ANTIBACTERIAL ACTIVITY AND SYNERGISM OF ANTIBIOTICS AND ANTI-INFLAMMATORY DRUGS

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1 INTRODUCTION

Alexander Fleming's 1928 discovery of penicillin ushered in the "Age of Antimicrobials." Following this, years of successful treatment against different kinds of bacteria were made possible by the creation of numerous novel antimicrobial compounds that were either natural, synthetic, or semi-synthetic in nature (AMINOV, 2017).

But as Charles Darwin once said, adaptation is the fundamental tenet of evolution, and bacteria have evolved sophisticated microbial resistance (MR) systems over time. The indiscriminate and improper use of antibiotics in the community, in agriculture, in animal husbandry, and in health services are significant risk factors for the emergence and spread of MR. This type of resistance is a problem that impacts the entire society and is caused by a number of factors (HOLMES, 2016).

Perez (2019) highlights that the increasing prevalence of multidrug-resistant Gram-positive and Gram-negative bacilli presents a major challenge to healthcare systems, requiring coordinated multidisciplinary efforts to develop effective strategies for their prevention and control.

Globally, the rising resistance of pathogenic microorganisms to conventional antibiotics has become a major public health concern. Understanding the global epidemiology of diseases linked to multidrug-resistant microbes is crucial since this issue is exacerbated by the extraordinary intensity of the movement of people and goods, which also encourages the transmission of microorganisms (WHO, 2015).

Therefore, the development of new antibiotics is urgently needed to combat drug-resistant infections (Chan *et al.*, 2017). However, significant scientific and regulatory barriers have hindered the advancement of novel antimicrobial agents, reinforcing the importance of alternative therapeutic strategies to manage bacterial

infections. Alternative methods of controlling bacterial infections are desperately needed because, in reality, only two new families of antibiotics with unique mechanisms of action have been created in recent decades (daptomycin [authorized in September 2003] and linezolid [approved in April 2000]).

Drug repurposing and synergistic drug screening have emerged as viable strategies in the fight against diseases brought on by bacteria that are resistant to several drugs. Since they can potentially overcome issues related to the weak activity of individual medications, such strategies are thought to be effective for treating serious infections. For instance, statins act on the bacterial membrane, making methicillin-resistant *Staphylococcus aureus* (MRSA) more sensitive to antibiotic therapies (ZHANG *et al*., 2021).

Thus, nonsteroidal anti-inflammatory drugs (NSAIDs), which primarily act by inhibiting the synthesis of pro-inflammatory mediators, may represent a promising adjunctive strategy for bacterial infections. Chan *et al.* (2017) reported that NSAIDs, when combined with antibiotics, can enhance antimicrobial efficacy, delay resistance development, and broaden antibacterial activity compared to monotherapy.

This chapter aims to review and contextualize existing evidence on the synergistic antibacterial activity of combining antibiotics with non-steroidal anti-inflammatory drugs (NSAIDs), particularly against *Escherichia coli* and *Staphylococcus aureus*. Emphasis is placed on literature-based findings that suggest enhanced antimicrobial efficacy through such combinations, highlighting their potential role as complementary therapeutic strategies in the face of rising antibiotic resistance

2 ANTIBACTERIAL ACTIVITY

Since ancient times, humans and bacteria have coexisted, and since then, microorganisms have undoubtedly caused diseases in humans (SOUZA *et al*., 2003). As a result, antibiotic therapy was a therapeutic revolution, and antibiotics remain one of the most prescribed medicine classes worldwide (SILVA, 2006).

Antibiotics are naturally derived or semi-synthetic compounds capable of inhibiting the growth or replication of microorganisms at low concentrations (SILVA, 2006).

Multidrug-resistant bacteria have become much more common and have spread all across the world. Because of this, government organizations and the medical community are taking increasingly effective steps to prevent and control harmful germs. Antimicrobial susceptibility tests (AST), which measure the overall sensitivity, reduced sensitivity, or resistance of bacteria to antimicrobials in vitro, must be properly carried out in order to identify this multidrug resistance (BRASIL, 2020).

The guidelines for conducting the Antimicrobial Susceptibility Test (AST) are established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and the Clinical and Laboratory Standards Institute (CLSI), two international groups. In Brazil, Ordinance No. 64, of 12/11/2018, of the Ministry of Health, regulates that the guidelines of the Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST), which is a Brazilian version of the EUCAST document, must be followed. Access is free to all BrCAST documents through the website www.brcast.org.br.

