# Lack of synergy between amoxicillin and cefazolin against *Enterococcus faecalis*: Reassessment of a proposed emperical regimen for infective endocarditis

N. Enslinger[1], S. Glöckner[2,3], S. Hagel[1], O. Makarewicz[1], M. Pletz[1,3], S. Madzgalla[1]

[1] Institute for Infectious Diseases and Infection Control, [2] Institute for Medical Microbiology, [3] IKARUS Studygroup
Jena University Hospital, Friedrich-Schiller-University Jena, Germany

## Background

A recent SPILF-AEPEI (*Société de Pathologie Infectieuse de Langue Française* and *Association pour l'Étude et la Prévention de l'Endocardite Infectieuse*) position statement recommended amoxicillin (AMX) plus cefazolin (CFZ) as empirical treatment for native valve and late-onset prosthetic valve infective endocarditis (IE), diverging from ESC guidelines (1). This recommendation was based on a single study by Peiffer-Smadja *et al*. reporting in-vitro synergy of AMX–CFZ against *Enterococcus faecalis* (2).

## Methods

Twenty clinical *E. faecalis* bloodstream isolates, including endocarditis strains, were tested in duplicate using checkerboard assays. Fractional Inhibitory Concentration Indices (FICI) for AMX/CFZ were calculated using the first non-turbid well. Synergy was defined as FICI <0.5, additive or indifferent effects as 0.5–4.0, and antagonism as >4.0. To evaluate potential method-related discrepancies, time–kill curve assays were performed for five isolates at ¼ MIC AMX and different CFZ concentrations. Synergy was defined as a ≥2-$\log_{10}$ reduction in CFU/mL at 24 hours compared with the most active single agent.

$$\mathrm{FICI} = \frac{\mathrm{MIC}_{\mathrm{A(combi)}}}{\mathrm{MIC}_{\mathrm{A(alone)}}} + \frac{\mathrm{MIC}_{\mathrm{C(combi)}}}{\mathrm{MIC}_{\mathrm{C(alone)}}}$$

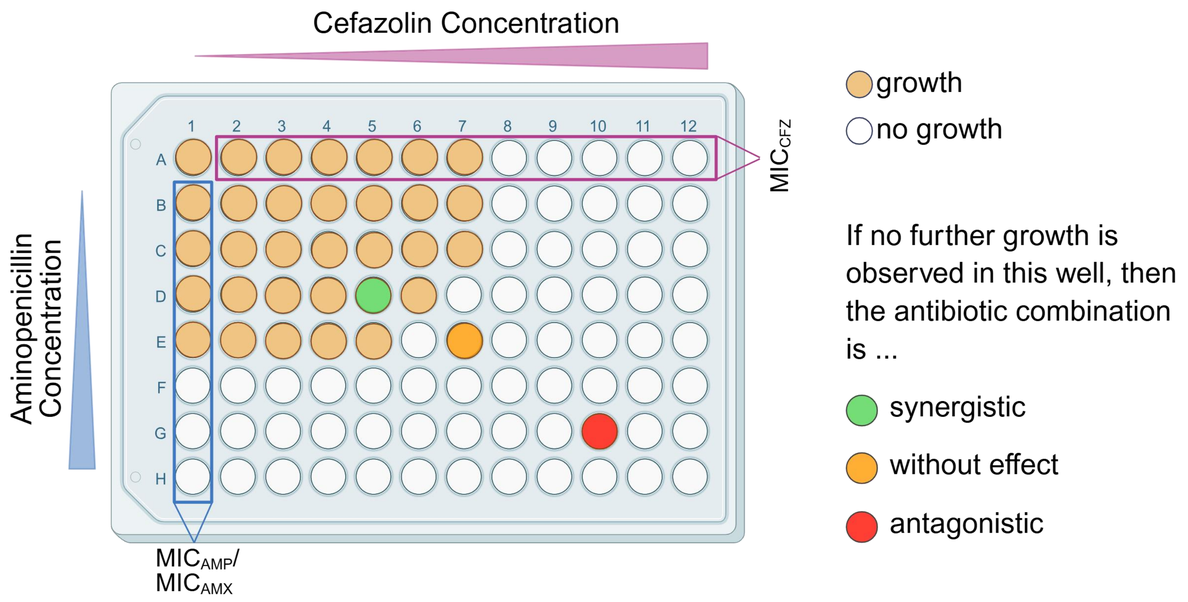


**Fig. 1**: **Schematic representation of a checkerboard assay well plate**. Column 1 serves as the MIC for the aminopenicillins, while row A serves as the MIC for cefazolin. Well 1A remains free of antibiotics as a growth control. Dpending on whch areas of the 96-well plate show growth, different effects can be observed: synergy (green), no effect (orange) or antagonism (red).

## Results

Checkerboard assays showed no evidence of synergy for AMX/CFZ  in any isolate.
Time–kill curve assays also showed no synergistic interactions, with only one isolate exhibiting a ≥2-$\log_{10}$ CFU/mL reduction. When the fixed CFZ concentration (37 mg/L) as described in the single study reporting a synergy was used, bactericidal activity was observed; however, this CFZ concentration exceeded the MIC for most isolates, indicating that the effect was attributable to cefazolin alone, rather than a true synergistic interaction with AMX. A CFZ-alone control curve confirmed this finding.

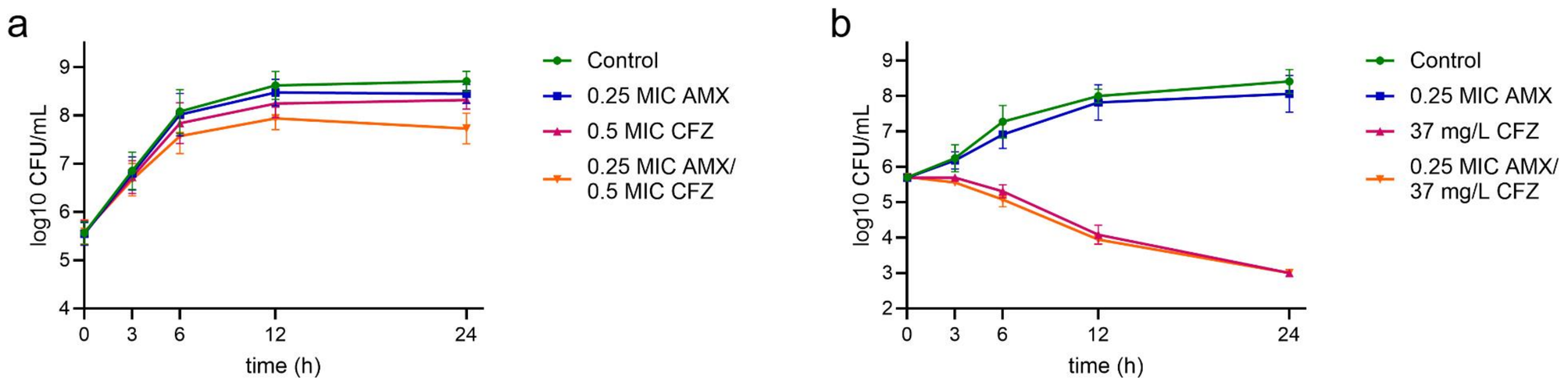


**Fig. 2**: **Mean Time Kill Curves of amoxicillin (AMX) and cefazolin (CFZ). Error bars represent SEMs of duplicate experiments**. **a** for five *E. faecalis* isolates, including three endocarditis isolates and two isolates from bloodstream infections. AMX was tested at 0.25xMIC and CFZ at 0.5xMIC. **b** for three *E. faecalis* isolates, including two endocarditis isolates and the ATCC 51299 quality control strain. AMX was tested at 0.25xMIC and CFZ at a fixed concentration of 37 mg/L.

## Conclusion

In contrast to previous reports, we found no in-vitro synergy between AMX combined with CFZ against *E. faecalis.* The effect that was observed in a single study likely reflected supratherapeutic CFZ concentrations rather than synergy. Given that such concentrations are not clinically achievable without toxicity, our data do not support using AMX–CFZ for targeted treatment of *E. faecalis* IE, though CFZ-based empirical regimens remain reasonable to optimize *S. aureus* coverage.

References:
1 Le Moing V. *et al*. Antibiotic therapy and prophylaxis of infective endocarditis–A SPILF-AEPEI position statement on the ESC 2023 guidelines. Infectious Diseases Now. 2025;55(1):105011.
2 Peiffer-Smadja N. *et al*. In vitro bactericidal activity of amoxicillin combined with different cephalosporins against endocarditis-associated Enterococcus faecalis clinical isolates. Journal of Antimicrobial Chemotherapy. 2019;74(12):3511-4.

Freistaat Thüringen
Ministerium für Wirtschaft, Wissenschaft und Digitale Gesellschaft
Kofinanziert von der Europäischen Union
Thüringer Aufbaubank Die Förderbank.