



Review

Reactive Oxygen Species (ROS)-Mediated Antibacterial Oxidative Therapies: Available Methods to Generate ROS and a Novel Option Proposal

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Abstract: The increasing emergence of multidrug-resistant (MDR) pathogens causes difficult-to-treat infections with long-term hospitalizations and a high incidence of death, thus representing a global public health problem. To manage MDR bacteria bugs, new antimicrobial strategies are necessary, and their introduction in practice is a daily challenge for scientists in the field. An extensively studied approach to treating MDR infections consists of inducing high levels of reactive oxygen species (ROS) by several methods. Although further clinical investigations are mandatory on the possible toxic effects of ROS on mammalian cells, clinical evaluations are extremely promising, and their topical use to treat infected wounds and ulcers, also in presence of biofilm, is already clinically approved. Biochar (BC) is a carbonaceous material obtained by pyrolysis of different vegetable and animal biomass feedstocks at 200–1000 °C in the limited presence of O₂. Recently, it has been demonstrated that BC's capability of removing organic and inorganic xenobiotics is mainly due to the presence of persistent free radicals (PFRs), which can activate oxygen, H₂O₂, or persulfate in the presence or absence of transition metals by electron transfer, thus generating ROS, which in turn degrade pollutants by advanced oxidation processes (AOPs). In this context, the antibacterial effects of BC-containing PFRs have been demonstrated by some authors against *Escherichia coli* and *Staphylococcus aureus*, thus giving birth to our idea of the possible use of BC-derived PFRs as a novel method capable of inducing ROS generation for antimicrobial oxidative therapy. Here, the general aspects concerning ROS physiological and pathological production and regulation and the mechanism by which they could exert antimicrobial effects have been reviewed. The methods currently adopted to induce ROS production for antimicrobial oxidative therapy have been discussed. Finally, for the first time, BC-related PFRs have been proposed as a new source of ROS for antimicrobial therapy via AOPs.

Keywords: multidrug-resistant pathogens; antimicrobial oxidative therapy; reactive oxygen species (ROS); biochar (BC); biochar-derived permanent free radicals (PFRs); advanced oxidation processes (AOPs)



Citation: Alfei, S.; Schito, G.C.; Schito, A.M.; Zuccari, G. Reactive Oxygen Species (ROS)-Mediated Antibacterial Oxidative Therapies: Available Methods to Generate ROS and a Novel Option Proposal. *Int. J. Mol. Sci.* **2024**, *25*, 7182. <https://doi.org/10.3390/ijms25137182>

Academic Editor: Irena Maliszewska

Received: 23 May 2024

Revised: 22 June 2024

Accepted: 27 June 2024

Published: 29 June 2024



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1. Introduction

The incessant and rapid increase of multidrug-resistant (MDR) pathogens causes the emergence of difficult-to-treat infections with long-term hospitalizations, high costs, and a frightening incidence of death. In the United States, more than 2.8 million antibiotic-resistant infections occur each year, resulting in 35,000 deaths [1]. Resistant bacteria are becoming an uncontrollable worldwide hazard to both humans and animals [2,3]. Management of antimicrobial resistance through available antibiotics is a global public health problem and represents a daily challenge for experts in the field. To limit the global antibiotic resistance crisis, the main solution would be to reduce the volume of the unscrupulous use of antibiotics both in medicine, agriculture, and the environment [4], as well as to perform an efficient infection control strategy to prevent the spread of contagions.

Anyway, the development of novel antimicrobial drugs remains urgent and mandatory [1]. Although several new agents based on existing classes of antibiotics are being developed, there has been little advancement in the exclusive discovery of novel agents [4].

Moreover, the capability of several bacterial and fungal species to form biofilms is an alarming mechanism through which pathogens develop a very complex form of resistance. Biofilm-producing pathogens represent a significant problem in many clinical settings since biofilm further increases their tolerance towards conventionally prescribed antimicrobials [5,6]. The high-dose use of antibiotics in biofilm conditions to treat chronic wounds, burns, chronic respiratory diseases, cystic fibrosis, and recurrent cystitis leads to intense selective pressure, which paradoxically drives further antibacterial resistance [4].

In this alarming scenario, the need for the development of optional per se effective therapeutic approaches or of alternative treatments that can improve the antimicrobial efficacy of existing drugs as well as biofilms is imperative [7]. This second strategy would reduce the amount of antibiotics to be used, thus limiting the emergence of resistance in pathogens. Entirely novel antimicrobial instruments characterized by a unique mechanism of action are represented by reactive oxygen species (ROS) induced by different methods [4].

1.1. Reactive Oxygen Species (ROS)

ROS have demonstrated in vitro and in vivo a significant antimicrobial action against a wide spectrum of Gram-positive and Gram-negative organisms, including MDR isolates and biofilm-producing pathogens [8]. The use of ROS could represent a new therapeutic approach for topical use on skin, mucosal membranes, or internal tissue that may be colonized with microbial inhabitants and biofilms [9].

Treatments involving ROS as antimicrobial agents are already available for topical application, are clinically approved to treat infected wounds, and are being developed for clinical use in other settings [10].

As mentioned above, ROS is the well-known acronym used in several sectors, including medicine, to indicate reactive oxygen species, including radical and not radical oxygen-containing atoms and molecules, such as superoxide anion (O_2^-), singlet oxygen (1O_2), peroxide (O_2^{-2}), hydrogen peroxide (H_2O_2), hydroxyl radicals ($OH\bullet$), and hydroxyl anions (OH^-), that are constantly being formed as byproducts of the physiologic aerobic metabolism of cells [11]. Additionally, ROS can react with NO produced by cells from intracellular L-arginine via the action of epithelial nitrogen oxide synthetase (NOS), neuronal NOS, and inducible NOS, forming other reactive species such as $NO\bullet$ and $ONOO^-$, while $NO\bullet$ in combination with O_2 provides $ONOO\bullet$. These molecules are referred to as reactive nitrogen species (RNS) [11].

1.1.1. Oxidative Stress (OS) by ROS

In normal conditions, ROS and RNS generation is kept under control by the antioxidant defenses and repair systems of cells [12]. On the contrary, when overproduced, the detoxification systems of cells fail to maintain ROS and RNS physiological levels, which accumulate, thus causing the onset of oxidative stress (OS) and inflammation. Irreversible damage to DNA, lipids, and proteins occurs, thus promoting aging, age-related diseases, and several degenerative human disorders [13].

1.1.2. Oxidative Stress (OS) by ROS Is the Cause of Diseases in Humans

Collectively, OS is a cascade of events that frequently triggers and accompanies molecular/cellular pathogenic events. It is responsible for several human disorders, including carcinogenesis [14,15], atherosclerosis, cardiovascular, and neurodegenerative diseases [16,17].

1.1.3. Oxidative Stress (OS) by ROS Is the Cause of Diseases in Microorganisms

As occurs in humans and also in pathogens, OS builds up when prooxidants overpower antioxidants. Therefore, ROS get accumulated in the microorganism's cell, thus exceeding the cell's capacity to readily detoxify them [18].

As examples, the host immune response as well as several antimicrobials counteract infections by inducing ROS accumulation. While the interaction between the host and pathogens causes exogenous OS in bacteria, intracellular redox reactions, antibiotics, and uncontrolled aerobic respiration contribute to endogenous OS [19]. ROS cause multiple damages to the bacterial cells, including double-stranded breaks in DNA by oxidizing dCTP and dGTP pools, which results in the misincorporation of bases into DNA. Additionally, ROS induces lipid peroxidation and protein carbonatization [20], thus exerting a very rapid bactericidal activity. It has been reported that ROS were able to cause a 3 log CFU reduction in 30 min and total eradication in 2 h when used against *Staphylococcus aureus* [21]. Several conventional and alternative antibiotics, including metal nanoparticles and natural molecules, exert their antimicrobial properties by inducing ROS hyperaccumulation in pathogens [22]. Other methods to exert ROS-mediated antimicrobial effects include photodynamic therapy (PDT), honey reactive oxygen (HRO) therapy, and hyperbaric oxygen treatment (HBOT).

1.1.4. ROS as a New Weapon against Pathogens

On these considerations, ROS could really represent an effective option for eradicating MDR pathogens. While procedures to induce ROS in microbial cells, such as HBOT and PDT, are traditional, the use of nanomaterials and engineered medical honey are rather novel and promising methods. Nevertheless, nanotoxicology is a field that is still not clearly defined and lacks sufficient and unequivocal epidemiologic data, information, and regulation. Furthermore, such methods may induce ROS formation in host cells as well. To make possible an enlargement of the clinical use of ROS to counteract MDR pathogens and related biofilm, the development of other delivery strategies for increasing the selectivity of ROS for microbial pathogens over the host tissue is necessary. In this regard, based on our recent studies on biochar (BC) [23,24], we profit from this review to propose a possible innovative method to be studied to produce ROS from a natural, low-cost source. Biochar (BC) is a carbonaceous material obtained by pyrolysis of different vegetable and animal biomass feedstocks and waste at 200–1000 °C in the limited presence or absence of oxygen. Due to its strong adsorption capacity, BC can exert a plethora of beneficial effects, including the removal of environmental pollutants and xenobiotics, thus preventing their uptake in plants, animals, and humans [25–28]. In microbiology, BC has been demonstrated to be helpful in limiting antimicrobial resistance by degrading/removing residual antibiotics from soil and water [24]. The so-called environmentally persistent free radicals (EPFRs) are known to exist in significant concentrations in atmospheric particulate matter (PM) and are primarily emitted from the combustion and thermal processing of organic materials. While their existence in combustion has been known for over half a century, only recently has their presence in environmental media and their healthy and/or hazardous effects been researched [29]. Nowadays, it has been demonstrated that BC can also contain persistent free radicals (PFRs) bound to the external or internal surfaces of its solid particles [29]. PFRs are reactive species due to unpaired electrons that can persist for several months, in contrast to traditional transient radicals [24]. Studies reported that PFRs are the main reason for BC's capacity to degrade organic pollutants through the generation of active oxygen species (ROS) and sulfate radicals [23]. It was reported that the generated ROS, including radical ($\bullet\text{OH}$, $\bullet\text{O}_2^-$, $\bullet\text{O}_2\text{H}$, $\text{SO}_4^{\bullet-}$) and non-radical species ($^1\text{O}_2$), successfully degraded several organic pollutants, hormones, and eDNA by advanced oxidation processes (AOPs). Interesting, PFRs-mediated ROS showed antibacterial effects against *Escherichia coli* and *S. aureus* [30–32], thus supporting the idea of the possible use of BC-derived PFRs as a novel method to induce ROS generation for antimicrobial oxidative therapy. In the following sections, all that was introduced in this section will be reviewed and discussed in greater depth.

2. Reactive Oxygen Species (ROS) and Oxidative Stress (OS)

2.1. Physiological and Pathological Origins of ROS

The following Figure 1 schematizes the main endogenous processes by which ROS can form in cells and the detrimental effects they can have on health [11], including DNA damage, lipids, and protein peroxidation, telomere reduction, aging, and death.

