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Chapter

Photodynamic Treatment of *Staphylococcus aureus* Infections

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Abstract

Introduction: *Staphylococcus aureus* is a Gram-positive coconut that causes various life-threatening infections and, in turn, represents a major producer of healthcare-associated infections. This pathogen is highly resistant to antibiotics, which has made it difficult to eradicate in recent decades. Photodynamic therapy is a promising approach to address the notable shortage of antibiotic options against multidrug-resistant *Staphylococcus aureus*. This therapy combines the use of a photosensitizing agent, light, and oxygen to eradicate pathogenic microorganisms. The purpose of this study is to provide relevant bibliographic information about the application of photodynamic therapy as an alternative antimicrobial therapy for *Staphylococcus aureus* infections. Methods: This review was achieved through a bibliographic search in various databases and the analysis of relevant publications on the subject. Results: A large body of evidence demonstrates the efficacy of photodynamic therapy in eliminating biofilm- or biofilm-producing strains of *Staphylococcus aureus*, as well as antibiotic-resistant strains. Conclusion: We conclude that photodynamic therapy against *Staphylococcus aureus* is a recommended antibacterial therapy that may complement antibiotic treatment.

Keywords: photodynamic therapy, *Staphylococcus aureus*, antibiotic resistance

1. Introduction

Staphylococci are a large group of gram-positive cocci, whose diameter varies from 0.5 to 1.5 μm whose grouping resembles grape Clusters. To date, 35 known species and 17 subspecies of the genus *Staphylococcus* have been reported [1]. *Staphylococcus aureus* (*S. aureus*) is a bacterium with wide dissemination; Although it is part of the human body's commensal microbiota, it can cause severe skin infections, localized abscesses, and also may cause osteomyelitis, endocarditis, and other life-threatening diseases. Also, *S. aureus* has become a significant cause of healthcare-associated infections (HAIs) [2]. Besides, *S. aureus* acts as an early colonizer, creating a favorable environment for the adhesion and colonization of bacteria producing biofilms (BF). BFs consist of an array of proteins and polysaccharides that form an extracellular matrix (**Figure 1**). This matrix is considered an essential virulence factor of *S. aureus* strains, as it functions as a barrier against antimicrobial agents and the host's immune system, helping to maintain bacterial colonization [3, 4].

Since the discovery of antibiotics and their application, many bacterial infections have been successfully treated. However, in recent years the resistance of bacteria to antibiotics is emerging and increasing rapidly. *S. aureus* has progressively
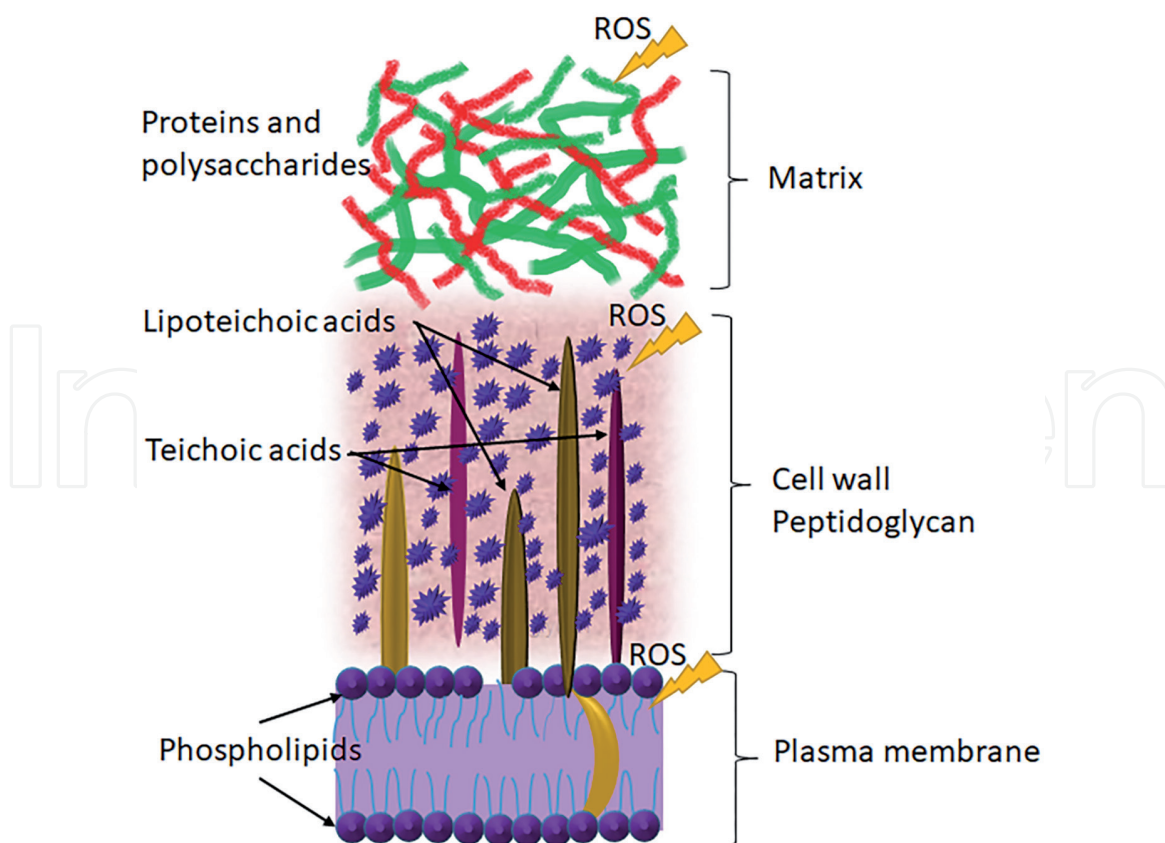
Figure 1.
Target structures from the Gram+ envelope for Photodynamic inactivation. Photodynamic therapy generates ROS that acts unspecifically on macromolecules present in the envelope of Gram + bacteria, such as lipids and proteins of the plasma membrane, peptidoglycan of the cell wall, and the array of proteins and polysaccharides macromolecules of the matrix that forms the biofilm.

gained multiple resistance to antibiotics, such as penicillin, methicillin, and other multiple drugs, leading to infections with frustrated or ineffective antibiotic therapies [5, 6]. The scarce development of new antibiotic added to the progressive increasing in multidrug-resistance of this and other clinically relevant bacteria has been considered by the World Health Organization (WHO) as one of the most pressing global threats to human health in the 21st century and described the situation as a global crisis and impending catastrophe of a return to the pre-antibiotic era. In this regard, the WHO published a list of microorganisms that should be investigated with priority to generate new antimicrobial drugs [7]. According to this list, *S. aureus,* with resistance to methicillin or vancomycin, is ranked second with high priority [7].

S. aureus displays various resistance mechanisms; for example, resistance to penicillin is mediated by hydrolytic enzymes called beta-lactamases. Beta-lactamases confer resistance to all penicillins except isoxazolyl penicillins (oxacillin, methicillin, cloxacillin, and nafcillin), as well as sensitivity to combinations of beta-lactams with beta-lactamase inhibitors (clavulanic acid, tazobactam, and sulbactam), or cephalosporins and carbapenems [8]. Resistance to methicillin, nafcillin, and oxacillin is independent of beta-lactamase production. This resistance is mediated by the *mec*A gene acquisition, which is translated into a new penicillin-binding protein (PBP2a). PBP2a decreases *S. aureus*'s affinity for methicillin and, therefore, allows it to survive treatments with this antibiotic [9]. Strains of *S. aureus* that express the *mec*A gene are called methicillin-resistant *S. aureus* (MRSA). MRSA strains are resistant to all beta-lactams (except 5th generation cephalosporins) and usually to aminoglycosides, erythromycin, clindamycin, tetracyclines, sulfonamides, quinolones, and rifampicin. While colonization of MRSA in a healthy

individual is generally not serious, it can be life-threatening for patients with deep wounds, intravenous catheters, or other invasive instruments, as well as secondary infections in patients with a weakened immune system. Following the guidelines of the Clinical and Laboratory Standards Institute (CLSI), there are strains of *S. aureus* that present low-level or borderline resistance, for example, to oxacillin (BORSA) or vancomycin (VISA). The BORSA is characterized by a minimum inhibitory concentration (MIC) of oxacillin at the resistance cut-off point (4 mg / L) or a dilution above it [8]. *S. aureus* may present resistance to glycopeptides when it presents a MIC of vancomycin (VAN) of 4–8 mg/L. Furthermore, it is considered resistant with a MIC of NPV $\geq$ 16 mg/L [10]. The MIC should be determined using the broth microdilution method, according to CLSI.

Due to all those mentioned above, there is a challenge in urgently searching for new antimicrobial approaches to treat bacteria without producing resistance to antibiotics. Several new strategies have been developed, such as metallic nanoparticles, cationic polymers, peptidoglycans, nanocarriers, photothermotherapy and photodynamic therapy (PDT). Due to its demonstrated antitumor activity, PDT has been strongly developed to treat cancer, although not so much in its antimicrobial activity. Some studies have shown that PDT successfully reduces the biological activity of specific virulence factors produced by Gram-negative strains, and therefore, the analysis of the efficacy of this therapy in Gram-positive bacteria is essential [3, 4].

PDT is based on the use of photosensitizer molecules (PS) that produce local cytotoxicity after being activated by light (photo-oxidative stress). PS compounds absorb energy from visible light of a specific wavelength and transfer it to molecular oxygen, producing reactive oxygen species (ROS). **Figure 2** shows the mechanism for ROS production, which could be by electron transfer (e-) to produce superoxide ($O_2^{\bullet-}$) or by energy transfer, which produces highly reactive singlet oxygen ($^1O_2$). ROS production induces nonspecific bacterial death [12].

Very few initiatives have studied the information described to date on PDT antimicrobial therapy against *S. aureus* infections, and a bibliographic exploration of this strategy is relevant. The present review is a bibliographic study of the information available on the application of PDT against strains of *S. aureus* with particular
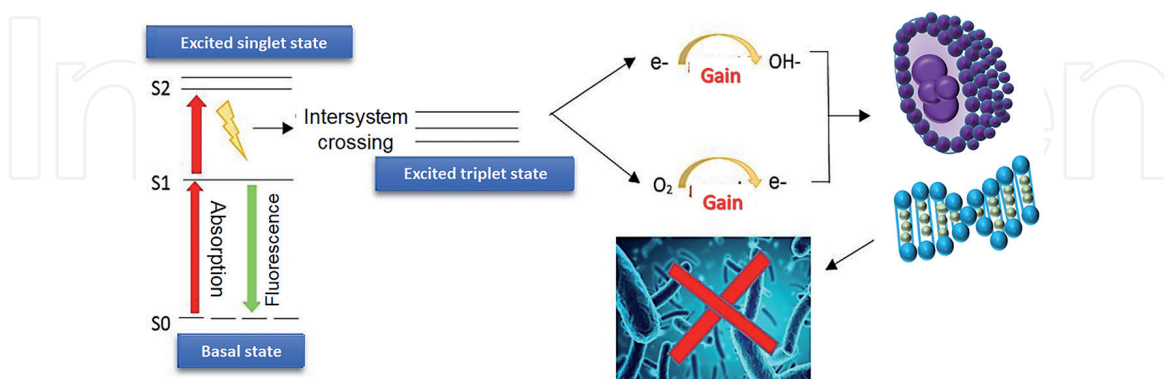
Figure 2.
The extraordinary power of the PSs. The basal state of PS is with two opposite lower energy molecular spin e-. The irradiation must penetrate the tissues deep enough to deliver enough energy to excite an e- to a higher energy orbital. An intersystem crossover can reach the excited state to a triplet state, where the excited e- spin is reversed. Excited triplet can produce two types of reactions: Type I and Type II. Type I: the excited triplet state can gain an e- from a nearby reducing agent, oxidizing it, producing H₂O₂. Type II: the excited triplet state transfers its energy directly to molecular oxygen, forming ¹O₂. The type II reaction provides most of the photooxidative stress. ¹O₂ is a ROS that produces concerted addition reactions to groups of alkenes present in organic molecules such as proteins, lipids o nuclear acids leading to nonspecific bacterial death. The generation of ¹O₂ will be effective, taking into account the PS, where its excited states have a longer half-life. It is essential to improve the probability of interacting with triple oxygen and producing ¹O₂ [11].

emphasis on *S. aureus* sensitive to multiple drugs (MDSSA); *S. aureus* multi-drug resistant strains (MDRSA); Methicillin-sensitive *S. aureus* (MSSA) and MRSA.

2. Photosensitizers

PS are non-toxic molecules capable of absorbing a specific wavelength's energy and transferring it to oxygen molecules present in biological solutions to produce the activated forms of $O_2^{\bullet-}$ and $^1O_2$. Both forms can produce ROS, which has the ability to promote bacterial cell death through the oxidation of closer organic macromolecules such as membrane components, proteins, lipids, and nucleic acids. In Gram positive bacteria, Pactivated PSs may produce ROS that acts unspecifically on macromolecules present in the envelope, such as lipids and proteins of the plasma membrane, peptidoglycan, and the array of proteins and polysaccharides macromolecules of the matrix (**Figure 1**). A PS has the property of being inert during administration and can be activated by being subjected to a specific wavelength.

2.1 Most used photosensitizer for PDT over *Staphylococcus aureus*

Table 1 summarized some of the more recent efforts to eradicate *S. aureus* by PDT. Porphyrins are an important class of natural macrocyclic molecules found in biological compounds and play an essential role in the metabolism of living organisms. The best known natural porphyrins are the heme group and chlorophyll. The heme group is a porphyrin-iron complex that is part of many active sites of different proteins, such as hemoglobin, myoglobins, and cytochromes. Uroporphyrin and coproporphyrin are the oxidation products of their respective porphyrinogens, which are the proper substrates in the biosynthetic pathway. The basic structure of porphyrin is formed by four pyrrole units interconnected by their alpha carbons linked by methyl bridges. The most commonly used photosensitizer drug in the last decade is porphyrin derivatives, such as the synthetic protoporphyrin Diarginate, T4 Porphyrin, Protoporphyrin IX, Coproporphyrin III, Porphyrin Formulation, among others. However, there are a few studies that have tried to verify whether these natural or synthetic molecules have a photo-oxidative activity with bactericidal action. The vast majority of these studies used porphyrin derivatives under irradiation with the red light of a wavelength range of 618–780 nm. PDT mediated by porphyrin derivatives increased antimicrobial efficacy and significantly reduced bacterial viability [10, 11, 14, 15, 40].

One of the initial studies on this PS was the series by Grinholc *et al*. [13, 16], who evaluated the bactericidal efficacy of PDT mediated by protoporphyrin diarginate over MRSA and MSSA strains in a large number of clinical isolates. They observed a reduction of 0–3 log10 of MRSA strains and 0.2 to 3 log10 for MSSA strains. Although this study's results were not significant, they paved the way for studying and developing PSs [13]. The 5-aminolevulinic acid (5-ALA), a prodrug that becomes protoporphyrin IX (PP IX) in the target cells, was used as a photosensitizer. Compared to other PSs, 5-ALA is only a natural intermediate in the heme biosynthetic pathway and can be removed rapidly from target cells. More importantly, it is small enough to penetrate the matrix and accumulate in target cells with less toxicity. The antimicrobial activity of 5-ALA-PDT was demonstrated on MRSA's planktonic strain in vitro by Huang *et al*. [37]. Their results showed that the number of living cells decreased as the concentration of the compound augmented. In control groups with solely 5-ALA or light, most of the bacterial cells were alive. Consequently, the photodynamic activity of 5-ALA is dose-dependent [37].

Bacteria	PS	Technique	Study	Application	Author and year
MSSA MRSA	PP Diarginate	Red light	In vitro	Clinical isolates	Grinholc et al. [13]
MSSA MRSA	TMP	Red light	In vitro	Biofilm-producing bacteria	Di poto et al. [10]
MRSA	MB and TMP	Red light	Ex vivo	Wound infections	Donelly et al. [14]
MRSA	TMP	Blue light	In vitro	Antibiotic resistant bacteria	Dosselli et al. [15]
MSSA MRSA	PP IX	Red light	In vitro	Antibiotic resistant bacteria	Grinholc et al. [16]
MRSA	HYP	Red light	In vitro In vivo	Biofilm-producing bacteria	Nafee et al. [17]
MDSSA	Hypocrelin B	Blue light LED	In vitro	Bacterial isolates	Yuan Jiang et al. [18]
S. aureus S. epidermidis S. haemolyticus	5-ALA	Red light	In vitro	Wound infections	Barra et al. [19]
MDSSA	HYP with NAC	Yellow light LED	In vitro	Biofilm-producing planktonic bacteria	Kashef et al. [20]
MDSSA	NnPs of gold with MB	Red light LED	In vitro	Skin infections (Impetigo)	Tawfik et al. [21]
MRSA MDRSA	Chlorophyll derivative	Red light LED	In vitro Ex vivo	Clinical isolates	Winkler et al. [22]
S. aureus P. acnes	ZnPc	Red light	In vitro In vivo	Skin infections	Chen et al. [23]
MDSSA	TBO	Red light	In vitro	Bacterial infections	Gandara et al. [24]
MSSA MRSA	TBO	Red light	In vitro	Antibiotic resistant bacteria	Hoorijani et al. [25]
MSSA MRSA	MB and TBO	Red light	In vitro	Biofilm-producing bacteria	Kashef et al. [26]

Bacteria	PS	Technique	Study	Application	Author and year
MDSSA	MB and RB	Red and green light	In vitro	Bacterial isolates	Pérez-Laguna et al. [27]
MDSSA MDRSA	S-PS	Red light	In vivo	Skin infections (burns and wounds)	Mai et al. [28]
MRSA	Cur	Blue light	Ex vivo	Bone tissue infections	Araujo et al. [29]
MDSSA	ZnPc	Red light	In vitro	Biofilm-producing bacteria	Gao et al. [30]
MRSA	ICG	Infrared light LED	In vitro	Skin infections (diabetic foot)	Li et al. [31]
MRSA	Resveratrol	Blue light LED	In vitro In vivo	Bacterial isolates	Dos Santos et al. [32]
MRSA	Silica NnPs conjugated with TBO	Red light LED	In vitro	Biofilm-producing bacteria	Anju et al. [33]
MDSSA	TBO	Red light LED	In vitro	Dental titanium implants	Zhiyu Cai et al. [34]
MDSSA	Iodide IR780	Infrared light	In vitro In vivo	Orthopedic titanium implants	Mu Li et al. [35]
MRSA	TBO	Red light	In vitro	Skin infections (Burns)	Mahmoudi et al. [3]
MRSA	Riboflavin	Blue light LED	In vitro	Bacterial isolates	Makdoumi et al. [36]
MDSSA MDRSA	S-PS	Red light	In vitro	Biofilm-producing planktonic bacteria	Jia et al. [3]
MRSA	5-ALA	Red light	In vitro	Biofilm-producing planktonic bacteria	Huang et al. [37]
MDSSA	NnPs of polydopamine conjugated with ICG	Infrared light	In vitro In vivo	Dental titanium implants	Yuan et al. [38]
P. aeruginosa S. aureus	TBO Conjugated Carbon Nanotubes	Red light	In vitro	Biofilm-producing bacteria	Anju et al. [33]
MRSA	Porphyrin formulation	White light LED	In vitro Ex vivo	Skin infections	Braz et al. [11]

Bacteria	PS	Technique	Study	Application	Author and year
MSSA MRSA	Cur	Blue light LED	In vitro	Biofilm-producing bacteria	Geraldo et al. [39]
MDSSA MDRSA	TBO	Red light	In vitro	Skin and mucous infections (periodontitis, burns and diabetic foot)	Liu et al. [6]
MRSA	CP III	Blue light LED	In vitro	Skin infections	Walter et al. [40]

MSSA: methicillin-sensitive Staphylococcus aureus; *MRSA: Methicillin-resistant* Staphylococcus aureus; *MSSA: methicillin-sensitive* Staphylococcus aureus; *MRSA: Methicillin-resistant* Staphylococcus aureus; *MDSSA: Multi-drug sensitive* Staphylococcus aureus; *MDRSA: Multi-drug resistant* Staphylococcus aureus; *S. aureus:* Staphylococcus aureus; *S. epidermidis:* Staphylococcus epidermidis; *S. haemolyticus: Staphylococcus haemolyticus; P. acnés:* Propionibacterium acnes; *P. aeruginosa:* Pseudomonas aeruginosa; *PS: photosensitizer; PP: protoporphyrin; TMP: porphyrin T4 (meso tetra (N-4-methyl pyridyl)); MB Methylene blue; HYP: Hypericin; NAC: N-acetylcysteine; 5-ALA: aminolevulinic acid; NnPs: nanoparticles; ZnPc: Zinc Phthalocyanine; TBO: Toluidine blue; RB: Bengal rose; S-PS: sinoporphyrin sodium; Cur: Curcumin; ICG: indocyanine green; CP: coproporphyrin; LED: Light-emitting diode.*

Table 1.
List for research and development of S. aureus *PDT.*

The second most prominent PS is Toluidine Blue (TBO), a hydrophilic cationic PS of phenothiazine dyes with a high $^1O_2$ quantum yield and strong absorption bands in the 620–660 nm region. Also, it has a high affinity for bacterial membranes and has been approved for clinical use in PDT, and is considered an effective and membrane-damaging PS [6]. In all studies where TBO act as a PS, irradiation with red LED light in the range of 630–635 nm was used. This combination increased the antibacterial efficacy of PDT and significantly reduced bacterial viability [3, 6, 24, 25, 33, 34]. One of the most prominent studies was carried out by the group of Zhyhyu Cai et al. [19], whose objective was to evaluate how effective the disinfection by combining antiseptics with PDT is in *S. aureus* BF present on the titanium surface is. The results indicate that the administration of antiseptics such as chlorhexidine or H_2O_2 with PDT was the most effective protocol, producing a reduction of approximately 3–4 log10 in the number of adhering bacteria compared to any treatment alone. In addition to bacterial reduction, it was the first study *in vitro* to evaluate the antibacterial effects of the concurrent application of antiseptics with PDT against *S. aureus* BF presented on the surface of Titanium [34].

