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*Review*

# The emergence of common bacterial community-acquired pneumonia (*Streptococcus pneumoniae*) resistance against antimicrobials; B-lactams, Macrolides and Fluoroquinolones

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**Simple Summary:** One bacteria species that commonly cause community-acquired pneumonia (CAP) is *Streptococcus pneumoniae.* β-lactams, macrolides, and fluoroquinolones are used to kill the bacteria. However, the effectiveness of these drugs is declining due to the emergence of resistance of *S. pneumoniae* to these antimicrobials. In addition, although pneumococcal vaccines and artificial intelligence (AI) have been developed, people cannot depend on them constantly. Therefore, understanding the resistance mechanism of *S. pneumoniae* might help researchers to create a more potent antibiotic in the future.

**Abstract:** *Streptococcus pneumoniae* is among the common bacteria responsible for community-acquired pneumonia (CAP). CAP is a type of pneumonia where people get infected outside the hospital. It is the primary source of mortality and morbidity worldwide. B-lactams, macrolides, and fluoroquinolones are the major anti-pneumococcus agents used to combat *S. pneumoniae*. However, antibiotic resistance in *S. pneumoniae* has emerged as a result of antibiotic overuse, raising concerns around the globe. Furthermore, in 2017, the World Health Organization (WHO) declared *S. pneumoniae* as one of the 12 preceding pathogens. Although pneumococcal vaccines have been established, people cannot depend on them solely. The artificial intelligence (AI) that is currently growing for antimicrobial resistance (AMR) also requires much work to be done. Therefore, understanding the resistance mechanism of *S. pneumoniae* in these anti-pneumococcus agents might help researchers to develop a better potent antimicrobial against *S. pneumoniae* in the future.

**Keywords:** *Streptococcus pneumoniae*; community-acquired pneumonia (CAP); antimicrobials, β-lactams; macrolides; fluoroquinolones; resistant; artificial intelligence (AI)

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## 1. Introduction

Pneumonia is an acute respiratory illness that affects the lungs, where a person's alveoli is filled with pus and fluid [1]. One of the frequent varieties of pneumonia is community-acquired pneumonia (CAP). It is an infection that a person acquires outside the hospital and is a major source of death and illness worldwide. World Health Organization (WHO) has ranked lower respiratory tract infections (LRTIs), including CAP, as the third highest cause of death [2,3]. More than 100 pathogens, including bacteria, viruses, and fungi such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Staphylococcus aureus*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae,* and *Legionella pneumophilia* are responsible for the development of CAP. However, *S. pneumoniae* continues to be the most prevalent cause of bacterial CAP [4,5].

*S. pneumoniae* or often referred to as pneumococcus is an opportunistic, Gram-positive bacteria that colonize the human mucosal surfaces of the upper respiratory tract (URT) [6]. The bacteria is correlated with high rates of morbidity and death, particularly among children under age two, immunocompromised individuals, and the elderly [7]. Although *S. pneumoniae* is a commensal bacterium, it can cause severe illness once it leaves its main reservoir (mucosal surfaces) and spreads to the other sterile site, such as the lungs resulting in pneumonia. β-lactams, macrolides, and fluoroquinolones are the major antimicrobials used to kill *S. pneumoniae*. However, the effectiveness of these antimicrobials is declining due to the emergence resistance of *S. pneumoniae* against these agents. Furthermore, in 2017, the WHO ranked *S. pneumoniae* as one of the 12 bacteria for which new therapies are urgently required [6,8].

In a recent WHO research on antibiotic resistance released in 2014, pneumococcus was classified as one of the nine microbes of global concern [9]. Bacteremia, otitis media, and meningitis are some of the other illnesses brought by pneumococcus. Pneumococcus is correlated to mortality rates in bacterial meningitis ranging from 16 – 37%. About 30 - 50% of adult survivors still experience persistent residual symptoms [10,11]. According to Van Boeckel et al. 2014 [12], from 2000 – 2010, the world's antibiotics usage increased by more than 30%, from approximately 50 billion to 70 billion standard units. In 2020, penicillin, macrolides, and cephalosporins were the antibiotics that were frequently used. India consumed 13 billion standard units of antibiotics in 2010, followed by China with 10 billion and the United States with 7 billion standard units [12] (a standard unit is the number of doses sold: the IMS Health database identifies a dose as pill, capsule, or ampoule).

