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# *Staphylococcus aureus bacteriophage: Antimicrobial potent agent for biocontrol*

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## Abstract

Nowadays, the world is witnessing the threat of the ineffectiveness of currently prescribed antibiotics to treat pathogenic bacteria. as a result of the intensive use of antibiotics in medicine and agricultural fields. Therefore, bacteria have developed a resistance to antibiotics. *Staphylococcus aureus* was the first bacterium to show resistance to antibiotics in the mid-1940s against penicillin. Later on, *S. aureus* developed resistance to many different antibiotics, such as methicillin-resistant *S. aureus* (MRSA). Therefore, bacteriophages are good, effective alternatives, and they demonstrate a high ability to kill bacteria, including *S. aureus* bacteriophages and are considered excellent biotic mortality agents and a novel biocontrol method versus antibiotics. In this review paper, we highlighted the pathogenicity of *S. aureus* and its ability to resist various types of antibiotics, as well as the application of different phages against *S. aureus* bacteria.

**Keywords:** *Staphylococcus aureus*, Antibiotic resistance, Bacteriophages, Phage therapy.

## Introduction

Antibiotics are considered one of the most important discoveries in medicine. Antibiotics have helped save millions of lives since the production of the first antibiotic. Penicillin was the first invented antibiotic, and it was produced on a large scale during World War II and used to treat injured soldiers[1]. Since that time, large-scale production of effective antibiotics has been intensively used, which has led to the development of resistance to a wide range of antibiotics[2].

*Staphylococcus aureus* is one of the most significant pathogens that has the ability to infect different parts of the human body and cause serious diseases. This bacterium can develop rapid resistance to antibiotics. Methicillin-resistant *Staphylococcus aureus* (MRSA), for example, is a strain of *S. aureus* that has developed multiple drug resistance against beta-lactam antibiotics[3]. Therefore, scientists are working hard to find effective alternatives to eliminate bacterial infections. Bacteriophages are a good example of active alternatives that have been studied intensively[4]. Therefore, many bacteriophages have been isolated and well characterized, and they could be a promising biocontrol agent. Some of them have reached Phase I and II clinical trials[5].

## *Staphylococcus aureus* bacteria

*Staphylococcus* is a gram-positive, sphere-shaped, non-spore-forming bacterium around 0.5–1.5 µm in diameter[6]. It grows under aerobic conditions, but it is also a facultative anaerobe, which means it has the ability to grow without oxygen under anaerobic conditions by fermentation[7]. It can grow at a temperature range of 15–45 °C, and the optimum temperature is 37 °C[8].

The *Staphylococcus* name came from Greek names: staphyle means bunch of grapes, and kokkos refers to a berry[10]. *S. aureus* was first observed in 1881 in a surgical abscess by Alexander Ogaston, who was a Scottish surgeon[9]. The *Staphylococcus aureus* bacterium was first isolated as a pure culture in 1886 by a German surgeon named Anton J. Rosenbach, who gave the name "aureus" to the bacteria according to the bacterial colony color, where "aureus" in Latin refers to "gold-colored".

## Pathogenicity of *Staphylococcus aureus*

*Staphylococcus aureus* is present as normal flora in humans and animals[11]. However, it can cause a variety of infections for both humans and animals[6]. It could be cause simple infections such as superficial skin infections like scalded skin syndrome, boils, abscesses, Wound infections[14] and cellulitis[12]. *S. aureus* causes many other infections, such as food poisoning, urinary tract infections, respiratory tract infections, osteomyelitis, and endocarditis. In some cases, it can lead to deadly infections[13].

## Virulence factors of *Staphylococcus aureus*

*Staphylococcus aureus* causes a wide spectrum of infections in humans and animals due to its virulence characteristics that allow it to cling to surfaces, penetrate or escape from the host immune system, and create detrimental toxic effects on the host[15,16]. It produces a variety of virulence factors that can be mainly classified into different categories, such as toxins and exoenzymes[16].

### - Toxins and superantigens

*S. aureus* creates different types of cytotoxins, such as α-toxin, which play a role in the lysis of several cell types, like erythrocytes, endothelial cells, platelets, and epithelial cells. The bicomponent pore-forming toxins, LukAB, and phenol-soluble modulins (PSMs), are other types of cytotoxin. On the other hand, T cell superantigens (SAgs) and B cell superantigen-protein A (SpA) are examples of staphylococcal superantigens.

### - Exoenzyme

The virulence factors that are produced by *S. aureus* with enzymatic characteristics can also be divided into two categories:

## I. Cofactors

Cofactors play a role in the activation of host zymogens, for instance, staphylokinase (Sak), coagulase (Coa), and von Willefactor binding protein (vWbp). Staphylokinase (Sak) plays a role as a cofactor in activating plasminogen, which degrades fibrin clots.

## II. Enzymes

*S. aureus* produces many different enzymes that are correlated with bacterial pathogenicity, such as nucleases, proteases, hyaluronidase, and lipases that break down host cellular components into small parts(Figure 1) [16].

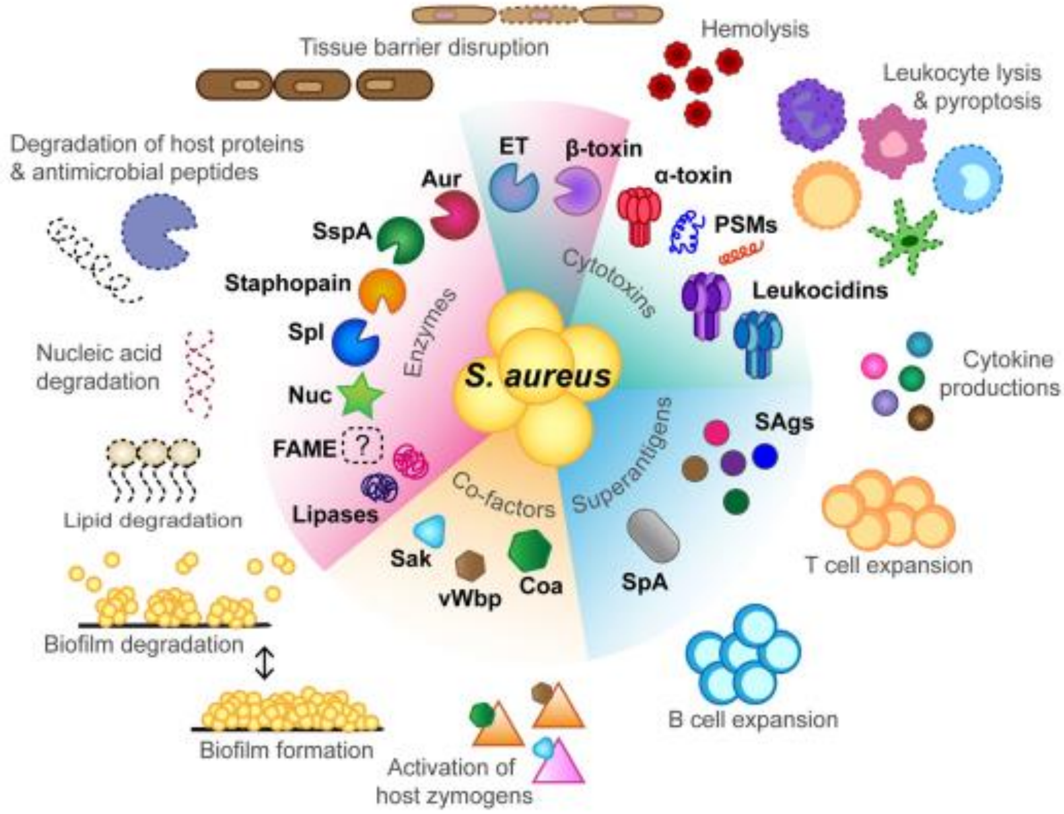


**Figure 1:** Virulence factors of *Staphylococcus aureus* [16].

## Treatment of *Staphylococcus aureus* and antibiotic resistance

Several different antibiotics have been used to treat *S. aureus* infections. Penicillin was the first antibiotic used to cure a disease caused by *S. aureus*. Later on, many different antibiotics have been developed and are commercially produced[17]. *S. aureus* is one of the most important organisms in terms of resistance to antibiotics. *S. aureus* has progressively developed variegated resistance mechanisms since the 1940s. It was the first bacterium that developed resistance to penicillin at that time (Figure 2). Later on, *S. aureus* has shown resistance to a variety of antibiotics, such as β-lactams, aminoglycosides, tetracyclines, fluoroquinolones, trimethoprim-sulfamethoxazole, clindamycin, vancomycin, linezolid, and daptomycin (Table 1).

