

In vitro Comparative Study of Different Anti-Bacterial Drugs Against Enterococcus Faecalis

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ABSTRACT

Background: To compare the in vitro efficacy of three antibacterial drugs, Ampicillin, Linezolid, and Daptomycin, against Enterococcus faecalis, in order to determine their relative potency and potential as treatment options for Enterococcus faecalis infections.

Materials and Methods: Bacterial Strains: Enterococcus faecalis strains were obtained from clinical isolates. Antibiotics: Ampicillin, Linezolid, and Daptomycin were procured in pharmaceutical grade powders. Preparation of Antibiotic Solutions: Prepare solutions of each antibiotic at clinically relevant concentrations. Minimum Inhibitory Concentration (MIC) Assay: MICs of each antibiotic against Enterococcus faecalis strains were determined using broth microdilution method following CLSI guidelines. Comparison of MICs: Compare the MIC values of each antibiotic to determine their relative potency against Enterococcus faecalis. Time-Kill Kinetics: Time-kill assays were conducted to evaluate the bactericidal activity of each antibiotic.

Results: MIC Results: Ampicillin exhibited MICs ranging from 0.280 – 2 µg/ml, Linezolid from 0.205-1.50 µg/ml, and Daptomycin from 0.125-1 µg/ml against Enterococcus faecalis strains. Time-Kill Kinetics: All three antibiotics demonstrated concentration-dependent bactericidal activity against Enterococcus faecalis. However, Daptomycin showed the most rapid and potent bactericidal effect, followed by Linezolid and then Ampicillin. Discussion: Daptomycin's superior bactericidal activity against Enterococcus faecalis suggests its potential as a preferred treatment option for infections caused by this pathogen, especially in cases where rapid bacterial clearance is crucial. However, Linezolid and Ampicillin remain viable options, particularly in cases where Daptomycin may not be suitable due to factors such as resistance or contraindications.

Conclusion: In vitro comparative analysis revealed that Daptomycin exhibited the most potent bactericidal activity against Enterococcus faecalis, followed by Linezolid and Ampicillin. These findings suggest that Daptomycin may be a preferred choice for the treatment of Enterococcus faecalis infections, although clinical studies are warranted to validate these results and determine their clinical relevance.

Keywords: Enterococcus Faecalis, daptomycin, Linezolid, Ampicillin.

INTRODUCTION

Enterococcus faecalis is a bacterium commonly associated with dental infections, particularly in cases of root canal-treated teeth or failed endodontic treatments.^[1] Dental infections

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involving *Enterococcus faecalis* can present significant challenges in clinical management due to the bacterium's ability to survive and persist in the root canal system despite standard endodontic procedures.^[2]

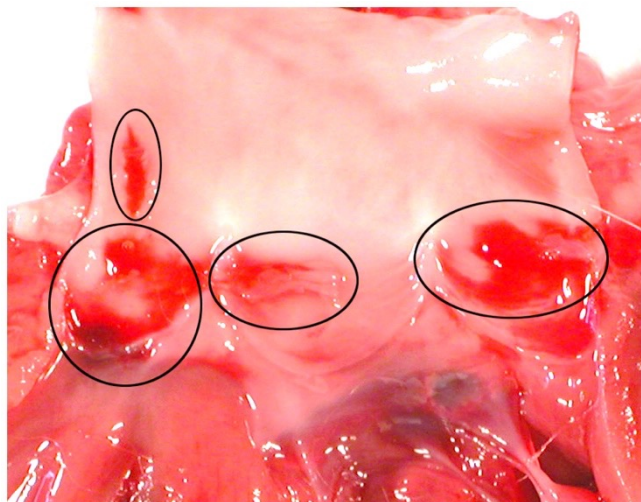


Figure 1: *Enterococcus faecalis* associated with dental infections^[3]

Enterococcus faecalis is a gram-positive, facultative anaerobic bacterium that inhabits the gastrointestinal tracts of humans and animals. While it is considered a commensal organism under normal circumstances, *Enterococcus faecalis* can also act as an opportunistic pathogen, causing a range of infections, particularly in individuals with compromised immune systems or in healthcare settings. Enterococcal infections can manifest as urinary tract infections, bacteremia, endocarditis, and intra-abdominal infections, among others, posing significant challenges in clinical management due to its intrinsic resistance to many antibiotics and its ability to acquire resistance mechanisms rapidly.^[4]

In recent years, the increasing prevalence of multidrug-resistant *Enterococcus faecalis* strains has further complicated treatment options, necessitating the exploration of alternative antibiotics and treatment strategies. In this context, the comparative evaluation of different antibacterial drugs in vitro against *Enterococcus faecalis* is of paramount importance to identify the most effective agents for combating infections caused by this pathogen.^[5]