Penicillin G has been the cornerstone treatment for pneumococcal disease for more than 40 years. The penicillin-resistant pneumococci, which was initially noted in the 1960s, has increased alarmingly over the past ten years [13–15]. About one-third of pneumococci had developed penicillin resistance by 1997 [15]. Unfortunately, tetracyclines, clindamycin, chloramphenicol, and trimethoprim-sulfamethoxazole (TMP-SMX) are frequently ineffective against penicillin-resistant bacteria [13–15]. Antibiotic use patterns are reflected in pathogens' ability to avoid being killed by particular types of drugs [16]. Pneumococcus resistance to β-lactams and macrolides is extremely concerning and may be exceed 20% in Southern Europe's countries [17,18].

Most penicillin-sensitive and penicillin-resistant *S. pneumoniae* are vulnerable to rifampicin, fluoroquinolones, and carbapenems [14–16,19]. Most strains are vulnerable to carbapenems-more than 90% [15]. However, 12 cases of pneumococcus losing fluoroquinolone sensitivity appear even though recent fluoroquinolones effective against more than 99% of isolates [15]. Vancomycin is still effective against all types of pneumococci, but some strains of *S. pneumoniae* have developed tolerance to it, raising the possibility that vancomycin resistance will soon appear [14,15].

Managing pneumococcus is difficult because of the widespread use of antibiotics, the development of numerous resistant clones, serotype replacement and capsular

modification, and the horizontal transmission of antibiotic resistance genes [7]. This review shed light on the *S. pneumoniae* resistance against these anti-pneumococcus agents.

## 2. Etiology

*S. pneumoniae* is a facultative anaerobic Gram-positive bacterium which could be alpha- or beta-hemolytic (under aerobic or anaerobic conditions, respectively), belonging to the Streptococcus genus. Other bacteria that have been identified in CAP include *Staphylococcus aureus*, *Hemophilus influenzae*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* as well as anaerobes. It is important to note that the frequency of *S. pneumoniae* appears to be gradually dwindling with the increased adoption and utilization of pneumococcal vaccines. Atypical pathogens such as *Chlamydophila pneumoniae* and *Mycoplasma pneumoniae* are, on the other hand, slowly emerging as major pathogens especially among young adults [20].

Certain risk factors that have been identified in patients with CAP include immunosuppression, excessive consumption of alcohol, age above 70 years, asthma, prolonged stay in overcrowded conditions, among others [21,22].

## 3. Pathogenesis

The occurrence of bacterial pneumonia typically begins with the entry of a causative pathogen (e.g., *S. pneumoniae, Hemophilus influenzae*) into a host's respiratory tract. The pathogen reaches the alveoli, multiply, and evoke host responses. The lower respiratory tract of the host could be accessed by the pathogen via aspiration, inhalation, direct inoculation and hematogenous or contiguous spread from an adjacent focus. Although possible, it is largely unusual for direct inoculation involving a penetrating thoracic injury or contiguous spread from an infection site (e.g., mediastinitis) to occur [23]. In intravenous drug abusers with tricuspid endocarditis, hematogenous spread may occur. Nevertheless, the major potential routes include small volume aspiration of bacterial pathogen into the host's oropharynx (which could ensue while sleeping) and through contaminated droplets inhalation. Microaspiration can happen frequently even in healthy people, but it is uncommon for pneumonia to develop. The development of pneumonia largely depends on aspirate volume, pathogenic bacterial inoculum, aspiration frequency, and the virulence of aspirated bacteria in relation to the host immune system [2]. In addition to mechanical protections, both innate and adaptive host defenses are essential for protection against such incidents [24].

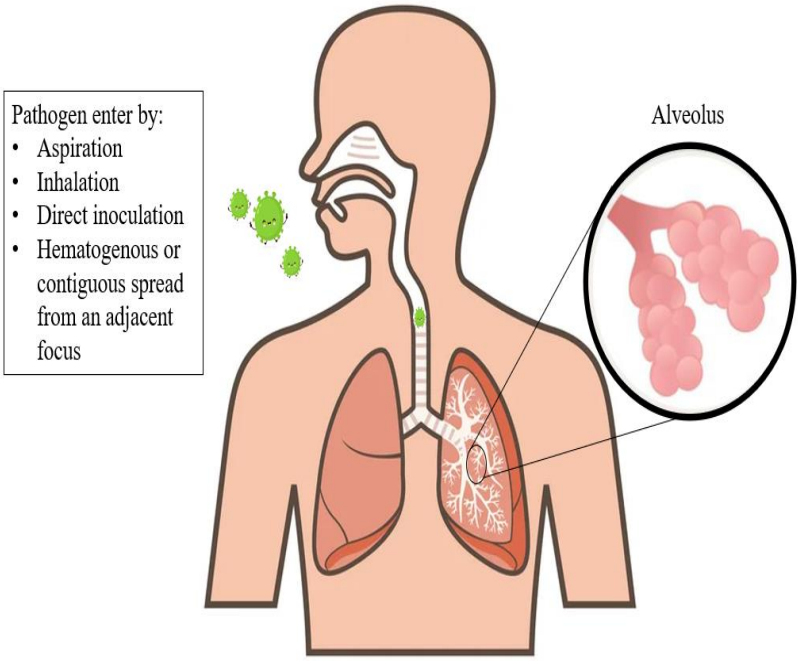


**Figure 1.** Pathogenesis of pneumonia. The host can become infected by the pathogen in a number of ways, such as through aspiration, direct inoculation, breathing in, and hematogenous or contiguous spread from a nearby focus. Once the pathogen reaches the alveoli, it multiplies and evokes a host response.

Early defenses against invading pathogens include nasal hairs and turbinates, functional cough and gag reflexes, and a branching tracheobronchial tree with an effective mucociliary clearance mechanism [23]. Pathogens that successfully reach the alveoli are further kept at bay by surfactant proteins and alveoli macrophages. If these defenses fail, and the pathogen persists and multiply, the host elicit inflammatory responses that result in many of the signs and symptoms observed in pneumonia patients. Excessive secretion of proinflammatory cytokines could worsen the disease, resulting in sepsis, shock, organ failure, or even death. Inflammatory mediators such as tumor necrosis factor (TNF), interleukin-1 (IL-1), interleukin-8 (IL-8), and granulocyte-colony stimulating factor are thought to give rise to fever and the release and attraction of neutrophils to the lung [23]. Leakage in alveolar capillary may ensue, leading to filled alveoli with possible hypoxemia. In severe conditions, patients may die from secondary changes in lung volume and compliance [25].

Overall, pneumonia establishes in patients following different stages of tissue changes. In the early stage, edema resulting from the occurrence of proteinaceous exudate in the alveoli occurs and is followed by a red hepatization stage which is caused by the accumulation of red cells. Next is the gray hepatization phase characterized by the lysis and degradation of the red cells with fibrin and neutrophil deposition. This is followed by the resolution stage involving the actions of macrophages, clearance of debris and decline in inflammatory responses [23].

## 4. What is antibiotic?

Antibiotics are drugs that help prevent bacterially-induced illnesses. To accomplish this, they either get rid of the microorganisms or stop them from replicating or reproducing. Antibiotic usually means against life [26]. A drug is classified as an antibiotic if it kills bacteria within the body. When the first antibiotics were created in the 1920s, strep throat was among the frequent mild bacterial diseases that resulted in mortality [27].

Additionally, surgery was hazardous. However, after the discovery of antibiotics in the 1940s, individuals could survive formerly fatal illnesses, live longer, and undergo safer treatments. The majority of microorganisms in the human body are benign. Some even assist in many human body functions, but practically every organ may get infected by microorganisms. Antibiotics can only treat bacterial illnesses which means they cannot be used to treat diseases caused by viruses. However, sometimes it is difficult to determine if an illness is bacterial or viral. Some antibiotics are effective against many types of bacteria and are called broad-spectrum. Others only attack certain microorganisms, called narrow-spectrum [28].

## 5. Antibiotic resistance and its effects

When administered appropriately and correctly, antibiotics become a potent weapon against bacteria [28]. However, up to 50% of all antibiotic usage is unnecessary. One of the causes of resistance to antibiotics is their overuse. Over time, bacteria evolve and transform into super bacteria or superbugs. They alter them to the point that antibiotics are no longer effective. Because there are no medications to eliminate them, they represent a serious concern. The superb method to prevent the spread of super bacteria is to use antibiotics appropriately [27].

Antibiotics are used to disallow and cure bacterial infections. Resistance to antibiotics occurs as bacteria has adjusted to the use of antibiotic. Neither humans nor animals acquire antibiotic resistance, only bacteria. These bacteria can infect humans and animals, and their illnesses are more challenging to treat than non-resistant ones [29]. Antibiotic resistance increases death, hospital stays, and medical costs. Therefore, antibiotic prescribed and consumption globally must be reformatted promptly. Even with the development of new medications, antibiotic resistance will still be a significant threat to the world. Additionally, in order to prevent the spread of infections, behavior adjustments must concentrate on enhancing food hygiene, engaging in safer sex, hand hygiene, and receiving vaccinations [27,30].

Antibiotic resistance is reaching intolerably high rates throughout the world. The emergence and worldwide dissemination of new resistance mechanisms threaten the capacity to cure widespread infectious illnesses. As antibiotics lose effectiveness, many diseases such as gonorrhea, tuberculosis, blood poisoning, and pneumonia, become harder to treat and sometimes immedicable [26]. In addition, antibiotics that can be purchased over-the-counter for human and animal usage hasten the development and spread of resistance. Similarly, doctors and veterinarians often overprescribe antibiotics in countries without standardized treatment recommendations, and the general population frequently overuses them. Without immediate action, the current situation will enter a post-antibiotic world where common illnesses and minor wounds may become fatal once more [31,32].

## 6. Prevalence of antimicrobial resistance of *S. pneumoniae*

*S. pneumoniae*, which has been identified globally as a major pathogen in CAP, is, unfortunately, resistant to many antibiotics. The initial report of *S. pneumoniae* resistance to penicillin and subsequent resistance to other classes of drugs has made the choice of antibiotic therapy difficult. The vulnerability of *S. pneumoniae* to β-lactams and macrolides has steadily decreased [33]. Resistance to fluoroquinolones, tetracycline, and trimethoprim-sulfamethoxazole (TMP-SMX) is also increasingly documented [8].

According to Cherazard et al. 2017 [8], the data obtained in the United States reported that the β-lactams prevalence rates resistance in *S. pneumoniae* range from <1 – 41.8% depending on the drugs of β-lactams itself. Penicillin varies between 13.8 – 41.8%. Cephalosporins vary between <1 – 29.9%, cefuroxime (29.9%), ceftriaxone (11.7%), ceftaroline (0 – 1%) and imipenem (23.8%). Besides that, macrolide resistance was reported to vary between 20 – 40%. Whereas the fluoroquinolones resistance in *S. pneumoniae* was between <1 – 2%, the lowest rate compared to the other two antimicrobials (Table 1).

**Table 1:** The prevalence of antimicrobials resistance in *S. pneumoniae* and the process behind its development.

| Antimicrobial agent/class | Resistance prevalence | Resistance mechanisms |
|---|---|---|
| Beta-Lactams | Penicillins<br>PCN G: 13.8%<br>PCN V: 41.8%<br>Cephalosporins: <1 - 29.9%<br>Cefuroxime: 29.9%<br>Ceftriaxone: 11.7%<br>Ceftraroline: 0 - <1% | 1) Mutation of penicillin-binding proteins<br>2) non-*pbp* genes, *murM* gene<br>3) Destruction by beta-lactamase enzymes |
| Macrolides | 20 - 40% | 1) Ribosomal alteration<br>2) Active efflux pumps |
| Fluoroquinolones | <1 - 2% | 1) Mutations in *gyrA*, *parC* and *parE* regions<br>2) Efflux pumps<br>3) Acquisition of plasmid-encoded genes |

According to a report that was obtained in the United States, the rate of resistance to β-lactams was the highest, followed by the resistance to macrolides and then fluoroquinolones. The resistance mechanisms of each drug are listed above.

## 7. Action and resistance mechanisms for β-lactams, macrolides and fluoroquinolones among *S. pneumoniae*

### a. β-lactams

A four-membered, nitrogen-containing beta-lactam ring is the structural core of β-lactam antibiotics. The terminal D-Ala-D-Ala peptide sequence, the cell wall transpeptidases' substrate, has a shape similar to that of this ring. There are four main β-lactam subgroups currently [34]. These medications target and suppress cell wall formation by attaching to the enzymes involved, and the beta-lactam ring is essential to their mechanism of action. Penicillin-binding proteins (PBPs) are the collective name for this set of enzymes attached to the cell membrane. PBPs are classified into 4-6 types based on the bacterial species. The PBPs (transpeptidases) responsible for cross-linking cell wall are usually crucial for survival [35,36].

The D-Ala-D-Ala peptide terminus, which acts as the natural substrate for transpeptidase action during cell wall peptidoglycan formation, has a three-dimensional structure mimicked by the 4-member ring of β-lactam antibiotics. These β-lactam medicines prevent the formation of cell walls by tightly binding to the transpeptidase active site [37]. The cell dies due to osmotic instability brought on by improper cell wall production, or the β-lactam binding to PBP may set off a chain of events that leads to autolysis and cell death. β-lactam medicines work against Gram-positive and Gram-negative bacteria. However, their efficacy varies due to differences in the cell walls of the two types of bacteria (for example, Gram-negative bacteria have an outer membrane, whereas Gram-positive bacteria do not) [34,38].

β-lactams antibiotics which includes penicillin, cephalosporins and carbapenems were once among the most effective antimicrobials used to treat patients infected with *S. pneumoniae* after its discovery in 1928 by Alexander Flaming. It used to be an antimicrobial that is highly susceptible to *S. pneumoniae*. However, in 1967, there was the first case reported that *S. pneumoniae* became resistant to penicillin in Australia [39]. The β-lactams' minimum inhibitory concentrations (MICs) increased over time, and numerous reports regarding β-lactams-intermediate and -resistant *S. pneumoniae* have been published. The scenario has decreased the use of β-lactams as a treatment option [40].

It has been hypothesised that at least two steps are responsible for the resistance development in pneumococcus and commensal streptococcus. The steps are the resistant

commensal streptococci selection with mutations point in their *pbp* genes and portions shifting of these resistance genes to competent pneumococci via homologous recombination (Table 1) (Figures 2 and 3) [41]. The mutations accumulate in three transpeptidases responsible in cell wall biosynthesis, namely PBPs. The overuse of the drug has led to a mutation that changes the amino acid sequences of the PBP2x, PBP2b, and PBP1a penicillin-binding proteins [42]. Even small varied fragments resulting from mutations would have a big effect in modifying the *pbp* genes, resulting in the β-lactams resistance in commensal streptococcus and *S. pneumoniae.* The mutated bacteria gain an advantage in the presence of antibiotics due to these sequence changes, which reduce the transpeptidases' affinity for the drug without affecting the enzyme's functionality [43].

In comparison to the similar regions of susceptible pneumococcus, the sequences of *pbp* genes in resistant pneumococcus strain exhibit a mosaic pattern, with variable lengths of sequences block that may diverge by up to 20% and 10% at the DNA levels, respectively [43]. The mosaic structure is due to the intraspecies and interspecies gene transfer, particularly from *Streptococcus mitis* and *Streptococcus oralis* (putative donors) which share the nasopharynx as their ecological niche. Exogenous DNA from Streptococcus that are resistant to β-lactams and occupy the same niche is integrated into the chromosome (Figure 3) [41,44].

Additionally, there is a hypothesis reported that non-*pbp* genes like the *murM* gene may have something to do with *S. pneumoniae's* resistance to β-lactams. This operon encodes transferases extending the peptidoglycan stem's L-Lys residue with an L-Ala-L-Ala cross-bridge. However, uncertainty exists regarding the exact mechanism by which these elongations contribute to β-lactam resistance [45,46]. Another route of resistance is the destruction of β-lactams by bacteria that produce beta-lactamase enzymes [47]. When beta-lactamases open the beta-lactam ring, the medication loses its ability to bind to PBPs and ceases to block the formation of cell walls. However, not all β-lactams can be hydrolyzed by all beta-lactamases. For instance, staphylococcal beta-lactamase quickly hydrolyzes penicillin and its derivatives, but it is unable to do so with several cephalosporins, including imipenem [34,35].

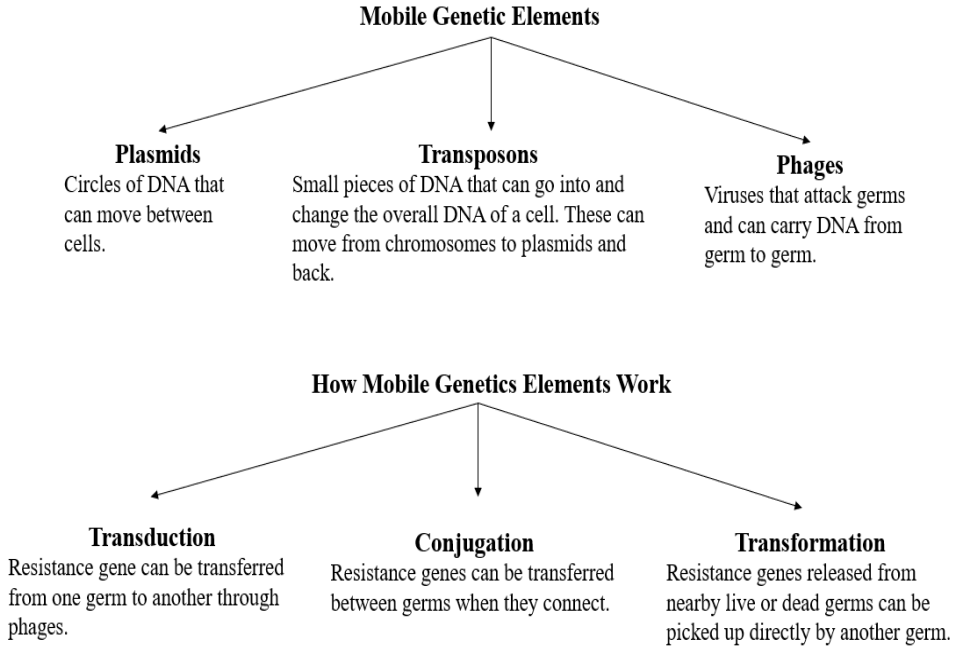


**Figure 2.** How antibiotic resistance moves directly from germ to germ. Resistance traits are transmissible from generation to generation. They can be transmitted directly between bacteria via mobile genetic components like plasmids, transposons and phages. The genetic component work by transduction, conjugation and transformation.

**b. Macrolides**

After the development of penicillin resistance in the pneumococcus, macrolides became another option to treat the pneumococcus infection. Macrolides such as erythromycin, azithromycin and clarithromycin are bacteriostatic antibiotics that bind to the 50S ribosomal subunit to suppress protein synthesis. [48,49]. The initial source of macrolides was the soil-borne bacterium *Saccharopolyspora erythraea*, also known as *Streptomyces erythreus*. Due to problems with absorption caused by Gram-negative outer membranes, macrolides are ineffective against most Gram-negative bacteria species (except enterococci). They are also effective against Legionella species, *Campylobacter* spp., *Mycoplasma* spp., *Treponema* spp., *Bordetella* spp., *Chlamydia* spp., *Chlamydophila* spp., and *Borreli* spp. [31,50].

The 23S ribosomal RNA molecule, among other ribosomal proteins, serves as a particular target for the macrolides' attachment to the 50S ribosomal subunit [51]. All macrolides suppress bacterial protein synthesis; however, they all work at various stages. The peptidyl transfer process is inhibited by 16-membered molecules, whereas the translocation of peptidyl-tRNA is blocked by 14-membered macrolides. According to the most current theory, protein synthesis is inhibited by all macrolides because they cause peptidyl-tRNA to dissociate from ribosomes during the elongation phase [52,53].

However, a rise in macrolide resistance in *S. pneumoniae* is linked to the extensive use of macrolides. It is believed that two mechanisms mainly mediate the pneumococcus resistance to macrolides; alteration of the ribosome by an enzyme encoded by the erythromycin-resistance methylase (*erm*B) gene and active efflux pumps encoded by macrolide efflux (*mef*E/*mef*A/mel) genes (Table 1) (Figure 3) [7].

**b(i). Ribosomal alteration**

*S. pneumoniae's* ribosomal methylase is encoded mainly by *erm*B, whose gene product demethylates the 23S rRNA target site. It is the most prevalent macrolide-resistance determinant gene in *S. pneumoniae* [54]. Ribosomal methylation by ErmB makes macrolide, lincosamide and streptogramin B ($MLS_B$ phenotype) become resistant. In addition, the phenotype is also responsible for a significant level of macrolide resistance [7]. In a few instances, the alteration of the binding site for macrolide and lincosamide by an *erm*B gene variant confers complete cross-resistance to clindamycin [55].

The induction of *erm*B permits high-level *ermB* translation when inducers like erythromycin are present [56]. The regulatory region situated upstream of the *erm*B gene in pneumococcus determines whether *erm*B expression is inducible or constitutively produced at high levels. The regulatory gene is comprised of a short leader peptide, known as *erm*BL, with its ribosome binding site (RBS1), a non-translational loop-stream structure, some erm*B* (*erm*B') coding sequences and its ribosome binding site (RBS2) [54,57]. Furthermore, *erm*BL2 is an additional leader peptide that is needed for erythromycin mediated induction of gene expression [58]. When *erm*B expression is inducible, it is believed that stalling of ribosome and stability of mRNA regulate its expression [59–61]. Ribosome stalling triggered by erythromycin at the tenth codon (Asp) of *erm*BL promotes a conformational transition in the mRNA, making the *erm*B ribosome binding site (RBS2) accessible to the ribosome for translation of *erm*B [57].

Through a translational attenuation, the oppression erm genes expression occurs when no inducing drug exists [54]. Stem-loop structure concealed the ribosome binding site 2 (GGAG) and AUG initiation codon of the *erm*B mRNA. Meanwhile, in the presence of erythromycin, an alternative stem-loop structure is altered, revealing the RBS2 and start codon of the *erm*B gene and inducing the *erm*B expression [57].

**b(ii). Active efflux pumps**

Antibiotic efflux is a process by which bacteria pump drugs from the inside to the outside environment utilizing efflux pumps (Figure 2) [62]. Sometimes, increased

antibiotic concentrations may get over this process, but the resistance rate is increasing [8]. Efflux pumps in pneumococcus are encoded by the macrolide efflux (*mef*E/*mef*A/*mel*) genes [7,54]. In general, the *mef*A gene encodes the formed resistance mechanism. In the past, this usually conferred a low level of resistance [8].

