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REVIEW

Antibiotic combination therapy: a widely used but poorly understood approach

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Abstract

Antibiotic combination therapy is widely used in clinical practice; however, its application often lacks a clear understanding of the underlying pharmacological principles. This review critically examines the rationale, benefits, and limitations of combination therapy by integrating evidence from *in vitro* studies, animal models, and the relevant clinical literature. Elicit was used as a support tool to assist in the organization and analysis of the selected studies. While combinations may broaden antimicrobial coverage, enhance bactericidal activity, or prevent resistance, they can also result in additive or antagonistic interactions that limit their clinical effectiveness. This review emphasizes that antibiotic combinations should not be guided by empirical intuition but approached as a rational strategy supported by well-defined therapeutic goals and strong scientific evidence to optimize clinical outcomes and address antimicrobial resistance with greater precision.

Keywords: drug Combinations, drug synergism, pharmacodynamics, anti-bacterial agents, therapeutic use.

Terapia combinada con antibióticos: un enfoque ampliamente utilizado pero poco comprendido

Resumen

La terapia combinada con antibióticos es ampliamente utilizada en la práctica clínica, aunque su aplicación frecuentemente carece de un entendimiento profundo de sus fundamentos farmacológicos. Esta revisión analiza críticamente los principios, beneficios y limitaciones de esta estrategia, integrando información de estudios *in vitro*, modelos animales y literatura clínica relevante. Para facilitar el análisis, se utilizó Elicit como herramienta de apoyo en la organización de los estudios seleccionados. Aunque estas combinaciones pueden ampliar el espectro antimicrobiano, mejorar la actividad bactericida o prevenir la aparición de resistencia, también pueden presentar interacciones aditivas o antagónicas que limitan su eficacia clínica. Se concluye que el uso racional de combinaciones antibióticas debe basarse en objetivos terapéuticos bien definidos y en evidencia científica sólida, más allá de la intuición empírica, con el fin de optimizar los resultados clínicos y enfrentar con precisión la resistencia antimicrobiana.

Palabras clave: Combinación de medicamentos; sinergismo de medicamentos; farmacodinamia; agentes antibacterianos; uso terapéutico

Introduction

Antibiotic combination therapy is increasingly used in clinical practice, particularly when monotherapy is perceived to have limitations. While rooted in the therapeutic principle of *primum non nocere* ("first, do no harm"), its empirical use can lead to unintended consequences, including toxicity, pharmacological interactions, and uncertain clinical benefits[1,2].

Brad Spellberg has described this strategy as involving "the good, the bad, and the ugly," referring to its potential therapeutic advantages, associated risks, and the ambiguity surrounding its real-world effectiveness[3]. In general, antibiotic combinations are prescribed when single-agent therapy may be insufficient owing to low potency, poor tissue penetration, toxicity, or antimicrobial resistance, all of which can compromise clinical outcomes[4]. However, the pharmacological rationale for combination therapy remains poorly understood. Concepts such as synergy, antagonism, and additive effects are inconsistently defined, and in vitro findings do not always translate into improved clinical outcomes.

This review aims to provide a comprehensive overview of antimicrobial combination therapy by integrating evidence

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from *in vitro* studies, animal models, and clinical applications to elucidate its benefits, limitations, and critical considerations for the treatment of infectious diseases.

Materials and methods

This narrative review was based on a literature search performed in PubMed/MEDLINE to identify relevant articles published in English or Spanish between 1969 and 2025. The following search strategy was applied: (((antibiotic drug combinations) AND (Drug Synergism)) AND (in vitro OR pharmacodynamics OR pharmacokinetics)) AND (animal model OR preclinical study OR clinical study). The database search will be conducted between February 17 and March 11, 2025. PubMed/MEDLINE and manual reference list screening were used for study identification. Studies were included if they (i) evaluated antibiotic combinations for the treatment of bacterial infections, (ii) provided experimental data from *in vitro* or *in vivo* studies on synergism, pharmacodynamics, or pharmacokinetics, (iii) reported outcomes in animal models or relevant clinical studies, and (iv) were published in English or Spanish between 1969 and 2025. Studies were excluded if they (i) combined antibiotics with non-antibacterial agents, (ii) focused on viruses, parasites, or fungi, (iii) were published in languages other than English or Spanish, or (iv) lacked full-text access.

The collected references were processed using *Elicit,* a literature review assistant that facilitates the organization and summarization of the scientific articles. Although this is a narrative review, the methodology was guided by the Scale for the Assessment of Narrative Review Articles (SANRA) criteria, and elements of the PRISMA checklist were adapted to enhance transparency in reporting. Study selection and evaluation were conducted manually by two independent reviewers who applied the predefined inclusion and exclusion criteria. Any discrepancies regarding inclusion or data extraction were resolved by consensus. The extracted data included study design, objectives, primary outcomes, definitions of synergy and antagonism, and reported adverse events. The data were reviewed and analyzed using Microsoft Excel 2022. Figure 1 summarizes the article selection process used in this study.

Literature review

Historical Perspective of Antibiotic Combination Therapy

In 1947, the *New England Journal of Medicine* published one of the first reports of deliberate combination therapy: penicillin plus streptomycin for a case of endocarditis caused by a penicillin-resistant *Streptococcus*; the authors explicitly suggested that dual therapy might become the standard for such infections[5].

Laboratory exploration soon followed. In 1950, an *in vitro* study of penicillin–streptomycin activity against the organism then known as *Streptococcus faecalis* laid the groundwork for the formal definitions of synergy and antagonism, the latter exemplified by the classic penicillin–chloramphenicol interaction[6]. However, it was pulmonary tuberculosis that provided the decisive impetus for combined regimens: adding para-aminosalicylic acid (PAS) to streptomycin delayed the emergence of streptomycin resistance from 67 % with monotherapy to 10 % with the combination, even though radiological and microbiological improvement remained modest[7].

Methodological advances have paralleled clinical interest. In 1974, Victor Lorian introduced an agar diffusion technique, visually similar to the Etest used today, to evaluate the synergy of trimethoprim with sulfamethoxazole against *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis*[8]. Since then, multiple quantitative methods, including checkerboard assays and time-kill curves, have been developed to characterize drug interactions (Figure 2).

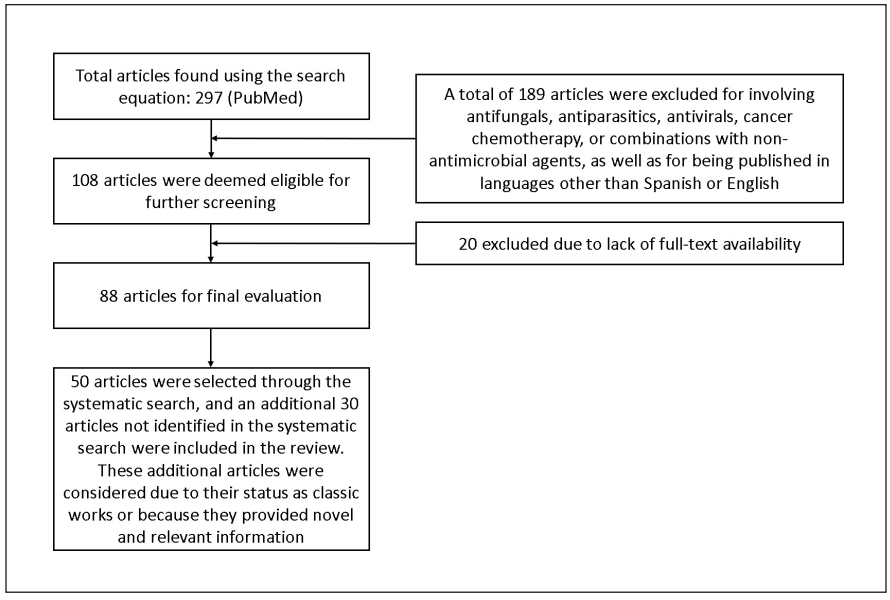

Figure 1. Literature review flowchart

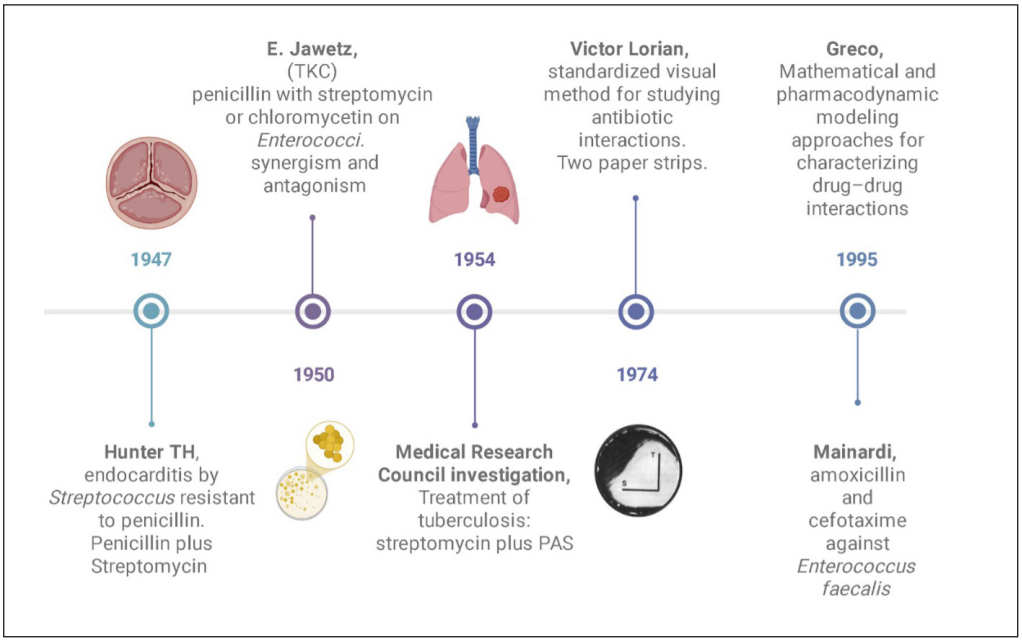

Figure 2. Timeline of key milestones in the evolution of antibiotic combination therapy. TKC = time-kill curves; PAS = para-aminosalicylic acid.