Root Canal Infections

Enterococcus faecalis is frequently isolated from root canal infections, especially in cases of persistent or refractory periapical lesions following root canal therapy.^[5]

The bacterium can colonize the root canal system, forming biofilms and resisting eradication by antimicrobial agents commonly used in endodontic treatment.^[6]

Factors contributing to the persistence of *Enterococcus faecalis* in root canals include its ability to survive in harsh environments, adapt to low pH conditions, and tolerate high concentrations of calcium hydroxide, a common intracanal medicament.^[7]

Clinical Manifestations

Enterococcus faecalis-associated dental infections may present with symptoms such as persistent or recurrent pain, swelling, tenderness to percussion, and the presence of sinus tracts or fistulas.

In some cases, patients may be asymptomatic, with the infection discovered incidentally during routine dental examinations or radiographic assessments.^[8]

Treatment Challenges

Eradicating *Enterococcus faecalis* from infected root canals poses significant challenges due to its resistance to conventional endodontic disinfectants and antimicrobial agents.

The bacterium's ability to form biofilms within the root canal system enhances its resistance to antimicrobial therapy and facilitates its survival despite mechanical instrumentation and irrigation.

Treatment failure or persistence of infection may necessitate retreatment of the root canal, apical surgery, or even tooth extraction in severe cases.^[9]

Significance of the Study

The emergence of antibiotic resistance among *Enterococcus faecalis* isolates presents a critical healthcare concern worldwide. Enterococcal infections are associated with high morbidity and mortality rates, particularly in vulnerable patient populations, such as those with underlying medical conditions or undergoing invasive procedures. Moreover, the ability of *Enterococcus faecalis* to

form biofilms further complicates treatment outcomes, as biofilm-associated infections are notoriously difficult to eradicate using conventional antibiotics.

Given the limited therapeutic options available for the management of *Enterococcus faecalis* infections, there is an urgent need to identify novel antimicrobial agents or optimize existing ones to improve clinical outcomes and reduce the burden of antimicrobial resistance. Through in vitro comparative studies, the efficacy and potency of different antibacterial drugs against *Enterococcus faecalis* can be systematically evaluated, providing valuable insights into their antimicrobial activity, spectrum of activity, pharmacokinetic properties, and potential synergistic effects.

Objectives of the Study

The primary objective of this study is to compare the in vitro efficacy of three commonly used antibacterial drugs, namely Ampicillin, Linezolid, and Daptomycin, against clinical isolates of *Enterococcus faecalis*. Specifically, the study aims to:

1. Determine the minimum inhibitory concentrations (MICs) of Ampicillin, Linezolid, and Daptomycin against *Enterococcus faecalis* strains using standardized broth microdilution methods.
2. Evaluate the time-kill kinetics of Ampicillin, Linezolid, and Daptomycin against *Enterococcus faecalis* to assess their bactericidal activity and killing kinetics over time.
3. Compare the relative potency and efficacy of Ampicillin, Linezolid, and Daptomycin in eradicating *Enterococcus faecalis* in vitro, providing insights into their suitability as treatment options for enterococcal infections.

Rationale for Anti-Bacterial Drugs Selection

The selection of Ampicillin, Linezolid, and Daptomycin for this comparative study is based on several factors, including their widespread use in clinical practice, differing mechanisms of action, and documented activity against *Enterococcus faecalis*.^[10]

Ampicillin, a beta-lactam antibiotic, exerts its bactericidal effect by inhibiting bacterial cell wall synthesis. While *Enterococcus faecalis* is inherently resistant to many beta-lactam antibiotics due to the production of penicillin-binding proteins with low affinity for beta-lactam agents, Ampicillin remains one of the primary treatment options for susceptible enterococcal infections, particularly in combination with an aminoglycoside.^[11]

Linezolid, a synthetic oxazolidinone antibiotic, inhibits bacterial protein synthesis by binding to the 23S ribosomal RNA of the bacterial ribosome, thereby preventing the formation of the initiation complex required for translation. Linezolid has demonstrated potent activity against a wide range of gram-positive pathogens, including *Enterococcus faecalis*, making it a valuable therapeutic option for enterococcal infections, especially those caused by multidrug-resistant strains.^[12]

Daptomycin, a cyclic lipopeptide antibiotic, disrupts bacterial cell membrane integrity by forming transmembrane channels, leading to depolarization and ultimately cell death. Daptomycin exhibits rapid bactericidal activity against various gram-positive bacteria, including *Enterococcus faecalis*, and is often reserved for the treatment of serious infections, such as bloodstream infections and endocarditis, particularly when other treatment options have failed or are contraindicated.^[13]