Several natural PS derived from plants are also highlighted to be used for PDT, such as the sinoporphyrin sodium (S-PS). For example, Mai *et al*. [28], and Jia *et al*. [4], used a similar methodology for activation of S-PS, employing red LED light irradiation. The results indicate that PDT therapy with S-PS has significant antibacterial activity on MDSSA and MDRSA bacteria [4, 28]. Also, the S-PS exceeds the solubility of other PS in a physiological environment and proves to be an ideal PS with low dark toxicity, as well as having high purity and easy extraction. Another natural PS widely used in *in vitro* and *in vivo* studies is hypericin (HYP), polycyclic quinine extracted from *Hypericum perforatum* (commonly known as St. John's wort) and derives its name from the plant. Previous interest in this herb has focused on its antidepressant effects. This plant has been evaluated and tested for its photo-oxidative activity against a series of bacterial and fungal strains in recent years. It has several desirable properties as PS, including a high $^1O_2$ generation quantum yield, a high extinction coefficient close to 600 nm, and relatively low dark toxicity. In studies carried out by the group of Nafee *et al*. [17], the quantity as low as 0.03 M of HYP inhibited *in vitro* 60–120% the growth of BF producing MRSA strains compared to planktonic cells, 55–75%. *In vivo* studies on rats showed higher wound healing potential, better epithelialization, and keratinization of the skin in infected wounds treated with HYP nanoparticles [17]. Finally, hypocrellin B, an active component of traditional Chinese medicine from the herb *Hypocrella bambuase*, was tested for PDT. Numerous studies have shown its antiviral, antibacterial, and antifungal effects and antitumor activity. Interestingly, this PS is also a strong ROS generator when activated by visible light. Yuan Jiang *et al*. [27] observed a significant decrease in the viability of *S. aureus* after LED light irradiation. Remarkable ultrastructural damage was also evidenced in bacterial cells due to the photodynamic action of hypocrellin B [18].

3. Photodynamic therapy in clinical isolates strains

Most of the studies on PDT for *S. aureus* infections found in the literature come from standard bacterial strains acquired from different microbiological laboratories for *in vitro*, *in vivo,* and *ex vivo* studies. The most used certified reference standard bacterial strains came from the American Type Culture Collection (ATCC). ATCC strains are certified microorganisms for quality control in microbiology. Also, their genotypic and phenotypic characteristics guarantee the identity of the microorganism, and by having this documentation, the laboratory will avoid carrying out

Isolations	Unit	Material	Resistance profile
MSSA 26	Surgery room	Respiratory sample	AM, EM, LE, MX, PG
MSSA 27	Surgery room	Lesion swab	_
MSSA 28	Surgery room	Lesion swab	_
MSSA 32	Internal Medicine	Lesion swab	_
MSSA 33	Dermatology	Lesion swab	CM, EM, TC
MSSA 35	Dermatology	Lesion swab	AM, LE, MX, PG
MRSA 36	Surgery room	Lesion swab	CM, EM, LE, MX
MRSA 37	Pneumology	Orin	CM, EM, LE, MX, TM
MRSA 38	Pneumology	Respiratory sample	CM, EM, LE, MX, TM
MRSA 40	Urology	Blood sample	CIP, CM, EM, FM, LE, MX, TC, TM
MRSA 41	Surgery room	Lesion swab	CM, EM, LE, MX, RI, TM
MRSA 42	Surgery room	Blood sample	CIP, CM, EM, LE, MX, TM
MRSA 43	Gynecology	Lesion swab	CM, EM, LE, MX, TM
MRSA 44	Ophthalmology	Lesion swab	CM, EM, LE, MX
MRSA 45	Internal Medicine	Lesion swab	CM, EM, GEM, LE, MX, TC, TM

MSSA: Methicillin-sensitive Staphylococcus aureus*; MRSA: Methicillin-resistant* Staphylococcus aureus*; AM: ampicillin; CIP: ciprofloxacin; CL: chloramphenicol; CM: clindamycin; EM: erythromycin; FM: fosfomycin; GEM gentamicin; LE: levofloxacin; MX: moxifloxacin; PG: penicillin G; RI: rifampicin; TC: Tetracycline; TM: Tobramycin.*

Table 2.
Diversity of the clinical isolates.

additional tests for the identification of the strains, which translates into saving time and resources. However, studies in which clinical isolates of *S. aureus* were used to demonstrate the PDT may be useful for bacteria from active infections.