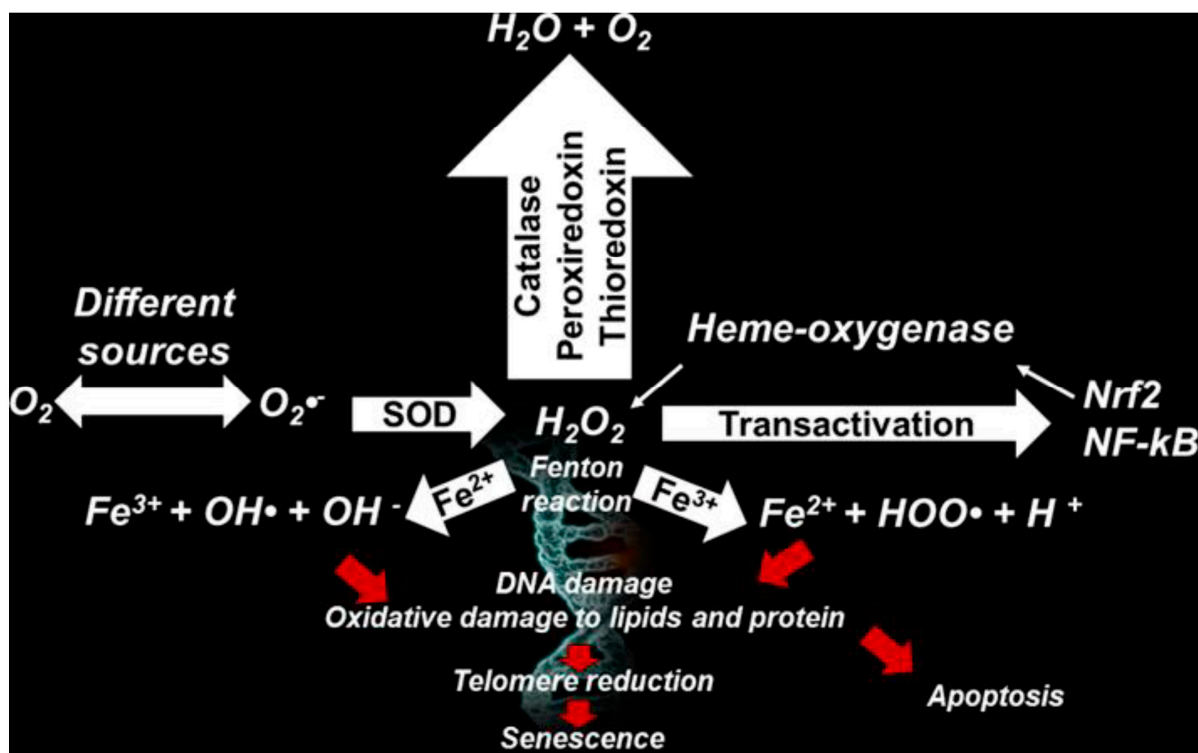


Figure 1. Schematic pathways of reactive oxygen species (ROS) production and their main effects on biological systems. Nrf2 = erythroid nuclear transcription factor-2; NF-kB = transcription factor involved in cellular responses to stimuli such as stress, cytokines, free radicals, heavy metals, ultraviolet irradiation, oxidized low-density lipoproteins (LDL), etc. Reproduced from our article [11].

On the other hand, the following Table 1 collects the endogenous molecules, organelles, and metabolic processes responsible for ROS production, dividing them into enzymatic and non-enzymatic ones. Also, it reports the sources, external to cells, that can induce ROS formation, and it refers to the main radical and non-radical oxygen and nitrogen reactive species that can form upon these events.

Based on the recently acquired knowledge and literature reports [33–36], with respect to the Table reported by us in 2020 [11], environmental persistent free radicals (EPFRs) and biochar-related persistent free radicals (BC-PFRs) have been included among the exogenous sources of ROS in Table 1. Upon their formation, according to reported processes and mechanisms, they can induce ROS formation by reacting with atmospheric or water dissolved O_2 , as well as with H_2O_2 and/or persulfate [24]. As shown in Figure 1, the molecular oxygen from different sources can be reduced to the radical superoxide anion ($O_2^{\bullet-}$), which is considered the primary ROS. Then, it reacts with other molecules through enzymatic or non-enzymatic metal-catalyzed processes, thus generating secondary ROS. In particular, phagocytic cells (neutrophils, monocytes, or macrophages) use NOX (Table 1) for one-electron reduction of molecular oxygen to the radical superoxide anion ($O_2^{\bullet-}$) during cellular respiration.

Table 1. Endogenous and exogenous sources of ROS and the main reactive species of both oxygen and nitrogen (RNS), which can be consequently produced.

Endogenous Sources		Exogenous Sources	Reactive Species
Enzymatic	Non-Enzymatic		
		Air	$O_2^{\bullet-}$
		Water pollution	H_2O_2
		Tobacco	$\bullet OH$
NOX	Mitochondria	Alcohol	$\bullet OOH$
MPO	Respiratory chain	Heavy/transition metals	ONOO \bullet
Cytochrome P450	Glucose auto-oxidation	Drugs	$NO_2\bullet$
Lipoxygenase	NAD \bullet	Industrial solvents	$NO\bullet$
Angiotensin II	Semiquinone radicals	Cooking	ONOOCO $_2^-$
Xanthine oxidase	Radical pyridinium	Radiation	NO^{2+}
Cyclooxygenase	Hemoproteins	EPFRs	ONOOH
FpH \bullet		BC-PFRs	N_2O_3
			ONOO $^-$
			ONOOCO $_2^-$
			$CO_3^{\bullet-}$

MPO = myeloperoxidase; NOX = NADPH oxidase; NAD = nicotinamide adenine dinucleotide; Fp = flavoprotein enzymes; EPFRs = environmental persistent free radicals; BC-PFRs = biochar-related persistent free radicals.

Then, $O_2^{\bullet-}$ is mainly transformed by superoxide dismutase (SOD) into hydrogen peroxide (H_2O_2), from which the highly reactive ROS hydroxyl ion ($\bullet OH$) and radical $HOO\bullet$ are formed through the Fenton or Haber–Weiss reactions in the presence of transition metals (Figure 1). $O_2^{\bullet-}$ is also produced from the irradiation of molecular oxygen with UV rays, photolysis of water, and by exposure of O_2 to organic radicals formed in aerobic cells such as NAD \bullet , FpH \bullet , semiquinone radicals, cation radical pyridinium, or hemoproteins (Table 1).

While the radical $O_2^{\bullet-}$ does not react directly with lipids, polypeptides, sugars, or nucleic acids, $\bullet OH$ and $HOO\bullet$ react especially with phospholipids in cell membranes and proteins, thus causing oxidative damage, DNA damage, telomere reduction, aging, and apoptosis [11] (Figure 1).

Furthermore, H_2O_2 can be converted by MPO (Table 1) to hypochlorous acid, which is particularly hazardous for cellular proteins [37].

Additionally, ROS can react with NO produced from intracellular *L*-arginine by cells as a defense mechanism, using three different kinds of NOS, such as epithelial NOS, neuronal NOS, and inducible NOS, thus forming reactive nitrogen species (RNS) such as $NO\bullet$ and $ONOO^-$. Finally, $NO\bullet$ in combination with O_2 , can provide $ONOO\bullet$, which induces lipid peroxidation in lipoproteins [11,38–40] (Table 1).

Some of the most representative oxygen and nitrogen reactive species reported in Table 1 have been correlated with their specific sources and with their physiological function in biological aerobic systems in Table 2.

Anyway, whatever their origin, both ROS and RNS cause indifferently detrimental oxidative modifications of cellular macromolecules such as carbohydrates, lipids, proteins, DNA, and RNA. Upon this damage, particular molecules are produced, which are considered markers of OS. The following Table 3 summarizes the main molecular targets of ROS and RNS, the reactions occurring during the damaging process, and the compounds that are consequently produced considered biomarkers of OS.

Table 2. The most representative radicals and non-radical reactive species produced in biological aerobic systems, with sources and functions.

Reactive Specie	Source	Function
$O_2^{\bullet-}$	Enzymatic process Autoxidation reactions Non-enzymatic electron transfer reactions	Reduces iron complexes such as cytochrome C Oxidizes ascorbic acid and α -tocopherol
$HOO\bullet$	Protonation of $O_2^{\bullet-}$	Initiates fatty acid peroxidation
$HO\bullet$	H_2O_2 via the Fenton reaction and HWR	Reacts with organic and inorganic molecules *
$NO\bullet$	<i>L</i> -arginine (substrate) NADPH (electron source) Nitric oxide-synthase	Intracellular second messenger Stimulates GC and PK Causes smooth muscle relaxation in blood vessels
$NO_2\bullet$	Protonation of $ONOO^-$ Homolytic fragmentation of $ONOOCO_2^-$	Acts on the antioxidant mechanism \downarrow Ascorbate and α -tocopherol in plasma
$ONOO\bullet$	Reaction of O_2 with $NO\bullet$	Oxidizes and nitrates methionine and <i>L</i> -tyrosine Oxidizes DNA to form nitroguanine
$CO_3^{\bullet-}$	(SOD)- Cu^{2+} reaction between $\bullet OH$ and HCO_3^-	Oxidizes proteins and nucleic acids
$ONOOCO_2^-$	Reaction of $ONOO^-$ with CO_2	Promotes nitration of oxyhemoglobin's tyrosine of the free radicals

HWR = Haber Weiss recombination; * DNA, proteins, lipids, carbohydrates; GC = guanylate cyclase; PK = protein kinases; \downarrow = reduced, lower.

Table 3. Oxidative modification of cellular macromolecules: reactions involved and produced markers of OS.

Cellular Macromolecules	Reactions	OS Biomarkers	Refs.
Proteins	RNS with <i>L</i> -tyrosine	NT	[41]
	Fenton reaction of ROS with <i>L</i> -lysine, <i>L</i> -arginine, <i>L</i> -proline, <i>L</i> -threonine	PC	[42]
Proteins/lipids	Michael-addition of aldehydic lipid oxidation products to <i>L</i> -lysine, <i>L</i> -cysteine, <i>L</i> -histidine	PC	[42]
Proteins/lipids	Complex oxidative process	Ox-LDL	[43]
Proteins/carbohydrates	Glyco-oxidation between <i>L</i> -lysine amino groups and <i>L</i> -arginine carbonyl groups linked to carbohydrates	AGEs	[44]
Lipids	$\bullet OH$ and $HOO\bullet$ mediated lipid peroxidation of poly-unsaturated fatty acids *	4-HNE, MDA, and F2-IsoPs	[41]
DNA	Mutagenic oxidation	2-Hydroxy adenine 8-Oxoadenine 5-Hydroxycytosine Cytosine glycol Thymine Glycol 8-OHGua 8-OHdG	[45]

AGEs = advanced glycation end products (*N*- ϵ -carboxymethyl-lysine pentosidine glucosepane); 4-HNE = 4-hydroxynonenal; MDA = malondialdehyde; F2-IsoP = F2-Isoprostanes; Ox-LDL = oxidized low-density lipoprotein; PC = protein carbonyl; 8-OHGua = 8-hydroxyguanine; 8OHdG = 8-hydroxy-2'-deoxyguanosine; * linoleic, arachidonic acids; NT = nitro tyrosine.

To counteract the detrimental effects reported in Table 3, cells have developed several repair systems able to restore or eliminate lipids, proteins, and DNA damaged by the

action of ROS and RNS. Particularly, cytosolic and mitochondrial enzymes, which include polymerases, glycosylases, and nucleases, repair the damaged DNA, while proteinases, proteases, and peptidases, which are part of the proteolytic enzymes, remove damaged proteins. In addition, biological systems have developed both physiological and biochemical mechanisms to limit free radicals' production and reactive species toxicity. At the physiological level, the microvascular system exerts the function of maintaining the levels of O₂ in the tissues, while at the biochemical level, a protective activity is exerted both by endogenous (enzymatic and non-enzymatic) and exogenous molecules, as reported in Tables 4 and 5. Collectively, GSH-Px, GR, and MSR are the main intermediaries in the processes for repairing oxidative damage.