Why Combine Antibiotics?

Antibiotic therapy may be initiated empirically or guided by microbiological confirmation with or without a corresponding susceptibility profile. In the empirical setting, appropriate selection depends on illness severity, clinical syndrome, likely pathogens, pharmacokinetic/pharmacodynamic (PK/PD) considerations, and expected resistance patterns. From a theoretical standpoint, seven scenarios justify the use of combination therapy:

Ensuring broad-spectrum coverage

This is critical for the empirical management of infections. In patients with septic shock, each hour of delay in initiating antibiotics increases mortality by 7%[9]. The rate of empiric antibiotic prescription errors ranges from 14.1% to 78.9%, and appropriate selection is associated with reduced 30-day mortality (RR 0.71; 95% CI: 0.62–0.82) (10). Empirical β-lactam combinations with aminoglycosides or fluoroquinolones have shown significant reductions in ICU mortality (35.7% vs. 28.8%; OR 0.75; 95% CI: 0.63–0.92; p = 0.0006) and in-hospital mortality (47.8% vs. 37.4%; OR 0.69; 95% CI: 0.59–0.81; p < 0.0001), as well as increased ventilator- and vasopressor-free days[2]. In Colombia, inappropriate antimicrobial initiation in febrile neutropenia increased mortality from 15.6% to 32.6%[11]. Thus, beyond synergy, the goal is to ensure at least one active agent.

Targeting distinct bacterial sub-populations and biofilm

This refers to the different growth phases and biofilm formation. The evidence for combining antibiofilm agents remains inconclusive, particularly for prosthetic-related infections. While two randomized controlled trials (1998 and 2020) showed mixed results, retrospective cohorts suggested that rifampin-based combinations may outperform monotherapy[12]. Similarly, tuberculosis treatment benefits from targeting heterogeneous mycobacterial populations, with rifampin being active against slowly replicating or dormant bacilli and pyrazinamide acting in acidic intracellular environments, thereby shortening therapy[3].

Enhancing the bactericidal effect

Multidrug-resistant (MDR) pathogens—methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococci (VRE), extended-spectrum β-lactamase/carbapenemase-producing Enterobacteriaceae, and "difficult-to-treat" *Pseudomonas aeruginosa*—may benefit from accelerated kill[13].

Enterococcus spp. are inherently tolerant to β-lactams, and monotherapy cures <40 % of bacteremia[14]. Adding an aminoglycoside (or a second β-lactam) yields 2-log reductions *in vitro* and clinical success rates of ~75 %[14–17].

For *P. aeruginosa*, penicillins plus aminoglycosides were synergistic in 23/45 strains, even when each drug was individually effective[18]. Nevertheless, a large clinical series found no survival benefit of dual definitive therapy once susceptibility was confirmed[2,19]. In bacterial endocarditis, combination therapy is preferred even when susceptibility is confirmed because of microbial tolerance and biofilm production. Aminoglycosides act synergistically with cell wall inhibitors to ensure bactericidal action and enable shorter treatment durations[20]. Consistent with these observations, a meta-analysis of *in vitro* studies on carbapenem-resistant gram-negative bacilli found the strongest bactericidal synergy for polymyxin–rifampicin in *A. baumannii*, polymyxin–fosfomycin in *K. pneumoniae*, and imipenem–amikacin in *P. aeruginosa*. These

combinations not only accelerated bacterial killing but also reduced bacterial regrowth compared with monotherapy, particularly for Klebsiella and Pseudomonas[21].

Restoring susceptibility via enzyme inhibition

Klebsiella pneumoniae is intrinsically ampicillin-resistant; sulbactam circumvents this by inhibiting the chromosomal β-lactamase[22]. New combinations—imipenem-cilastatin/relebactam, ceftolozane-tazobactam, ceftazidime-avibactam, meropenem-vaborbactam—likewise neutralize carbapenemases in *P. aeruginosa* and Enterobacteriaceae, thereby restoring the partner β-lactam's activity[23].

Preventing the emergence of resistance

Antibiotic exposure can suppress pathogenic organisms but also promote the selection of resistant strains. This phenomenon is well illustrated in *Mycobacterium tuberculosis*, where the *in vitro* spontaneous mutation rate for resistance is 2.25×10^{-10} for rifampin, 2.56×10^{-8} for isoniazid, 2×10^{-12} for the combination of rifampin and isoniazid, 2.56×10^{-7} for ethambutol, and 1×10^{-25} for a regimen combining three first-line drugs. Given that a tuberculous cavity may harbor approximately 10^8 bacilli, it is plausible that two to three organisms are already resistant to isoniazid[24]. Consequently, monotherapy may eradicate susceptible organisms while permitting the proliferation of resistant ones, ultimately leading to the stepwise acquisition of resistance.

In the case of rapidly replicating bacteria, a review involving 14,000 patients treated with various antimicrobial regimens reported the emergence of resistance in 5.6% of cases. Resistance was more frequently observed with monotherapy using penicillins or aminoglycosides than with carbapenems or combination therapies[25]. A Cochrane systematic review of patients with cystic fibrosis found no significant reduction in the emergence of resistance over 2–8 weeks when comparing combination therapy to monotherapy for *Pseudomonas aeruginosa* infections[26]. Similarly, a 2021 study comparing monotherapy and combination therapy for *P. aeruginosa* bacteremia found no significant differences in the emergence of β-lactam-resistant phenotypes in follow-up cultures within 30 days, nor resistance rates to antipseudomonal agents among gram-negative organisms, or isolation rates of MRSA or VRE.

Reducing toxicity

Although often invoked theoretically, deliberate dose reduction to mitigate toxicity is rare in clinical practice[27]. An instructive exception is multidrug-resistant TB: replacing standard 600 mg linezolid with 300 mg daily in a bedaquiline-pretomanid-linezolid regimen preserved efficacy while markedly lowering hematologic and neurologic toxicities[28].

Inhibiting toxin production

Protein synthesis inhibitors can curb exotoxin elaboration. In severe *Streptococcus pyogenes* infections, adjunctive clindamycin treatment diminishes the expression of M protein, superantigens, and streptolysins[29]. A multicenter US cohort (1956 patients) showed that β-lactam + clindamycin reduced in-hospital mortality versus β-lactam monotherapy (6.5 % vs. 11 %; adjusted OR 0.44, 95 % CI 0.23–0.81) even in patients without shock or necrotizing fasciitis[29].

Together, these rationales underscore that the decision to employ combination therapy must be anchored in a clear mechanistic objective rather than the assumption that "more drugs are always better."

General Definitions

A rigorous appraisal of combination therapy depends on clearly delineated terminologies.

Synergism is defined as a positive interaction in which the antibacterial effect of two agents together is substantially greater than would be expected from their individual activities. Operationally, synergy is demonstrated when, in time-kill assays, the combination produces a reduction of at least 2 $\log_{10}$ CFU/mL relative to the most active single drug, or—when checkerboard methods are used—when the fractional inhibitory concentration index (FICI) is ≤ 0.5. Bactericidal activity, whether of a single antibiotic or a combination, is defined as a ≥ 3-$\log_{10}$ reduction in the original inoculum (time 0) in CFU/mL (Δ log 3) within a defined period[1,27,30–35].

Conversely, *antagonism* denotes a negative interaction in which the combined effect is less than that of the individual drugs. Experimentally, this corresponds to an increase of ≥ 2 $\log_{10}$ CFU/mL in time–kill curves or a FICI > 4 in checkerboard assays[1,33,36–40].

An *indifferent* or *additive* interaction is observed when the combined activity neither exceeds nor diminishes the summed individual effect. Here, the change in bacterial burden is < 2 $\log_{10}$ CFU/mL in time-kill studies, and the FICI lies between 0.5 and 4[1,4,27,32,36].

A related but distinct concept is *potentiation*. Potentiation occurs when the presence of a second drug lowers the effective dose 50 (ED_{50}) of the first drug by at least 50 % without altering the maximal attainable effect (E_{max})[15]. In pharmacodynamic terms, this is best appreciated by fitting Hill's equation to dose-response data: the curve for the combination shifts leftward (reflecting greater potency) but reaches the same plateau as the single-agent curve.

Figure 3 schematically illustrates these four interaction patterns—synergy, potentiation, antagonism, and indifference—by comparing the complete dose-response curves for a reference drug and the corresponding two-drug combination.

Mechanisms Underpinning Synergism

Synergism can arise through different mechanisms depending on the microorganism involved and the specific mode of action of the antibiotics used[27]. These mechanisms, both synergistic and antagonistic, are illustrated in Figure 4.