By comparing the antimicrobial activity of these three antibiotics against *Enterococcus faecalis* in vitro, this study aims to provide valuable insights into their relative efficacy, potency, and potential clinical utility in the management of enterococcal infections. Additionally, the findings of this study may inform antibiotic selection strategies, treatment guidelines, and antimicrobial stewardship practices, ultimately contributing to improved patient outcomes and the mitigation of antimicrobial resistance in healthcare settings.^[14]

The comparative evaluation of different antibacterial drugs against *Enterococcus faecalis* is crucial for informing evidence-based treatment decisions and optimizing therapeutic outcomes in patients with enterococcal infections. Through rigorous in vitro studies such as the one proposed herein, researchers and clinicians can gain valuable insights into the antimicrobial activity, efficacy, and

potential clinical utility of various antibiotics, thereby enhancing our armamentarium against this challenging pathogen.^[15]

MATERIAL AND METHODS

Collection of Bacterial Strains

Obtain clinical isolates of *Enterococcus faecalis* from reputable culture collections or clinical laboratories. Strains should be characterized for species identification and antimicrobial susceptibility profiling using standardized methods such as disk diffusion or broth microdilution.

Collection of Antibiotics

Procure pharmaceutical-grade powders of Ampicillin, Linezolid, and Daptomycin from reliable sources. Ensure that antibiotics are stored according to manufacturer recommendations and reconstituted in appropriate solvents to achieve desired concentrations.

Preparation of Culture Media

Prepare Mueller-Hinton broth according to established protocols. Ensure sterility of media by autoclaving before use. Below are the preparation methods for Mueller-Hinton broth:

Preparation of Mueller-Hinton Broth:

Ingredients Requirements:

- Beef extract: 2 g
- Acid hydrolysate of casein: 17.5 g
- Starch: 1.5 g
- Agar: (for Mueller-Hinton agar only, not for broth)
- Distilled water: 1 liter
- pH adjusted to 7.3 ± 0.1 at 25°C

Procedure:

1. Dissolve the beef extract, acid hydrolysate of casein, and starch in distilled water.
2. Heat while stirring until the mixture boils to ensure complete dissolution of the ingredients.

3. Adjust the pH of the solution to 7.3 ± 0.1 at 25°C using 1N sodium hydroxide (NaOH) or 1N hydrochloric acid (HCl) as necessary.
4. Dispense the broth into sterile containers or tubes in appropriate volumes for storage and future use.
5. Sterilize the broth by autoclaving at 121°C for 15 minutes.
6. After autoclaving, allow the broth to cool to room temperature before use.
7. Store the prepared Mueller-Hinton broth at $2-8^{\circ}\text{C}$ until needed. Avoid repeated freeze-thaw cycles.

Ensure that the pH of the prepared Mueller-Hinton broth or agar is within the specified range (7.3 ± 0.1). Perform sterility checks by incubating a portion of the prepared media at $35-37^{\circ}\text{C}$ for 24-48 hours and confirming the absence of microbial growth.

Minimum Inhibitory Concentration (MIC) Determination

- Prepare antibiotic stock solutions at concentrations ranging from 0.125 to 2 $\mu\text{g/mL}$ for Ampicillin, Linezolid, and Daptomycin.
- Perform MIC assays using the broth microdilution method following Clinical and Laboratory Standards Institute (CLSI) guidelines.
- Inoculate standardized bacterial suspensions (0.5 McFarland standard) into microtiter plates containing serial dilutions of antibiotics.
- Incubate plates at 35°C for 18-24 hours.
- Determine the MIC as the lowest concentration of antibiotic that inhibits visible growth of *Enterococcus faecalis*.

Time-Kill Kinetics Assay

- Prepare bacterial inoculum by diluting an overnight culture of *Enterococcus faecalis* to a concentration of approximately 10^6 CFU/mL in Mueller-Hinton broth.

- Divide the bacterial suspension into aliquots for each antibiotic concentration to be tested
- Add antibiotics at predetermined concentrations to bacterial suspensions in separate tubes.
- Incubate tubes aerobically at 35°C with shaking.
- Remove aliquots at specified time intervals (e.g., 0, 2, 4, 6, and 24 hours) for viable count determination.
- Plate serial dilutions of aliquots onto Mueller-Hinton broth plates and incubate for 24-48 hours.
- Enumerate colony-forming units (CFUs) and calculate bacterial killing kinetics.

