie-aph(2")-Ia* gene was performed using previously published primer sequences, yielding an expected amplicon of 369 bp (29). Amplification products were resolved on 2% agarose gels stained with ethidium bromide. A confirmed HLGR clinical isolate served as the positive control; *E. faecalis* ATCC 29212 served as the negative control.

2.4 Antimicrobial Agents and MIC Determination

Daptomycin (DAP) and ceftaroline fosamil (CPT) reference powders were obtained from accredited commercial suppliers. Working dilutions were prepared freshly in cation-adjusted Mueller–Hinton broth (CAMHB) supplemented with calcium chloride to a final concentration of 50 mg/L, as required for accurate daptomycin MIC determination (30,31). MICs were determined by broth microdilution in 96-well microtitre plates across a concentration range of 0.125–128 µg/mL according to CLSI M07 guidelines (30). All assays were performed in duplicate on at least two independent occasions. The MIC was recorded as the lowest concentration at which no visible turbidity was observed after 18–24 hours of incubation at 37°C.

2.5 Checkerboard Synergy Assay

Synergistic activity between daptomycin and ceftaroline was evaluated using the two-dimensional broth microdilution checkerboard method. Daptomycin was prepared across a concentration range of 0.125–16 mg/L along one axis of a 96-well microtitre plate, while ceftaroline was prepared across 0.0156–8 mg/L along the perpendicular axis, both using two-fold serial dilution increments. Each well was inoculated with a standardized bacterial suspension to achieve a final inoculum of approximately 5×10^5 CFU/mL in cation-adjusted Mueller–Hinton broth (CAMHB). The plates were incubated at 37 °C for 24 h, after which bacterial growth inhibition was assessed visually. Synergy between the two antibiotics was quantified using the fractional inhibitory concentration index (FICI) calculated as:

$$FICI = \frac{MIC_{DAP\ combo}}{MIC_{DAP\ alone}} + \frac{MIC_{CPT\ combo}}{MIC_{CPT\ alone}}$$

The interaction was interpreted according to standard criteria as synergy (FICI ≤ 0.5), additive or partial synergy (0.5 < FICI ≤ 1.0), indifference (1.0 < FICI < 4.0), or antagonism (FICI ≥ 4.0) (8,15). The lowest FICI value obtained among all

tested concentration combinations was considered for the overall interaction classification. All experiments were performed in duplicate, and *Enterococcus faecalis* ATCC 29212 was included as the quality control strain to ensure methodological accuracy

2.6 AI-Assisted Computational Analysis

A structured AI-assisted analytical workflow was applied to the 14 experimentally derived concentration–FICI data pairs to complement conventional microbiological interpretation and enhance visualisation of the drug interaction landscape. The workflow consisted of three analytical components.

A continuous response surface was fitted to the discrete FICI data using Radial Basis Function (RBF) interpolation with a thin-plate spline kernel applied to $\log_2$-transformed concentration axes (Python implementation: *scipy.interpolate.RBFInterpolator*, smoothing parameter = 0.03). This approach generated a smooth three-dimensional interaction surface and enabled identification of the synergy valley, representing the concentration region associated with the lowest FICI values, with greater spatial resolution than conventional point-wise FICI tables.

Experimental FICI values were projected onto a two-dimensional colour-coded matrix, with daptomycin and ceftaroline concentrations represented along the respective axes. A diverging colour scale (deep blue indicating synergy and amber–red indicating indifference) was applied to facilitate visual identification of interaction gradients and transition zones across the tested concentration matrix.

A Random Forest classifier was trained on $\log_2$-transformed concentration pairs (n = 14) to categorise each observation into one of three interaction classes: synergy, additive, or indifferent. The model was configured with 300 decision trees, a maximum tree depth of 3, and a fixed random state to ensure reproducibility. Model performance was evaluated using leave-one-out cross-validation (LOOCV). Feature importance scores were extracted to quantify the relative contribution of each antibiotic concentration to the classification outcome. Owing to the limited dataset size, the classifier is presented as an exploratory pattern-recognition tool rather than a predictive model.