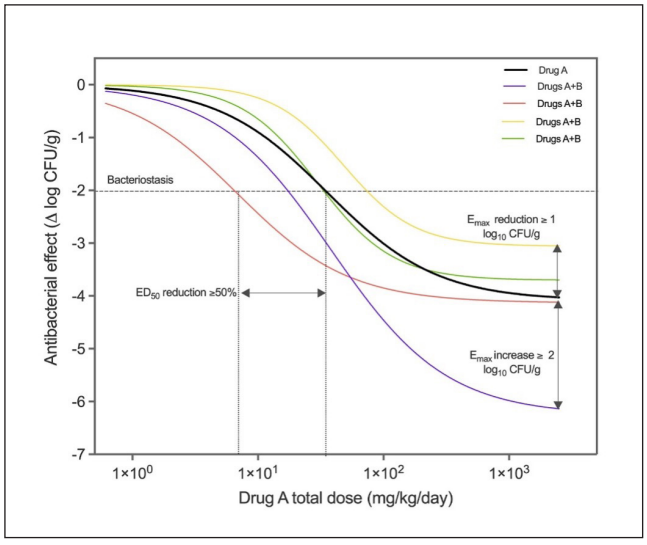

Figure 3. Dose-response curves illustrate different types of *in vivo* pharmacodynamic interactions between Drug A **(black)** and the combination of Drugs A + B (colored curves). Synergism, E_{max} ≥ 2 log_{10} CFU/g increase of the combination compared to the single drug **(purple)**; potentiation, reduction ED_{50} ≥50% without significant changes in E_{max} **(red)**; antagonism, E_{max} reduction ≥ 1 log_{10} CFU/g in the combination compared to the single drug **(yellow)**; and indifferent, without changes of E_{max} or ED_{50} **(green)**. The dashed horizontal line indicates the bacteriostatic threshold. The x-axis is on a logarithmic scale.

Synergistic interactions can be classified as specific or promiscuous. Specific synergy occurs when the enhanced effect is directly attributable to well-characterized mechanisms of action of each antibiotic. In contrast, promiscuous synergy results from nonspecific, off-target pharmacological interactions. A typical example is a drug combination in which one agent prolongs the half-life of the other, thereby increasing systemic exposure and enhancing the overall antibacterial effect[1].

In Vitro Techniques for Quantifying Synergism

A range of *in vitro* methods (Table 1) is available to assess the interactions between two or more antibiotics. These techniques aim to determine whether a given combination exhibits synergistic, additive, indifferent, or antagonistic effects against a specific bacterial isolate.

Checkerboard Assay

The checkerboard method involves preparing serial dilutions of two antimicrobial agents in a two-dimensional microdilution plate format using concentrations that are proportional to each agent's minimum inhibitory concentration (MIC) (Figure 5b)[41]. The resulting interactions are interpreted algebraically and categorized as synergistic, indifferent, or antagonistic, depending on whether the combination achieves greater, equivalent, or reduced antibacterial activity compared to the individual drugs[41].

Growth inhibition was assessed visually. The interaction was quantified by calculating the Fractional Inhibitory Concentration Index, as shown in Equation[1]:

$$FIC\ index = FIC_A + FIC_B = \frac{A}{MIC_A} + \frac{B}{MIC_B} \quad (1)$$

Where A and B are the MICs of each agent in combination and MIC_A and MIC_B are the MICs of each agent alone.

Compound interactions based on the FIC index are considered synergistic (FIC < 0.5) when inhibition is enhanced, additive or indifferent (FIC 0.5–4) when there is little to no change, and antagonistic (FIC > 4) when activity is reduced or MIC increases.

In simpler terms, synergy can also be defined as a ≥ fourfold reduction in the MICs of both agents when used in combination compared to when each is used alone[33,34].

However, the checkerboard technique has some important limitations. It does not evaluate bactericidal activity because it only detects visible growth inhibition. Moreover, both FICI and isobolograms presuppose linear dose-response relationships, which are not always accurate[41]. As the assay provides data from a single time point, it offers a static snapshot of drug interactions rather than a dynamic assessment over time.

Time–Kill Curves

The time-kill curve (TKC) assay was employed to investigate the dynamic nature of synergism or antagonism in antibiotic combinations. This technique measures the number of viable bacteria at multiple time points following exposure to each agent individually and in combination (Figure 5a)[1,42–45]. Unlike other *in vitro* methods, the TKC evaluates bacterial killing, not just growth inhibition, and provides a temporal profile of antimicrobial activity, typically over 24 h.

The interpretation of the TKC results was based on changes in colony-forming units per milliliter. Synergism was defined as a ≥2 log_{10} CFU/mL reduction in the bacterial count for the combination compared to the most active single agent. Antagonism is indicated by a ≥2 log_{10} CFU/mL increase, while no interaction is defined as a change of <2 log_{10} CFU/mL[2,15,46,47].

This approach provides a more realistic and dynamic view of antimicrobial interactions than static assays, such as checkerboard testing. However, it has limitations in mimicking *in vivo* pharmacokinetics. Specifically, the drug concentration remained constant throughout the experiment, unlike in the human body, where absorption, distribution, metabolism, and excretion alter drug levels over time. Furthermore, the assay begins with a standardized, unchanging bacterial inoculum that does not fully replicate the complexities of a live infection scenario[30].

Agar Diffusion Assays

Agar diffusion assays can be performed using two main techniques: disk diffusion and E-test, or a combination of both techniques. In the disk diffusion method, two paper disks

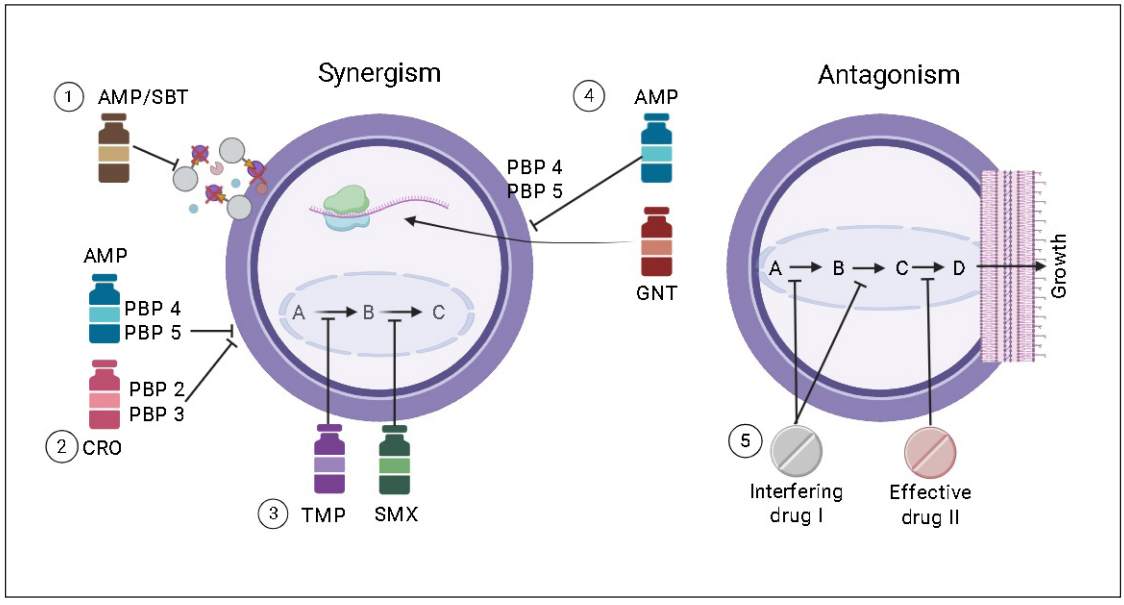

Figure 4. Mechanisms of Antibiotic Synergism and Antagonism: *synergism* can be achieved through several mechanistic pathways: **1.** β-lactamase inhibition – For example, ampicillin–sulbactam inhibits the β-lactamase produced by *Klebsiella pneumoniae*, thereby overcoming its intrinsic resistance and restoring susceptibility to ampicillin. **2.** Saturation of penicillin-binding proteins (PBPs) – The combination of ampicillin and ceftriaxone leads to complete saturation of PBPs in *Enterococcus faecalis*, resulting in lethal disruption of bacterial cell wall integrity. **3.** Sequential blockade of metabolic pathways – A classic case is trimethoprim-sulfamethoxazole, which inhibits consecutive steps in folate biosynthesis, producing a cumulative bactericidal effect. **4.** Facilitated aminoglycoside entry following cell-wall damage – A cell-wall-active agent (e.g., a β-lactam) compromises the bacterial envelope, allowing aminoglycosides to penetrate. This mechanism is seen in *Enterococcus*, *Streptococcus*, *Staphylococcus*, *Listeria monocytogenes*, and many Gram-negative bacilli (36). ***Antagonism*** occurs when: **5.** One drug interferes with the optimal action of another – Drug I may inhibit a metabolic process (A → B → C) that is essential for Drug II to exert its full bactericidal effect. This is often seen when bacteriostatic agents slow down bacterial growth, thereby reducing the efficacy of bactericidal agents that require active replication for their action. Abbreviations: Abbreviations: AMP: ampicillin; AMP/SBT: ampicillin–sulbactam; CRO: ceftriaxone; GNT: gentamicin; PBP: penicillin-binding protein; TMP: trimethoprim; SMX: sulfamethoxazole.

impregnated with different antimicrobial agents are placed on the surface of an agar plate inoculated with the bacterial strain of interest. The plates were then incubated until bacterial growth was observed. Synergistic activity was inferred by comparing the inhibition zones of each agent alone to those produced by the combination[1,48].

The E-test follows the same basic principle but uses commercial strips impregnated with continuous antibiotic concentration gradients. The strips were placed on the inoculated agar surface. After incubation, an elliptical zone of inhibition appeared around the strip, and the minimum inhibitory concentration (MIC) was determined at the point where the zone intersected with the strip scale[1].

Limitations of Antibiotic Combinations and Their Assessment Methods

Although many antibiotic combinations show synergistic effects *in vitro*, these results do not always translate into *in vivo* efficacy, and there is often a poor correlation with the clinical trial outcomes. *In vitro* models maintain constant drug concentrations over time and use planktonic bacteria that readily express antimicrobial targets. These models evaluate only the interaction between drugs and pathogens in isolation[2].

In contrast, *in vivo* settings involve multiple additional variables that affect therapeutic outcomes, including dynamic drug pharmacokinetics (i.e., fluctuating concentrations), the presence of biofilms that alter bacterial behavior, and the host immune response. These factors imply that some synergistic pairs have compatible pharmacokinetic profiles that support clinical translation, while others do not[1] (Figure 6).

Moreover, external pharmacological interactions should be considered. For instance, ketoconazole combined with rifampicin has synergistic effects against *Mycobacterium tuberculosis*. However, ketoconazole is a CYP3A4 inhibitor, whereas rifampicin is a CYP3A4 inducer, creating a pharmacokinetic conflict that renders co-administration strongly contraindicated despite the observed antimicrobial synergy[43].

There are important limitations inherent to the methodologies used. The two most commonly employed techniques, checkerboard assays and time-kill curves, do not yet have a universally accepted gold standard. Efforts have been made to establish correlations between the two, but the experimental findings have sometimes been inconsistent. It is important to note that these methods assess fundamentally different phenomena: checkerboard assays evaluate growth inhibition, whereas the TKC measures the rate and extent of bacterial

Table 1. *In vitro* methods for assessing antibiotic synergy, antagonism, and indifference

Method (references)	Synergism	Antagonism	Indifference	Comment	Clinical Application
Checkerboard [1,41]	FICI ≤ 0.5	FICI > 4	FICI between 1 and 4	Measures inhibition of growth (bacteriostasis); does not quantify bactericidal activity.	Used for initial *in vitro* screening of antibiotic interactions, especially in research or complex multidrug-resistant infections.
Time–Kill Curves[1,33,36,42–45]	Reduction ≥ 2 $\log_{10}$ CFU/mL or ≥ 100-fold increase in bactericidal activity	Increase ≥ 2 $\log_{10}$ CFU/mL or ≥ 100-fold reduction in bactericidal activity	Reduction < 2 $\log_{10}$ CFU/mL compared to most active drug	Measures bactericidal activity; constant drug exposure; does not reflect *in vivo* kinetics; inoculum is static; time frame limited to 24–48 hours.	Gold standard for evaluating synergy in serious infections; used in research and occasionally for clinical decision-making in refractory infections.
Disk Diffusion (solid agar)[1,41]	Increased inhibition zone or "bridge" connecting the halos of both antibiotics	Reduced or truncated inhibition near the junction of halos	Two independent inhibition zones	Qualitative method.	Useful for quick, visual detection of synergy; applied in resource-limited settings or as preliminary screening.
Paper Strip Diffusion (with membrane/ cellophane transfer)[8]	No bacterial growth inside the angle formed by the two strips	Indentation of growth into the angle	No change at the intersection zone	Historical interest only; no longer in routine use.	Obsolete; only used for educational or historical demonstration purposes.
E-test[49]	FICI ≤ 0.5	FICI > 4	FICI between 1 and 4	Commonly used in microbiology laboratories; uses gradient strips to determine interaction.	Widely used in clinical microbiology labs for synergy testing when standardization is needed, especially in multidrug-resistant infections.
Broth Disk Elution, (CLSI M100, 35th edition)	Absence of turbidity in broth	–	–	Applied to determine the susceptibility of Enterobacteriaceae and *Stenotrophomonas maltophilia* to CAZ/AVI + aztreonam combinations.	Specifically applied for evaluating β-lactam + β-lactamase inhibitor combinations, especially in MBL-producing Gram-negatives.

Abbreviations: CAZ/AVI: Ceftazidime/Avibactam; **CFU/mL:** Colony-Forming Units per Milliliter; **CLSI**: Clinical and Laboratory Standards Institute; **FICI:** Fractional Inhibitory Concentration Index; **MBL**: Metallo-β-Lactamase

killing. While some investigators have reported good agreement between the methods[40,49–51], other studies have found poor correlation[38,39,52–54]. One proposed strategy to enhance concordance involves tailoring the antibiotic concentrations used in time-kill assays based on the checkerboard results[55]. Overall, the choice of method can significantly influence the interpretation of a given antimicrobial combination as synergistic, indifferent, or antagonistic.

Combination of Bacteriostatic and Bactericidal Agents

As previously explained, there are antibiotic combinations in which the results are antagonistic, particularly those that involve the pairing of bacteriostatic and bactericidal drugs. This phenomenon was described by Eagle in 1951 and later by Jawetz in 1954[56], who proposed that Drug (I) could interfere with the maximal effect of Drug (II) by blocking a metabolic pathway (A → B → C) that is necessary for the optimal action of the effective agent (II) (Figure 5).

In this context, if Drug (I) is bacteriostatic and slows down the exponential growth of bacteria, which is required for bactericidal agents to achieve their greatest effect, then the activity of the latter is reduced. Thus, it has been suggested that combinations involving protein synthesis inhibitors targeting the 30S ribosomal subunit and cell wall synthesis inhibitors, protein synthesis inhibitors targeting the 50S subunit and DNA gyrase inhibitors, or inhibitors of cell wall synthesis combined with folate synthesis inhibitors frequently result in antagonism[57].

This effect was documented in a clinical study on pneumococcal meningitis[58], where mortality was compared between patients treated with penicillin monotherapy (30%) and those treated with penicillin plus aureomycin (a tetracycline) (79%), the latter of which has bacteriostatic activity.

However, this interpretation has recently been questioned, as the sequential use of a bacteriostatic agent followed by a bactericidal agent has demonstrated synergism[59]. One explanation is that pre-exposure to a bacteriostatic agent before the use of gentamicin prevented the emergence of resistance, a phenomenon that could also explain the effect observed with β-lactams in preventing the emergence of β-lactamase-producing organisms.

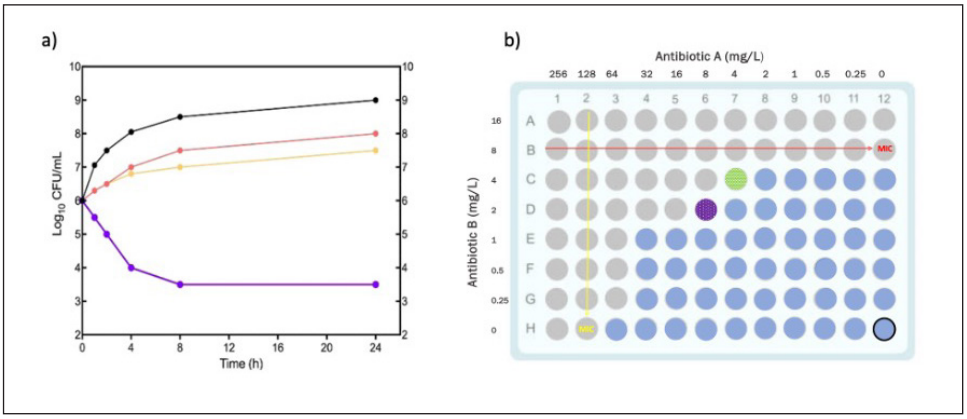

Figure 5. *In vitro* methods for assessing synergism. a) Time-kill curves showing bacterial growth (log_{10} CFU/mL) over 24 hours under different treatments. Black: untreated control; red: Antibiotic A at ½× MIC; yellow: Antibiotic B at ½× MIC; purple: a combination of A + B (each at ½× MIC), resulting in synergistic killing (~3 log_{10} CFU/mL at 24 h). **b)** Synergy checkerboard assay showing 2-fold serial dilutions of Compound A (columns 1–11) and Compound B (rows A–G). Single-agent controls are included in column 12 (Compound B) and row H (Compound A) to determine MICs and calculate FIC values. Gray circles indicate no growth; blue circles indicate growth. A bold-outlined blue circle marks the growth control (H12). Well, D6 indicates synergy (FIC < 0.5), while C7 shows an additive or indifferent effect (FIC = 0.5–4).

Adverse Effects Associated with Antimicrobial Combination Therapy

As previously mentioned, the use of antimicrobials is not without risk to the human body. The potential for adverse effects can vary considerably depending on the specific combination employed. For instance, in patients with febrile neutropenia, combination therapy with piperacillin plus tobramycin resulted in adverse effects, such as allergic reactions, nephrotoxicity, and ototoxicity, in 20% of cases, compared to 8% in those treated with ceftazidime alone[60].

Certain combinations are associated with a higher incidence of toxicity than others. For example, the combination of piperacillin-tazobactam and vancomycin has been linked to a significantly increased risk of acute kidney injury (AKI) when compared tovancomycin combined with cefepime or meropenem[61].

In the treatment of infections caused by *Enterococcus* spp., the combination of ampicillin and ceftriaxone is associated with fewer adverse events (1%) than the traditional regimen of ampicillin and gentamicin (25%), despite similar clinical efficacy[17]. This underscores the importance of considering the safety profiles when selecting combination regimens.

Another major concern is the risk of *Clostridioides difficile* infection, which increases with cumulative antibiotic exposure. Agents such as clindamycin, β-lactams, cephalosporins, and fluoroquinolones have been strongly associated with this complication[2].

Recently, increasing attention has been paid to the impact of antibiotics on human microbiota. These agents can profoundly disrupt the richness and diversity of microbial species, particularly in the gastrointestinal tract[3]. Antibiotics with broad activity against commensal anaerobes, such as β-lactams and metronidazole, are especially prone to causing deep and lasting alterations in the gut microbiome.

Antibiotic Combination in Animal Models

Animal models have proven extremely valuable for determining (1) the relationship between serum and tissue drug concentrations, (2) the temporal kinetics of antimicrobial activity *in vivo*, (3) the pharmacokinetic/pharmacodynamic (PK/PD) indices that correlate with therapeutic efficacy, and (4) the magnitude of those PK/PD indices required to achieve optimal outcomes. Compared to *in vitro* systems and clinical trials, animal models offer the distinct advantage of allowing more precise identification of the PK/PD index that is most closely associated with therapeutic success[62].

Animal models enable exploration beyond the MIC, incorporating pharmacokinetic parameters such as the area under the curve (AUC), maximum serum concentration (C_{max}), serum half-life ($T_{1/2}$), and time above a certain concentration. They also permit the assessment of pharmacodynamic effects by quantifying the relationship between tissue drug levels and therapeutic or toxic outcomes. Key pharmacodynamic variables include the maximum effect (E_{max}), effective dose 50 (ED_{50}), and bacteriostatic dose (*in vivo* correlate of the MIC) [63,64].

Together, drug concentration and antimicrobial effect help determine which PK/PD index, whether AUC/MIC, %T>MIC, or C_{max}/MIC, best predicts the treatment outcome[62,65]. Animal studies also allow the full characterization of dose-response relationships, making it possible to accurately define the magnitude of the PK/PD index that correlates with efficacy[63].

Several animal models have been proposed (Table 2). Although

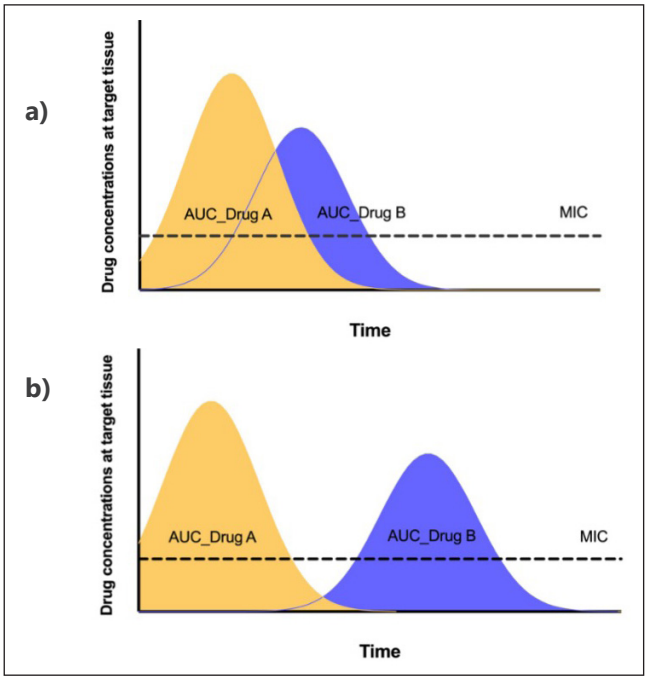

Figure 6. Discordant synergistic partners. (a) Synergistic drug combinations with aligned pharmacokinetic profiles achieve sustained co-exposure at the target site, ensuring drug concentrations remain above the MIC over time (AUC_drug A + AUC_drug B). (b) Conversely, pharmacokinetically discordant synergistic agents exhibit minimal co-exposure at the target tissue, impairing the translation of *in vitro* synergy into *in vivo* efficacy. AUC = Area under the curve; MIC = Minimum Inhibitory Concentration. Figure adapted from reference 41.

these are designed to approximate human pharmacodynamics, host-specific differences must be considered when evaluating animal data. A key distinction lies in drug clearance, which can be up to nine times faster in small animal species than in humans[64,66].

A novel approach for assessing *in vivo* antibiotic combinations involves generating complete dose-response curves and classifying interactions based on changes in pharmacodynamic parameters, specifically E_{max} (used to define synergism or antagonism) and ED_{50} (used to define potentiation). This method demonstrated that the *in vivo* effect of the ampicillin + ceftriaxone combination, which is widely considered synergistic against *Enterococcus faecalis,* is better described as potentiation in a murine thigh infection model. The observed antibacterial effect depends on the dose location along the exposure-response curve[15].

From *In Vitro* and Animal Models to Clinical Application

The ultimate goal of antimicrobial therapy is to prevent fatal outcomes secondary to the infectious disease being treated. Many antibiotic combinations begin with a foundation in *in vitro* studies, where synergism is demonstrated, and subsequently progress to *in vivo* models and eventually to clinical applications. A paradigmatic example is the management of *Enterococcus faecalis* infection. Initial studies have revealed high failure rates with monotherapy in severe infections. Subsequently, it was shown that the combination of ampicillin and gentamicin significantly reduced therapeutic failure. However, the emergence of high-level aminoglycoside resistance (HLAR) necessitates the development of alternative regimens[67].

This prompted Mainardi to explore new combinations using *in vitro* checkerboard assays. He identified synergism between two β-lactams, ampicillin and cefotaxime, against *E. faecalis*[16]. Building on this, Gavaldà conducted animal studies using an experimental endocarditis model, showing that ampicillin plus ceftriaxone reduced the MIC of ampicillin by a factor of 1–4 and significantly lowered the bacterial load in cardiac vegetations[17].

Based on these findings, Gavaldà et al. conducted a clinical observational study in patients with *E. faecalis* endocarditis. The study evaluated 21 patients with HLAR and 22 without HLAR, all treated with ampicillin plus ceftriaxone. The overall cure rate was 67%. This was followed by a randomized clinical trial comparing ampicillin plus gentamicin and ampicillin plus ceftriaxone. Mortality rates were similar between the groups (22% vs. 21%), but adverse effects were significantly lower in the ceftriaxone group[14].

This case illustrates how findings from basic science and animal models can be successfully translated into clinical practices. Table 3 shows how treatment choices have been informed by sequential evidence from *in vitro* synergy testing, validation in animal infection models, and finally, supported by clinical studies.

Multiple Antibiotic Combinations: Is More Better Than One?

In antibiotic therapy, combinations can involve more than two agents when clinically justified. However, outcomes vary depending on the pathogen and specific antimicrobial combination employed.

In carbapenem-resistant Enterobacteriaceae (CRE) infections, a meta-analysis of 1,441 patients found no significant difference in mortality between triple and double antibiotic therapy (HR 0.99; 95% CI: 0.85–1.14; P = 0.85)[75]. This suggests that the addition of a third agent does not always enhance clinical outcomes.

In contrast, Parveen et al. reported better microbiological cure rates with triple therapy (colistin + cefoperazone/sulbactam + tigecycline) than with dual therapy (colistin + minocycline) for multidrug-resistant *Acinetobacter baumannii* infections[68]. However, this study had a limited sample size, which may affect the generalizability of its findings.

For carbapenem-resistant *Pseudomonas aeruginosa* producing metallo-β-lactamases such as IMP, VIM, or NDM, time-kill curve (TKC) studies have demonstrated synergy with combinations involving colistin, rifampicin, and a β-lactam (e.g., meropenem or imipenem)[76]. Among the dual combinations, colistin-rifampicin produced the lowest viable bacterial cou-

nt (2.25 log_{10} CFU/mL at 1× MIC and 3.71 log_{10} CFU/mL at 0.5× MIC). In contrast, all triple combinations reduced bacterial counts to zero (0 log_{10} CFU/mL) at both concentrations, highlighting the potential of triple regimens against highly resistant organisms.

Nevertheless, another study found that although colistin–rifampicin was synergistic against NDM-producing *Klebsiella pneumoniae*, adding meropenem provided minimal additional benefit[77]. This indicates that not all triple combinations produce additive or synergistic effects, and their success may be context-specific.

An illustrative clinical case was reported by Mularoni et al.[73]. involved sternal osteomyelitis caused by VIM-producing *P. aeruginosa*. The patient underwent surgical debridement and was treated with a triple regimen of ceftazidime-avibactam, aztreonam, and amikacin. This combination was selected based on the TKC results, which showed strong synergism at 1× MIC and led to complete clinical cure.

As shown by these examples, triple-antibiotic combinations are typically reserved for highly drug-resistant pathogens and are often selected even when individual agents show resistance in susceptibility testing. However, these regimens must be judiciously tailored, and the concept that "more is better" should not be generalized. The decision to use multiple antibiotics must be based on mechanistic rationale, synergistic data, and patient-specific factors.

Future perspectives

It has long been proposed that adherence to specific pharmacokinetic/pharmacodynamic (PK/PD) indices for individual antimicrobials can predict therapeutic success. While this has been demonstrated for monotherapy, the behavior of PK/PD indices in the context of antibiotic combinations is unclear. As early as 2018, Couet posed a pivotal question: *What would be the appropriate PK/PD targets for a combination of two concentration-dependent antibiotics, each with individual targets of Cmax/MIC > 8?* The general tendency, he suggested, would likely be to maintain the optimal dosing of each agent individually[78].

With the increasing prevalence of multidrug-resistant (MDR) pathogens, novel β-lactamase inhibitors have been developed. While the PK/PD index that best predicts the efficacy of β-lactams is %T>MIC (the percentage of time the drug concentration remains above the MIC), it remains uncertain whether this same index should be used to evaluate the inhibitor or β-lactam–inhibitor combination. Most β-lactamase inhibitors have limited intrinsic antibacterial activity, and defining the PK/PD index that governs their efficacy is relatively complex[78]. The situation becomes even more complicated because the inhibitor alters the MIC of the β-lactam it is paired with[79]. Therefore, the ideal PK/PD index for β-lactam/inhibitor combinations should account for the contribution of the inhibitor and be able to define the magnitude of exposure required to ensure the β-lactam's effectiveness. This led to the proposal of a new parameter, the critical concentration (T>threshold), which is best understood as the specific inhibitor concentration required to protect the β-lactam[80].

In combination therapy, the MIC is not a static value. Based on this observation, a new PK/PD index has been proposed to capture fluctuating susceptibility: the time above the instantaneous MIC (MICi)[62]. However, much remains to be understood about the most appropriate and predictive PK/PD parameters for antibiotic combinations.

Table 2. Comparative overview of animal infection models used in studies of antibiotic combination therapy

Infection Model (references)	Description	Efficacy Measurement	Advantages	Limitations
Mouse, rat, or rabbit thigh infection[15,63,64,66]	Intramuscular injection of bacteria into the thigh of mice or rats.	Change in log_{10} CFU/thigh after 24 hours, comparing treated animals to baseline (time 0) or no treatment controls; Log_{10} Base-10 Logarithm	Highly reproducible and enables evaluation of combination therapies	Limited extrapolation to lung, CNS, or bloodstream infections. The neutropenic mouse model does not reflect the role of host immunity.
Infective endocarditis in rabbits, mice, or rats[14,67,68]	Catheter implantation in the aortic valve followed by infection.	Decrease in log_{10} CFU per gram of vegetation and number of animals with sterile vegetation.	Enables evaluation of combination therapies in high-inoculum infections.	Technically complex; differences in pharmacokinetics between rabbits and humans.
Pulmonary infection model in mice[69,70]	Infection via aerosol exposure or direct intratracheal inoculation.	Reduction in bacterial burden in lung tissue (log_{10} CFU/g), survival rate, and lung histopathology.	Allows measurement of drug concentrations in epithelial lining fluids.	Some antibiotics have uneven penetration into lung tissue or alveolar space, complicating efficacy interpretation.
Galleria mellonella[38,71]	Infection via intra-hemocoelic injection or through the cuticle.	Number of surviving larvae at the end of the experiment; changes in melanization as an indicator of severity.	Economical; rapid reproduction; suitable for preliminary screening.	Survival outcomes can vary significantly based on maintenance conditions; and lack of vertebrate immune components.
Peritonitis model[42,43,66]	Direct intraperitoneal bacterial inoculation or cecal perforation.	Number of surviving mice.	Simulates secondary peritonitis.	Does not measure bacterial burden in tissues or correlate with pharmacokinetic data.

Abbreviation: CFU/g: Colony-Forming Units per Gram; **CNS**: Central Nervous System; **log_{10}:** Base-10 Logarithm

Table 3. Examples of treatment selection based on *in vitro*, animal model, and clinical evidence

Pathogen	Study Type (reference)	Technique	Antibiotics	Primary Outcome
E. faecalis	*In vitro* study[16]	Checkerboard assay	Ampicillin + cefotaxime	MIC_{50} of amoxicillin decreased from 0.5 µg/mL to 0.06 µg/mL in the presence of 4 µg/mL cefotaxime; MIC_{50} of cefotaxime dropped from >256 µg/mL to 1 µg/mL with 0.06 µg/mL amoxicillin.
	Animal model[65]	Time–kill curves and rabbit endocarditis model	Ampicillin + ceftriaxone	Residual bacterial titers in aortic valve vegetations were significantly lower with the combination compared to ampicillin alone.
	Clinical study[17]	Multicenter, observational, non-randomized cohort	Ampicillin + gentamicin vs. ampicillin + ceftriaxone	Mortality: 22% vs. 21%; relapses: 2 vs. 4; adverse events: 25% vs. 1%, significantly higher in the gentamicin group (P < .001).
***P aeruginosa* (MBL-producing)**	*In vitro* study[72]	Time–kill curves	Ceftazidime–avibactam + aztreonam	5 isolates resistant to aztreonam, aztreonam–avibactam, and ceftazidime-avibactam; triple combination restored bactericidal activity in 4/5 isolates (80%).
	In vitro + in vivo study[73]	Time-kill Curves and Clinical Application	Ceftazidime–avibactam + aztreonam + amikacin	Triple combination at 1× MIC showed strong synergy within 8 hours, achieving 99.9% bacterial reduction sustained up to 48 hours. Clinical cure achieved.
Listeria monocytogenes	*In vitro* study[45]	Time–kill curves	Penicillin or ampicillin + gentamicin or streptomycin	Combinations showed ≥2 log_{10} reduction in bacterial counts.
	In vivo study (MONALISA)[74]	Retrospective cohort	Ampicillin + gentamicin or ampicillin + TMP/SMX	Mortality reduction in bacteremia or neurolisteriosis with combination therapy: TMP/SMX: OR 0.49 (0.26–0.92), p = 0.027; aminoglycosides: OR 0.60 (0.38–0.94), p = 0.024; active β-lactams: OR 0.10 (0.04–0.26), p < 0.0001.

Abbreviation: CFU: Colony-Forming Units; **MBL:** Metallo-β-Lactamase; MIC**:** Minimum Inhibitory Concentration; **MIC_{50}**: Minimum Inhibitory Concentration for 50% of isolates; **OR**: Odds Ratio; **P**: P-value; **TMP/SMX**: Trimethoprim/Sulfamethoxazole

Discussion

This review shows that antibiotic combination therapy, despite its widespread clinical use, often relies on empirical rather than mechanistic rationales. Evidence from experimental and clinical studies demonstrates that synergistic effects, such as β-lactamase inhibition or sequential metabolic blockade, can enhance bacterial killing and delay resistance; however, these outcomes depend strongly on the pathogen type, pharmacokinetic compatibility, and dosing strategy. The frequent mismatch between in vitro synergy and clinical efficacy highlights the need for pharmacodynamically guided, rather than empirically driven, combination design.

However, the current evidence has important limitations. *In vitro* and animal models only partially reproduce human pharmacokinetics and immune responses, and the lack of standardized synergy testing complicates comparisons across studies. Moreover, as this was a narrative review, potential selection bias cannot be excluded.

To advance this field, future research should adopt standardized PK/PD frameworks and harmonized testing protocols, focusing on defining predictive pharmacodynamic indices and validating them through well-designed clinical trials. These efforts are essential for translating preclinical findings into rational, evidence-based combination therapies.

In conclusion, combination antibiotic therapy should be regarded as a sophisticated pharmacological strategy rather than the simple co-administration of two antimicrobial agents. Its clinical implementation demands a thorough understanding of the underlying rationale, specific therapeutic goals, such as spectrum broadening, enhanced bactericidal efficacy, resistance suppression, or toxicity reduction, and supporting scientific evidence.

Decisions regarding combination therapy must not rely solely on empirical intuition or theoretical appeal but should instead be anchored in well-established pharmacodynamic principles, mechanistic plausibility, and comprehensive data from *in vitro* models, preclinical studies, and clinical trials. Each regimen should be evaluated within its specific microbiological and pharmacokinetic context, incorporating validated synergy assessments and critically appraising safety profiles.

In summary, the rational use of antibiotic combinations, guided by defined therapeutic objectives and substantiated by robust scientific evidence, is essential to optimize clinical outcomes, minimize harm, and address the growing threat of antimicrobial resistance with scientific rigor and precision.

Ethical considerations

Protection of persons. The authors declare that no experiments involving human beings or animals were conducted in this narrative review.

Protection of vulnerable populations. Not applicable.

Confidentiality. Not applicable, as no access to medical records was required due to the nature of the manuscript.

Privacy. Not applicable.