Both qualitative and quantitative approaches can be used for TSA. Qualitative approaches reveal whether the microbe is resistant to the tested antimicrobial agent (R), susceptible to increased exposure (I) (formerly "intermediate"), or sensitive (S). The amount of antibiotic needed at the infection site to achieve a sufficient clinical response is what distinguishes "S" from "I" (BRASIL, 2020).

The "disc diffusion" approach is the most popular qualitative technique in everyday laboratory work. The minimum inhibitory concentration (MIC), or the lowest concentration in milligrams per milliliter of the antimicrobial agent needed to prevent observable bacterial growth in vitro, is also determined by quantitative methods in addition to providing qualitative information. Three approaches can be used to determine the MIC: agar dilution, broth dilution or by means of antimicrobial concentration gradient strips. The choice of method may vary depending on the microorganism and the antimicrobial, so correct identification of the microorganism to be tested is necessary (BRASIL, 2020).

According to their morphology, the majority of bacteria are categorized as rods, cocci, or spirals (ALBERTS *et al.*, 2017). They are further divided into two major types, gram-positive and gram-negative, which are distinguished by the Gram staining method. Gram-positive bacteria make up 50% of the cell wall, whereas gram-negative bacteria only make up 5% (TRABULSI and ALTERTHUM, 2017). The cell walls of Gram-positive bacteria are primarily composed of thick peptidoglycan layers, which differ significantly in structure and composition from those of Gram-negative bacteria (CARVALHO and RECCO-PIMENTEL, 2007).

Teichoic acids found in gram-positive bacteria's cell walls provide them antigenic specificity, whereas endotoxins (lipopolysaccharide [LPS]—which is made up of lipid A and antigen O) give gram-negative bacteria this ability. The periplasmic space, also known as the periplasm, is located between the plasma membrane and the complex outer layer of polysaccharides, lipoproteins, and phospholipids that characterizes gram-negative organisms. In certain species, this space is home to the β-lactamase enzymes that break down penicillin and other β-lactams (LEVINSON, 2016).

Gram staining is a fundamental microbiological technique used to classify bacteria and guide initial antimicrobial therapy. Gram-positive bacteria stain blue, while gram-negative bacteria stain red. This method, which involves four steps, involves a heat-fixed bacterial smear with the reagents crystal violet, Lugol's, alcohol, and fuchsin (TRABULSI

and ALTERTHUM, 2017). The Gram staining technique differentiates bacteria based on cell wall composition: Gram-positive bacteria retain the crystal violet stain and appear purple, whereas Gram-negative bacteria lose the initial stain and take up safranin, appearing red or pink (LEVINSON, 2016).

Understanding the distinctions between gram-positive and gram-negative bacterial walls is crucial for researching antibiotic and chemotherapeutic mechanisms of action, pathogenicity, and numerous other topics that are closely linked to the chemical makeup and structure of bacterial walls (TRABULSI and ALTERTHUM, 2017). In addition to defending against bacteriophages, which are viruses that target bactéria (JUNQUEIRA and CARNEIRO, 2018), the cell wall also fends off lysis by osmotic swelling and is a target of host antibacterial proteins like lysozyme and antibiotics like penicillin (ALBERTS *et al.*, 2017).

Gram staining is useful for identifying a variety of bacteria since it affects the selection of antibiotics because gram-positive bacteria are typically more vulnerable to penicillin G than gram-negative bacteria. However, not all bacteria can be seen using Gram staining for a variety of reasons. For instance, Mycoplasma pneumoniae does not have a cell wall, and its high lipid content hinders the dye's ability to penetrate microbacteria like M. tuberculosis, which are identified using different microscopic techniques (LEVINSON, 2016).

While some, like mycobacteria, are bacilli, the majority of gram-positive bacteria are cocci. Pneumococci, streptococci, and staphylococci (like *Staphylococcus aureus*) are the three gram-positive bacteria that are most crucial to medicine. According to MENEZES E SILVA and NEUFELD (2006), these organisms' capsules have the potential to induce acute pyogenic (pus-producing) diseases, including meningitis, pharyngitis, pneumonia, and boils.

Bacilli and diplococci are the most significant gram-negative bacteria in medicine, and many of them, including *Escherichia coli* and *Helicobacter pylori*, are long-term inhabitants of the gastrointestinal system. These microorganisms are the primary source of infection in the gastrointestinal system, lungs, meninges, urinary tract, and wounds (MENEZES E SILVA and NEUFELD, 2006).