Quality Control

- Include quality control strains such as ATCC Enterococcus faecalis strains to validate assay performance.
- Ensure reproducibility by repeating experiments on different days and under standardized conditions.

Limitations

- Acknowledge potential limitations of the study, such as the use of in vitro models that may not fully replicate in vivo conditions.
- Address any constraints related to assay methodology, bacterial strains, or antibiotic concentrations.

Reproducibility

- Provide detailed protocols and procedures to facilitate the reproducibility of the study by other researchers.
- Share raw data and materials upon request to support transparency and verification of results.

RESULT AND DISCUSSION

Minimum Inhibitory Concentration (MIC) Results

The MIC values of Ampicillin, Linezolid, and Daptomycin against Enterococcus faecalis were determined using the broth microdilution method, following standardized protocols recommended by the Clinical and Laboratory Standards Institute (CLSI) are shown in figure 2.



Figure 2: Broth dilution method; (a) Inhibition zone made by Daptomycin, (b) Inhibition zone made by Linezolid, (c) Inhibition zone made by Ampicillin

Comparison of MIC Values

The MIC values of Ampicillin, Linezolid, and Daptomycin against Enterococcus faecalis strains indicate differences in the susceptibility of the bacterium to each antibiotic. Daptomycin exhibited the lowest MIC values, suggesting greater potency and effectiveness in inhibiting the growth of Enterococcus faecalis compared to Ampicillin and Linezolid (Table 1). This is consistent with the bactericidal mechanism of action of Daptomycin, which disrupts bacterial cell membrane integrity, leading to rapid cell death.

Ampicillin exhibited MICs ranging from 0.280 – 2 µg/ml, Linezolid from 0.205-1.50 µg/ml, and Daptomycin from 0.125-1 µg/ml against Enterococcus faecalis strains (figure 3, 4). These MIC values represent the lowest concentrations of each antibiotic that inhibited visible growth of Enterococcus faecalis under the experimental conditions.

Table 1: Comparison of MIC Values of Ampicillin, Linezolid, and Daptomycin

S. N o.	Anti-Bacterial Drugs	Range (µg/ml)	MIC ₅₀ (mg/l iter)	MIC ₉₀ (mg/l iter)	% Sensitive	% Resistant
1	Daptomycin	0.12 5-1	0.34	0.78	100	0
2	Linezolid	0.20 5-1.50	0.40	1.41	95	3.5
3	Ampicillin	0.28 0 – 2	0.60	2	89	10.2

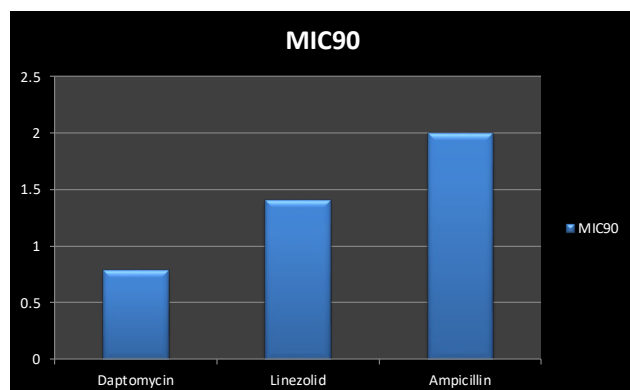


Figure 3: MIC₅₀ Values of Ampicillin, Linezolid, and Daptomycin

Linezolid, a synthetic oxazolidinone antibiotic, also demonstrated potent activity against *Enterococcus faecalis*, with MIC values comparable to those of Daptomycin. The ability of Linezolid to inhibit bacterial protein synthesis by binding to the 23S ribosomal RNA of the bacterial ribosome contributes to its efficacy against gram-positive pathogens, including *Enterococcus faecalis*.

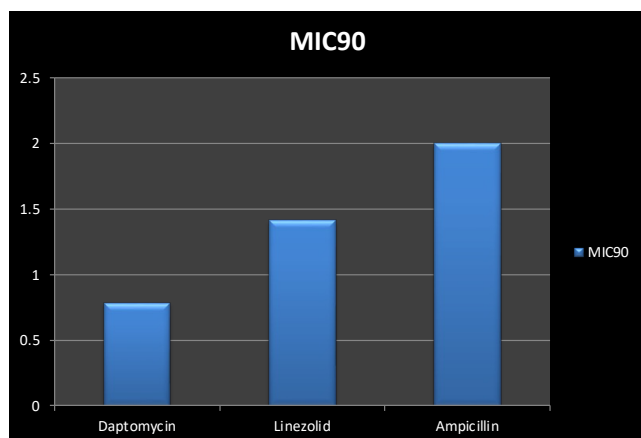


Figure 4: MIC₉₀ Values of Ampicillin, Linezolid, and Daptomycin

In contrast, Ampicillin, a beta-lactam antibiotic, exhibited higher MIC values against *Enterococcus faecalis* strains, indicating reduced potency and efficacy compared to Linezolid and Daptomycin (figure 3, 4). *Enterococcus faecalis* is known to possess intrinsic resistance mechanisms against beta-lactam antibiotics, including the production of penicillin-binding proteins with low affinity for beta-lactam agents, which may contribute to the higher MIC values observed for Ampicillin.