3. Results

3.1 Isolate Identification and HLGR Confirmation

All study isolates were confirmed as *E. faecalis* by VITEK 2 and biochemical tests. HLGR phenotype was confirmed in all isolates by absence of inhibition around the 120 µg gentamicin disc. PCR amplification of the *aac(6')-Ie-aph(2")-Ia* gene produced the expected 369 bp amplicon in all HLGR isolates, confirming the genotypic basis of resistance. No amplification was observed in the negative control, consistent with published data confirming *aac(6')-Ie-aph(2")-Ia* as the predominant molecular determinant of HLGR in clinical enterococcal isolates (32,33).

3.2 Individual MIC Profiles

Tested individually, all HLGR *E. faecalis* isolates exhibited MIC ≥ 16 mg/L for daptomycin and MIC ≥ 8 mg/L for ceftaroline, indicating resistance to each agent as monotherapy. Gentamicin MICs were confirmed at ≥ 500 µg/mL, consistent with the HLGR phenotype. Quality control results with *E. faecalis* ATCC 29212 were within the acceptable ranges specified by CLSI 2024 (14).

3.3 Checkerboard and FICI Results

The checkerboard assay demonstrated a clear shift in the growth inhibition boundary toward lower antibiotic concentrations when daptomycin and ceftaroline were used in combination, indicating pharmacodynamic cooperation between the two agents. Figure 1 illustrates the visual growth inhibition matrix of the checkerboard assay, in which green-shaded wells correspond to complete bacterial inhibition and orange-shaded wells indicate visible bacterial growth. Rows represent daptomycin concentrations ranging from 0.125 to 16 mg/L, while columns represent ceftaroline concentrations ranging from 0.0156 to 8 mg/L. The inhibition boundary shifts toward lower concentrations within the combination region of the matrix, demonstrating a synergistic interaction pattern between the two antibiotics.

Representative microtitre plate images further supported these observations. Figure 2 presents the 96-well microtitre plate photograph obtained after 24 h incubation at 37 °C together with the threshold-segmented image used for objective analysis. The plate photograph shows a cluster of clear wells located in the upper-left region of the matrix,

corresponding to growth-inhibited wells at lower combined antibiotic concentrations. To reduce subjectivity in visual interpretation, the plate image was additionally processed using threshold-based grayscale segmentation. In the segmented image, black regions represent turbid (growth-positive) wells, whereas white regions represent optically clear (growth-inhibited) wells, confirming the visual classification of bacterial growth endpoints. This segmentation analysis provided objective validation of the inhibition pattern across the tested concentration ranges of daptomycin (16–0.125 mg/L) and ceftaroline (8–0.0156 mg/L).

Selected fractional inhibitory concentration index (FICI) values across the tested concentration matrix are presented in Table 1. The minimum FICI recorded was 0.375 at daptomycin 2 mg/L combined with ceftaroline 2 mg/L, indicating strong synergy. The synergy threshold (FICI = 0.500) was also observed at daptomycin 4 mg/L plus ceftaroline 2 mg/L. Both synergistic combinations corresponded to ≥4-fold reductions in the MIC of each antibiotic compared with its standalone value. Several additional concentration pairs produced additive or partial synergy effects (0.5 < FICI ≤ 1.0). Importantly, no antagonistic interaction (FICI ≥ 4.0) was detected across any of the tested antibiotic combinations.

Table 1. FICI Values for Daptomycin–Ceftaroline Combinations Against HLGR *Enterococcus faecalis* Isolates

DAP (mg/L)	CPT (mg/L)	FICI	Interaction
16	8	2.000	Indifferent
16	4	1.500	Indifferent
16	2	1.250	Indifferent
8	8	1.500	Indifferent
8	4	1.000	Additive/Partial
8	2	0.750	Additive/Partial
4	8	1.250	Indifferent
4	4	0.750	Additive/Partial
4	2	0.500	Synergy (threshold)
2	8	1.125	Indifferent
2	4	0.625	Additive/Partial
2	2	0.375	Synergy (best)
1	4	0.563	Additive/Near-synergy
0.5	4	0.531	Additive/Near-synergy

DAP = daptomycin; CPT = ceftaroline; FICI = fractional inhibitory concentration index. Synergy: FICI ≤ 0.5; Additive/partial: 0.5 < FICI ≤ 1.0; Indifferent: 1.0 < FICI < 4.0.