2.1 THE BACTERIA *Staphylococcus spp.*

The microbiota of humans and animals is composed of bacteria of the genus *Staphylococcus*, which also inhabit the skin and mucous membranes. These bacteria are opportunistic pathogens and are associated with mild infections, such as skin infections and food poisoning, but they can also cause serious, sometimes fatal infections, such as infections related to invasive medical devices, bacteremia, meningitis and pneumonia. Among the species, *Staphylococcus aureus* is the most pathogenic because it has a variety of virulence factors (DALEN; PESCHEL and SORGE, 2020).

Staphylococcus aureus causes abscesses, various pyogenic infections (e.g., endocarditis, septic arthritis, and osteomyelitis), food poisoning, scalded skin syndrome, and toxic shock syndrome. *S. aureus* is one of the most common causes of hospital-acquired pneumonia, septicemia, and surgical wound infections. It is an important cause of skin infections such as folliculitis, cellulitis, and impetigo, and is the leading cause of bacterial conjunctivitis (LEVINSON, 2016).

Clusters of staphylococci, which resemble bunches of grapes, are spherical gram-positive cocci. With a minimum pH of 4.2, a maximum of 9.3, an optimum of 7.0-7.5, a minimum temperature of 6°C and a maximum temperature of 48°C, with an ideal of 37°C, they can thrive in a wide range of dry conditions and are facultative anaerobes that are not picky or mobile (TRABULSI and ALTERTHUM, 2015).

All staphylococci contain catalase, which breaks down hydrogen peroxide. This is a crucial virulence component because it allows the bacteria to withstand the deadly effects of H_2O_2 inside neutrophils. The fact that *S. aureus* generates coagulase, an enzyme that induces plasma coagulation, in addition to catalase sets it apart from other staphylococci species (LEVINSON, 2016).

The two types of coagulase, conjugated coagulase and free coagulase, have distinct characteristics. Known as a clumping factor, conjugated coagulase is discovered attached to the cell wall. Extracellular secreted free coagulase forms a complex with a plasma-based protein known as Coagulase Reaction Factor (CRF) and combines with fibrinogen to create fibrin (clots) (MENEZES E SILVA and NEUFELD, 2006).

The cell wall of *S. aureus* contains protein A, a key virulence factor, and the carotenoid pigment staphyloxanthin, which imparts a golden color to colonies and enhances resistance to oxidative stress within neutrophils. Additionally, two other important characteristics are the fermentation of mannitol and hemolysis of red blood cells, whose iron from hemoglobin is used by the bacteria for growth and synthesis of cytochrome enzymes for energy production (LEVINSON, 2016). The primary features of *S. aureus* pathogenesis are displayed in Table 1.

Type of pathogenesis	**Typical disease**	**Predisposing factor**	**Prevention method**
Toxigenic (superantigen)	Toxic shock syndrome	Nasal or vaginal packing	Reduce the time you use the cap
	Food poisoning	Improper food storage	Refrigerate food
Local pyogenic (abscess)	Skin infection (e.g., impetigo and surgical wound infections).	Poor skin hygiene; failures in aseptic procedures.	Cleanliness; hand washing; reduction of nasal carrier status.
Disseminated pyogenic (abscess)	Sepsis and endocarditis	Use of intravenous drugs.	Reduce the use of intravenous drugs.

Table 1. Important features of the pathogenesis of *S. aureus.*

Source: Adapted from Levinson (2016).

S. aureus has multiple systems that regulate gene expression, but the AGR (Accessory Gene Regulator) system is the most well-studied and is crucial to the species' virulence expression. This system has a quorum sensing function, meaning that it can determine the concentration of other identical bacterial cells in its environment based on the actions of a single cell (TRABULSI and ALTERTHUM, 2015).

The enzyme β-lactamase, which breaks down a number of penicillins, is encoded by plasmids found in over 90% of *S. aureus* strains. Methicillin/oxacillin-resistant *S. aureus* (MRSA/ORSA) and naphyllin-resistant *S. aureus* (NRSA) are two common names for *S. aureus* strains that are resistant to β-lactamase-resistant penicillins, including methicillin and naphyllin (LEVINSON, 2016). More recently, *S. aureus* with intermediate sensitivity to vancomycin (VISA) and vancomycin-resistant *S. aureus* (VRSA) have also been isolated (MENEZES E SILVA and NEUFELD, 2006).

Along with producing β-lactamases, *S. aureus* also expresses penicillin binding proteins (PBPs), PBP1, PBP2, PBP3, and PBP4, which confer resistance to several beta-lactam antibiotics. The alternative version PBP2a, which is expressed by MRSA, has the ability to transpeptide the N-acetyl-glucosamine and N-acetyl-muramic chains, preserving the peptidoglycan structure and, in turn, the bacterial cell wall. VISA resistance is brought on by thicker cell walls, which are caused by changes in about 30 different genes and are typically triggered by long-term, inappropriate vancomycin use (ALTERTHUM and TRABULSI, 2015).