PDT is an approach that has shown promise in treating skin and soft tissue infections, one of the most recent studies of Mahmoudi *et al*. [3], used clinical isolates of *S. aureus* from samples of burn wounds of 95 patients with symptomatic infection. The viability of *S. aureus* isolates was significantly reduced to 40% after 30 sec of exposure to a LED light, with minimal risk of development of resistance [3]. Another study developed by Tawfik *et al*. [21] was carried out over clinal isolates of *S. aureus* from a population of twenty children aged 3 to 5 years diagnosed with impetigo. The PDT was compared for light-irradiated gold, methylene blue (MB), and gold -MB conjugate nanoparticles. It was shown that the maximum inhibitory effect on *S. aureus* was obtained with the gold nanoparticle-MB conjugate [21]. The 5-ALA has also been used for PDT over clinical isolates of BF of MSSA and MRSA strains of *S. aureus* [41]. The results over isolates from samples of adult patients with chronic rhinosinusitis with or without nasal polyps showed a robust bactericidal effect that increased when the PDT was combined with antibiotics treatment [41]. Finally, one of the most relevant studies carried out by Winkler *et al*. [22] tested the effectiveness of the chlorophyll derivative (Ce6) combined with a red light to eradicate *in vitro* a diverse set of clinical isolates of MSSA and MRSA. Those bacterial isolates, their biological sample, origin, and their susceptibility profiles are listed in **Table 2**, showing the diversity of the strains. The *in vitro* study demonstrated that all clinical isolates of MSSA and MRSA were inactivated by PDT when bacterial cells were previously incubated with $\geq$128 μM Ce6 [22].

4. Synergism with antibiotics or other drugs

Although PDT presents positive expectations for the treatment of MDRSA, several researchers have wanted to anticipate the generation of resistance, and they began the search for an antimicrobial strategy that generates greater potency and better results. Therefore, a new research sub-field has been opened, combining PDT with antibiotic treatment in *S. aureus*.

Gentamicin (GEN) is one of the most widely used antibiotics for treating various HAIs. The GEN is an aminoglycoside, which inhibits protein synthesis binding to the 30S subunit of the bacterial ribosome and causes protein mistranslation and bacterial death. GEN is a broad-spectrum antibiotic used for clinical treatment, although its frequent use has generated a high resistance level. Several authors have evaluated the synergy of combining the GEN with PDT for the antibacterial treatment of *S. aureus*. Most of these studies use a similar methodology, consisting of pre-treatment of the bacteria in the dark with different tested PS concentrations. This is followed by the transfer of treated bacteria in suspensions to microtiter plates containing different GEN concentrations and irradiated with different doses of light. Finally, the bacterial plaque count is performed in the dark to calculate bacterial viability. In this way, a diminution in the GEN-MIC when combined with PDT is determined [6, 19, 42, 44]. One of the most representative and updated studies, developed by the group of Liu *et al*. [6], verified the synergistic effects of PDT by combining TBO with GEN. This combination has a better antibacterial activity on MDSSA compared to MDRSA bacteria. The authors observed a dose-dependent effect with a maximum of 9 μg / mL GEN decreased of up to 1.8 log10 the survival of MDSSA bacteria. However, no bactericidal effect was observed on MDRSA at a GEN concentration of up to 150 μg/mL. Suggesting the MDSSA strains are more sensitive to PDT-GEN therapy than MDRSA [6].

Another widely used antibiotic to treat infections caused by multidrug-resistant bacteria is Linezolid (LN). Linezolid is a bacteriostatic antibiotic that binds to bacterial ribosomal RNA, inhibiting protein translation of Gram-positive bacteria. A large body of evidence shows that PDT significantly increases the effectiveness of LN treatment synergistically for different strains of *S. aureus* [26, 27]. Special mention deserves the study by Kashef *et al*. [31], who observed that the combination of TBO and MB in PDT with LN is useful in eradicating *S. aureus* BF in chronic diabetic foot ulcers. By itself, PDT therapy with MB or TBO did not decrease the bacterial viability of any of the *S. aureus* tested strains. However, the combination of MB-PDT and antibiotics resulted in a 1.2 log10 reduction in viability comparing to the antibiotic treatment alone (0.6 log10 reductions) [31]. The ciprofloxacin (CIP) is an antibiotic agent of the quinolone family, which binds to the DNA gyrase-DNA complex. The DNA gyrase allows the DNA to unwind and rotate freely within the cell; thus, CIP produces bacterial death. PDT studies showed synergism with CIP [45, 46]. For example, Ronqui *et al*. [46] observed a significant antibacterial increase, reducing bacterial survival 5.4 log10 in *S. aureus* BF and approximately 7 log10 for *Escherichia coli* BF combining PDT treatment with MB and CIP [46].

One of the most explored resistance mechanisms in the last two decades has been that of vancomycin-resistant *S. aureus* (VRSA). VAN acts on the synthesis of the bacterial cell-wall, inhibiting the formation of peptidoglycans. Not only the permeability of the cytoplasmic membrane but also the RNA synthesis is altered. Strains of VRSA can transfer their resistance mechanism to other pathogenic bacteria such as *Enterococcus faecalis,* causing a significant number of HAIs outbreaks [47]. For this reason, PDT has been studied as a therapeutic option that enhances the bactericidal action of VAN [10, 41]. Di poto et al. [10], demonstrated that pre-treatment of clinical MSSA and MRSA strains producers of BF with PDT, followed by the addition of NPV, causes disruption of the BF matrix and allows complete elimination of the bacteria. Synergism with PDT improved the antimicrobial activity of VAN with a five-fold decrease in bacterial viability compared to samples treated with PDT alone [10].

The synergism of PDT has also been explored with other drugs such as mucolytics, anticoagulants, antiseptics, and disinfectants. In general, these studies present encouraging results. All showed decreased bacterial viability in combined therapy with these different compounds [11, 20, 31, 34, 35]. For example, Braz *et al*. [11] showed both *in vitro* and *ex vivo* the efficacy of PDT mediated by a formulation based on a non-separate mixture of cationic porphyrins (FORM) in combination with potassium iodide (KI) or povidone-iodine (PVP-I) for photoinactivation of MRSA in the skin. The in vitro results demonstrated that the FORM + KI combination was an effective therapy in a dose-depended manner. Results *ex vivo*, shown a reduction of 3.1 Log10 using FORM + KI or FORM + PVP-I under irradiation [11].

5. Effects of photodynamic therapy on *S. aureus* biofilms

S. aureus is one of the most important etiologic agents of HAIs, in part, due to the ability of *S. aureus* to form BF. The BF provides a microenvironment that protects bacteria from the immune system's action and antibiotics, providing an extended virulence to the strain [10]. The BF formed by *S. aureus* are communities of microorganisms integrated into a matrix of extracellular polymers. The matrix comprises adhesion polysaccharides and extracellular enzymes, which have shown aggressive behavior [48].

Infections by organisms that produce BF are an important challenge in medical practice, leading to new therapeutic strategies. PDT has been a central focus and

shows mixed results in the literature. Studies using TBO as PS to eradicate *S. aureus* BF have shown a significant reduction in bacterial viability [33, 34, 48]. Noteworthy Anju *et al*. [25], used silica nanoparticles to enhance the antimicrobial efficacy of TBO. The authors evaluated the anti-BF efficacy of the photoactivated TBO silica nanoparticles against *Pseudomonas aeruginosa* (*P. aeruginosa*) as well as *S. aureus*. The results showed that the PDT reduced the viability in *P. aeruginosa* by $66.39 \pm 4.22\%$ and the viability of *S. aureus* by $76.22 \pm 3.45\%$. Regarding the controls, the use of TBO alone resulted in an inhibition of 27.28 ± 1.87 and $48.52 \pm 1.91\%$ for BF formation by *P. aeruginosa* and *S. aureus*, respectively [25]. A modification in the encapsulation of TBO for PDT was achieved employing carbon nanotubes, which were useful and showed improved results over BF of *P. aeruginosa* and *S. aureus* [33]. The anti-BF activity of TBO with nanotubes after exposure to light was 69.94% and 75.54% for *P. aeruginosa* and *S. aureus*, respectively. Compared to the study by Anju *et al*. [25], the photoinactivation of bacteria was much higher, and cell viability and exopolysaccharide production were more reduced [33].

Authors using indocyanine green (ICG) as PS observed mixed results [29, 31]. For example, Li *et al*. [31] compared the effect of adding EDTA to ICG for PDT on planktonic and BF bacteria. The results showed that PDT induced by ICG -EDTA combination has a more pronounced antibacterial effect in *S. aureus* and *P. aeruginosa* than PDT with ICG alone. In turn, *P. aeruginosa* was more sensitive to ICG -EDTA PDT than *S. aureus*. Also, PDT combined with antibiotic treatment contributed significantly to eradicating bacteria and disrupting the BF structure. Different results were obtained when combining polydopamine nanoparticles with ICG for PDT of orthopedic titanium implants [29]. Evaluations demonstrated that PDT-mediated ROS and nor hyperthermia were sufficient by themselves to achieve a significant bactericidal effect on *S. aureus* BF. However, both effects, local hyperthermia and ROS production, were synergistic and effectively inhibited most *S. aureus* BF [29].

The photodynamic activity of Curcumin (Cur) by high photooxidation was demonstrated to efficiently abolishing *S. aureus* BF [30, 39]. The group of Geraldo *et al*. [30] established the efficacy of Cur -PDT over MSSA and MRSA BF. The results showed that concentrations as low as 20–40 μM resulted in 1log10 reduction of MSSA BF, but the effect reaches 3log10 inactivation at 80 μM. For MRSA BF, it was observed that at 20 μM of Cur produced a reduction of 1log10, and similarly higher concentrations, 40 and 80 μM, decreased the bacterial survival to 2 log10 in a dose-dependent activity [39]. Hypericin (HYP) is one of the natural derivatives widely used in PDT for the elimination of *S. aureus* BF [17, 20]. However, its bactericidal effect is only achieved in combination with N-acetylcysteine (NAC) [20]. The combination of HYP-NAC in PDT is able to interrupt the preformed BF of *S. aureus* (ATCC 25923), reducing the bacterial viability between 5.2 to 6.3 log10. The treatment for clinical isolates demonstrated similar bactericidal activity, decreasing the viability by 5.5–6.7 log10 [20]. Gao *et al*. [49] showed that zinc phthalocyanine (ZnPc) generates ROS during the PDT treatment of *S. aureus* BF. According to his flow cytometric studies, the bacterial DNA was severely damaged [49]. Finally, combining iodine IR780- PDT with thermal phototherapy (PTT) is effective both *in vitro* and *in vivo* [35]. The authors observed that antibacterial treatment applying only PDT or PTT is not effective in completely eradicating already formed BF [35].

6. Modulation in gene expression by photodynamic therapy

Without considering prophages, plasmids, and transposons, the *S. aureus* genome core is a circular chromosome of approximately 2,800 Kb. The genes that encode virulence factors in *S. aureus* may be contained in the core genome and in

accessory elements. Genes encoding virulence factors can be transferred between different staphylococci strains or transferred to bacteria from other species, including Gram-negative bacteria. In *S. aureus*, the expression of virulence genes is controlled by several regulatory genes; the most studied is the *agr* gene (accessory genetic regulator). The *agr* gene has become associated with a quorum-sensing (QS) system. The RNAIII gene is the main effector of the *agr* system. It acts as a small RNA that regulates the expression of many virulence factors, including most of the genes that encode proteins associated with the cell wall and extracellular structures [50]. These factors are also associated with the formation of BF. Given its importance, the *agr* system can be a good therapeutic target for treating acute and chronic infections associated with the formation of BF. [23]. Therefore, the researchers emphasize the use of PDT can interfere with these systems' actions by inhibiting the spread of BF-forming strains [16, 23, 24].

Pourhajibagher *et al*. [50], evaluated in multiple species, including *S. aureus*, the cell viability of bacterial BF subjected to ICG as a photosensitizer. The gene expression of the QS system and the *arg* gene was determined and compared to untreated bacteria. In both *S. aureus* and other bacteria, *agr* gene and QS gene expression levels decreased after PDT. The *agr* gene expression was reduced approximately 3.7 times, as well as the bacterial viability of *S. aureus* decreased between 42 and 82%, revealing an association of the gene with bacterial BF [23]. In another study, Mahmoudi *et al*. [3] determined the ICA operon gene expression changes in bacteria subjected to sub-lethal doses of PDT in clinical isolates from wound infections in patients with burns. The ICA gene regulates *S aureus* BF production. A significant decrease in the expression of the ICA gene was observed in all *S. aureus* isolates after treatment, suggesting that the inactivation of virulence factors through interference in the expression of the ICA gene by PDT may reduce the pathogenesis of *S. aureus* [3].

One of the objectives that PDT seeks is to modulate the virulence of multi-resistant strains by repressing the expression levels of genes involved in bacterial resistance. An example was that of Huang *et al*. [37], who studied the expression response of a specific MRSA gene (*nuc* gene). The *nuc* gene encodes the expression of a thermally stable extracellular nuclease produced by *S. aureus*. The authors observed that this gene's transcription, ordinarily high, was down-regulated after PDT, suggesting the treatment interferes with its expression [37].

7. Discussion

S. aureus is the main pathogenic species of its genus, and a common cause of various superficial and internal infections. Although some of this infection can be quickly resolved, this pathogen's current interest derives from its high frequency in life-threatening diseases such as sepsis, meningitis, or pneumonia by MDR-bacteria. The variability of *S. aureus* and the rapid adaptive response to changes in the environment and its continuous acquisition of antibiotic resistance determinants have made it a habitual resident of the hospital habitat and an important agent of HAIs. Since the main consequence of bacterial resistance is antibiotic therapy failure, the increase in morbidity and mortality, and the increase in medical care costs, it is essential to containing the problem. As MDR-infections with *S. aureus* increases worldwide to dangerous levels, the urgent search for new antimicrobial strategies is required. Complementary therapies and antimicrobial treatment options may help relieve pressure from multidrug-resistant bacteria on healthcare systems. PDT then promises to be very useful to complement antibiotic treatment. The PDT is a noninvasive strategy, which uses inert compounds that need to be activated locally [6].

As we see in **Figure 1**, PDT's mechanism can change the internal and external structural integrity of bacterial cells and cause unspecific cell death. This process is closely related to the formation of ROS, without the generation of resistance. **Table 1**, shows the most widely used and explored SP are those derived from porphyrins since these present a high decrease in bacterial viability when irradiated. It should be noted that the irradiation to activate the photo-oxidative effect of PS is essential since the effectiveness of the treatment depends on this. The wavelength in the ranges of 620 to 700 nm is considered the most efficient technique as the red light manages to penetrate deep enough into the target tissue to produce its activity.

The BFs are important to point considering the pathogenicity of *S. aureus*. The PDT achieved the eradication of the BF in most investigations, but this disruption capacity was variable and is highly dependent on the technique used (the type of PS, type of irradiation, combination with antibiotics, among others). The decrease in bacterial survival in BF after PDT was much lower than observed for planktonic bacteria. The difference may radicate in the cell wall composition and growth rate, and the matrix components that hinder the photosensitizer absorption and light penetration.

The synergy with antimicrobials in combination therapy effectively increases microorganisms' sensitivity to the antibiotics of choice. In addition to avoiding a large amount of antibiotic use, this strategy minimizes the spread of resistance. On the other hand, a lower drug concentration can be used during combined therapy to reduce the side effects.

Genetics plays an important role, and PDT showed that it might generate a modulation in the genes associated with virulence. Promoting the silencing of gene expression, the PDT significantly decreases bacterial viability. In turn, the *agr* gene has been assigned a central role in the pathogenesis of *S. aureus*. Its down-regulation may affect colonization factors, components of the microbial surface, and the formation of BF that are regulated by *agr* gene.

8. Conclusions

Based on the above, we can conclude that PDT treatment is highly recommended to strengthen antibacterial therapies. The PDT generates unspecific photo-oxidative effects that improve an effective elimination of *S. aureus* strains without generation of resistance. The information presented here could help develop standardized protocols for managing infections caused by *S. aureus*, particularly those with antimicrobial resistance. We believe it is necessary to expand future studies of this therapy *in vivo,* including clinical trials since, according to what has been presented, there are several *in vitro*, *in vivo,* and *ex vivo* evidence that proves that PDT is secure and its bactericidal effect does not produce resistance.

Conflict of interest

The authors declare no conflict of interest.

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