In 2015, methicillin-resistant *S. aureus* (MRSA), the most common antibiotic-resistant bacterium, was blamed for over 148,000 infections, including 7000 deaths, within the EU[17]. A study on antibiotic susceptibility of *S. aureus* showed several isolates were multi-drug resistant (MDR)[18].

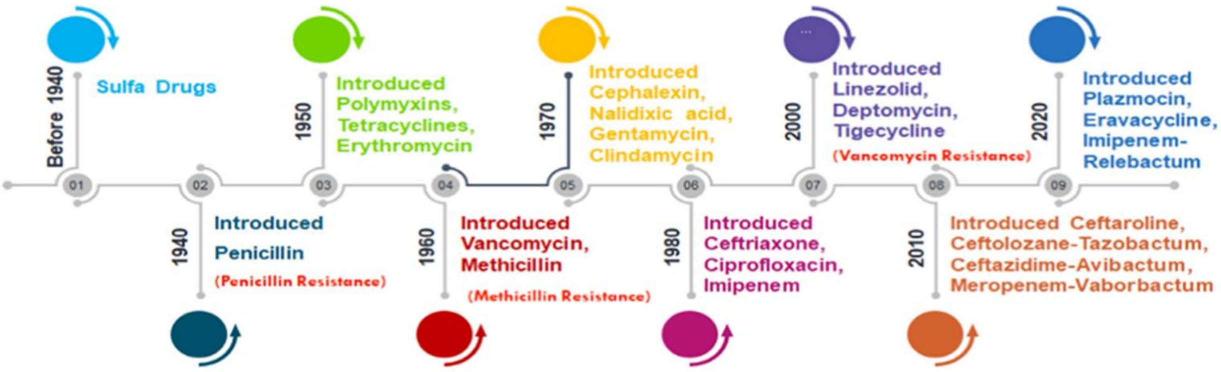
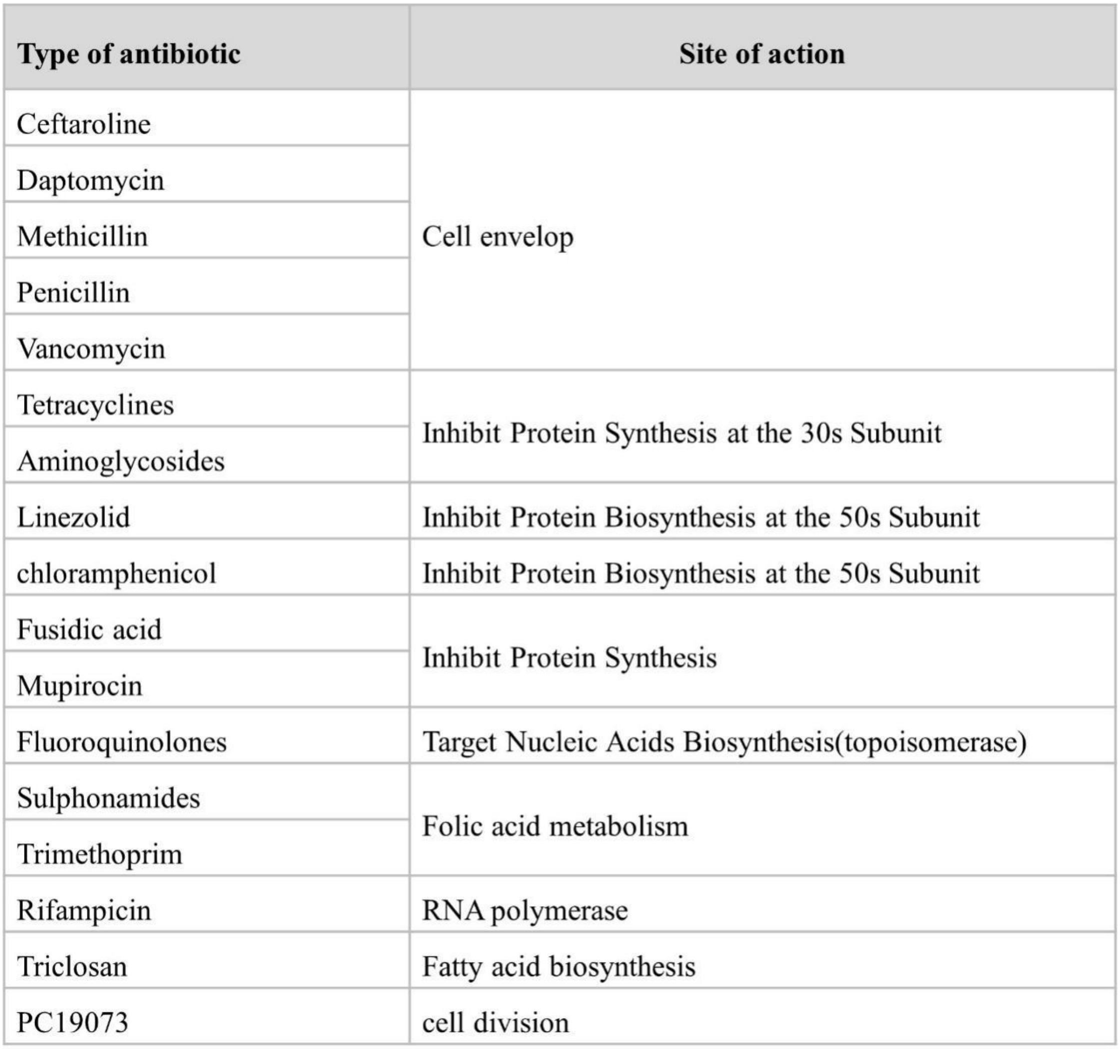


**Figure 2:** a schematic the development of antimicrobial drugs over time, followed by the evolution of resistance[19].

## Methicillin-resistant *S. aureus* (MRSA)

As new *S. aureus* clones continue to appear widely, the prevalence and epidemiology of methicillin-resistant *S. aureus* (MRSA) are constantly shifting. Through horizontal gene transfer, some S. aureus strains have been able to get the staphylococcal cassette chromosome mec (SCCmec), which has allowed them to become methicillin-resistant and evolve into MRSA[3]. The *mecA* gene, which encodes the modified penicillin-binding protein (PBP2a), is found in all SCCmec types. So far, twelve SCCmec types (II-XIII) have been described, among them types I, II, and III are the larger types and carry additional resistance genes against several antibiotics, which are predominantly associated with hospital-acquired MRSA strains[20]. Along with *mecA* gene, *mecC* has been reported in various *S. aureus* isolated from both humans and animals. The *mecC* gene was first described in MRSA strain LGA251, and it encodes a distinct protein called PBP2aLGA[21]. In addition, *mecB* gene was detected in a plasmid in MRSA isolated from a patient[22].

Table 1: List of antibiotics that have been used against *S. aureus*[17,23]



| Type of antibiotic | Site of action |
|---|---|
| Ceftaroline | Cell envelop |
| Daptomycin | |
| Methicillin | |
| Penicillin | |
| Vancomycin | |
| Tetracyclines | Inhibit Protein Synthesis at the 30s Subunit |
| Aminoglycosides | |
| Linezolid | Inhibit Protein Biosynthesis at the 50s Subunit |
| chloramphenicol | Inhibit Protein Biosynthesis at the 50s Subunit |
| Fusidic acid | Inhibit Protein Synthesis |
| Mupirocin | |
| Fluoroquinolones | Target Nucleic Acids Biosynthesis(topoisomerase) |
| Sulphonamides | Folic acid metabolism |
| Trimethoprim | |
| Rifampicin | RNA polymerase |
| Triclosan | Fatty acid biosynthesis |
| PC19073 | cell division |

## Bacteriophage

Bacteriophages (Phages) are indeed the simplest and most abundant biological creatures on the planet. There are approximately $10^{31}$-$0^{32}$ viral particles on the Earth. Therefore, they are the world's largest repository of genetic material[24]. However, phages, like other viruses, cannot replicate on their own; they need a host. Therefore, as phages, they use bacterial cells exclusively as a host. Regarding their structure, phages are generally composed of genome DNA or RNA (double or single stranded) encased in a capsid protein shell[25]. The majority of them are composed of a basic head and tail. Despite their basic structure, bacteriophages have a broad range of morphology, size, and genetic variation. Some of them have spherical, icosahedral, or cubic heads that are connected to tails, while others are filamentous but less common[26]. Phages, despite their appearance, are non-motile, and they are only transmitted through Brownian motion[25]. With regard to phage specificity, some phages can infect very limited numbers of bacterial hosts, while others can infect a wide range of hosts. The major factor that determines the phage's specificity is receptors on the host cell surface[27].

## Phage therapy

Phages are the most interesting antibiotic alternatives that can be employed in medicine and other fields[4]. According to the National Institutes of Health in the United States, phages are promising methods for fighting antimicrobial resistance (AMR)[28]. Phages do not infect humans, animals, or plants; therefore, it is considered safe to use as antibacterial agents. In the last two decades, intensive studies have been done on bacteriophages, especially after the rise of the global antibiotic resistance problem[29]. These studies included isolation and characterization of new bacteriophages as well as studies on their effectiveness, specificity, safety, and toxicity[29]. The FDA and European Medicines Agency (EMA) reported that the phage production have to be produced according to GMP standards[29].

## *Staphylococcus aureus* phages

*Staphylococcus aureus* is infected by numerous viruses known as bacteriophages (phages), most of which possess a double-stranded DNA genome that belongs to the order Caudovirales[30,31]. The Caudovirales order is divided into three families: *Siphoviridae*, *Myoviridae*, and *Podoviridae*, as shown in Figure 2. The *Siphoviridae* family is widely available and characterized by an icosahedral capsid and a non-contractile tail varying from 130 to 400 nm. Phages belonging to the *Siphoviridae* family have a moderate genome size of around 40 kb. In contrast, the Phages classified under the second family *Myoviridae* have an icosahedral capsid, a long contractile tail, and a large genome of more than 125 Kb. The last family is called *Podoviridae*, which has a small isosahedral capsid and a short, non-flexible, and non-contractile tail. The phages belong to this family, characterized by a small genome size, approximately < 20 Kb[32].

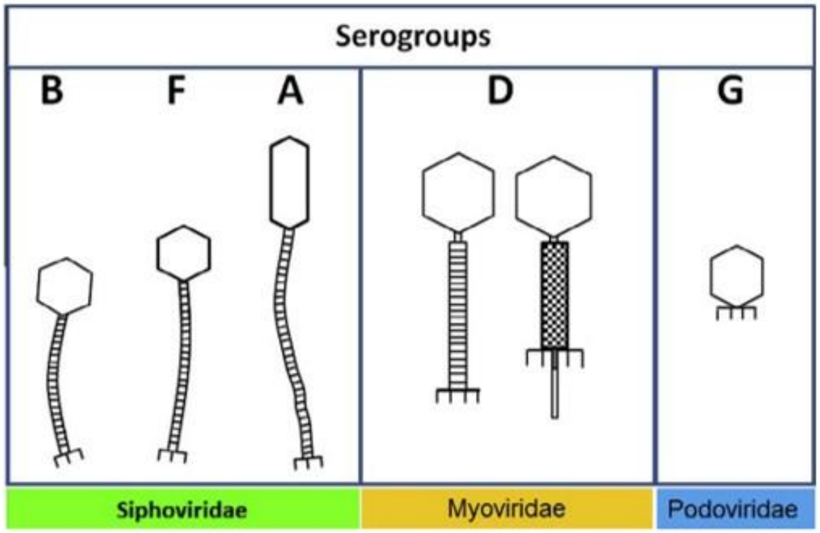


**Figure 3:** *Staphylococcus* phage families[33].

Until now, numerous phages belonging to three *S. aureus* have been isolated from different environments. These isolated phages have been well characterized and used to treat *S. aureus* infections in animals and humans. The phage features vary according to the family to which they belong. For instance, phages belong to *Siphoviridae*, characterized by an infective limit host number, which means these phages have a narrow host range. In addition, the phage integrates into the host genome; consequently, the phage does not lyse the host, and is called a temperate phage. As wll as *Podoviridae* family members are prophage integrated into host genome. In contrast, the *Myoviridae* family members have a wide host range and have lysed the host; for this reason, the phages belonging to this family prefer to use them in phage therapy[34]. Several bacteriophages belong to different families that have been used *in vivo* (in animals and humans) to treat *S. aureus*. Among the *Myoviridae* family, include Phage-Sa36, phage Sa83, phage Sa87, phage StaPh_1, StaPh_3, StaPh_4, StaPh_11, StaPh_16, and phage JD007[35-37]. Phages belonging to the *Siphoviridae* family include phage MR11 and phage MSa[38,39]. As well, the *Podoviridae* family, such as phage S13′[40].

## Phage-based strategies against *Staphylococcus aureus*

### I. Genetically engineered phages

Recent advancements in genomic and synthetic biology have significantly enhanced the development of bacteriophage therapies against *S. aureus*[41,42]. Kiga The CRISPR-Cas system used to alter phage genomes[43]. The CRISPR-Cas9 system has been used to target resistance *mecA*, *aacA*, and *grlA*, *grlB* genes in *S. aureus*, which lessen the resistance of bacteria to various antibiotics[44]. Engineered phages with the CRISPR-Cas system capable of killing cultures of S. aureus for about six hours[45].

### II. Phage-antibiotic synergy

Phages combined with antibiotics increase bacterial killing rate and alleviate resistance progress[46]. A research paper reported that the combination of vancomycin with phage K decreases the MIC 16- fold[47]. In another study, a combination of oxacillin with phage Sb-1 enhances the antimicrobial activity against MRSA[48].

### III. Biofilm disruption by phages

Biofilm formation of *S. aureus* complicates treatment because of its resistance to antibiotics. Bacterial biofilms can be penetrated by phages, which lead to biofilm degradation and bacterial lysis. depolymerases are enzymes produced by some phages, which lyse the extracellular matrix and eventually lead to bacterial abolition[49].

## Challenges of Phage Therapy

Phage therapy challenges before applying in clinical trials are as follows:

### I. Bacterial resistance

Development of bacterial resistant mechanisms, such as phage receptor altering, engaging CRISPR-Cas systems to lyse the phages, and production of proteins that inhibit phage replication. To overcome this problem, a phage cocktail could be used to lessen the development of bacterial resistance development[50].

### II. Manufacturing and stability

Preparations of therapeutic phages for bacterial eradication pose significant difficulties to ensure consistency, purity, and potency. Bacterial endotoxins removal is one of the issues to prevent side effects in patients. Furthermore, preserving the constancy of phage preparations during storage and transport needs to optimize conditions such as pH and temperature to maintain phage viability[51].

### III. Host immune response

The human immune system has the ability to recognize bacteriophage as foreign antigens. After administration of phages into the human body (orally, intravenously, topically, or inhalation), they may trigger the innate immune response such as phagocytosis and the activation of complement system, which participated in rapid clearance of phages[52]. Prolonged and repeated exposure can enhance an adaptive immune system leading to the production of antibodies (IgG and IgM), which neutralize phage particles[53].

These limitations can be passed by encapsulation, phage cocktail, and engineering of phages to be less immunogenic[54].

## Conclusions

Antibiotics are considered a crucial factor in fighting pathogenic bacteria. Therefore, they have been widely used to treat bacterial infections for a long time. Recently, most of these antibiotics have lost their effectiveness as a result of the development of resistance by some bacterial strains. A million people are infected with antibiotic-resistant bacteria every year. *S. aureus* is a good example of such bacteria that have been shown to have resistance to many different antibiotics. For instance, methicillin-resistant *S. aureus* (MRSA) is multidrug resistant and has become a real problem, especially in hospitals. Therefore, phage therapy at the moment is worthwhile to focus on to find a good alternative to curing multidrug-resistant bacteria. Presently, many projects around the world are running to find active and safe phage products that comply with the requirements of the USA Food and Drug Administration (FDA). Currently, we have finished working on isolation and characterization of a promising and novel bacteriophage that has shown potency toward *S. aureus* and, to a lesser extent, other gram-positive and gram-negative bacteria.

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## Author’s Declaration

- We hereby confirm that all the Figures and Tables in the manuscript are original and have been created by us.
- We have obtained ethical clearance for our study from the local ethical committee at Al-Nahrain University/College of Biotechnology. This approval underscores our commitment to ethical research practices and the well-being of our participants.
- Ethical Clearance: The project was approved by the local ethical committee at Al-Nahrain University/College of Biotechnology, ensuring adherence to ethical standards and the protection of participants' rights and welfare.

## Author’s Contribution Statement

All authors participated in the collection of literature reviews, wrote the draft of the review article, and revised it.

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