2.2 THE BACTERIA *Escherichia coli*

The most frequent cause of sepsis and urinary tract infections linked to gram-negative bacteria is Escherichia coli. It is the agent most commonly linked to "traveler's diarrhea," a watery diarrhea, and one of the two major causes of neonatal meningitis. Some strains of *E. coli* are enterohemorrhagic and cause bloody diarrhea (LEVINSON, 2016).

The Enterobacterales order is composed of Gram-negative bacilli belonging to the families Enterobacteriaceae, Morganellaceae, Yersiniaceae, among others. This order is a diverse group of Gram-negative bacilli with high clinical importance because they are associated with several community-acquired and healthcare-related infections. These bacteria can present multidrug-resistant phenotypes to antimicrobials, which is due to different resistance mechanisms. This order includes the bacterium *Escherichia coli* (LÓPEZ *et al.*, 2019).

The most prevalent facultative anaerobic organism in the colon and feces is *E. coli*, a rectal gram-negative bacillus. *E. coli* differs from *Shiguella* and *Salmonella*, the two primary intestinal infections, in that it ferments lactose. The O antigen of the cell wall, the H antigen of the flagellum, and the K antigen of the capsule are the three antigens found in *E. coli* (LEVINSON, 2016).

E. coli can cause sickness because of a number of components, including pili, a capsule, endotoxins, and three exotoxins (enterotoxins), two of which produce watery diarrhea and one of which causes hemolytic-uremic syndrome and bloody diarrhea (LEVINSON, 2016).

Enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC), enterohemorrhagic *E. coli* (EHEC), and enteropathherent *E. coli* (EAEC) are the five categories into which *E. coli* that cause diarrhea are now divided. The virulence mechanism of EPEC is linked to the illness known as "infantile diarrhea" and has nothing to do with the discharge of normal enterotoxins. Enterotoxins are produced in ETEC, which is commonly referred to as "traveler's diarrhea." The strains of EHEC that cause hemolytic uremic syndrome and hemorrhagic colitis do not ferment sorbitol. EAEC adheres to cells, does not produce enterotoxins, is noninvasive, and induces mild diarrhea (MENEZES E SILVA and NEUFELD, 2006). Extraintestinal Pathogenic *E. coli* (ExPEC) is a sixth categorization that is further described by Trabulsi and Alterthum (2015).

2.3 PENICILLIN

Penicillin was discovered by Alexander Fleming in 1928 and is a member of the β-lactam class. These antimicrobials inhibit transpeptidation, the final reaction of peptidoglycan synthesis, which is largely responsible for the rigidity of the bacterial cell wall (OSAWA *et al.*, 2017). One of the most frequent penicillins used in therapy is amoxicillin.

Penicillin-binding proteins (PLPs or PBPs) are a group of related targets for the actions of penicillins and cephalosporins, even though the inhibition of the transpeptidase described has been shown to be significant. Many of these proteins are present in all bacteria; for instance, *Staphylococcus aureus* has four PBPs, whereas *Escherichia coli* has at least seven. PBPs vary in their affinity for different β-lactam antibiotics, however the contacts become covalent (MENEZES E SILVA and NEUFELD, 2006).

β-Lactam antibiotics are a class of antibiotics that have in common in their structure the presence of the β-lactam ring (6-aminopenicillanic acid). The manipulation of the side chains of this nucleus allowed the development of numerous semisynthetic penicillins, with amoxicillin standing out. Penicillins are very unstable, and spontaneous degradation of these drugs occurs. The β-lactam group is mainly responsible for this instability, as the opening of the β-lactam ring can occur under different conditions (temperature, pH and enzymatic degradation). One of the major challenges in determining the pharmacokinetics of these drugs is how to maintain their stability under different storage conditions (ROBERTS *et al.*, 2014).

Amoxicillin (AMOX), also known as alpha-amino-p-hydroxybenzyl penicillin, is an antibiotic that was introduced in 1970. AMOX is similar to ampicillin, both having a broad spectrum of antimicrobial activity, differing in that AMOX is more absorbed when administered orally. It is used in the treatment of respiratory tract infections, such as otitis media, bronchitis and bacterial sinusitis, and is also used in the treatment of urinary tract infections (WRIGHT, 1999). The structural formula of the amoxicillin trihydrate molecule is shown in figure 1.

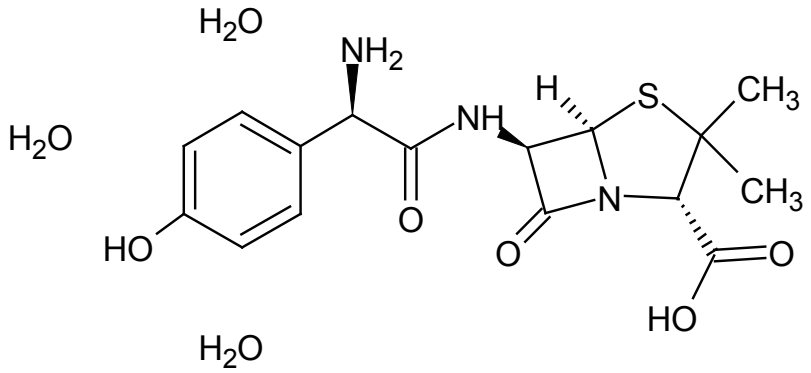

Figure 1 - Amoxicillin trihydrate's structural formula.

Source: Created by the authors (2025).

Amoxicillin is a broad-spectrum antibiotic indicated for the treatment of bacterial infections caused by microorganisms sensitive to the action of amoxicillin. The strains of microorganisms sensitive to the bactericidal action of amoxicillin *in vitro* are: Gram-positive: *Enterococcus faecalis, Streptococcus pneumoniae, Streptococcus pyogenes, Streptococcus viridans, Staphylococcus aureus sensitive to penicillin, Corynebacterium species, Bacillus anthracis, Listeria monocytogenes* and *Clostridium*. Gram-negative Aerobes: *Haemophilus influenzae, Escherichia coli, Proteus mirabilis, Salmonella, Shigella, Bordetella pertussis, Brucella, Neisseria gonorrhoeae, Neisseria meningitidis, Pasteurella septica, Vibrio cholerae and Helicobacter pylori*. Amoxicillin is susceptible to manipulation by beta-lactamase enzymes, therefore, the spectrum of activity of amoxicillin does not cover the microorganisms that produce it, that is, it does not include resistant *Staphylococcus* and all strains of *Pseudomonas*, *Klebsiella* and *Enterobacter* (ANVISA, 2023).

2.4 CIPROFLOXACIN

By binding to the topoisomerase complex (II and IV) during double-strand uncoiling for supercoiling relaxation, Ciprofloxacin, a fluoroquinolone, inhibits bacterial DNA replication by targeting DNA gyrase and topoisomerase IV, ultimately leading to cell death (LOURENÇO *et al.*, 2017). Quinolones inhibit gyrase-mediated DNA superhelicalization at concentrations that are well correlated with those required to inhibit bacterial growth. Mutations in the gene encoding the A subunit polypeptide can confer resistance to these drugs (MENEZES E SILVA and NEUFELD, 2006).

Figure 2 displays the ciprofloxacin molecule's structural formula.

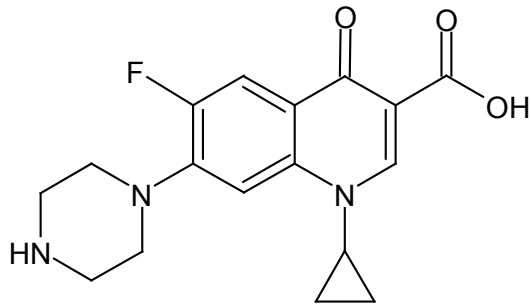

Figure 2 - Ciprofloxacin's structural formula.

Source: Created by the authors (2025).

Ciprofloxacin is prescribed for a number of infections and exhibits a wide range of activity against both Gram-positive and Gram-negative bacteria. Microorganisms resistant to cephalosporins, penicillins, and aminoglycosides are typically not resistant to ciprofloxacin because this medication does not interact with these antibiotics. It is indicated for the treatment of complicated and uncomplicated infections caused by sensitive microorganisms: of the respiratory tract (many microorganisms, e.g. *Klebsiella, Enterobacter, Proteus, E. coli, Pseudomonas, Haemophilus, Moraxella, Legionella,* and *Staphylococcus*, react very sensitively to ciprofloxacin), of the middle ear, paranasal sinuses, eyes, kidneys and/or efferent urinary tract, reproductive organs, abdominal cavity, skin and soft tissues, bones and joints, and even generalized infections (ANVISA, 2023).

2.5 NON-STEROIDAL ANTI-INFLAMMATORY DRUGS

One of the most commonly prescribed medicine groups globally is non-steroidal anti-inflammatory drugs (NSAIDs) (BJARNASON *et al*., 2018). These medications are used to treat inflammatory illnesses such rheumatic diseases, fever, and acute or chronic pain because of their analgesic and anti-inflammatory properties (SOSTRES *et al*., 2010).

However, there are significant gastrointestinal side effects linked to their use, including bleeding, obstructions, and perforations of peptic ulcers. This is because traditional NSAIDs act by inhibiting the two isoforms of the enzyme cyclooxygenase (COX). COX-1 is responsible for lowering the amounts of gastroprotective prostaglandin synthesis, causing gastric and intestinal injury. Most tissues respond to inflammatory stimuli by inducing COX-2 (RAFANIELLO *et al*., 2016). Figures 3, 4, and 5 illustrate NSAIDs, which include ibuprofen, nimesulide, and diclofenac sodium.

Ibuprofen is one of the best-selling NSAIDs in the world with analgesic, antipyretic and anti-inflammatory action used in the treatment of arthritis, muscle tension, headache and others (AHMADI *et al.*, 2014; BAUMANN, BAXENDALE, 2016). Ibuprofen was discovered in 1961 and in 1969 it began to be sold with a prescription in the United Kingdom to treat rheumatic diseases. In 1970, several trials were carried out with approval for the marketing of this drug without a prescription (HALFORD *et al.*, 2012).

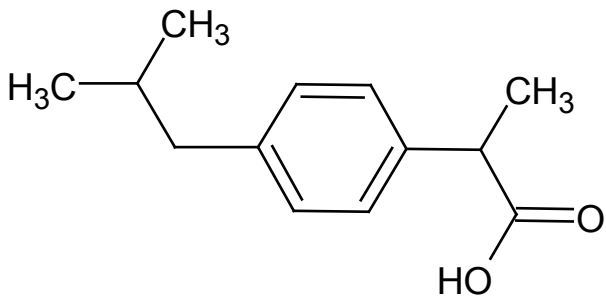

Figure 3 - Ibuprofen's structural formula.

Source: Created by the authors (2025).

Nimesulide (4'-nitro-2'-phenoxymethanesulfonanilide) is a sulfonanilide class nonsteroidal anti-inflammatory medication (NSAID) that has analgesic, antipyretic, and anti-inflammatory properties. 42 hospitalized children, ages 6 months to 8 years, with fever and acute respiratory tract infections participated in a double-blind trial to examine nimesulide's antipyretic properties. Antibiotics such as erythromycin or amoxicillin were administered concurrently to both groups. Temperature readings prior to and throughout the six hours following the initial nimesulide dosage decreased, but no changes were noted in the placebo group. Nimesulide was accepted well. According to the findings, nimesulide has an instant antipyretic effect, which could be helpful in clinical settings prior to initiating antibiotic treatment (ANVISA, 2023).

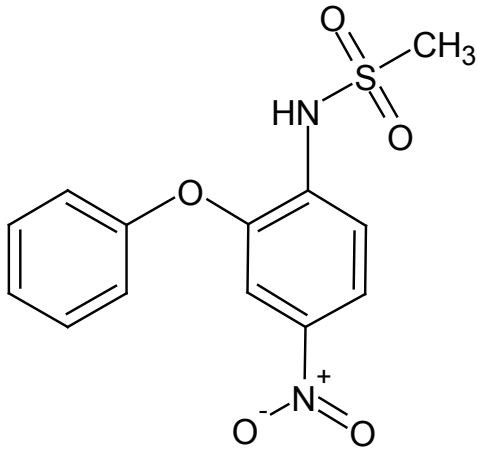

Figure 4 - Nimesulide's structural formula.

Source: Created by the authors (2025).

A white or slightly yellowish crystalline powder that dissolves slightly in water at 25°C, diclofenac sodium is a derivative of benzeneacetic acid. 2-[(2,6-dichlorophenyl)amino] benzeneacetic acid is the chemical name. Diclofenac is an analgesic, antipyretic, and anti-inflammatory drug. Like other NSAIDs, diclofenac sodium works by inhibiting cyclooxygenase (COX-1 and COX-2). The exact mechanism of action is unknown. Diclofenac may work by reducing prostaglandins in peripheral tissues because it is a strong inhibitor of prostaglandin production, which mediates inflammation (ANVISA, 2023).

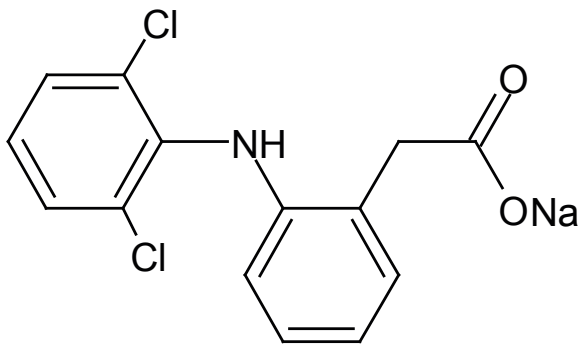

Figure 5 – Diclofenac sodium's structural formula.

Source: Created by the authors (2025).

2.6 DRUG SYNERGY AND ANTIBIOTIC THERAPY

The high level of antibiotic resistance in harmful bacteria, which is mostly caused by the population's overuse of these medications, means that antibiotic therapy is not always successful in treating these organisms (SILVA, 2006; OSTROSKY *et al.*, 2008).

Antibiotic resistance represents a major global health challenge, associated with increased morbidity, mortality, treatment costs, and prolonged hospital stays. Drugs lose their effectiveness and infections continue as a result of MR, which raises the possibility of transmission. The inappropriate and overuse of antimicrobials is speeding up MR (BRASIL, 2020).

Through processes such cytoplasmic membrane disintegration, Proton Motive Force (PMF) instability, electron flow, active transport, and coagulation of cell contents, compounds can affect bacteria. Certain sites may be impacted by various mechanisms, and not all mechanisms of action act on certain targets (BURT, 2004). Escherichia coli, a multipurpose species that can cause intestinal and urinary infections, septicemia, meningitis, and other conditions, is one form of Gram-negative bacterium whose growth is challenging to suppress (ALBERT *et al.*, 1995).

The current state of diseases brought on by bacteria that are resistant to drugs is strange and alarming. Multidrug-resistant bacterial infections will be the leading cause of death worldwide unless significant changes are made. By 2050, these infections will claim the lives of about ten million people annually, surpassing the deaths from other diseases like diabetes and cancer (O'NEILL, 2014).

The World Health Organization (WHO) recently released a list of 12 families of bacteria that pose a hazard to human health and are considered "priority pathogens" for the study and creation of novel antimicrobials. The bacterium *Staphylococcus aureus*, which is resistant to methicillin (oxacillin) and has intermediate sensitivity or resistance to vancomycin, is in priority category 2 (high), while the bacterium Escherichia coli, which is resistant to carbapenems and third-generation cephalosporins, is in priority category 1 (critical) (WHO, 2017).

Studies that assessed the synergy of antibiotics and nonsteroidal anti-inflammatory medications in antimicrobial action against different bacteria were developed in this MR setting. Chen *et al.*'s study from 2023 showed that using ibuprofen in combination with common antibiotics has synergistic antimicrobial effects against microorganisms that cause cystic fibrosis (CF). The findings imply that ibuprofen and widely used antibiotics work together to exhibit synergistic antibacterial action against clinical bacterial strains that are resistant to drugs in vitro. In an in vivo pneumonia model, the combination of ceftazidime and ibuprofen was shown to be effective against resistant P. aeruginosa; aspirin and ibuprofen are two NSAIDs that work in concert with cefuroxime and chloramphenicol to combat methicillin-resistant *Staphylococcus aureus* (MRSA).

According to the authors of the aforementioned study, ibuprofen's anti-inflammatory qualities and ability to mitigate the deterioration in lung function by inhibiting bacterial growth account for the results of its trials for cystic fibrosis (CF). They therefore suggest that ibuprofen and other NSAIDs, such aspirin and naproxen, have a synergistic bactericidal effect by sensitizing microorganisms that are resistant to antibiotics. Ibuprofen and perhaps other NSAIDs have dual anti-inflammatory and antibacterial properties that make them perfect for treating CF patients' lung infections and hyperinflammation.

According to a study by Chan *et al.* (2017), aspirin and ibuprofen, two non-steroidal anti-inflammatory drugs (NSAIDs), have a synergistic effect on the antibacterial activity of cefuroxime and chloramphenicol against methicillin-resistant *Staphylococcus aureus*. The study's findings also showed that combined therapy of an antibiotic and an NSAID as an adjuvant molecule is an effective treatment option for infections and inflammatory conditions.

Nonsteroidal anti-inflammatory drugs (NSAIDs) may exhibit antibacterial activity and have synergistic effects with antibiotics. As demonstrated by Altaf *et al.* (2019), who assessed the antibiotic susceptibility profile and synergistic effect of NSAIDs on the antibacterial activity of the antibiotics oxytetracycline and gentamicin against methicillin-resistant *Staphylococcus aureus* (MRSA) in cases of goat mastitis, such therapy can be used to treat animals.