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References

1. Gómara M, Ramón-García S. The FICI paradigm: Correcting flaws in antimicrobial in vitro synergy screens at their inception. Biochem Pharmacol. mayo de 2019;163:299-307.
2. Tamma PD, Cosgrove SE, Maragakis LL. Combination Therapy for Treatment of Infections with Gram-Negative Bacteria. Clin Microbiol Rev. julio de 2012;25(3):450-70.
3. Spellberg B. Principles of Antiinfective Therapy. En: Blaser M Martin J, Cohen M Jeffrey I, Holland M Steven M, Doi P Yohei, MD, Falsey M Ann R, Garrett P Wendy S, MD, et al., editores. Mandell, Douglas, and Bennett's Principles and Practice of Infectious Diseases [Internet]. Tenth Edition. 2026. p. 218-228.e3. Disponible en: https://www.clinicalkey.es/#!/content/3-s2.0-B9780323934992000177
4. Tyers M, Wright GD. Drug combinations: a strategy to extend the life of antibiotics in the 21st century. Nat Rev Microbiol. marzo de 2019;17(3):141-55.
5. Hunter TH. Use of streptomycin in the treatment of bacterial endocarditis. Am J Med. mayo de 1947;2(5):436-42.
6. Jawetz E, Gunnison JB, Speck RS. Antibiotic Synergism and Antagonism. N Engl J Med. 20 de diciembre de 1951;245(25):966-8.
7. TREATMENT of pulmonary tuberculosis with streptomycin and para-aminosalicylic acid; a Medical Research Council investigation. Br Med J. 11 de noviembre de 1950;2(4688):1073-85.
8. Lorian V, Fodor G. Technique for Determining the Bactericidal Effect of Drug Combinations. Antimicrob Agents Chemother. junio de 1974;5(6):630-3.
9. Pak TR, Young J, McKenna CS, Agan A, DelloStritto L, Filbin MR, et al. Risk of Misleading Conclusions in Observational Studies of Time-to-Antibiotics and Mortality in Suspected Sepsis. Clin Infect Dis. 30 de noviembre de 2023;77(11):1534-43.
10. Marquet K, Liesenborgs A, Bergs J, Vleugels A, Claes N. Incidence and outcome of inappropriate in-hospital empiric antibiotics for severe infection: a systematic review and meta-analysis. Crit Care. diciembre de 2015;19(1):63.
11. Flórez Riaño AF, Rojas Castro OJ, Ospina S, Ramírez-Sánchez IC. Association between inappropriate empirical antimicrobial therapy and mortality in gram-negative bloodstream infections in patients with febrile neutropenia and hematological malignancy. J Infect Chemother. febrero de 2025;31(2):102538.
12. Karlsen ØE, Borgen P, Bragnes B, Figved W, Grøgaard B, Rydinge J, et al. Rifampin combination therapy in staphylococcal prosthetic joint infections: a randomized controlled trial. J Orthop Surg. diciembre de 2020;15(1):365.
13. Zhang W, Guo Y, Li J, Zhang Y, Yang Y, Dong D, et al. In vitro and in vivo bactericidal activity of ceftazidime-avibactam against Carbapenemase–producing Klebsiella pneumoniae. Antimicrob Resist Infect Control. 21 de noviembre de 2018;7(1):142.
14. Beganovic M, Luther MK, Rice LB, Arias CA, Rybak MJ, LaPlante KL. A Review of Combination Antimicrobial Therapy for Enterococcus faecalis Bloodstream Infections and Infective Endocarditis. Clin Infect Dis. 2 de julio de 2018;67(2):303-9.
15. Jimenez-Toro I, Rodriguez CA, Zuluaga AF, Otalvaro JD, Vesga O. A new pharmacodynamic approach to study antibiotic combinations against enterococci in vivo: Application to ampicillin plus ceftriaxone. Chang YF, editor. PLOS ONE. 8 de diciembre de 2020;15(12):e0243365.
16. Mainardi JL, Gutmann L, Acar JF, Goldstein FW. Synergistic effect of amoxicillin and cefotaxime against Enterococcus faecalis. Antimicrob Agents Chemother. septiembre de 1995;39(9):1984-7.
17. Fernández-Hidalgo N, Almirante B, Gavaldà J, Gurgui M, Peña C, De Alarcón A, et al. Ampicillin Plus Ceftriaxone Is as Effective as Ampicillin Plus Gentamicin for Treating *Enterococcus faecalis* Infective Endocarditis. Clin Infect Dis. 1 de mayo de 2013;56(9):1261-8.
18. Heineman HS, Lofton WM. Unpredictable Response of *Pseudomonas aeruginosa* to Synergistic Antibiotic Combinations In Vitro. Antimicrob Agents Chemother. mayo de 1978;13(5):827-31.
19. Babich T, Naucler P, Valik JK, Giske CG, Benito N, Cardona R, et al. Combination versus monotherapy as definitive treatment for *Pseudomonas aeruginosa* bacteraemia: a multicentre retrospective observational cohort study. J Antimicrob Chemother. 15 de julio de 2021;76(8):2172-81.
20. Delgado V, Ajmone Marsan N, De Waha S, Bonaros N, Brida M, Burri H, et al. 2023 ESC Guidelines for the management of endocarditis. Eur Heart J. 14 de octubre de 2023;44(39):3948-4042.
21. Scudeller L, Righi E, Chiamenti M, Bragantini D, Menchinelli G, Cattaneo P, et al. Systematic review and meta-analysis of in vitro efficacy of antibiotic combination therapy against carbapenem-resistant Gram-negative bacilli. Int J Antimicrob Agents. mayo de 2021;57(5):106344.
22. Barriere SL. Bacterial Resistance to β-Lactams, and Its Prevention With Combination Antimicrobial Therapy. Pharmacother J Hum Pharmacol Drug Ther. 10 de septiembre de 1992;12(5):397-402.
23. Tamma PD, Heil EL, Justo JA, Mathers AJ, Satlin MJ, Bonomo RA. Infectious Diseases Society of America 2024 Guidance on the Treatment of Antimicrobial-Resistant Gram-Negative Infections. Clin Infect Dis. 7 de agosto de 2024;ciae403.
24. Dheda K, Mirzayev F, Cirillo DM, Udwadia Z, Dooley KE, Chang KC, et al. Multidrug-resistant tuberculosis. Nat Rev Dis Primer. 24 de marzo de 2024;10(1):22.
25. Fish DN, Piscitelli SC, Danziger LH. Development of resistance during antimicrobial therapy: a review of antibiotic classes and patient characteristics in 173 studies. Pharmacotherapy. 1995;15(3):279-91.
26. Holland P, Jahnke N. Single versus combination intravenous anti-pseudomonal antibiotic therapy for people with cystic fibrosis. Cochrane Database Syst Rev. 23 de junio de 2021;6(6):CD002007.
27. Eliopoulos' CT. Antibiotic Combinations: Should They Be Tested? CLIN MICROBIOL REV. 1988;1.
28. Lee M, Lee J, Carroll MW, Choi H, Min S, Song T, et al. Linezolid for Treatment of Chronic Extensively Drug-Resistant Tuberculosis. N Engl J Med. 18 de octubre de 2012;367(16):1508-18.
29. Babiker A, Li X, Lai YL, Strich JR, Warner S, Sarzynski S, et al. Effectiveness of adjunctive clindamycin in β-lactam antibiotic-treated patients with invasive β-haemolytic streptococcal infections in US hospitals: a retrospective multicentre cohort study. Lancet Infect Dis. mayo de 2021;21(5):697-710.
30. Karakonstantis S, Ioannou P, Kofteridis DD. In search for a synergistic combination against pandrug-resistant A. baumannii; methodological considerations. Infection. junio de 2022;50(3):569-81.
31. Naghavi M, Vollset SE, Ikuta KS, Swetschinski LR, Gray AP, Wool EE, et al. Global burden of bacterial antimicrobial resistance 1990–2021: a systematic analysis with forecasts to 2050. The Lancet. septiembre de 2024;404(10459):1199-226.
32. Wang Y, Li H, Xie X, Wu X, Li X, Zhao Z, et al. In vitro and in vivo assessment of the antibacterial activity of colistin alone and in combination with other antibiotics against Acinetobacter baumannii and Escherichia coli. J Glob Antimicrob Resist. marzo de 2020;20:351-9.
33. Ma XL, Guo YZ, Wu YM, Gong WT, Sun J, Huang Z. In vivo bactericidal effect of colistin–linezolid combination in a murine model of MDR and XDR Acinetobacter baumannii pneumonia. Sci Rep. 15 de octubre de 2020;10(1):17518.
34. Ribes S, Pachón-Ibáñez ME, Domínguez MA, Fernández R, Tubau F, Ariza J, et al. In vitro and in vivo activities of linezolid alone and combined with vancomycin and imipenem against Staphylococcus aureus with reduced susceptibility to glycopeptides. Eur J Clin Microbiol Infect Dis. noviembre de 2010;29(11):1361-7.
35. Friedland IR, Paris M, Ehrett S, Hickey S, Olsen K, McCracken GH. Evaluation of antimicrobial regimens for treatment of experimental penicillin- and cephalosporin-resistant pneumococcal meningitis. Antimicrob Agents Chemother. agosto de 1993;37(8):1630-6.
36. Domenech A, Ribes S, Cabellos C, Taberner F, Tubau F, Domínguez MA, et al. Experimental study on the efficacy of combinations of glycopeptides and β-lactams against Staphylococcus aureus with reduced susceptibility to glycopeptides. J Antimicrob Chemother. 1 de octubre de 2005;56(4):709-16.
37. Kaouah Z, Buyck JM, Pichon M, Burucoa C, Prouvensier L, Moreau J, et al. In vitro efficacy of combinations of antibiotics used in clinical