Table 4. Non-enzymatic endogenous and exogenous molecules that can counteract free radicals and reactive species toxicity. ↓ Means decrease/reduction.

Process	Endogenous Molecules	Actions	Exogenous Molecules	Effect
NE	Vitamin E	Interact with ROS and RNS and terminate the free radical chain reactions	Vitamin C	↓ O ₂ • ↓ •OH
	Vitamin C		Vitamin E	↓ Lipid peroxidation
	Carotenes			
	Ferritin			
	Ceruloplasmin		Resveratrol	↓ O ₂ • ↓ •OH
	Selenium		Phenolic acids	↓ Lipid peroxidation
	GSH		Flavonoids	
	Manganese		Oil	↓ O ₂ • ↓ •OH
	Ubiquinone			
	Zinc		Lecithin	↓ Lipid peroxidation
Flavonoids	Selenium	Antioxidant		
Coenzyme Q				
Melatonin	Zinc	Antioxidant		
Bilirubin				
Taurine	Acetylcysteine	Antioxidant		
Cysteine				
Albumin				
Uric acid				

NE = not enzymatic; GSH = reduced glutathione.

Table 5. Enzymatic endogenous molecules that can counteract free radicals and reactive species toxicity. ↓ Means decrease/reduction.

Enzymes	Actions	Effects
SOD	Converts O ₂ to H ₂ O ₂ ↓ Hydroxyl radical production	
CAT	Decomposes H ₂ O ₂ to H ₂ O + O ₂ ↓ Hydroxyl radical production	
GSH-Px	Converts peroxides and hydroxyl radicals into nontoxic forms by the oxidation of GSH into GSSG	
GR	Converts glutathione disulphide to GSH	↓ Hydroxyl radical production ↓ Oxygen reactive species ↓ Nitrogen reactive species ↓ OS
GSTs	Catalyzes the conjugation of GSH to xenobiotic substrate	
G6PD	Catalyzes the dehydrogenation of G6P to 6-phosphoglucono-Δ-lactone	
Nrf2	Regulates the expression of antioxidant proteins	
ARE	Encodes for detoxification enzymes and cytoprotective proteins	
NQO1	Catalyzes the reduction of quinones and quinonoids to hydroquinone molecules	
MSR	Carries out the enzymatic reduction of the oxidized form of methionine to methionine	

SOD = superoxide dismutase; CAT = catalase; GSH-Px = glutathione peroxidase; GR = glutathione reductase; GSH = reduced glutathione; Nrf2 = erythroid nuclear transcription factor-2; ARE = antioxidant response element; NQO1 = NAD (P)H quinone oxidoreductase 1; GSTs = glutathione S-transferases; G6P = glucosio-6-fosfato; G6PD = glucosio-6-fosfato dehydrogenase; GSSG = glutathione disulphide; MSR = methionine sulfoxide reductase.

2.2. Pathogen Responses to OS

As reported previously in the introduction, ROS cause multiple damages to the bacterial cells [22]. Anyway, bacteria can evade OS by several means, including detoxifying methods using enzymes such as catalase, alkyl hydroperoxide reductase, thioredoxin, and superoxide dismutase (SOD). Additionally, they use pigments such as carotenoids, metal homeostasis, and repair devices including DNA restoration, general stress response, and SOS response [18,46]. All these mechanisms are regulated by gene networks [46]. *E. coli* reacts with OS, mainly producing SOD and catalase, which convert $O_2^{\bullet-}$ to H_2O_2 (SOD) and, in turn, H_2O_2 into H_2O and O_2 (catalase). While mammalian cells possess two types of SOD, *E. coli* owns three isoforms of SOD characterized by different metal cores. Particularly, *sodA* contains Mn, *sodB* includes Fe, and *sodC* comprises both Cu and Zn. *E. coli* also has two types of catalases, namely hydro-peroxidase I and hydro-peroxidase II [47]. Also, *E. coli* has several major regulators activated during OS, such as OxyR, SoxRS, OhrR, and RpoS. OxyR and SoxR control the catalase and SOD transcription in relation to the $O_2^{\bullet-}$ and H_2O_2 concentrations. They undergo conformation changes when oxidized in the presence of hydrogen peroxide and superoxide radicals, respectively, and subsequently control the expression of cognate genes [48]. In contrast, the RpoS regulon is induced by an increase in RpoS levels. It is a specialized sigma factor that governs the expression of genes that lead to general stress resistance in cells [49]. These genes may be involved in eliminating oxidative agents, repairing systems of affected biomolecules, and maintaining normal cellular physiologic circumstances. Despite the enormous genomic diversity of bacteria, OS response regulators present in *E. coli* are functionally conserved in a wide range of bacterial groups. Bacteria have developed complex, adapted gene regulatory responses to OS, probably due to the high level of ROS produced endogenously through their basic metabolism. Additionally, several bacterial pathogens prevent the increase of ROS by directly inhibiting the synthesis of NADPH oxidase [50]. Iron homeostasis and remodeling of metabolism are two other methods by which bacteria lessen the damage caused by ROS. Bacteria can remodel their metabolism via upregulation of the glyoxylate shunt, thus reducing endogenous ROS formation, or by redirecting the metabolism toward the pentose phosphate pathway and augmenting the production of NADH, which refills the level of antioxidants. Ketoacids such as pyruvate and α -ketoglutarate can decarboxylate in the presence of ROS, thus originating toxic molecules and diminishing damage caused by ROS [51]. Iron, which is also involved in ROS generation by Fenton reactions, is crucial for the growth and survival of bacteria, and paradoxically, iron acquisition by siderophore action is pivotal to counteracting OS [52]. Siderophores are compounds that bacteria produce when intracellular iron concentrations are low to facilitate their uptake [53]. Two siderophores, namely Staphyloferrin A and B, have been found to enhance the resistance to OS in *S. aureus*, while *E. coli* produces an enterobactin siderophore to alleviate damage from OS [51,52]. It was demonstrated that OS in turn regulates bacterial siderophore production [53]. When *E. coli* was exposed to H_2O_2 and paraquat, the expression of enterobactin increased in the presence of a high concentration of iron, which reduced the sensitivity of the isolate to both H_2O_2 and paraquat [53]. Similarly, when methicillin-resistant *S. aureus* (MRSA) was exposed to the antimicrobial surface coating AGXX[®], siderophore biosynthesis genes were highly upregulated. Some bacterial species produce biofilm as a highly specialized and organized form of resistance in which bacteria cooperate and stay protected by a self-produced biomass [54]. Persisters are bacterial cells in a dormant state with low metabolic activity existing in biofilm that showcase high antibiotic tolerance, can recolonize post-therapy [55], are less sensitive to ROS, and have demonstrated increased expression of efflux pumps. Efflux pumps major expression is another mechanism to react to OS, which allows bacteria to pump out the ROS-damaged proteins [56].

3. Antimicrobial Oxidative Therapies: Available Methods to Induce ROS Formation

As previously reported in the Introduction, the induction of high levels of reactive oxygen species (ROS) by several procedures, thus causing OS detriment to bacterial cells,

has been extensively studied to inhibit several species of Gram-positive and Gram-negative bacteria, viruses, and fungi. The use of ROS represents a new therapeutic approach for topical use on skin, mucosal membranes, or internal tissue that may be colonized with microbial inhabitants and biofilms [57]. It was found that the antibacterial effect of several conventional and alternative antibiotics, metal nanoparticles, and natural molecules is also based on their capability of inducing ROS hyperaccumulation in pathogens [22]. Therefore, other methods have been developed, are clinically applied, or are in clinical trials to produce ROS finalized for antimicrobial oxidative therapy. They include photodynamic therapy (PDT), honey reactive oxygen (HRO) therapy, and hyperbaric oxygen treatments (HBOT) [57].

3.1. ROS Formation Induced by Conventional, Alternative, and Natural Antimicrobials

Before reviewing the main antimicrobial therapies based on ROS induction, such as PDT, HRO, and HBOT, in this section we have reviewed other methods to provoke ROS improvement for antimicrobial uses. Table 6 reports information concerning conventional and alternative antimicrobials, including some antibiotics, nanoparticles, and natural compounds, which exert their effects by generating ROS.

Table 6. Conventional and alternative antimicrobials, including some antibiotics, nanoparticles, and natural compounds, exert their effects by generating ROS.

Type	Name	Mechanism of Action	Clinical Application	Target	Refs.
Antibiotics	Nitrofurantoin	Autooxidation of nitroaromatic anion radicals * in the presence of O ₂ provides O ₂ ⁻ and then ROS, thus causing OS and toxicity to bacteria	UTI	<i>E. coli</i>	[18]
	Polymyxin B	Accumulation of OH•	Untreatable infections	<i>Acinetobacter baumannii</i> <i>Pseudomonas aeruginosa</i> <i>Enterobacteriaceae</i> **	[58]
	Nalidixic acid	Mutagenesis by oxygen free radical generation	Gastroenteritis Enteric fever Bacteremia	<i>Salmonella typhimurium</i>	[59]
	Norfloxacin				
	Norfloxacin Ampicillin Kanamycin Fluoroquinolones β-Lactams Aminoglycosides	↑ Superoxide levels ↑ H ₂ O ₂ ↑ Lethality by accumulation of OH•	UTI	<i>E. coli</i>	[60]
	Nalidixic acid Trimethoprim Ampicillin Aminoglycoside	Post-stress ROS-mediated toxicity			[61,62]

Table 6. Cont.

Type	Name	Mechanism of Action	Clinical Application	Target	Refs.		
Organo Metals	OSECs	Ebselen ***	By inhibiting TrxR in bacteria lacking glutathione thus triggering OS.	Skin infections Bacteremia Endocarditis Food poisoning Pneumonia TSS	<i>S. aureus</i>	[63]	
				Tuberculosis	<i>M. tuberculosis</i>	[64]	
Alternative antimicrobials	Nanomaterials	MSNP-maleamic	↑ ROS (40% <i>E. coli</i> , 50% <i>S. aureus</i>)	UTI	<i>E. coli</i>		
		NPs	MSNPs-maleamic-Cu	↑ ROS (40% <i>E. coli</i> , 30% <i>S. aureus</i>)	Skin infections Bacteremia Endocarditis Food poisoning Pneumonia TSS	<i>S. aureus</i>	[65]
					Skin infections Bacteremia Endocarditis Food poisoning Pneumonia TSS UTI	<i>E. coli</i> <i>S. aureus</i> <i>S. epidermidis</i> <i>Photobacterium hosphoreum</i>	[64]
		Metal oxide NPs	↑ ROS ↑ RNS				
NZs	AgPd0.38	By ROS produced on Ag and Pd bimetallic alloy	Severe infections	<i>S. aureus</i> <i>Bacillus subtilis</i> <i>E. coli</i> <i>P. aeruginosa</i>	[66]		
Metal based	AgRuSCs	AGXX®	By ROS catalytical production	UTI Skin infections Bacteremia Endocarditis Food poisoning Pneumonia TSS	<i>Enterococcus faecalis</i> MRSA	[54,67–69]	
Natural Compounds	Allicin		OS by ↑ ROS via ↓ of low MW thiols	Skin infections Bacteremia Endocarditis Food poisoning Pneumonia TSS	<i>S. aureus</i>	[70]	

UTI = urinary tract infections; * by a NADH-dependent reduction; ** carbapenemase-producing; ↑ means increase; ↓ means decrease/reduction; TSS = toxic shock syndrome; NPs = nanoparticles; *** used also in combination with ROS-producing antimicrobials such as silver nanoparticles (AgNPs); NZs = nanozimes; OSECs = organo-selenium compounds; AgRuSCs = silver and ruthenium-based surface coatings; MRSA = methicillin-resistant *S. aureus*; MW = molecular weight.

3.1.1. ROS Formation Induced by Antibiotics

Clinically approved antibiotics such as erythromycin, by protein synthesis inhibition, and rifampicin, by inhibiting RNA synthesis, are effective against *Rhodococcus equi*, while vancomycin is antibacterial against *R. equi*, *Mycobacterium tuberculosis*, and *S. aureus*, by inhibiting cell wall synthesis inhibition. Moreover, norfloxacin, by inhibiting DNA gyrase, is effective against *R. equi*, *S. aureus*, and *E. coli*. Clofazimine, by DNA replication inhibition, and ethambutol and isoniazid, by cell wall synthesis inhibition, are antibacterial against *M. tuberculosis*. Finally, quinones, by different cellular targets, are active on *Enterococcus* sp., *Streptococcus* sp., *Staphylococcus* sp., and *Moraxela catarrhalis* [64]. Anyway, studies observed that antibiotics functioning with a primary mode of action not correlated with OS, interfering with some bacterial cell targets, as above reported, were found to cause bacterial damage while also generating ROS [71,72]. As shown in Figure 2, the interaction of antibiotics with bacterial cell targets can cause both ROS hyperproduction and cell damage. The damage and disease induced by the initial ROS hyperproduction cause, in turn, additional ROS induction and production. The self-sustained growth of ROS concentration in bacterial cells goes out of control, thus causing irreversible OS and lethally amplifying cellular damage, leading to bacteria death.

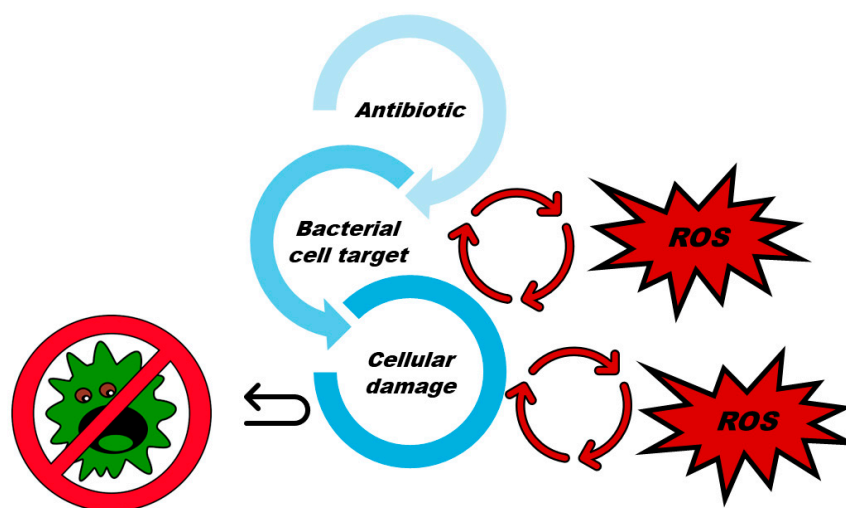


Figure 2. ROS induction by antibiotics as a secondary mechanism of their antibacterial effects.

Particularly, some antibiotics generate ROS through overstimulation of electrons via the tricarboxylic acid cycle and the release of iron from the iron-sulfur clusters, thus activating the Fenton chemistry. Nitrofurantoin and Polymyxin B are two commonly used ROS-mediated antibiotics [22]. Nitrofurantoin, used to treat urinary tract infections by *E. coli* [18], acts through a NADH-dependent reduction, producing nitroaromatic anion radicals. The autooxidation of these anion radicals in the presence of O_2 produces O_2^- , which ROS generate, thus causing OS and toxicity in bacteria [18]. Polymyxin B (PMB) is part of the family of antimicrobial peptides and is active mainly on Gram-negative bacteria such as *A. baumannii*, *P. aeruginosa*, and carbapenemase-producing *Enterobacteriaceae* [18,73]. Due to its neurotoxic and nephrotoxic properties, PMB is advised to be used only as a last resort antibiotic [18]. Sampson et al. demonstrated that PMB, in addition to being a membrane disruptor [74], induced cell death in Gram-negative bacteria by the accumulation of $OH\bullet$ [58]. Anyway, Arriaga-Alba and co-workers were the first authors to report oxidative stress induction as a part of the mechanism of action of nalidixic acid and norfloxacin in *Salmonella typhimurium*. [59]. Antibiotics were shown to upregulate many oxidative stress genes in *P. aeruginosa* [22]. Wang and Zhao found out that norfloxacin was more lethal in *E. coli* deficient in the catalase gene *katG* than in its isogenic mutants, thus confirming a potential pathway linking hydroxyl radicals to antibiotic lethality [60]. Also, ampicillin and kanamycin showed increased lethality in an alkyl hydroperoxide reductase

ahpC *E. coli* mutant, lacking the defense system to contrast hydroxyl radicals. These studies evidenced increased superoxide levels in the bacterium, which were the source of H₂O₂, which in turn generated the highly toxic hydroxyl radical responsible for the improved lethality of antibiotics [60]. Hong et al. demonstrated that *E. coli* exposed to lethal oxidative stressors caused by antibiotics including nalidixic acid, trimethoprim, ampicillin, and aminoglycosides did not die only during the actual treatment but also post-treatment, after the removal of the initial stressor, due to post-stress ROS-mediated toxicity [61,62]. Anyway, the connection between antibiotic action and ROS is not yet clearly demonstrated. Since it was demonstrated that antibiotics also work under anoxic conditions, some reports oppose the idea that the generation of ROS contributes to their lethality, which is instead influenced by the bacterial metabolism, iron homeostasis, and iron-sulfur proteins [75–77]. Although contradictory studies exist concerning the possible ROS influence on antibiotic lethality, it has been established that numerous alternative antimicrobials work by inducing ROS-mediated OS in bacteria. Unfortunately, some ROS-producing antibiotics, such as aminoglycosides, fluoroquinolones, and β -lactam antibiotics, may induce host cellular damage in specific tissues, such as the renal cortex or tendons, by generating OS, which is anyway manageable by specific antioxidant molecules [64].

3.1.2. ROS Formation Induced by Alternative Antimicrobials

Several novel alternative antimicrobials are under development whose primary mode of action seems to be through the generation of ROS-causing OS in bacteria. Generally, these antimicrobials often target the redox defenses, such as the thiol-dependent enzyme thioredoxin reductase (TrxR) in bacteria [63]. Examples of ROS-mediated antimicrobials include Ebselen, nanoparticles, nanozymes, and AGXX[®]. Even if not reported in Table 6, due to their photodependent capability to produce ROS, emerging nanomaterials such as carbon dots (CDs), produced by different sources, have demonstrated antimicrobial properties by ROS induction.

Ebselen is an organo-selenium-based antioxidant drug endowed with anti-inflammatory, antioxidant, and cryoprotective effects. It acts by inhibiting TrxR in bacteria lacking glutathione, thus triggering OS thus being lethal to these pathogens. Ebselen was recently shown to efficiently inhibit in vitro the growth of MDR *S. aureus*, to improve wound healing in rats, and to reduce the bacterial load in *S. aureus* skin lesions in rats [63]. Ebselen has been reported to inhibit *M. tuberculosis*. Importantly, Ebselen could also be combined with other ROS-stimulating compounds that block the antioxidant defenses of bacteria, such as silver nanoparticles (NPs) [64].

Mainly due to their small size (<100 nm), nanoparticles (NPs) can cause hyperproduction of ROS, which can cause carbonylation of proteins, peroxidation of lipids, DNA/RNA breakage, and membrane structure destruction, thus damaging cells [78]. Among NPs, those made of silver, such as silver oxide NPs (AgNPs), titanium dioxide, silicon, copper oxide, zinc oxide, gold, calcium oxide, and magnesium oxide NPs, have been ported to have antibacterial effects against both Gram-positive and Gram-negative pathogens [57]. Mesoporous silica NPs (MSNPs) containing a maleamato ligand (MSNPs-maleamic) and others containing also copper (III) coordinator ions (MSNPs-maleamic-Cu), synthesized by Diaz-Garcia et al., demonstrated antibacterial activity against *E. coli* and *S. aureus* by OS induction [65]. The minimum inhibitory concentration values (MICs) of MSNPs-maleamic and MSNPs-maleamic-Cu established that both preparations performed better against *E. coli* than on *S. aureus* and that MSNPs-maleamic (MIC = 62.5 μ g/mL) was more effective than MSNPs-maleamic-Cu (MIC = 125 μ g/mL) against *E. coli* [65]. Both preparations caused a significant increase of ROS in both species (30–50% more than in control), and the NPs that caused the major increase displayed lower MICs (MSNPs-maleamic, 50% in *S. aureus*, and 40% in *E. coli*), thus confirming that ROS and OS generation contribute to the antibacterial mechanism of action of MSN-maleamic and MSN-maleamic-Cu [65]. As recent members of the nanomaterial family, carbon dots (CDs) have demonstrated photoluminescence, easy surface functionalization modification, simple preparation, low toxicity, low

side effects, and a lower probability of developing resistance, showing great antibacterial and antiviral potential [79]. Although the specific antibacterial mechanism of CDs needs to be strengthened, several studies have associated their antimicrobial effects with ROS improvements. Rabe et al. prepared positively charged CDs with different surface passivation layer thicknesses using polyethylene imine (PEI) of different molecular weights, which demonstrated strong antibacterial activity by photogenerated ROS [80]. Bing et al. prepared both positively charged SC-CDs (+27.6 mV), negatively charged CC-CDs (−19.5 mV), and neutrally charged GC-CDs (0.946 mV) [81]. When tested on *E. coli*, these CDs demonstrated antibacterial effects based on ROS aggregation, cell apoptosis, and bacterial cell membrane destruction, which led to programmed bacterial death [81]. Moreover, when CDs with a high negative surface charge (-75 ± 4 mV) were used to treat *S. aureus* and MRSA, they showed antibacterial activity under laser irradiation. Particularly, upon CD adhesion to the cell surface, production of ROS and cell wall damage occurred, protein structure and function changed, with the subsequent death of bacteria [82,83]. Finally, but many other examples exist, Wang et al. synthesized graphene-based Cl-doped CDs, which, due to the high content of defect sites caused by Cl doping, were capable of producing ROS under visible light irradiation and were exploitable for antibacterial applications [84].

The major drawback of ROS-based antibacterial therapy is the low selectivity of ROS, which is detrimental to human cells as well. Recently, nanomaterials possessing enzyme-like characteristics and referred to as nanozymes (NZs), have been reported to produce surface-bound ROS that were selective in killing bacterial cells over mammalian ones [66]. Particularly, ROS bound on silver and palladium bimetallic alloys (AgPd0.38) efficiently killed antibiotic-resistant bacteria, including *S. aureus*, *Bacillus subtilis*, *E. coli*, and *P. aeruginosa* (MBC = 4–16 $\mu\text{g}/\text{mL}$), without developing drug resistance and inhibiting biofilm formation [66].

AGXX[®] is a silver and ruthenium-based antimicrobial surface coating that can be coated or deposited on various carriers, such as cellulose, plastics, ceramics, or metals. AGXX[®] demonstrated low levels of toxicity to mammalian cells [85] and no tendency to develop resistance due to its multiple modes of action [67]. ROS are produced catalytically by AGXX[®], which has been demonstrated to inhibit the growth of *Enterococcus faecalis* and MRSA, as well as to prevent biofilm formation in MRSA [54,68]. In both species, AGXX[®] affected oxidative stress defenses such as superoxide dismutase (SodA), catalase (KatA), alkyl hydroperoxide reductase (AhpCF), thioredoxin/thioredoxin reductase (Trx/TrxR), disulfide reductase (MerA), and oxidized bacillithiol (BSH) [69]. AGXX[®]-induced OS, general stress, heat shock (expression of Clp proteases), and copper stress [54,68]. In MRSA, it affected iron homeostasis and upregulated several siderophore biosynthesis (sbn) genes [54,69], while in *S. aureus*, increased protein-thiol oxidations, protein aggregations, and a BSH redox state were observed [67,69]. The mechanism of action of AGXX[®] was assessed in *S. aureus* by observing two interconnected redox cycles by which AGXX[®] simultaneously exerts ROS-mediated (superoxide anion, hydrogen peroxide, and highly toxic HO●) antimicrobial effect and achieves self-renewal.

3.1.3. ROS Formation Induced by Natural Compounds

In addition to synthetic compounds, natural compounds can exert ROS-mediated antimicrobial effects. It is the case of allicin and honey, with the latter being the main ingredient of a clinically approved gel formulation for topical administration. Honey is used in honey antimicrobial therapy, which has been discussed here in a dedicated section. There are many other secondary metabolites produced by plants that may elicit oxidative stress in bacteria, such as catechins, ferulic acid, and their derivatives [64]. The combination of other ROS- and RNS-generating antimicrobials with these compounds may lead to the development of promising therapeutic strategies against different intracellular bacterial pathogens [64]. Particularly, allicin is a constituent of garlic, which works as a thiol-reactive compound, decreasing the levels of low molecular-weight thiols, which should function

as a defense against ROS. Their increase causes OS in bacterial cells, thus inhibiting their growth [70].

3.2. ROS Formation Induced by Antibacterial Photodynamic Therapy

In a study on the toxicity of small concentrations of acridine red on *Paramecium* spp., it was recognized that the observed toxicity was dependent on the time of day and the amount of daylight [86]. Later, von Tappeiner and Albert Jesionek clinically applied this approach to treat skin carcinomas and coined the term “photodynamic phenomenon” [87–89]. Thus, anticancer photodynamic therapy was born. In those years, the successful photodynamic inactivation of bacteria was also described [86]. Anyway, while anticancer PDT has been clinically applied for 25 years, at least in the treatment of actinic keratosis or basal cell carcinoma, its application as an antimicrobial option has only more recently been rediscovered to manage the emergence of the first drug-resistant infections in the healthcare sector during the early 1990s [90,91].

3.2.1. Basic Principles of Photodynamic Therapy

PDT is based on the combination of three elements, including a non-toxic compound referred to as a photosensitizer (PS), light in a spectral range appropriate for exciting the PS (typically from the visible to near infrared (NIR) spectrum), and molecular oxygen [91]. The mechanism of PDT is described by the Jablonski diagram reproduced in Figure 3.

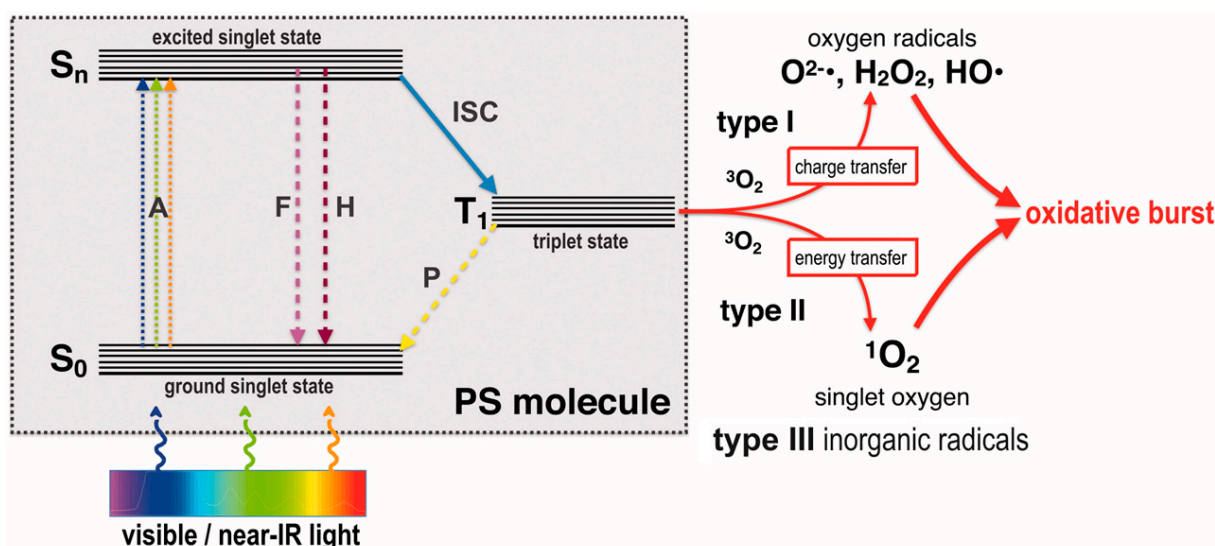


Figure 3. Jablonski diagram showing the photochemical and photophysical mechanisms of antimicrobial photodynamic therapy (PDT). S_0 : ground singlet state of the PS molecule; S_n : excited singlet state of the PS molecule; T_1 : triplet excited state of the PS molecule; A: absorption of light; F: fluorescence emission; H: heat generation (internal conversion); ISC: inter-system crossing; P: phosphorescence emission; 3O_2 : ground state oxygen; 1O_2 : singlet oxygen; $O_2^{\cdot-}$: superoxide anion; $HO\cdot$: hydroxyl radical; H_2O_2 : hydrogen peroxide. The image is an adaptation from an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0/> accessed on 22 May 2024), which permits unrestricted use, distribution, and reproduction in any medium [86].

Briefly, upon absorption of a photon (A), the PS moves from its ground singlet state (S_0) to an excited singlet state (S_n). In this status, PS can lose energy, thus returning to S_0 by emitting fluorescence (F) or heat (H) via internal conversion. On the contrary, it can pass to a longer-living excited triplet state PS (T_1) through an inter-system crossing (ISC) process. Following, it can either return to the S_0 state by phosphorescence emission (P) or by generating reactive oxygen species (ROS) by two mechanisms [92]. When a type I mechanism is followed, electrons are transferred to surrounding substrates, thus forming

superoxide radical anions ($O_2^{\bullet-}$), that undergo dismutation into hydrogen peroxide (H_2O_2), from which the highly reactive hydroxyl radical ($HO\bullet$) derives via Fenton-like reactions. Differently, in the type II mechanism, energy and not charge are transferred directly to the ground-state molecular oxygen (3O_2), leading to the emergence of singlet oxygen (1O_2), which is nothing else than energized molecular oxygen [93]. At this point, PS is returned to its S_0 status, ready to begin another cycle with the production of additional ROS. Interesting, one PS molecule can generate thousands of molecules of 1O_2 before being destroyed. The singlet oxygen quantum yield describes the amount of type II mechanism [93]. During antibacterial PDT, type I and type II reactions can occur simultaneously, and the ratio between these processes depends on the type of PS utilized, its chemical structure, and the specific microenvironment in which the PDT is implemented. The interplay between type I and type II mechanisms is a critical factor to consider for an optimized treatment and understanding its underlying photochemical processes [94–96]. As already reported, ROS are detrimental for bacteria by targeting several vital microbial molecules, such as proteins, lipids, and nucleic acids, thus determining bacterial death. An additional type III reaction has been recently incorporated into the familiar categories of type I and II mechanisms. In this novel process, free radicals of inorganic compounds are generated, regardless of the presence of oxygen, which would participate in the photoinactivation of microorganisms [97].

3.2.2. Photosensitizers Used in Clinical Trials

The main photosensitizers used in clinical trials are listed in Table 7. They include phenothiazinium, porphyrin, chlorin, phthalocyanine, xanthene derivatives, fullerenes, phenalenones, riboflavin, curcumin, hypericin, and 5-amino-lavulinic acid [98,99]. In this context, porphyrins are aromatic macrocycles that exhibit a characteristic absorption spectrum with a strong $\pi-\pi^*$ transition of ≈ 400 nm (Soret band) and four Q bands in the visible region. They are endowed with a strong 1O_2 generation efficiency and an excellent fluorescence property. Particularly, Photofrin is recorded as the first-generation PS for PDT. Unfortunately, Photofrin suffers from poor water solubility and a low extinction coefficient in the NIR region [100]. Phthalocyanines (PCs) represent the second-generation of PSs for PDT. Compared with porphyrins, PCs have an exact molecular structure and better photophysical and photochemical properties. PCs exhibit a strong absorption band in the red region, and the presence of metal atoms, such as Zn, Al, and Si, yields a long T1 lifetime and a high 1O_2 generation quantum yield [101]. Unfortunately, drawbacks such as a strong tendency to aggregate in aqueous solutions and a too slow clearance in vivo should be solved before their massive application in clinical PDT [100]. Organic dyes, such as indocyanine green (ICG), IR-825, and IR-780, showed considerable application in fluorescence imaging and PDT due to their near-infrared (NIR) absorption and excellent biocompatibility. Curcumin is a photoactive, polyphenolic compound derived from the turmeric root. Curcumin shows excellent phototoxicity to cancer cells and cytoprotectivity to normal cells. However, its poor water solubility and rapid clearance from the living body prevents its use in vivo. In this regard, the introduction of electron-donating groups to the curcumin skeleton can redshift the fluorescence wavelength, while modification with glycosylated ligands can significantly enhance its water solubility [102,103]. Fullerenes have peculiar electronic properties and biological activities. Specifically, C60 is an extremely efficient 1O_2 generator with a quantum yield close to 100% [104]. However, its PDT application is limited because of its hydrophobic surface and extremely poor water solubility. Therefore, the development of novel methods to improve the water dispersibility of C60 has received considerable attention for the past few years [100].

Table 7. Most common photosensitizers used in clinical trials.

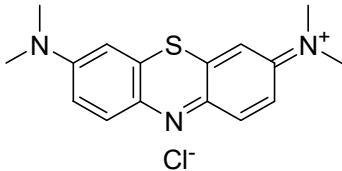
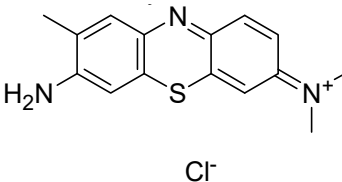
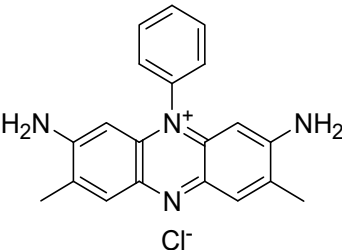
Class	Compound	Description	Target	Abs _{max} (nm)	Impact in the Field	Refs.
	 <p>Methylene Blue</p>		Dental plaque	632		[105]
Phenothiazinium derivatives	 <p>Toluidine Blue</p>	3-ring p-system Auxochromic side groups Single positive charge SOQY < 0.5 Type I reactions	<i>Streptococcus mutans</i>	410	1st clinically approved PS (dentistry)	[106]
	 <p>Safranin O</p>		<i>F. nucleatum</i> <i>P. gingivalis</i>	520	Standard PS in vitro	[107]
						[108]

Table 7. Cont.

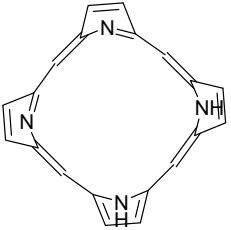
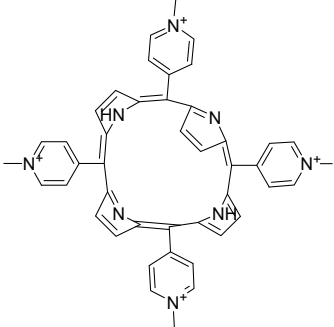
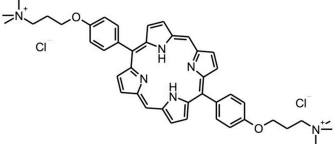
Class	Compound	Description	Target	Abs _{max} (nm)	Impact in the Field	Refs.
	 <p>Porphyrin</p>		<i>S. aureus</i> <i>P. aeruginosa</i> <i>E. faecalis</i>	446	Widely used as standard PS in vitro	[109–111]
Porphyrin derivatives	 <p>TMPyP *</p>	Four pyrrole cycles Up to eight positive charges SOQY = 0.5–0.8 Occurring in nature Type II reactions	MRSA ESBL <i>K. pneumoniae</i>	421	N.R.	[112]
	 <p>XF-73 *</p>		Staphylococci Enterococci Streptococci <i>S. aureus</i> biofilm	380–480		[113]

Table 7. Cont.

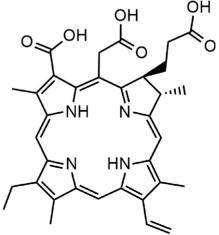
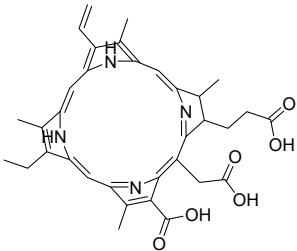
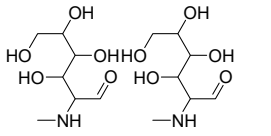
Class	Compound	Description	Target	Abs _{max} (nm)	Impact in the Field	Refs.
Chlorin derivatives			<i>S. aureus</i> <i>E. coli</i>	660 (neutral)		[114]
	Chlorin e6	Like heterocyclic-macrocyclic compounds	<i>E. coli</i>	532 (cationic)		[115]
		Neutral or up to eight positive charges SOQY = 0.5–0.8 3 pyrrole and 1 pyrroline subunit Type II reactions			N.R.	
			MRSA MSSA	660		[116]
	Photodithazine® **					

Table 7. Cont.

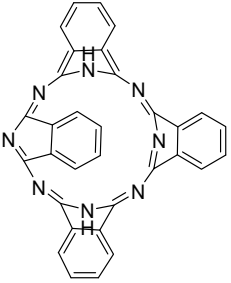
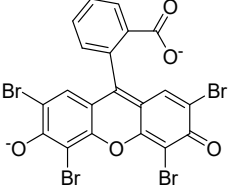
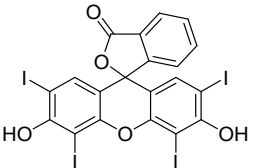
Class	Compound	Description	Target	Abs _{max} (nm)	Impact in the Field	Refs.
Phthalocyanin derivatives	 <p>Phthalocyanine</p>	4 pyrrole cycles Hydrophobic and uncharged Type II reactions	<i>A. hydrophila</i>	670	N.R.	[117]
Xanthene derivatives	 <p>Na⁺Na⁺ Eosin Y</p>	Anionic xanthene dyes Fluorescein derivatives SOQY = 0.5–0.6 Type II reactions	MRSA MRSA biofilm <i>S. aureus</i>	N.R.	Sparse studies in recent years	[118]
	 <p>Erythrosine</p>		<i>S. mutans</i> <i>Lactobacillus casei</i> <i>Candida albicans</i>			470

Table 7. Cont.

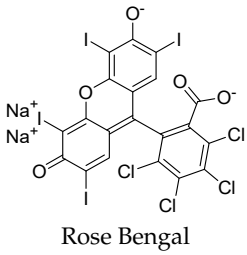
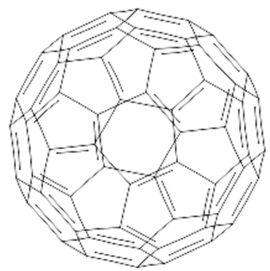
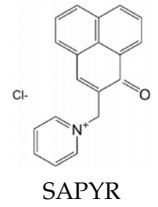
Class	Compound	Description	Target	Abs _{max} (nm)	Impact in the Field	Refs.
Xanthene derivatives	 <p>Rose Bengal</p>	<p>Anionic xanthene dyes Fluorescein derivatives SOQY = 0.5–0.6 Type II reactions</p>	<p><i>E. faecalis</i> <i>P. aeruginosa</i></p>	532	Sparse studies in recent years	[120]
Nanomaterials	 <p>Fullerene C60 ***</p>	<p>Soccer-ball-shaped cage molecules Made exclusively from carbon atoms Neutral Extended p-conjugated system Type I and II reactions</p>	<p><i>S. aureus</i> <i>E. coli</i></p>	532	Unique class of PS Sparse studies on effect on biofilms	[121,122]
Phenalenones	 <p>SAPYR</p>	<p>Biosynthesized by plants to defend against pathogens using the sun to generate singlet oxygen SOQY > 0.9 Positively charged pyridinium-methyl moiety Type II reactions</p>	<p><i>E. faecalis</i> <i>Actinomyces naeslundii</i></p>	360–420	First water-soluble exclusive type-II PS	[123]

Table 7. Cont.

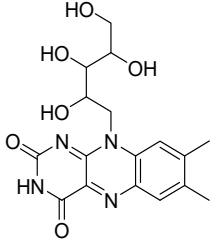
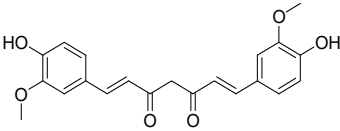
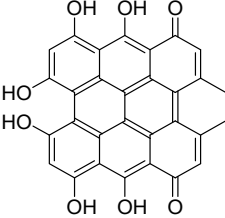
Class	Compound	Description	Target	Abs _{max} (nm)	Impact in the Field	Refs.
Riboflavin derivatives	 <p>Vitamin B2</p>	In PDT, cationic derivatives with up to eight positive charges SOQY = 0.7–0.8 Type II reactions	MRSA EHEC	450 (cationic)	N.R.	[124]
Curcumins	 <p>Curcumin (neutral)</p>	Naturally occurring yellow dye from the rootstocks of <i>Curcuma longa</i> Approved as a food additive (E100) For use in PDT, positive charges have been included in derivative structures Type I reactions	<i>S. mutans</i> <i>L. acidophilus</i>	547	Novel positively charged derivatives with enhanced water solubility	[125,126]
Natural compounds	 <p>Hypericin (neutral)</p>	Naturally occurring For use in PDT, positive charges have been included in derivative structures Type II reactions	<i>S. aureus</i> <i>E. coli</i>	593	N.R.	[127,128]

Table 7. Cont.

Class	Compound	Description	Target	Abs _{max} (nm)	Impact in the Field	Refs.
5-ALA	 5-aminolevulinic acid	<p>δ-amino acid in which the hydrogens at the γ position are replaced by an oxo group</p> <p>Metabolized to protoporphyrin IX</p>	MRSA	410	Optical imaging agent	[129]

SOQY = singlet oxygen quantum yield * derivatives of porphyrin; ** derivatives of chlorine e6; S = Abs max, Soret band; Q = Q band; *** functionalized in multiple ways by adding positively charged moieties; ESBL = extended spectra β -lactamases; MRSA = methicillin-resistant *S. aureus*; MSSA = methicillin-sensitive *S. aureus*; MRSE = methicillin-resistant *S. epidermidis*; EHEC = Enterohemorrhagic *E. coli*; 5-ALA = 5-aminolevulinic acid.

The most common PSs clinically used in anticancer PDT have also been extensively studied for their antibacterial properties and effectiveness in the treatment of various bacterial infections. Many published studies have determined that phenothiazinium PSs such as MB and TB are effective on planktonic bacteria. Furthermore, some studies also tested the efficacy of phenothiazinium against biofilm structures [98]. Recently, new derivatives such as dimethyl methylene blue (derived from MB) and EtNBS (N-ethylpropylsulfonamido) have been studied. These dyes possess a high cationic charge, which makes them more effective against bacterial cells [98]. Rose Bengal is an anionic synthetic xanthene dye that has shown promise in several clinical trials for PDT, particularly in the treatment of localized bacterial infections. Other synthetic anionic xanthene dyes derived from Fluorescein are Eosin Y and Erythrosine (ERY). All these dyes have an absorption peak in the green wavelength range (480–550 nm). The attachment and uptake of anionic PSs by the bacterial cells are lower than cationic ones [98]. Amino-levulinic acid (ALA) is a prodrug that can be converted to protoporphyrin IX (PpIX), clinically used (in the form of hydrochloride salt) in combination with blue light illumination for the treatment of minimally to moderately thick actinic keratosis of the face or scalp. It has been demonstrated to be effective in the treatment of bacterial infections, including periodontal infections. Additionally, a variety of evidence has proven that 5-aminolevulinic acid-based photodynamic therapy (ALA-PDT) is clinically effective in the management of *Acne vulgaris* and is recommended as an alternative treatment modality for severe acne [130]. Concerning chlorins, mainly cationic derivatives of chlorin-e6 are used for PDT. Photodithazine[®] is a commercially available chlorin-e6 derivative with two positive charges. Chlorine e6 (Ce6) and various phthalocyanines have strong antibacterial properties and have been used in preclinical studies and in some early clinical trials for antibacterial PDT. Curcumin is a natural compound found in *Curcuma longa*, and its cationic derivatives have also been investigated as possible PSs. Collectively, the use of specific PSs in clinical trials strongly varies depending on the target bacterial infection and the research objectives of each study [98,131,132].

3.2.3. Antibacterial PDT vs. Antibiotics

Unfortunately, as reported in the following Table 8, even if the advantages of using antibacterial PDT (APDT) compared to antibiotics are significant, APDT has certain limitations that need careful consideration.

Table 8. Advantages and limitations of APDT compared to conventional antibiotics.

Advantages	Limitations	Discussion on Limitations
↑ BS action than antibiotics including bacteria, protozoa, fungi	Light limited penetration capabilities	Possible problems in reaching deep seated infections by scattering phenomena [133] Bacterial colonies located beneath the skin's surface or within organs could be difficult to reach * [134]
↓ Adverse effects and damage to the host tissue	Potential lack of target specificity	↑ Selectivity and efficiency of PSs toward bacteria and ↓ toxicity on mammalian cells can be achieved using proper vectors or by co-administration, conjugation, or incorporation with polycationic materials, bacterial-targeting peptides, polymers, antibiotics, or antibodies [135–138]
Bactericidal effects are independent of antibiotic resistance pattern	Risk of antibiotic inactivation	When in combination with certain antibiotics, APDT can inactivate the antibiotics [134]
No resistance following multiple sessions of therapy	Potential side effects	Emergence of skin sensitivity, redness, and pain at the treatment site [139]

* In this case, the correct choice of the wavelength and PS is pivotal; PS = photosensitizer; BS = broad spectrum; ↑ means increase, more; ↓ means decrease/reduction, less, or minor.

Despite promising results that have been observed in some diseases, the clinical translation of PDT for bacterial infections has progressed slower than for cancer treatment and is less widespread than anticipated. Therefore, its widespread adoption in medical practice remains limited. Among the limitations, the modest capability of light to penetrate skin, tissues, and organs hampers the application of APDT for systemic infection and reduces its effectiveness in topical treatments. The penetration of light depends on the optical properties of the tissue and the wavelength of the light used. There is heterogeneity between tissues and even within a tissue. These inhomogeneity sites (e.g., nuclei, membranes, etc.) cause light scattering, reflecting, transmitting, or absorption [133]. Light within the 620–850 nm spectrum range achieves optimum tissue penetration and PDT applications [133]. Mainly concerning potential side effects, understanding and effectively managing them is critical for optimizing patient outcomes. To fully harness the potential of antibacterial PDT as a valuable therapeutic strategy for combating bacterial infections, it is imperative to address these drawbacks through ongoing research efforts and comprehensive approaches [131,140]. The successful outcome of antibacterial PDT depends on the selected PS. To enhance PS selectivity over host tissue while maintaining nonselective activity against microbial species represents a pivotal challenge. The optimal PS should target both Gram-positive and Gram-negative species, accounting for their differential treatment responses. Several very recent studies have described the latest advancements in the field over the past years, emphasizing new PS systems relevant for antibacterial PDT's methodologies [86,141–143], which have recently been reviewed by Sébastien Clément and Jean-Yves Winum [134].

3.2.4. Light Sources

Different light sources have been and are employed in PDT, each with advantages and disadvantages, as reported in Table 9. In addition to the light sources reported in Table 9, “non-coherent” or “non-thermal” light sources without giving details of the actual light source used have been reported.

Table 9. Main light sources employed to activate PSs.

Light source	LASER		Monochromatic, coherent, and collimated light High irradiance Couplable into optical fiber bundles Expansive, cumbersome	Refs.
	Argon	Diode		
		Neodymium doped	Yttrium Aluminum Garnet lasers	[144]
	Light-emitting diodes (LEDs)		Deliver a slightly wider emission spectrum than LASER Low costs No monochromatic, no coherent	[144]
	Gas-discharge lamps	Quartz-tungsten-halogen lamps Xenon-discharge lamps Sodium lamps		They can be spectrally filtered to match any PS No efficiently couplable into optical fiber bundles Cause more heating as compared to LASERs and LEDs
		Daylight		Broad-spectral range from UV to IR region Free of cost Can illuminate a very large area with high uniformity Variable in irradiance, radiant exposure is poorly controlled

LASER = Light Amplification by Stimulated Emission of Radiation.

Additionally, there are also occasional reports of other light sources such as endoscopy systems, photopolymerisers, and supra-luminous diodes (SLD) [144]. All in all, for the irradiation of a given PS, parameters such as radiant exposure, light irradiance, power output, spectral emission, intensity of the respective light source, as well as the mode of light delivery (via optical fiber or directly), are more important than the type of the light source itself [144].

3.2.5. Clinical Trials

Due to the multi-faceted nature of the antimicrobial photodynamic process and its diverse targets, the emergence of resistance in microbes becomes highly improbable. Consequently, the anti-bacterial PDT should be considered promising as a potent and innovative approach to combating bacterial infections [147]. In the last few years, the volume of research focused on PDT as a therapeutic device to inhibit a wide range of microorganisms, including bacteria, has remarkably increased. Anyway, the antibacterial PDT has yet to attain the capability to tackle systemic infections. On the contrary, it has demonstrated high potential for addressing localized infections sustained by MDR bacteria, including hospital-acquired pathogens such as *E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* spp., which together constitute the group ESKAPE [148]. Additionally, in vitro and in vivo studies have demonstrated the capability of PDT to eradicate or significantly reduce biofilms, thus finding applications in dental diseases, skin infections, and orthopedic implant treatment [149,150]. The ongoing advancement of antibacterial PDT systems is also marked by the continuous refinement of the strategies, particularly through synergistic combinations of diverse chemicals. Antibacterial PDT has been applied in about 40 clinical trials for the treatment of dermatological disorders and oral infections. Clinical trials on antibacterial PDT have focused on evaluating its safety and efficacy in treating specific bacterial infections, especially those that are challenging to manage with traditional antibiotics [91]. Some of the key findings from these trials include those obtained in dermatological disorders such as acne vulgaris and bacterial skin conditions, where antibacterial PDT studies have shown positive outcomes in reducing the severity of acne lesions [151–154]. Some studies have also explored applications for treating infected wounds, especially those associated with MDR bacteria. The results suggest that antibacterial PDT can be beneficial in promoting wound healing and controlling bacterial growth [155,156]. In the field of periodontal diseases, clinical trials have shown promising results for treating chronic periodontitis, a common bacterial infection that affects the gums and supporting structures of the teeth [157–160]. Other clinical trials have also examined the use of antibacterial PDT in managing bacterial infections related to medical implants, such as catheters and prosthetic devices [161,162].

3.3. ROS Formation Induced by Antibacterial Honey Therapy

Honey was used in traditional medicine mainly to treat wounds due to its antimicrobial effects and healing properties. The bactericidal efficacy of honey was reported more than a century ago by Van Ketel [163], whose findings prompted extensive research on honey over the next decades. With the advent of modern medicine, interest in honey and its medical use decreased [163]. Nowadays, honey is undergoing a revival in its consideration for antimicrobial and wound healing applications, due to the rising global antibiotic resistance, which makes the development of novel alternative therapies to combat infections necessary. Several factors can contribute to its effective antimicrobial activity, which can strongly vary depending on different microbial strains, including the geographical and botanical source, its harvesting, processing, and storage conditions. It has been demonstrated that a range of both Gram-positive and Gram-negative bacteria, including MDR strains, biofilms, fungi, and viruses, can be inhibited by honey. Furthermore, susceptibility to antibiotics can be restored when used synergistically with honey. Table 10 reports the antibacterial effects of honeys from different geographical sources and the related target bacteria.

Table 10. Antimicrobial effect of honey from different geographical locations and target pathogens.

Country of Origin	Honey Sample	Organisms	Refs.
Australia			
New Zealand	Manuka	<i>S. aureus</i> , <i>P. aeruginosa</i>	[164]
New Zealand	Manuka	<i>S. aureus</i> , MRSA, MSSA Coagulase-negative <i>S. epidermidis</i> <i>K. pneumoniae</i> , ESBL <i>E. coli</i>	[165]
Australia	<i>Leptospermum</i> based honey	<i>S. aureus</i>	[166]
North America			
Canada	Canadian honey	<i>E. coli</i> , <i>Bacillus subtilis</i>	[167]
Cuba	Christmas vine, Morning glory Black mangrove Linen vine, Singing bean	<i>S. aureus</i> , <i>P. aeruginosa</i> <i>E. coli</i> , <i>B. subtilis</i>	[168]
South America			
Chile	Ulmo honey	MRSA, <i>E. coli</i> , <i>P. aeruginosa</i>	[169]
Argentina	Algarrobo, citrus and multifloral honey	<i>S. aureus</i> , <i>E. faecalis</i> , <i>E. coli</i> <i>Morganella morganii</i> <i>P. aeruginosa</i>	[170]
Europe			
Scotland	Blossom, heather, Highland, Portobello Orchard	<i>Acinetobacter calcoaceticus</i> <i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i>	[171]
Northwest Spain	Rubus honey	<i>S. aureus</i> , <i>S. epidermidis</i> <i>Micrococcus luteus</i> , <i>E. faecalis</i> <i>B. cereus</i> , <i>Proteus mirabilis</i> , <i>E. coli</i> <i>P. aeruginosa</i> <i>Salmonella typhimurium</i>	[172]
Denmark	Heather, raspberry, rapeseed, hawthorn White clover	<i>S. aureus</i> , <i>P. aeruginosa</i> , <i>E. coli</i>	[173]
Slovakia	Honeydew honey	<i>P. aeruginosa</i> , <i>S. aureus</i>	[174]
Asia			
China	Buckwheat honey	<i>S. aureus</i> , <i>P. aeruginosa</i>	[164]
Saudi Arabia	Sider honey	<i>S. aureus</i> , <i>Streptococcus pyogenes</i> <i>Corynebacteria pseudotuberculosis</i> <i>K. pneumoniae</i> , <i>P. aeruginosa</i> <i>E. coli</i>	[175]
Africa			
Algeria	Astragalus, wall-rocket, eucalyptus Legume, peach, juniper, buckthorn multifloral	<i>Clostridium perfringens</i> , <i>S. aureus</i> , <i>E. coli</i> , <i>B. subtilis</i> .	[176]
Nigeria	Wildflower and bitter leaf honey	<i>S. typhimurium</i> <i>Shigella dysenteries</i> , <i>E. coli</i> <i>B. cereus</i> , <i>S. aureus</i>	[177]
Egypt	Cotton, blackseed, orange, eucalyptus Sider, clover honey	<i>E. coli</i> , <i>S. aureus</i> <i>Streptococcus mutans</i> , <i>P. mirabilis</i> <i>P. aeruginosa</i> <i>K. pneumoniae</i>	[178]
Egypt	Acacia, citrus, clover, coriander, cotto palm honey	<i>S. aureus</i> , <i>S. pyogenes</i> <i>C. pseudotuberculosis</i> <i>K. pneumoniae</i> , <i>P. aeruginosa</i> , <i>E. coli</i>	[175]

ESBL = extended spectrum β -lactamase.

3.3.1. Medical Application of Honey

Although the knowledge of the antibacterial compounds involved in the antibacterial effects of honey remains incomplete, the information on honey has remarkably expanded in recent years. Otherwise, despite the variability of the antibacterial activity of honey, which limits its extensive applicability in medicine, several honeys have been approved for clinical application [179]. Currently, honey is mainly used as a topical antibacterial agent in wound applications. Its high viscosity grants an effective, hydrated, and protective barrier between the wound site and the external environment. Honey has been used to treat wounds such as burns, trauma, and chronic wounds, where the complex wound healing process could be interrupted by infection or specific disease states (e.g., diabetes), thus limiting the development of irreversible chronic wounds, recurrent infections, amputation/limb salvage, and life-threatening conditions [163]. For mild to moderate superficial and partial-thickness burns, honey was more effective than conventional treatment for reducing microbial colonization and improving wound healing [180,181]. In a study, the application of honey to tunneled cuffed hemodialysis catheters resulted in a comparable bacteremia-free period compared with that obtained with mupirocin treatment [182]. Honey has also been widely explored as a tissue-regenerative agent. In this case, it has been applied directly to the wound or in combination with traditional wound dressings, which allow honey to remain in direct contact with the wound bed, thus providing a persistent and long-term release of antimicrobial agents to contribute to all stages of wound healing. Furthermore, the presence of reactive oxygen species (ROS) such as H_2O_2 has been shown to promote wound healing by promoting cellular repair processes and tissue regeneration [10,183]. Anyway, some limitations exist, such as being absorbed by the dressing, poor penetration into the wound site, and short-term antimicrobial action. To address these issues, tissue engineering approaches have been developed, such as its formulation in electrospun fibers and hydrogels [184–188]. Collectively, the use of honey, honey-derived, and honey-inspired products in tissue engineering applications, combined with other biomaterials, may enable its use in a variety of other clinical situations outside wound care, where the combination of antimicrobial properties and tissue regeneration is desirable.

The Case of Surgihoney Reactive Oxygen (SHRO)

The first therapeutic agent based on the oxidative activity of ROS was a pharmaceutical honey gel for treating wounds, referred to as Surgihoney Reactive Oxygen (SHRO). Particularly, SHRO is an engineered, sterilized honey created to act as a preventive antimicrobial agent for soft tissue infections. SHRO, deriving from natural organic honey from different origins, is capable of providing a constant level of ROS over a prolonged period of time when topically applied to a wound. Subsequently, ROS induce OS due to $\bullet OH$ production and inhibit the essential metabolic procedure for bacterial growth [189]. As discussed in more in the subsequent sections, the antimicrobial activities of SHRO are mainly due to the generation of H_2O_2 [190]. Other formulated honey prototypes (PT1 and PT2) were designed to further increase the generation of H_2O_2 . Subsequently, honey ROS-based antibiotic agents differently formulated, such as sprays, nebulizers, and infusions, that employ this mechanism are being developed and may be particularly useful for delivering ROS to other clinical sites. Nowadays, there are many types of therapeutic honey on the market (e.g., Paterson's curse, Rosemary, Manuka, Thyme, Revamil, Rewa Rewa, heather honey, Khadi, Kraft honey, Multifloral, and Medihoney) [191,192]. Table 11 summarizes a clinical register using SHRO in various complex and severe infections [4].

Table 11. Summary of a clinical register of the use of SHRO in various complex infections.

Clinical Site	Dosing Regimen	Clinical Details	Outcome	Adverse Reports
Respiratory tract	Daily nebulized SHRO in respiratory nebulizer	Bronchiectasis, several patients One patient—recurrent exacerbations with secondary infection with <i>Mycobacterium avium</i>	Reduction in bacterial load and temporary eradication of <i>M. avium</i> (1 patient)	None
Scalp	Daily topical application for 6 weeks	Fungal kerion, <i>T. tonsurans</i> Patient intolerant to oral antifungals	Complete resolution	None
Intraperitoneal	50–100 g daily via abdominal drain	Severe four-quadrant peritonitis following intraabdominal infection and corrective surgery Patients also on systemic antibiotics Often polymicrobial infections with MDR strains and <i>Candida</i> spp.	Variable, but general peritonitis Control	N.R. to SHRO use
Abdominal wall Deep soft tissue	SHRO into an open cavity with each dressing	Used both prophylactically and therapeutically in around 20 patients	Prevention of infection and effective therapy in infected cavities	None
Prosthetic joints	Single dose around prosthetic joint at surgery Numerous patients	Mixed microbiology including <i>S. ludenensis</i> Given in conjunction with systemic antibiotics	Good adjunct to existing management	None
Prepatellar bursitis	Single application at debridement	On immunosuppression for psoriasis <i>M. malmoeense</i> isolated from prepatellar pus Put on clarithromycin, rifampicin and ethambutol + SHRO topically	Complete healing and no further isolation of <i>M. malmoeense</i>	None
Bladder	Twice-weekly instillation via suprapubic catheter	Several patients with long-term urethral or suprapubic catheters	Reduction in urosepsis	None
External auditory canal	Daily with wick or cotton wool	<i>Pseudomonas</i> otitis externa	Resolved	None
Oral infections	Daily oral application of 10 g SHRO	Recurrent aphthous ulcer, gingivitis, geographic tongue. No microbiology	Reported reduction in the duration of symptoms	None
<i>Helicobacter gastritis</i>	Once daily 10 g SHRO for 10 days	Confirmed <i>Helicobacter pylori</i> gastritis MDR strain and no response to antibiotic eradication regimens	Continuation of symptoms Therapeutic failure	None

N.R. = Not related.

With the rising age of the population in many countries, and the global epidemic of obesity and type 2 diabetes [4], the disease of chronic soft tissue lesions is becoming enormous. Most chronic breaks in the skin often become colonized with bacteria [193], which regardless of their pathogenicity, play an essential role in slowing tissue healing, establishing biofilm, and resulting in wound slough and an unpleasant odor [193]. As reported in Table 11, in these cases where often available antibiotics are no longer functioning,

the effects of SHRO on bioburden and biofilm [189] can be of great help. In fact, the early use of ROS in such lesions can control bioburden and biofilm, thus sparing conventional antibiotic use and supporting infection control [189,194,195]. In clinical studies, ROS therapy via SHRO has demonstrated satisfactory safety and tolerability and is clinically and cost-effective in practice [194,196]. Most outstandingly, SHRO has demonstrated impressive capacity to clean up bacterial bioburden and biofilm in chronic wounds, being also active on MDR bacteria such as *P. aeruginosa*, MRSA, and vancomycin-resistant enterococci (VRE) present in an ischemic ulcer [4].

Medical Grade Honey for Clinical Applications

Medical-grade honey (MGH) intended for clinical application must be sterilized by gamma irradiation to destroy potentially present bacterial spores, including those of *Bacillus* spp. and *Clostridium botulinum*, which could cause wound botulism or gangrene [197]. Several types of honey, including MGH, have recently been re-introduced into modern medicine. There is no clear definition of MGH, but according to a study by Hermann et al., MGH should satisfy the criteria reported in Figure 4 [198].

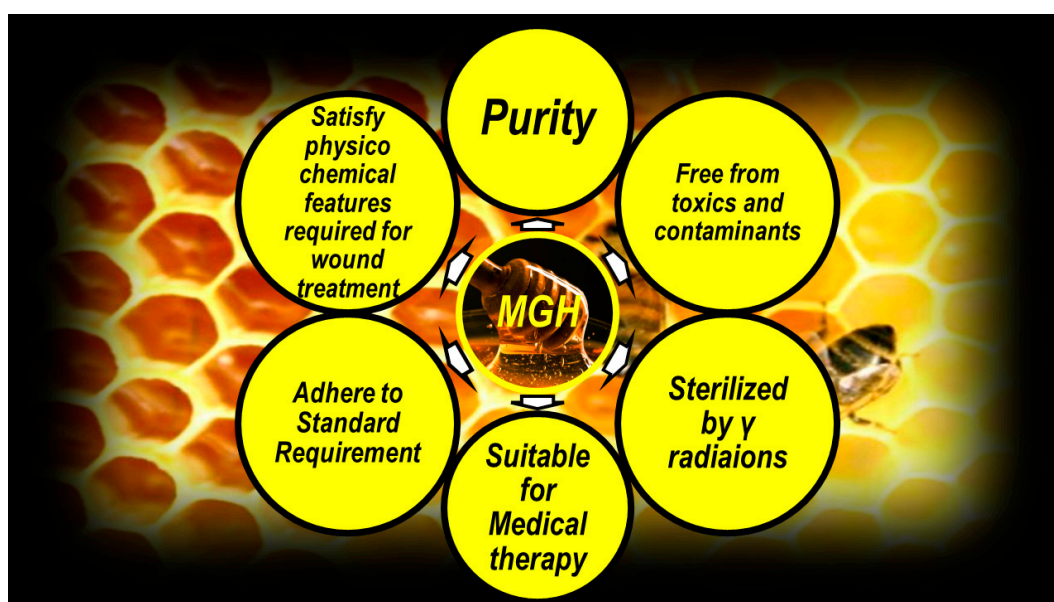


Figure 4. Characteristics and criteria that a medical-grade honey (MGH) should fulfill, according to Hermann et al. [198].

Manuka and Revamil[®] are the major medical-grade honeys currently approved for clinical application. Manuka honey is produced from the manuka bush (*Leptospermum scoparium*), a native of New Zealand and Australia. The raw honey used as a source for Revamil[®] is instead produced by a standardized process in greenhouses. Since Revamil[®] honey is registered as a medical device for applications in wound healing and not as an antimicrobial agent, the antimicrobial activity is not specified for individual batches of this honey. However, in a quantitative liquid bactericidal assay, both Revamil[®] and manuka honeys demonstrated potent bactericidal activity [199,200], with manuka honey being the most performant against *S. aureus*, *B. subtilis*, and *P. aeruginosa*. On the contrary, these honeys had identical bactericidal activity against *E. coli*. The following Table 12 reports the approved and already commercially available wound healing products based on honey.

Table 12. Commercially available honey-based wound healing products.

Product	Description	Indications	Mechanism of Action	Refs.	Clinical Evidence
Activon® Manuka Honey Tube AM	100% MGMH	Sloughy, necrotic wounds # Malodorous wounds #	Debrides necrotic tissue Can be used in dressings or directly into cavities	[201]	Blistering and cellulitis in a type 2 diabetic patient Pediatric burn Foot ulceration Grade 5 sacral wound [201]
Activon® Tulle AM	Knitted viscose mesh dressing with 100% MH	Granulating or shallow wounds Debriding or de-sloughing small areas of necrotic or sloughy tissue	Creates a moist healing environment Eliminates wound odor Antibacterial action	[201]	Over-granulated grade 3 and 4 pressure ulcers Extensive leg cellulitis Venous ulcer, chronic wound Infections, necrotic foot [201]
Algivon® Plus AM	Reinforced alginate dressing with 100% MH	Cavities, sinuses, pressure, leg, diabetic ulcers	Absorbs exudate Debrides, removes slough	[201]	Chronic wounds [202] Burn wound management [203]
Algivon® Plus Ribbon AM		Surgical, infected wounds Burns, graft sites Ideal for wetter wounds	Reduces bacterial load	[201]	Autoamputation of fingertip necrosis [204]
Aurum® ostomy bags WM	MGMH added to hydrocolloids	Stoma care	Kills bacteria Suppresses inflammation Promote healthy skin around the stoma	[205]	Pyoderma gangrenosum around ileostomy [206]
L-Mesitran® Border AME	Hydrogel + honey (30%) pad on a fixation layer	Chronic wounds \$	Exudate absorption Re-hydration of dry tissue Antibacterial properties	[207]	Pediatric minor burns and scalds [208]
L-Mesitran® Hydro AME	Sterile, semi-permeable hydrogel dressing ##	Chronic wounds * Superficial and acute wounds ** Superficial and partial-thickness burns *** Fungating wounds, donor sites	Donates moisture to rehydrate dry tissue Antibacterial properties	[207]	Pediatric minor burns and scalds [208] Fungating wounds [209]
L-Mesitran® Ointment AME	Ointment \$\$	Surgical wounds, cuts, and abrasions	Aids debridement and reduce bacterial colonization	[207]	Skin tears, irritation, and inflammation [209]
ManukaDress IG MPI	Wound dressing made with 100% <i>Leptospermum scoparium</i> @	Leg and pressure ulcers First- and second-degree burns Diabetic foot ulcers, surgical and trauma wounds	Osmotic activity that promotes autolytic debridement and helps maintain a moist wound environment	[163]	Burn management [163] