Antimicrobial Resistance and Novel Alternative Approaches to Conventional Antibiotics

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Abstract: Antimicrobial resistance is a significant public health issue. The unprecedented spread of antimicrobial-resistant organisms has been identified by the World Health Organization as one of the leading healthcare threats. Projections for annual worldwide deaths attributed to antimicrobial resistance approach 10 million by 2050, with an associated economic burden of USD 100 trillion. This paper reviews the mechanisms known to contribute to antimicrobial resistance and provides insight into potential available alternatives to conventional antibiotics. Antimicrobial approaches addressed include dual antibiotic therapy, antimicrobial peptides, monoclonal antibodies, bacteriophages, probiotics, nanomaterials, and cannabinoids. Key pathogens in need of antimicrobials referred to as the ESKAPE pathogens are discussed.

Keywords: antibiotics; resistance; peptide; bacteriophage; probiotics; nanoparticles; cannabinoids

1. Introduction

Antibiotics have been the mainstay of therapy for treating microbial infections since the discovery of penicillin by Alexander Fleming in 1929 [1]. Shortly after the first antibiotics were discovered and introduced to the market, antimicrobial resistance started to develop. The mechanisms of antibiotic resistance are shown in Figure 1 [2]. For several decades, the issue of antibiotic resistance was alleviated by the introduction of new classes of antibiotics. However, the development of novel antibiotics has slowed down significantly in recent years, and as a result, antimicrobial resistance has increased exponentially [3]. Furthermore, the misuse and excessive dispensing of antibiotics in various health and agricultural sectors has exacerbated the issue and led to the development of resistant strains [4]. Bacteria undergo genetic mutations to avoid lethal damage when exposed to a threat, showcasing genetic plasticity through both genome rearrangements and the acquisition of exogenous genes via horizontal gene transfer. This adaptability plays a crucial role in antimicrobial resistance [5]. Bacteria will continue to evolve and develop resistance strategies as long as antibiotics are employed, as it is an inevitable outcome of evolution [6].

In 2017, with the escalation of antibiotic resistance globally, the World Health Organization (WHO) published a list of bacterial pathogens that are resistant to numerous conventional antibiotics. The bacteria listed with the highest priority status included Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumonieae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species and are often referred to by the acronym ESKAPE [7,8]. The matter of antibiotic resistance is especially concerning in hospital settings because nosocomial infections involve bacteria that exhibit multidrug resistance (MDR), which refers to the ability of microorganisms to withstand the effects of at least three classes of antibiotics that are intended to kill or inhibit their growth [5]. A recent study involving more than 1 million hospitalized patients in the United States revealed that ESKAPE pathogens constituted 42.2% of the species isolated from bloodstream infections. The results demonstrated that patients infected with ESKAPE pathogens experienced a 3.3-day increase in the length of stay and a 2.1% increase in mortality rates compared to...
Those infected by non-ESKAPE pathogens [9]. These virulent ESKAPE pathogens encompass multidrug-resistant properties. Despite their genetic diversity, these bacteria share resistance mechanisms that contribute to the survival of these pathogens. Understanding the mechanisms underlying bacterial resistance is important for limiting the spread of ESKAPE pathogens, mitigating the spread of antimicrobial resistance, and designing novel strategies to overcome resistance patterns [7,8].

Bacteria utilize the following two primary classifications of resistance mechanisms to counter antibiotics: intrinsic and acquired resistance [10]. With innate resistance, specific bacterial species exhibit inherent structural characteristics that provide resistance to certain antibiotics. These groups of bacteria typically lack a target site for the specific antibiotic, rendering the drug ineffective. For example, *Mycoplasma* species do not have a cell wall; thus, they are resistant to β-lactam antibiotics and glycopeptides, which primarily target bacterial cell walls. On the other hand, acquired resistance is achieved either through genome modifications or through the transfer of genetic material that confers resistance from other bacterial strains through horizontal gene transfer [10–13]. An example of acquired resistance is seen in the development of the high-level aminoglycoside resistance (HLAR) phenotype. Some species of bacteria can produce aminoglycoside-modifying enzymes, including phosphotransferases, acetyltransferases, and nucleotidyltransferases. The enzymes are located on plasmids and are readily spread between bacteria. They alter the conformation of the antibiotic, hindering its ability to bind to its target [14]. Generally, bacterial strains that display innate or acquired resistance diminish the efficacy

**Figure 1.** Mechanisms of antimicrobial resistance. Schematic representation of a bacterium in which various antimicrobial resistance strategies utilized by MDR pathogens are highlighted. A generic antibiotic is represented as a yellow hexagon and is explored in six potential scenarios, as follows: (1) changes in drug permeability following bacterial cell wall modifications and the downregulation of porins; (2) drug target modifications which prevent the binding of the antibiotic to the bacterial cell; (3) drug inactivation by enzymes by hydrolyzing the antibacterial; (4) expression and activation of efflux pumps that expel antimicrobial compounds; (5) the formation of biofilms which makes it difficult for antimicrobials to reach the bacteria; and (6) plasmid-carrying genes conferring antibiotic resistance.
of a specific antibiotic via one or more of the following strategies: (1) limiting drug uptake by decreasing the permeability of the cell wall, (2) altering the drug target, (3) modifying enzymes to inactivate the drug, and (4) activating the drug efflux pump [11]. Another protective mechanism used by pathogenic bacteria to combat antibiotics involves the formation of biofilms [3,15]. Biofilms surround the bacterial cells with a thick adhesive matrix that contains polysaccharides, proteins, and extracellular DNA of the resident bacteria. The proximity of bacterial cells within a biofilm facilitates horizontal gene transfer, making it easier for the bacteria to share resistance genes. Furthermore, the bacterial cells in biofilms tend to be sessile, and therefore antibiotics that target growing or dividing bacteria are rendered ineffective. Overall, biofilms protect the bacteria from antimicrobial agents and provide protection from the host immune response. The matrix barrier allows resistant microorganisms to grow in the presence of an antibiotic at a concentration that would otherwise be inhibitory to growth [11,12,16]. Pathogenic microorganisms, especially the ESKAPE pathogens, employ all the previously mentioned mechanisms to combat antibiotics. As a result, bacterial susceptibility to antibiotics has decreased, making it extremely challenging for clinicians to treat patients suffering from infections associated with MDR pathogens. The development of innovative antibiotics is extremely challenging due to its time-consuming processes, high costs, and the rapid emergence of resistance upon the introduction of any new antibiotic. Due to these challenges, the current pipeline for developing new antibiotics includes only 27 drug candidates that target the ESKAPE pathogens. Most of these drug candidates are modifications of previous classes that already exhibit cross-resistance [17]. With the rise of antimicrobial resistance and the difficulties associated with antibiotic discovery, the pursuit of alternatives has become an urgent medical need. Current research is focusing on investigating novel alternatives to antibiotics that may involve targeting unique mechanisms of action or will work synergistically with current antibiotics to enhance their effects [18]. Some of the proposed options for antibiotic alternatives include dual antibiotic therapy, drug-adjuvant combinational therapy, antimicrobial peptides, monoclonal antibodies, bacteriophages, probiotics, nanomaterials or nanoparticles, and cannabinoids (Figure 2).

Figure 2. Schematic representation of antimicrobial approaches and their corresponding mechanisms of action.
2. Methods

A review of previously published studies was conducted. The search terms used included the following: antibiotic efficacy, antimicrobials, probiotics, antimicrobial biologics, biofilms, quorum sensing, drug delivery, etc. MEDLINE, EMBASE, related internet websites, and reference lists were searched with a date range of 2000 to 2023 to identify appropriate papers that addressed the objectives of this review. Publications were reviewed independently by two investigators. The investigators extracted the data and inspected each reference identified by the search and applied inclusion criteria. In cases in which the same studies were reported in more than one publication, the study’s results were accounted for only once. The electronic search was followed by extensive hand searching using reference lists from the identified articles. Publications written in English were reviewed exclusively.

3. Alternatives Methods to Antibiotics in the Face of Antibiotic Resistance

3.1. Dual Antimicrobial Therapy and Drug–Adjuvant Combinations

To date, various mechanisms of bacterial resistance have been reported in nearly all antibiotics on the market [19]. The relationship between the duration of antibiotic exposure and the development of antibiotic resistance indicates the importance of using drugs with favorable pharmacokinetic properties that enable rapid access to the intended target site. Unfortunately, many available treatment options do not have these properties and alternative options must be considered. One approach to improve the susceptibility of bacteria to conventional antibiotics is the utilization of combinational therapy, which involves combining two or more antibiotics based on their susceptibility pattern so that the two agents can work synergistically. This approach is favorable because it can provoke an enhanced response [20]. Three potential mechanisms of this approach include the inhibition of a mutual target and different pathways, the inhibition of different targets and the same pathway, and the inhibition of the same target and the same pathway [4].

The first mechanism can be understood by reviewing a four-drug regimen protocol used in the treatment of tuberculosis infections caused by *Mycobacterium tuberculosis*, which is known as RIPE therapy. These drugs each have a unique mechanism of action, as follows: rifampicin inhibits RNA polymerase and prevents replication, isoniazid inhibits InhA, a key component of fatty acid synthesis that is found in the cell wall of *Mycobacterium tuberculosis*, the mechanism of action of pyrazinamide is not well understood at this time, and ethambutol inhibits arabinosyl transferases, which are key players in synthesis of the bacterial cell wall. The RIPE regimen demonstrates the benefit of utilizing different pathways in combinational therapy; because there are at least three pathways involved in the RIPE regimen, if *M. tuberculosis* can resist one pathway, several other pathways will be restricted [6,21]. An example of a combinational therapy that inhibits two separate targets in the same pathway is Bactrim, which is made up of trimethoprim and sulfamethoxazole. The components of Bactrim each inhibit different steps in folic acid synthesis within the same pathway. They target dihydrofolate reductase and dihydropteroate synthase, respectively. On the other hand, the components of Synercid, quinupristin, and dalfopristin work toward a distinct target and inhibit a single pathway. The components of Synercid bind to adjacent sites on the 50S ribosomal subunit and exhibit bactericidal activity when they work synergistically. Studies have demonstrated that Synercid is 10–100 times more effective than either of its individual components, which emphasizes the benefits of combining antimicrobial agents [21,22].

Another method utilized to combat antibiotic resistance involves drug–adjuvant combinations. Adjuvants serve as molecules that enhance the antibacterial activity of an antibiotic by directly targeting resistance mechanisms, indirectly enhancing the antibacterial effects by inhibiting efflux pumps, or disrupting bacterial signaling pathways [20]. A commonly prescribed drug–adjuvant combination is Augmentin, which is composed of amoxicillin and clavulanic acid. Pathogenic bacteria have developed the β-lactamase enzyme to protect themselves against the β-lactam antibiotics. As resistance to β-lactam
antibiotics expanded, there was an increased need for alternative therapeutic options. The addition of clavulanic acid, which is a β-lactamase inhibitor, improves the efficacy of amoxicillin by inhibiting the β-lactamase enzymes that inactivate amoxicillin. However, it is important to note that not all β-lactamase enzymes are sensitive to β-lactamase inhibitors. Therefore, there is increased research toward developing advanced β-lactamase inhibitors such as BLI-489 and LK-157 molecules, which have demonstrated promising results for the inhibition of the extended-spectrum β-lactamase (ESBL) bacteria in preclinical studies [6].

*Pseudomonas aeruginosa* bacteria develop resistance against cephalosporins by encoding AmpC β-lactamase. Avibactam is useful in this context because it enhances the activity of ceftazidime due to its ability to inhibit the hydrolytic activity of AmpC [6,23,24]. In a study conducted by Levasseur et al., the results indicated that ceftazidime in combination with avibactam was superior to ceftazidime on its own. The susceptibility of *Pseudomonas aeruginosa* to ceftazidime was reported to be 65% when the cephalosporin was used alone. However, when ceftazidime was combined with avibactam, the susceptibility increased to 94%. Overall, the selection of dual antimicrobial therapy and antibiotic–adjuvant combinations allows for therapeutic options with a broad spectrum of activity, as well as improved efficacy against resistant bacterial pathogens. Some drawbacks of this method include the increased risks of systemic side effects and the possibility of pharmacokinetic and pharmacodynamic interactions [25].

### 3.2. Antimicrobial Peptides

In the face of growing antibiotic resistance, there is increased focus on the potential application of antimicrobial peptides (AMPs) for targeting pathogenic bacteria. AMPs are short, positively charged peptides that play a role in the innate immune systems of prokaryotes, insects, plants, and higher animals [18,26]. AMP-based therapies display broad-spectrum antibacterial, antiviral, and antifungal activity and are expected to emerge as alternatives to antibiotics. To date, the FDA has granted approval for several AMPs for antibacterial treatment, while additional AMPs are currently undergoing clinical development [27]. The promising potential of AMPs as antibacterial therapeutics is attributed to their broad-spectrum bactericidal activity, coupled with the low risk for the development of resistance against peptides [28]. AMPs exert their antimicrobial effects by targeting the bilayer structures within bacterial cell walls of both Gram-positive and Gram-negative bacteria. AMPs are positively charged and therefore can interact with the negatively charged polysaccharides in the cell membranes of Gram-negative bacteria, as well as the negatively charged lipoteichoic acid in the membranes of Gram-positive bacteria [18,28].

This mechanism makes AMPs favorable antimicrobials because bacteria rarely develop resistance against this mechanism. In addition, resistance is rare for antimicrobial peptides due to the unique characteristics of these peptides. Each AMP has a distinct structure and peptide-to-lipid ratio and unique lipid membrane properties, which allow for diverse mechanisms of action. Furthermore, with progress in sequencing technologies, these AMPs can be readily modified, thereby allowing them to serve as adjuvants to antibiotics and enhance their effectiveness by overcoming any developing resistance [22]. Several AMPs have been approved by the FDA for clinical use and include gramicidin, polymyxins, daptomycin, vancomycin, oritavancin, dalbavancin, and telavancin. Gramicidin D was approved by the FDA in 1955 as a component of Neosporin for the treatment of bacterial conjunctivitis. Since its approval, gramicidin has most often been utilized to treat nasal, ocular, and throat infections, as well as surface wound infections. Polymyxins are used topically to treat eye infections, for the selective decontamination of the digestive tract (SDD), and for the treatment of infections caused by drug-resistant Gram-negative bacteria. Colistin, a polymyxin, is a cyclic lipopeptide that was approved by the FDA in 1962. Colistin works against numerous Gram-negative bacteria, including *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter* species. Daptomycin, a cyclic AMP, was approved by the FDA in 2003 for the treatment of skin and soft tissue infections caused by Gram-positive bacteria such as *Staphylococcus aureus*. Cubicin, which is a derivative
of daptomycin, was approved for the treatment of skin and soft tissue infections, as well as bloodstream infections caused by *Staphylococcus aureus*. Oritavancin, dalbavancin, and telavancin are derived from vancomycin, which was FDA-approved as an oral solution in 1983. These derivatives have greater potency compared to vancomycin, and they are effective against vancomycin-resistant bacteria. Like cubicin, oritavancin, dalbavancin, and telavancin are used to treat skin and soft tissue infections caused by Gram-positive bacteria. They received FDA approval in 2014, 2014, and 2009, respectively [22,29].

### 3.3. Monoclonal Antibodies

Monoclonal antibodies (MABs) are produced by B cells and work by selectively targeting antigens. In recent years, MABs have gained appeal as therapeutic options due to their high specificity and favorable side effect profile [30]. They play a crucial role in the treatment of cancers, autoimmune disorders, and inflammatory disorders [31]. However, to date, only three monoclonal antibodies have received approval by the FDA for the treatment of bacterial infections. These include bezlotoxumab, raxibacumab, and obiltoxaximab [32]. These MABs exhibit their antimicrobial effects by neutralizing secretory toxins. Bezlotoxumab was approved in 2017, after the completion of two phase 3 clinical trials, for the treatment of recurrent *Clostridium difficile* infections. Bezlotoxumab works by neutralizing enterotoxin B. The results of clinical studies revealed that in patients infected with *Clostridium difficile*, those who received bezlotoxumab in conjunction with antibiotics experienced a 38% reduction in reinfection rates compared to patients who received only antibiotics. Raxibacumab and obiltoxaximab were approved for the treatment of inhalational anthrax. The safety profiles of both raxibacumab and obiltoxaximab have been investigated in healthy volunteers, but they have not yet been used in clinical practice. In practice, these MABs may be employed as adjuncts to antibiotics or may be used prophylactically, offering protection to high-risk groups [31,33].

### 3.4. Bacteriophages

Bacteriophages are viral entities that invade bacteria and can be genetically modified for various antibacterial applications. Although their clinical application dates back to the beginning of the 20th century, their popularity declined a few decades later with the rise of antibiotics in the mid-20th century. Today, as antibiotic resistance becomes more prevalent, there is a rising interest in utilizing bacteriophages to treat MDR infections (Figure 3) [34,35]. The mechanistic effects of bacteriophages are unique to other antibacterial agents. The first step in bacteriophage infection is adsorption to receptors on the host’s surface in a lock-and-key interaction. After adsorption, the phage expels its genetic material into the bacterial host cell [35]. Lytic phages undergo replication inside the host bacterial cells and have the capability to produce virolysins, or lytic enzymes, that degrade peptidoglycan in the bacterial cell wall. On the other hand, lysogenic phages can be genetically engineered to express specific proteins, enzymes, antimicrobial peptides, or toxins to disrupt the normal metabolic processes in bacteria [6,18]. Phage therapy is being extensively studied on a global scale for the development of optimized phages or combinations of these phages that can be used in clinical practice. Over the past decade, several in vitro and preclinical studies have been conducted and are now being considered for entry into clinical trials [22]. An interesting approach involves utilizing the endolysins produced by phages to break down bacterial cell walls. A study conducted by Wu et al., demonstrated that the phage PD-6A3, along with its endolysin Ply6A3, improved sepsis in rodents in approximately one-third of cases involving clinical MDR strains of *Acinetobacter baumannii* [36]. Ye et al. summarized some endolysins that are currently being studied as alternatives to antibiotics to treat MDR organisms [32]. For example, an endolysin, Ply6A3, demonstrated high antibacterial activity against *Escherichia coli*, *Acinetobacter baumannii*, and methicillin-resistant *Staphylococcus aureus* (MRSA). Another endolysin, rSAL-1, has demonstrated activity against MRSA and has been developed and tested as a drug known as SAL200 to treat *Staphylococcus aureus* infections. This drug has several favorable properties, including potent bacteriolytic
effects against MDR bacterial strains and hard-to-treat biofilms. In addition, it showed no serious adverse effects in phase 1 clinical trials. SAL200 is the first endolysin-based drug to be approved for treating human skin and soft tissue infections caused by methicillin-susceptible *Staphylococcus aureus* (MSSA) and methicillin-resistant *Staphylococcus aureus* (MRSA). Ye et al. reported that the endolysin, CF-301, has demonstrated activity against *Staphylococcus aureus* and has been developed and tested as a drug known as Exebacase, which is used for the treatment of *S. aureus* bloodstream infections and is currently in phase 3 clinical studies. During phase 2, it was found that in combination with antibiotics, Exebacase improved clinical outcomes including reductions in mortality, length of hospital stays, and in-hospital readmission rates in hospitalized patients suffering from *S. aureus* bloodstream infections and endocarditis [32,37,38].

Probiotics are microorganisms that confer health benefits when administered in adequate quantities [7]. Probiotics most often belong to the *Bifidobacterium*, *Lactobacillus*, *Saccharomyces*, or *Bacillus* genera. These microorganisms provide antibacterial effects via the following mechanisms: the production of antimicrobial bacteriocins, enhancement of mucosal barriers to prevent bacterial attachment and entry into the mammalian gut, elimination of toxins, restoration of gut dysbiosis, and induction of immunomodulation by promoting protective cytokines and suppressing pro-inflammatory cytokines [6,18]. In the past century, many studies have revealed the benefits of utilizing probiotics, ranging from direct inhibition of pathogenic microorganisms to improvements in host immune system functions, thereby influencing the survival of bacterial pathogens. A study conducted by Piewngam et al. indicates the potential for utilizing Bacillus strains as a probiotic therapy to eradicate *Staphylococcus aureus*. They found that Bacillus subtilis secreted lipopeptides known as fengycins. Fengycins display antibacterial activity through the inhibition of the *S. aureus* agr quorum-sensing system, which is vital for *S. aureus* survival [39]. Lee et al. focused on the HW01 bacteriocin, produced by the *Pediococcus acidilactici* probiotic, and its activity against *Pseudomonas aeruginosa*. They found that while HW01 bacteriocin did not affect *P. aeruginosa* in its planktonic state, the bacteriocin was successful in reducing the

![Figure 3. Bacteriophage antimicrobial mechanism of action. Schematic representation of a bacteriophage infecting a bacteria cell. Stages represented include bacteriophage attachment, DNA replication, transcription and translation of the bacteriophage genes, assembly of new phage, and lysis.](image-url)
viability of biofilm cells. HW01 decreased the motility of *P. aeruginosa*, reduced the production of virulence factors, and inhibited biofilm formation [40]. In an in vivo study that was conducted, 140 species of *Lactobacillus* were tested against *Klebsiella pneumoniae*. Thirteen of the tested strains were able to prevent the formation of biofilms. In addition, they found that 1 of the 13 strains, *Lactobacillus plantarum* CIRM653, was also able to disrupt *Klebsiella pneumoniae* preformed biofilms. Similarly, Viera et al. demonstrated that *Bifidobacterium longum* 5 effectively reduced the severity of *K. pneumoniae* infections in mice and reduced the mortality rates of the studied mice by 50%. The antibacterial mechanism of *Bifidobacterium longum* 5 involves stimulating the release of pro-inflammatory cytokines, neutrophil recruitment, and reducing the bacterial load in infected lungs [41]. In a study performed by Sikorska and Smoragiewicz, the results demonstrated that several bacteriocins produced by numerous *Lactobacillus* probiotics were effective in preventing MRSA biofilm formation [42]. Several studies have evaluated the efficacy of probiotic bacteriocins in combating bacterial pathogens. The results of experimental models and clinical trials reveal that probiotics serve as promising alternatives to antibiotics; however, further research is needed to determine the preservation of cell viability and dose optimization treatment with probiotics [43].

### 3.6. Nanomaterials and Nanoparticles

Recent studies have highlighted the benefits of utilizing nanomaterials, including nanoparticles (NPs) and nano-drug carriers, to treat a wide range of infections, especially those caused by multidrug-resistant (MDR) strains. The mechanisms of nanoparticles can be classified into the following two categories: enhancing the effects of current antibiotics and exerting new bactericidal effects that are independent of existing antibiotics. In the first category, nanomaterials can serve as vehicles to deliver antibiotics into bacterial cells while bypassing cellular barriers and delivering the antibiotic to exert its effects in the cytoplasm of the bacterial target. This mechanism is favorable because it allows for the timed release of antibiotics from encapsulated nanoparticles. The slow release of the antibiotic not only lowers the required dosage and associated side effect profile but also enhances the pharmacokinetics, therapeutic index, and cost-effectiveness of the drug [18,44]. Extensive research of nanoparticles in combination with commercially available antibiotics has been conducted to determine the optimal combinations against MDR microorganisms. The preparation of the nanoparticles to serve as vehicles involves the following two types of interactions: physical and chemical. Physical interactions are based on interactions, including hydrophobic and electrostatic interactions. On the other hand, chemical interactions involve the chemical bonding between functional groups of the nanoparticles and the antibiotic agent. For example, hydrogen-functionalized nanoparticles are conjugated with the drugs with an amine group, aldehyde-functionalized NPs are conjugated with the drugs with a hydrazide group, and gold NPs are conjugated with the drugs containing sulfhydryl groups [45]. In the second category, nanomaterials exert bactericidal effects through physical and biochemical processes [44]. Bacterial cell walls are typically negatively charged. Therefore, nanoparticles are usually designed to encompass a positively charged polymer coating to allow for electrostatic interaction when the NP encounters the anionic cell membrane. This interaction leads to destruction of the bacterial cell membrane, prevents bacterial proliferation, and prevents biofilms from forming. Nanoparticles are often fused with metals (e.g., silver, gold, aluminum, and copper) to induce these bactericidal effects. Gold, silver, and copper nanoparticles cause physical damage to the cell wall of the host followed by cell lysis. Silver nanoparticles produce reactive oxidative species that damage peptidoglycan structures found in bacterial cell walls [32]. Researchers are investigating the effectiveness of various nanoparticle-based metal alloys and nano-drug carries to combat MDR bacteria, as well as their potential to inhibit biofilm formation [18]. An in vitro study [22] revealed that chemically synthesized gold nanoparticles were effective against numerous drug-resistant bacterial strains, including *Staphylococcus aureus*, *Enterococcus faecium*, *Enterococcus faecalis*, *Escherichia coli*, *Vibrio cholerae*, and *Salmonella typhimurium*. Zinc oxide nanoparticles have shown antimicrobial activity against Gram-negative bacteria, including *Escherichia coli* and
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Pseudomonas aeruginosa, as well as Gram-positive bacteria, including Bacillus subtilis and Staphylococcus aureus [20]. Likewise, Tiwari et al. demonstrated that chemically synthesized zinc oxide nanoparticles were successful in inhibiting the growth of carbapenem-resistant Acinetobacter baumannii strains [46]. Titanium dioxide nanoparticles have been studied for their unique antimicrobial properties, as well. Studies have revealed that these NPs have strong antibacterial activity against Staphylococcus aureus and Escherichia coli [20]. Overall, researchers have identified antibiotic–nanoparticle conjugates as an innovative category of antimicrobial agents that address the challenges posed by MDR bacterial infections. The unique properties of nanoparticles allow for extended binding, the precise targeting of antibiotics at the target site, and shielding from lytic enzymes. As a result, when the antibiotic is delivered by an NP, a higher concentration of the drug reaches the bacterial cell, thereby eliminating higher dose requirements and reducing negative side effects [45].

3.7. Cannabinoids

As ongoing research focuses on developing new alternatives to antibiotics, compounds with previously described antimicrobial effects are being reassessed as potential therapeutic agents in treating infectious diseases. The antimicrobial characteristics of cannabinoids have been recognized since the middle of the 20th century [47]. Researchers have started to reinvestigate natural products as they work to address the prevailing antibiotic crisis because natural products have consistently served as a valuable source for the identification of antimicrobial drug candidates [48]. In recent years, there have been many studies conducted to evaluate the medicinal applications of cannabinoids, which are found in Cannabis plants (Figure 4). The results of numerous preclinical safety studies and clinical studies highlight the potential antimicrobial applications of multiple substances found in Cannabis plants [49]. Cannabinoids can be categorized as either endogenous or exogenous when produced synthetically. Both exogenous and endogenous cannabinoids interact with CB1 and CB2 receptors [50]. In the treatment of bacterial infections in animal models, the results demonstrated that exogenous cannabinoids, particularly tetrahydrocannabinol (THC), can minimize resistance to numerous pathogens, including Listeria monocytogenes, Treponema pallidum, Legionella pneumophila, and Staphylococcus aureus [51]. Another exogenous cannabinoid, cannabidiol (CBD), has been studied in numerous in vitro studies. The results of these studies demonstrated that CBD has both bacteriostatic, as well as bactericidal, activity against several bacteria, including methicillin-susceptible Staphylococcus aureus (MSSA), methicillin-resistant Staphylococcus aureus (MRSA), Streptococcus mutans, and Streptococcus faecalis [48,49]. Similarly, in a study conducted by Luz-Veiga et al., the results indicated that CBD displayed antimicrobial effects against Pseudomonas aeruginosa and Escherichia coli [52]. Cannabinoids are useful against MDR pathogens because they can prevent biofilm formation, minimize biofilm metabolic activity, and prevent bacterial cell survival within the biofilm [49]. In addition, cannabinoids can be useful for enhancing the activity of current antibiotics when used synergistically with antibiotics. For example, CBD exhibited synergistic effects when combined with ampicillin to combat MRSA biofilms. Likewise, when CBD was utilized as a synergistic agent with erythromycin, vancomycin, rifampicin, or colistin, CBD’s antimicrobial effects were amplified against target pathogens such as E. coli and MRSA. Cannabinoids have also been studied for their antifungal potential. Research indicates that Cannabis metabolites can also be used to inhibit pathogenic fungi; however, their antifungal properties have not been as extensively investigated as their antibacterial properties [47]. Overall, these results highlight the broad applications of cannabinoids as antimicrobial therapeutic alternatives.
4. Conclusions

Antimicrobial resistance is a serious global health problem. Several factors are responsible for the resistance, including the following: 1. the decline in the development of new novel antibiotics; 2. the large-scale use of triclosan in consumer products, including toothpaste and cleansers, has negatively impacted antibiotic efficacy; 3. the change in the gene expression of bacteria cells dispersed from a biofilm has resulted in the prevalence of more virulent microbes. Collectively, the mechanisms responsible for the increase in antimicrobial-resistant microbes clearly suggests that alternative antimicrobial therapeutic options need to be explored and developed. A focus is needed to develop biofilm-dispersing agents that will discharge planktonic microbial cells to a more vulnerable planktonic state. On the positive side, good progress has been made with natural products, including bacteriocins, probiotics, bacteriophages, and cannabinoids, as well as synthetic entities nanomaterials, and nanoparticles. A concerted effort is needed to transition the alternative antimicrobial platforms from research potential to patient use in a timely manner.

Funding: This research received no external funding.

Conflicts of Interest: The authors declare no conflict of interest.

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