practice on clinical isolates of *Helicobacter pylori*. Helicobacter. mayo de 2024;29(3):e13081.
38. Wei W, Yang H, Hu L, Ye Y, Li J. Activity of levofloxacin in combination with colistin against Acinetobacter baumannii : In vitro and in a Galleria mellonella model. J Microbiol Immunol Infect. diciembre de 2017;50(6):821-30.
39. Robertson GT, Scherman MS, Bruhn DF, Liu J, Hastings C, McNeil MR, et al. Spectinamides are effective partner agents for the treatment of tuberculosis in multiple mouse infection models. J Antimicrob Chemother. 20 de diciembre de 2016;dkw467.
40. Yu Y, Walsh TR, Yang RS, Zheng M, Wei MC, Tyrrell JM, et al. Novel partners with colistin to increase its in vivo therapeutic effectiveness and prevent the occurrence of colistin resistance in NDM- and MCR-co-producing Escherichia coli in a murine infection model. J Antimicrob Chemother [Internet]. 20 de octubre de 2018 [citado 11 de marzo de 2025]; Disponible en: https://academic.oup.com/jac/advance-article/doi/10.1093/jac/dky413/5139795
41. Lorian V, editor. Antibiotics in laboratory medicine. 5th ed. Philadelphia, PA: Lippincott Williams & Wilkins; 2005. 889 p.
42. Tang HJ, Chen CC, Lai CC, Zhang CC, Weng TC, Chiu YH, et al. In vitro and in vivo antibacterial activity of tigecycline against Vibrio vulnificus. J Microbiol Immunol Infect. febrero de 2018;51(1):76-81.
43. Bax HI, Bakker-Woudenberg IAJM, De Vogel CP, Van Der Meijden A, Verbon A, De Steenwinkel JEM. The role of the time-kill kinetics assay as part of a preclinical modeling framework for assessing the activity of anti-tuberculosis drugs. Tuberculosis. julio de 2017;105:80-5.
44. Kebriaei R, Rice SA, Singh KV, Stamper KC, Dinh AQ, Rios R, et al. Influence of Inoculum Effect on the Efficacy of Daptomycin Monotherapy and in Combination with -Lactams against Daptomycin-Susceptible Enterococcus faecium Harboring LiaSR Substitutions.
45. Moellering RC, Medoff G, Leech I, Wennersten C, Kunz LJ. Antibiotic Synergism Against *Listeria monocytogenes*. Antimicrob Agents Chemother. enero de 1972;1(1):30-4.
46. Li J, Yang X, Chen L, Duan X, Jiang Z. *In Vitro* Activity of Various Antibiotics in Combination with Tigecycline Against *Acinetobacter baumannii* : A Systematic Review and Meta-Analysis. Microb Drug Resist. diciembre de 2017;23(8):982-93.
47. Lee HJ, Bergen PJ, Bulitta JB, Tsuji B, Forrest A, Nation RL, et al. Synergistic Activity of Colistin and Rifampin Combination against Multidrug-Resistant Acinetobacter baumannii in an *In Vitro* Pharmacokinetic/Pharmacodynamic Model. Antimicrob Agents Chemother. agosto de 2013;57(8):3738-45.
48. Entenza JM, Moreillon P. Tigecycline in combination with other antimicrobials: a review of in vitro, animal and case report studies. Int J Antimicrob Agents. julio de 2009;34(1):8.e1-8.e9.
49. White RL, Burgess DS, Manduru M, Bosso JA. Comparison of three different in vitro methods of detecting synergy: time-kill, checkerboard, and E test. Antimicrob Agents Chemother. agosto de 1996;40(8):1914-8.
50. Oka' S, Goto' M, Kaji' Y, Kimara' S, Matsuda K, Asahi Y, et al. Synergic activity of imipenem/cilastatin combined with cefotiam against methicillin-resistant Staphylococcus aureus.
51. Sanderink D, Cassisa V, Chenouard R, Mahieu R, Kempf M, Dubée V, et al. Colistin–Glycopeptide Combinations Against Multidrug-Resistant *Acinetobacter Baumannii* in a Mouse Model of Pneumonia. Future Microbiol. mayo de 2019;14(7):581-6.
52. Norden CW. Experimental Osteomyelitis. IV. Therapeutic Trials with Rifampin Alone and in Combination with Gentamicin, Sisomicin, and Cephalothin. J Infect Dis. 1 de noviembre de 1975;132(5):493-9.
53. Vouillamoz J, Moreillon P, Giddey M, Entenza JM. In vitro activities of tigecycline combined with other antimicrobials against multiresistant Gram-positive and Gram-negative pathogens. J Antimicrob Chemother. febrero de 2008;61(2):371-4.
54. Zhuang L, Sy SKB, Xia H, Singh RP, Mulder MB, Liu C, et al. Evaluation of in vitro synergy between vertilmicin and ceftazidime against Pseudomonas aeruginosa using a semi-mechanistic pharmacokinetic/pharmacodynamic model. Int J Antimicrob Agents. febrero de 2015;45(2):151-60.
55. Perea EJ, Clavijo E, Nogales MC, Luque IG. Interaction of aminoglycosides and cephalosporins against *Pseudomonas aeruginosa* . Correlation between interaction index and killing curve. J Antimicrob Chemother. 1988;22(2):175-83.
56. Jawetz E, Gunnison JB, Coleman VR. Observations on the Mode of Action of Antibiotic Synergism and Antagonism. J Gen Microbiol. 1 de abril de 1954;10(2):191-8.
57. Ocampo PS, Lázár V, Papp B, Arnoldini M, Abel Zur Wiesch P, Busa-Fekete R, et al. Antagonism between Bacteriostatic and Bactericidal Antibiotics Is Prevalent. Antimicrob Agents Chemother. agosto de 2014;58(8):4573-82.
58. Lepper MH. TREATMENT OF PNEUMOCOCCIC MENINGITIS WITH PENICILLIN COMPARED WITH PENICILLIN PLUS AUREOMYCIN: Studies Including Observations on an Apparent Antagonism Between Penicillin and Aureomycin. AMA Arch Intern Med. 1 de octubre de 1951;88(4):489.
59. Gil-Gil T, Berryhill BA. Antibiotic killing of drug-induced bacteriostatic cells. Uhlemann AC, editor. Antimicrob Agents Chemother. 26 de marzo de 2025;e00156-25.
60. De Pauw BE, Deresinski SC, Feld R, Lane-Allman EF, Donnelly JP. Ceftazidime Compared with Piperacillin and Tobramycin for the Empiric Treatment of Fever in Neutropenic Patients with Cancer: A Multicenter Randomized Trial. Ann Intern Med. 15 de mayo de 1994;120(10):834-44.
61. Pan K, Li R, Li Y, Ding X, Li X, Lv Q. Vancomycin combined with piperacillin/tazobactam increases the risk of acute kidney injury compared with vancomycin plus other anti-pseudomonal beta-lactams: a systematic review and network meta-analysis. J Antimicrob Chemother. 3 de enero de 2025;80(1):47-58.
62. Jimenez-Toro I, Rodriguez CA, Zuluaga AF, Otalvaro JD, Perez-Madrid H, Vesga O. Pharmacokinetic/Pharmacodynamic Index Linked to *In Vivo* Efficacy of the Ampicillin-Ceftriaxone Combination against Enterococcus faecalis. Antimicrob Agents Chemother. 16 de febrero de 2023;67(2):e00966-22.
63. Zuluaga AF, Vesga O. Aportes del modelo en ratón neutropénico de infección del muslo al conocimiento sobre la farmacología de antibióticos.
64. Andes D, Craig WA. Animal model pharmacokinetics and pharmacodynamics: a critical review. Int J Antimicrob Agents. abril de 2002;19(4):261-8.
65. Gavaldà J, Torres C, Tenorio C, López P, Zaragoza M, Capdevila JA, et al. Efficacy of Ampicillin plus Ceftriaxone in Treatment of Experimental Endocarditis Due to *Enterococcus faecalis* Strains Highly Resistant to Aminoglycosides. Antimicrob Agents Chemother. marzo de 1999;43(3):639-46.
66. Zhao M, Lepak AJ, Andes DR. Animal models in the pharmacokinetic/pharmacodynamic evaluation of antimicrobial agents. Bioorg Med Chem. diciembre de 2016;24(24):6390-400.
67. Jiménez Toro I, Rodríguez CA, Zuluaga AF. Eficacia de las combinaciones antimicrobianas para el tratamiento de infecciones enterocócicas: una revisión crítica. Rev Chil Infectol. octubre de 2019;36(5):556-64.
68. Parveen N, Sikander RI, Zeeshan M, Muneeb Ali S, Hassan M. Comparison Between the Combination Therapy in Terms of Therapeutic Effectiveness and Anti-Microbial Efficacy in Extensive Drug-Resistant Acinetobacter Baumannii Infections. Life Sci. 4 de julio de 2023;4(3):6.
69. Story-Roller E, Maggioncalda EC, Lamichhane G. Synergistic Efficacy of β-Lactam Combinations against *Mycobacterium abscessus* Pulmonary Infection in Mice. Antimicrob Agents Chemother. agosto de 2019;63(8):e00614-19.
70. Andréjak C, Almeida DV, Tyagi S, Converse PJ, Ammerman NC, Grosset JH. Characterization of Mouse Models of Mycobacterium avium Complex Infection and Evaluation of Drug Combinations. Antimicrob Agents Chemother. abril de 2015;59(4):2129-35.
71. Skinner K, Sandoe JAT, Rajendran R, Ramage G, Lang S. Efficacy of rifampicin combination therapy for the treatment of enterococcal infections assessed in vivo using a Galleria mellonella infection model. Int J Antimicrob Agents. abril de 2017;49(4):507-11.
72. Lee M, Abbey T, Biagi M, Wenzler E. Activity of aztreonam in combination with ceftazidime–avibactam against serine- and metallo-β-lactamase–producing Pseudomonas aeruginosa. Diagn Microbiol Infect Dis. enero de 2021;99(1):115227.
73. Mularoni A, Mezzatesta ML, Pilato M, Medaglia AA, Cervo A, Bongiorno D, et al. Combination of aztreonam, ceftazidime–avibactam and amikacin in the treatment of VIM-1 Pseudomonas aeruginosa ST235 osteomyelitis. Int J Infect Dis. julio de 2021;108:510-2.
74. Charlier C, Perrodeau É, Leclercq A, Cazenave B, Pilmis B, Henry B, et al. Clinical features and prognostic factors of listeriosis: the MONALISA national prospective cohort study. Lancet Infect Dis. mayo de 2017;17(5):510-9.
75. Wang L, Tong X, Huang J, Zhang L, Wang D, Wu M, et al. Triple Versus Double Therapy for the Treatment of Severe Infections Caused by Carbapenem-Resistant Enterobacteriaceae: A Systematic Review and Meta-Analysis. Front Pharmacol. 30 de enero de 2020;10:1673.
76. Kim SH, Kim HM, Chung DR, Ko JH, Huh K, Cho SY, et al. Synergistic effects of colistin–rifampin-based triple antimicrobial combination therapy against Carbapenem-resistant *Pseudomonas aeruginosa* : a time-kill assay. J Antimicrob Chemother. 3 de marzo de 2025;80(3):738-45.
77. Lagerbäck P, Khine WWT, Giske CG, Tängdén T. Evaluation of antibacterial activities of colistin, rifampicin and meropenem combinations against NDM-1-producing *Klebsiella pneumoniae* in 24 h *in vitro* time–kill experiments. J Antimicrob Chemother. agosto de 2016;71(8):2321-5.
78. Couet W. Pharmacokinetics/pharmacodynamics characterization of combined antimicrobial agents: a real challenge and an urgent need. Clin Microbiol Infect. julio de 2018;24(7):687-8.
79. Sy SKB, Derendorf H. Experimental design and modelling approach to evaluate efficacy of β-lactam/β-lactamase inhibitor combinations. Clin Microbiol Infect. julio de 2018;24(7):707-15.
80. Assefa GM, Roberts JA, Mohammed SA, Sime FB. What are the optimal pharmacokinetic/pharmacodynamic targets for β-lactamase inhibitors? A systematic review. J Antimicrob Chemother. 2 de mayo de 2024;79(5):946-58.