Time-Kill Kinetics Results

The time-kill kinetics assays were conducted to evaluate the bactericidal activity and killing kinetics of Ampicillin, Linezolid, and Daptomycin against *Enterococcus faecalis* over time. Bacterial cultures were exposed to different concentrations of antibacterial agents and viable counts were determined at specified time intervals.

The results of the time-kill kinetics assays revealed concentration-dependent bactericidal activity for all three antibacterial against *Enterococcus faecalis*. However, distinct differences were observed in the rate and extent of bacterial killing over time.

Daptomycin demonstrated the most rapid and potent bactericidal effect, with a significant reduction in bacterial viability observed within the first few hours of exposure to the antibiotic. Linezolid exhibited moderate bactericidal activity, with a gradual decrease in bacterial counts over time. In contrast, Ampicillin showed the least potent bactericidal activity, with slower kinetics of bacterial killing compared to Linezolid and Daptomycin.

The time-kill kinetics assays provided insights into the bactericidal activity and killing kinetics of Ampicillin, Linezolid, and Daptomycin against *Enterococcus faecalis* over time. The results demonstrated concentration-dependent bactericidal activity for all three antibiotics, with Daptomycin exhibiting the most rapid and potent bactericidal effect, followed by Linezolid and then Ampicillin.

The rapid bactericidal activity of Daptomycin against *Enterococcus faecalis* is consistent with its mechanism of action, which involves the disruption of bacterial cell membrane integrity. This results in the rapid depolarization of the cell membrane and

subsequent cell death, leading to a significant reduction in bacterial viability within the first few hours of exposure to the antibiotic.

Linezolid also exhibited potent bactericidal activity against *Enterococcus faecalis*, albeit with slower kinetics compared to Daptomycin. The gradual decrease in bacterial counts over time observed with Linezolid reflects its mechanism of action, which involves the inhibition of bacterial protein synthesis. While Linezolid may take longer to achieve bactericidal effects compared to Daptomycin, its ability to inhibit protein synthesis ultimately leads to bacterial cell death and eradication of the infection.

In contrast, Ampicillin demonstrated the least potent bactericidal activity against *Enterococcus faecalis*, with slower kinetics of bacterial killing compared to Linezolid and Daptomycin. This is consistent with the higher MIC values observed for Ampicillin, indicating reduced efficacy in inhibiting bacterial growth and viability.

Clinical Implications

The findings of this comparative study have important clinical implications for the treatment of enterococcal infections, particularly those caused by *Enterococcus faecalis*. Daptomycin and Linezolid emerged as promising therapeutic options, with potent bactericidal activity against *Enterococcus faecalis* strains, including those with elevated MIC values.

The rapid bactericidal activity of Daptomycin makes it an attractive choice for the treatment of severe enterococcal infections, such as bloodstream infections and endocarditis, where rapid bacterial clearance is crucial for patient outcomes. Similarly, Linezolid offers a valuable alternative for the treatment of enterococcal infections, particularly in cases where Daptomycin may not be suitable due to factors such as resistance or contraindications.

While Ampicillin demonstrated lower potency and efficacy against *Enterococcus faecalis* compared to Linezolid and Daptomycin, it remains a viable treatment option for susceptible enterococcal infections, particularly in less severe cases or when combination therapy with other antibiotics is warranted.

CONCLUSION

In conclusion, the comparative study of Ampicillin, Linezolid, and Daptomycin against *Enterococcus faecalis* provides valuable insights into their relative potency, efficacy, and killing kinetics. Daptomycin and Linezolid demonstrated potent bactericidal activity against *Enterococcus faecalis* strains, with Daptomycin exhibiting the most rapid and potent bactericidal effect. These findings have important clinical implications for the treatment of enterococcal infections and underscore the need for judicious antibiotic selection and antimicrobial stewardship practices to optimize patient outcomes and mitigate the emergence of antibiotic resistance. This comparative analysis of different antibacterial drugs against *Enterococcus faecalis* provides valuable insights into their relative efficacy, potency, and potential clinical utility in the treatment of enterococcal infections.

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