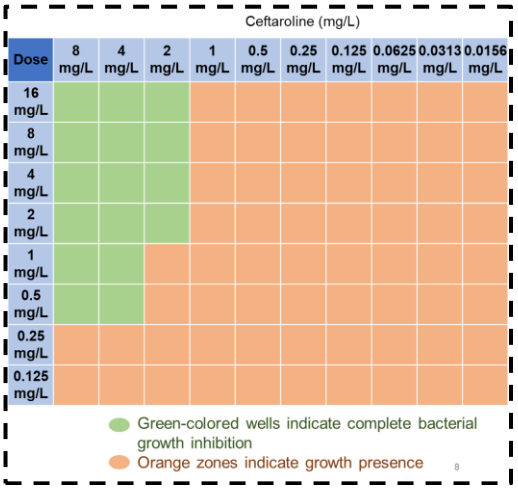

Figure 1. Visual Representation of the Checkerboard Assay — Growth Inhibition Matrix for Daptomycin and Ceftaroline Against HLGR Enterococcus faecalis

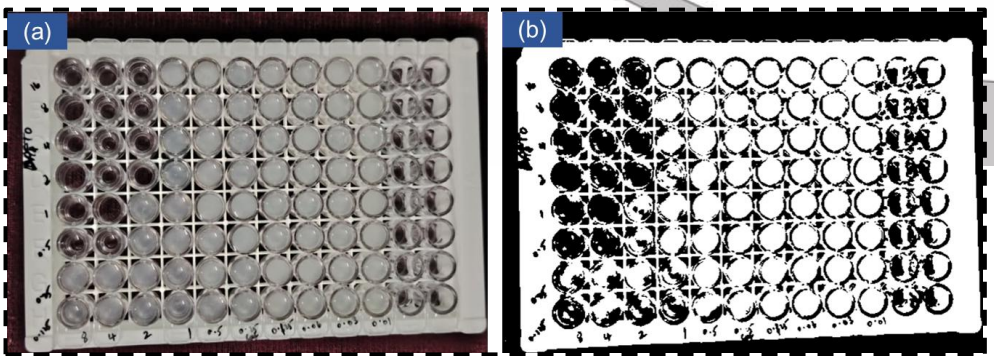

Figure 2. Microtitre Plate Photographs and Threshold-Based Image Segmentation of the Checkerboard Assay

3.4 AI-Assisted Response Surface and Classification Analysis

The AI-assisted analysis provided a quantitative visualisation of the concentration–interaction landscape derived from the checkerboard assay. Figure 3 shows the three-dimensional response surface generated by Radial Basis Function (RBF) interpolation of the 14 experimentally derived FICI values. The surface reveals a well-defined synergy valley located at daptomycin 2–4 mg/L in combination with ceftaroline 2 mg/L, where interpolated FICI values fall below the 0.5 synergy threshold. In contrast, the remainder of the surface plateaus at FICI values between 1.0 and 2.0 at higher individual antibiotic concentrations. The semi-transparent plane at FICI = 0.5 visually delineates the synergy region from the surrounding additive and indifferent interaction zones.

A complementary planar representation of the interaction landscape is provided in Figure 4, which displays the two-dimensional FICI heatmap of the checkerboard matrix. The colour gradient from deep blue (synergy) through teal (additive interaction) to amber–red (indifferent interaction) illustrates the progressive reduction in cooperative antibacterial activity as individual drug concentrations increase toward their respective monotherapy MICs. Synergistic wells are concentrated in the lower-left quadrant of the matrix, corresponding to low combined antibiotic concentrations, consistent with the synergy valley observed in the three-dimensional response surface.