The *mef*E/*mel* operon codes for pneumococcal macrolide efflux and happens via a process that is not fully understood [63]. For *S. pneumoniae* to become resistant to macrolides, both *mefE* and *mel* are necessary. Both genes are located on the macrolide efflux genetic assembly (Mega) element. Their expression is driven by a promoter that is induced by macrolides of 14- and 15-membered such as clarithromycin, azithromycin, and erythromycin [54,64].

*mef*E in *S. pneumoniae* encodes a 405 amino acid protein, a part of the major facilitator superfamily, which expels molecules out of the cells using proton motive force-driven efflux. *Mel*, also called *msr*D, is a homolog of the *S. aureus* gene *mrs*A. It codes for an ATP-binding cassette (ABC) transporter protein but lacks the usual hydrophobic and membrane-binding domains. *Mel* is expected to interact with chromosomally encoded transmembrane complexes [54].

*Mef*E and *mel* confer synergistically to make the bacteria resistant to macrolides. They also are the components of efflux pump in *S. pneumoniae*. The process for macrolide efflux in *S. pneumoniae* could be presented by the movement of macrolides from ribosomes by mel, which displace the macrolide molecules to *mef*E for efflux [54,65]. Furthermore, the *mef*E/*mel* promotes resistance to the human antimicrobial peptide LL-37 which induces the expression of efflux pumps. The finding may indicate that the activation of the efflux pump undergoes throughout the nasopharyngeal colonization [66].

**c. Fluoroquinolones**

Fluoroquinolones are strong antimicrobials targeting DNA gyrase and DNA topoisomerase IV enzymes [32,67]. Gyrase modifies DNA to include negative supercoils and relieving the torsional stress that is thought to accumulate prior to transcription and replication complexes [68]. Topoisomerase IV has significant decatenation action. Given that gyrase and topoisomerase IV are crucial enzymes, it makes sense that drugs blocking them will stop bacterial growth. The fluoroquinolones, on the other hand, actively harm cells by encasing the enzymes on DNA in drug/enzyme/DNA complexes where proteins hold dsDNA breaks together [69,70].

In order to speed up the division of daughter chromosomes, DNA gyrase adds negative superhelical twists to the double helix of bacterial DNA before the beginning of replication [71]. This procedure is essential for beginning DNA replication because it makes it possible for initiation proteins to bind. The two monomeric *GyrA* and two monomeric *GyrB* subunits that make up DNA gyrase are encoded by the *GyrA* and *GyrB* genes, respectively [72]. At the end of a replication cycle, segregation into two daughter cells is made possible by decatenation, or the elimination of daughter chromosomal interlinking, carried out by topoisomerase IV. Topoisomerase IV is made up of two *ParC* (*GrlA* in *S. aureus*) subunits and two *ParE* (*Grl B* in *S. aureus*) subunits. They are produced by *ParC* and *ParE* genes, respectively, [73].

The pneumococcus resistance to fluoroquinolones is lower compared to the other two agents because the use of this antimicrobial is limited due to the development of articular cartilage damage in weight-bearing joints in animal models [74,75]. There are three primary processes by which *S. pneumoniae* develops resistance to fluoroquinolones; accumulated mutations in the bacterial genome, enhanced efflux or bacteria picking up plasmid-encoded genes (Table 1) (Figures 2 and 3) [76].

**c(i). Mutations**

The *gyrA* and *gyrB* code for DNA *gyrase*, while *parC* and *parE* code for topoisomerase IV [77]. Mutations within *gyrA*, *parC*, and *pare* can happen impromptu or step by step

[76,78]. Consequently, results in changes to the quinolone-resistance-determinant region (QRDR) of *gyrA* and/or *parC*, altering the fluoroquinolone binding site and decreasing the attachment of the drug to the enzyme-DNA complex. Resistance in ciprofloxacin is caused solely by mutations occurring at *parC*. However, resistance to the recent fluoroquinolones requires *parC* and *gyrA* mutations [8,76,78].

**c(ii). Efflux and acquisition of plasmid-encoded genes**

Another way *S. pneumoniae* acquires resistance to fluoroquinolones is by upregulating the efflux mechanism due to changes in regulatory genes [79]. It is believed that *PmrA*, a well-described regulatory gene, controls the efflux pump expression, although *PatA* and *PatB* have also been linked to the development of fluoroquinolone resistance [78,79].