Altaf *et al.* (2019) found that NSAIDs and resistant antibiotics exhibited partial synergism when combined with either Meloxicam and Oxytetracycline or Gentamicin; Flunixin and Meglumine with Oxytetracycline demonstrated a synergistic effect, while Flunixin and Meglumine with Gentamicin demonstrated partial synergism. When combined with oxytetracycline, diclofenac sodium shown an additive effect; however, gentamicin exhibited an indifferent impact. The in vivo findings demonstrated the efficacy of combining gentamicin or oxytetracycline with meglumine, flunixin, or meloxicam. The study came to the conclusion that combining resistant antibiotics with NSAIDs can effectively battle resistance against zoonotic MRSA infections spread through milk.

According to Zhang *et al.* (2021), diclofenac at large dosages can stop MRSA from growing and does not quickly cause drug-resistant mutations. Low dosages of diclofenac, however, have the ability to "resensitize" bacteria to β-lactams, which helps to get around drug resistance and boosts the effectiveness of traditional antibiotics against bacteria. Additionally, MRSA-associated biofilm formation on implants is inhibited by low dosages of diclofenac when combined with β-lactams. Because diclofenac and β-lactams have multitarget effects against MRSA, the authors suggest that their synergistic impact may have promising uses in the prevention of perioperative infections.

Recent studies have evaluated NSAID-antibiotic combinations as a strategy against resistant pathogens. Table 2 presents selected investigations, outlining drug pairs, experimental models, target microorganisms, and synergistic outcomes.

Drug Combination	Experimental Model	Target Microorganisms	Type of Synergy	Main Findings	Author (Year)
Aspirin + Cefuroxime Ibuprofen + Chloramphenicol	*In vitro*	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Full synergy	NSAIDs enhanced the antibacterial activity of antibiotics against MRSA*	**Chan *et al.* (2017)**
Diclofenac + β-lactams	*In vitro* (biofilm and resistance assays)	Methicillin-resistant *Staphylococcus aureus* (MRSA)	Synergy and resensitization	Diclofenac inhibited biofilm formation and resensitized MRSA* to β-lactams	**Zhang *et al.* (2021)**
Ibuprofen + Ceftazidime	*In vitro* and *in vivo* (mouse pneumonia model)	Drug-resistant *Pseudomonas aeruginosa*	Full synergy	Combination significantly reduced bacterial load and lung inflammation	**Chen *et al.* (2023)**
Meloxicam / Flunixin + Oxytetracycline / Gentamicin	*In vitro* and *in vivo* (goat mastitis model)	Zoonotic MRSA	Partial or additive synergy	NSAIDs improved antibiotic efficacy; effects varied by combination	**Altaf *et al.* (2019)**

Table 2. Compilation of key studies investigating the synergistic potential of NSAIDs combined with antibiotics against multidrug-resistant bacteria.

*Methicillin-Resistant *Staphylococcus aureus*

Diclofenac is a widely used NSAID, known for its efficacy in managing postoperative and inflammatory pain with a favorable safety profile. It is a well-tolerated NSAID with minimal documented adverse effects. Furthermore, a number of researchers have proposed that diclofenac may prevent the growth of a wide range of bacteria, such as *Mycobacterium TB*, *S. aureus*, *Candida albicans*, *Listeria monocytogenes*, and *Escherichia coli* (ZHANG *et al.*, 2021).

When diclofenac was administered in conjunction with β-lactams, which are commonly utilized during the perioperative phase, antibacterial effects were noted (ZHANG *et al.*, 2021). However, there is no thorough research on the mechanism by which diclofenac resensitizes MRSA to β-lactams.

The development of bacterial resistance brought on by the careless use of antibiotics and/or the patient stopping treatment too soon because their symptoms have improved make antibiotic therapy a therapeutic issue. As a result, synergistic treatments that try to use less antibiotics to treat infections are highly legitimate. These challenges reinforce the relevance of exploring combined therapeutic strategies aimed at enhancing antimicrobial efficacy while minimizing resistance selection pressure.

The increasing prevalence of antimicrobial resistance highlights the urgent need for alternative therapeutic strategies to potentiate the efficacy of existing antibiotics. In this context, the combination of non-steroidal anti-inflammatory drugs (NSAIDs) with conventional antimicrobials represents a promising adjunctive approach against multidrug-resistant pathogens such as *Staphylococcus aureus.* Preliminary findings suggest enhanced antimicrobial activity and potential resensitization effects; however, further *in vivo* and clinical investigations are essential to validate these outcomes, elucidate mechanisms of action, and establish safe and effective therapeutic protocols.

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