To further explore patterns within the dataset, a Random Forest classifier was applied to the $\log_2$-transformed concentration pairs. The model achieved an overall leave-one-out cross-validation (LOOCV) accuracy of 78.6%, correctly classifying 11 of 14 observations. Classification of indifferent interactions was perfect (F1 = 1.00), while additive interactions were satisfactorily classified (F1 = 0.77). Synergistic interactions were not correctly classified under LOOCV, which is expected given the small minority class size (n = 2). Feature importance analysis demonstrated near-equal contributions from daptomycin (0.49) and ceftaroline (0.51) concentrations, reflecting the symmetric contribution of both antibiotics to the interaction outcome. The Random Forest decision boundary and LOOCV confusion matrix are presented in Figure 5, illustrating the classification regions within the $\log_2$-transformed concentration space.

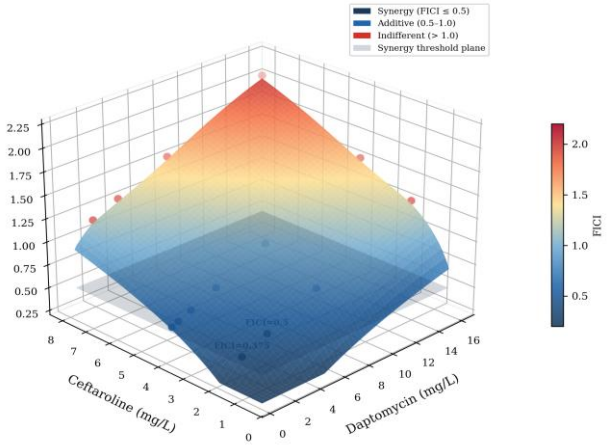

Figure 3. Three-Dimensional Response Surface of FICI Values Across Daptomycin–Ceftaroline Concentration Combinations Against HLGR Enterococcus faecalis

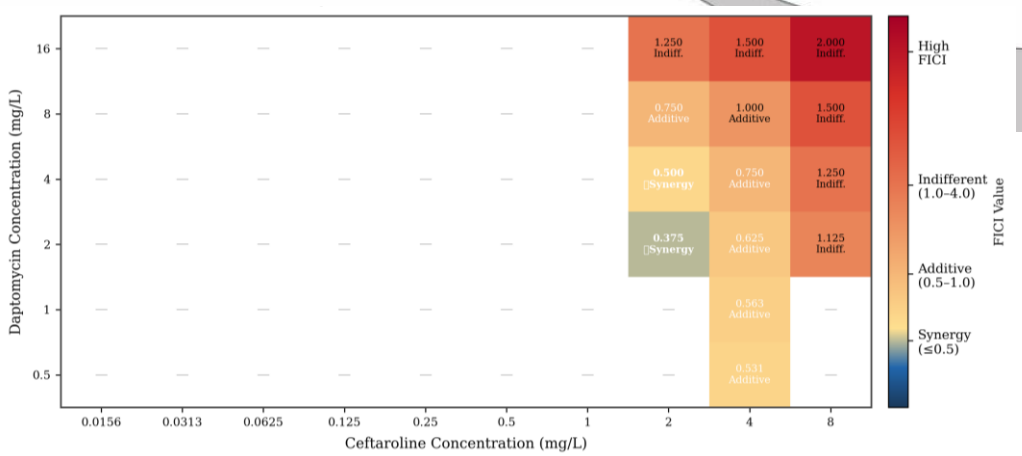

Figure 4. FICI Heatmap of the Daptomycin–Ceftaroline Checkerboard Matrix Against HLGR Enterococcus faecalis