Plasma-mediated resistance to quinolones happens via Qnr-protein-producing plasmid [8]. However, the mechanism of this transferrable resistance and the fluoroquinolone-resistance plasmids prevalence in clinical settings are unknown [80].

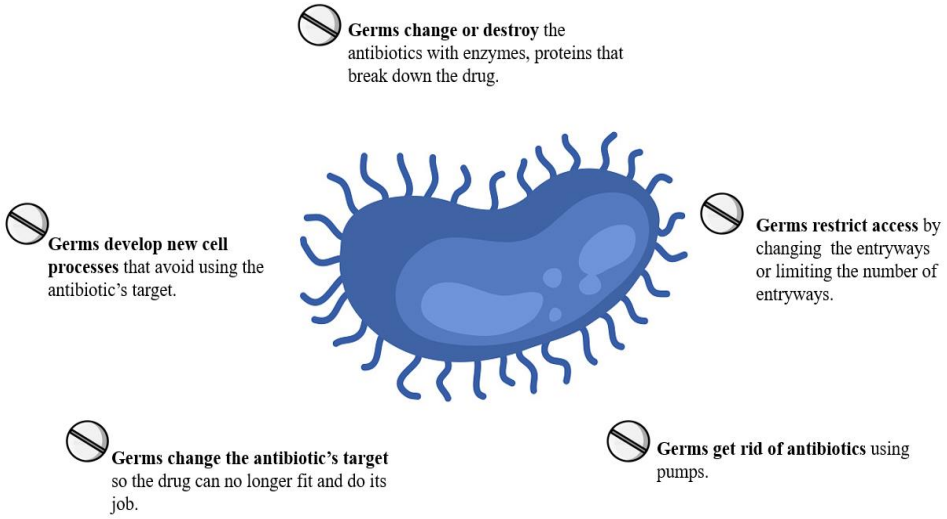


**Figure 3.** How bacteria fight back against antimicrobials. Antibiotics combat bacteria. However, bacteria adapt and discover new ways to survive. Their defensive strategies are known as resistance mechanisms.

## 8. Current trends versus new trends in terms of diagnostic

Currently, antimicrobial resistance (AMR) is typically diagnosed using two standard approaches; whole genome sequencing for antimicrobial susceptibility testing (WGS-AST) and direct AST. Unfortunately, the traditional technique of measuring antibiotic resistance levels, AST, is neither effective nor does it explain how AMR works [29,81]. WGS-AST offers a quick, reliable, and accurate diagnostic for AMR, but it needs big, high-dimensional datasets to successfully extract data. As a result, the following techniques are used to advance earlier ones using AI technology [82,83].

## 9. Role of artificial intelligence to overcome AMR

Artificial intelligence (AI) has demonstrated notable performance in controlling AMR recently. For example, AI applications based on sequencing have been used to examine AMR [84]. Additionally, obtaining clinical data to create clinical decision support systems might assist doctors in tracking AMR trends to boost the prudent use of antibiotics. Furthermore, research into synergistic medicine combinations and the development of novel antibiotics also makes extensive use of AI applications [85]. Random forests (RF),

naive Bayes (NB), decision trees (DT), support vector machines (SVM), and artificial neural networks (ANN) are some of the most used AI techniques for AMR [29,86].

Even though AI technology is growing in importance as a tool for AMR predictions, much more work has to be done in this area. Although the IR-spectrometer approach or AI-based FAST may speed up AST, their workflows are too complex for non-professional staff to employ [81]. Furthermore, generating a general WGS-AST model for multiple species with a small training dataset requires constructing a sizeable database capable of more sophisticated AI algorithms, like transfer learning and other methods [85]. This will be necessary for the future as the WGS-AST model necessitates a sizeable training dataset to optimize its essential parameters and can only be applied for particular species.

## 10. Conclusion

*S. pneumoniae* is bacteria that always find ways to evade being killed. Among the three significant antimicrobials, fluoroquinolones recorded the lowest rates for the emergence of *S. pneumoniae* resistance. There are numerous mechanisms that *S. pneumoniae* has developed to counterattack the antimicrobials (β-lactams, macrolides, and fluoroquinolones) activity. A genetic structural alteration in penicillin-binding proteins is believed to lead to the phenotypic manifestation of β-lactams resistance. The changes in the ribosomal target site and active efflux pumps are two mechanisms of macrolide resistance. Meanwhile, fluoroquinolone resistance is influenced by accumulating mutations in the bacterial genome, increased efflux, or the acquisition of plasmid-encoded genes. Although AI technology is growing for AMR, much work still needs to be done in this area. Therefore, establishing a new potent treatment is crucial to fight the bacteria.

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