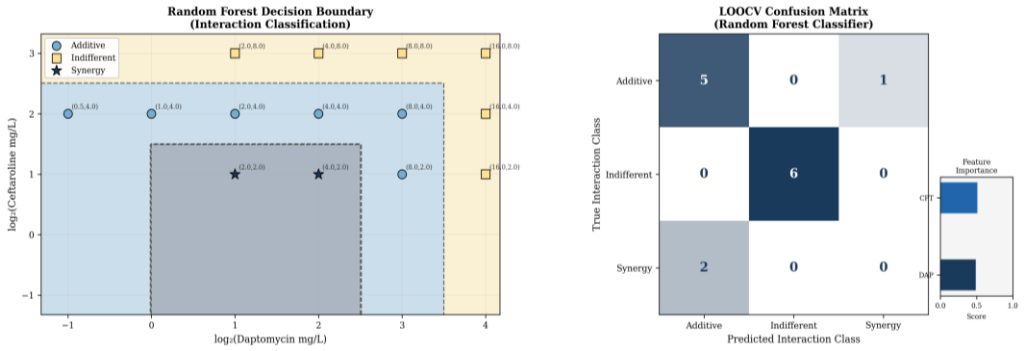

Figure 5. Random Forest Decision Boundary and LOOCV Confusion Matrix for Classification of Daptomycin–Ceftaroline Interaction Outcomes

4. Discussion

The results of this study demonstrate robust in vitro synergy between daptomycin and ceftaroline against HLGR *E. faecalis* clinical isolates, as evidenced by FICI values as low as 0.375 and ≥ 4-fold reductions in the individual MIC of each agent at the most favourable combination concentrations. The complete absence of antagonism across all tested concentration pairs further supports the pharmacological compatibility of this pairing. These observations are mechanistically consistent with the emerging understanding of how β-lactam-mediated cell-wall disruption potentiates the membrane-active bactericidal activity of daptomycin in resistant Gram-positive organisms.

The bactericidal mechanism of daptomycin involves calcium-dependent oligomerisation and insertion into the cytoplasmic membrane, causing membrane depolarisation and inhibition of macromolecular synthesis (34,35). Buttress et al. (18) recently confirmed that daptomycin additionally forms tripartite complexes with phosphatidylglycerol and lipid II cell-wall precursors within fluid membrane microdomains, disrupting peptidoglycan synthesis as a second, independent mode of killing. This dual mechanism is relevant to the combination strategy evaluated in this study, as ceftaroline acts upstream of both targets by compromising cell-wall structural integrity. Through high-affinity binding to PBP5 and PBPs 1–4, ceftaroline disrupts peptidoglycan cross-linking and alters the net surface charge of the bacterial envelope, lowering the electrostatic barrier to daptomycin membrane insertion (17,25). Smith et al. confirmed that ceftaroline reduced daptomycin MIC values more consistently than any other β-lactam tested across a collection of vancomycin-resistant *E. faecalis* and *E. faecium* strains, and that ceftaroline uniquely enhanced daptomycin membrane binding against daptomycin non-susceptible strains (8). Sakoulas et al. (24) provided fluorescence microscopy evidence that ceftaroline pre-treatment markedly increased daptomycin membrane binding in VRE strains and sensitised organisms to killing by cathelicidin LL-37, indicating that ceftaroline-induced surface remodelling engages both pharmacological and innate immune bactericidal pathways. Taken together, these data establish a mechanistically credible and internally consistent basis for the synergy observed in this study.

The clinical relevance of these in vitro findings is underscored by the therapeutic context of HLGR *E. faecalis* infections. The *aac(6')-Ie-aph(2")-Ia* gene was detected in all HLGR isolates in this study, consistent with prevalence data from multiple published cohorts (23,25). Once expressed, this enzyme irreversibly abolishes aminoglycoside–cell-wall-active-agent synergy, narrowing therapeutic options substantially. Sakoulas et al. reported the successful clinical use of the daptomycin–ceftaroline combination in recurrent HLGR *E. faecalis* left-sided endocarditis following failure of dual β-lactam therapy, achieving microbiological cure (26). The FICI values obtained in the present study are consistent with the in vitro synergy rationale underlying that clinical success and extend the observation to a panel of diverse clinical isolates from multiple infection sources. The ≥ 4-fold MIC reductions achieved at the synergistic combinations carry practical therapeutic implications: standard daptomycin dosing of 8–10 mg/kg once daily combined with ceftaroline 600 mg every 8 hours intravenously would be expected to produce concentrations exceeding the in-combination MIC at sites of infection. Furthermore, achieving equivalent antibacterial activity at lower effective individual drug concentrations may reduce the risk of daptomycin-associated myopathy and creatine phosphokinase elevation, which can limit sustained high-dose therapy.

The AI-assisted analytical framework applied in this study provided an additional interpretive dimension that complements standard FICI reporting. The three-dimensional response surface identified a spatially coherent synergy valley at low combined concentrations, confirming that the observed FICI values of ≤ 0.5 are not isolated data points but reflect a contiguous region of pharmacodynamic cooperation across the tested concentration matrix. This spatial perspective is not accessible from tabular FICI data alone and has the potential to guide concentration selection in future pharmacokinetic–pharmacodynamic modelling work. The Random Forest classifier demonstrated robust performance for the indifferent and additive interaction classes and confirmed near-equal pharmacodynamic contributions from both agents, as reflected by symmetric feature importance scores (0.49 for daptomycin; 0.51 for ceftaroline). This symmetry is mechanistically consistent with the dual-disruption model in which cell-wall compromise by ceftaroline and membrane depolarisation by daptomycin contribute cooperatively and comparably to the observed bactericidal synergy. The non-classification of the synergy class under LOOCV is attributable to the small minority class size (n = 2) and is transparently acknowledged as a limitation of this exploratory analysis. Expansion of the dataset through broader concentration ranges and additional isolates would be required to yield a generalisable predictive model.

Several limitations of this study warrant acknowledgement. The checkerboard assay assesses static pharmacodynamic interactions and does not capture time-dependent bactericidal kinetics; time-kill experiments are needed to confirm bactericidal rates and the durability of synergy over 24–48 hours. The planktonic in vitro model may not reflect bacterial behaviour within biofilm matrices or endocardial vegetations, which are clinically relevant contexts for HLGR *E. faecalis* infection. Pharmacokinetic–pharmacodynamic modelling in dynamic in vitro systems such as hollow-fibre infection models, and in vivo validation in animal models of endocarditis, are required before these data can directly inform clinical dosing recommendations.

5. Conclusion

This study provides systematic in vitro evidence of robust synergy between daptomycin and ceftaroline against clinical HLGR *E. faecalis* isolates with genotypically confirmed *aac(6')-Ie-aph(2")-Ia*-mediated resistance, achieving a minimum FICI of 0.375 and ≥ 4-fold MIC reductions at key combination concentrations, with no antagonism at any tested concentration pair. The integrated AI-assisted analytical framework — comprising three-dimensional response surface modelling, FICI heatmap, and Random Forest classification with leave-one-out cross-validation — provided an enhanced and reproducible visualisation of the pharmacodynamic interaction landscape, identifying a spatially coherent synergy valley at low combined concentrations. Taken together with existing mechanistic and clinical evidence, these findings support the daptomycin–ceftaroline combination as a scientifically sound rescue strategy for refractory HLGR enterococcal infections and provide a foundation for further investigation through time-kill kinetics, biofilm studies, pharmacokinetic–pharmacodynamic modelling, and prospective clinical evaluation.

Declarations

Ethics Approval

This study involved clinical microbiological data generated during routine diagnostic workup at SRM Medical College Hospital and Research Centre. Ethics approval was obtained from the Institutional Ethics Committee. All data were anonymised prior to analysis.

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No funding was received.
Conflict of Interest
Conflict of Interest Statement: No declarations of interest were made.
Author Contributions
PT: conceptualisation, methodology, investigation, data curation, computational analysis, writing — original draft. MJ: supervision, project administration, writing — review and editing. STJ: methodology, validation. LKV: supervision, resources, writing — review.

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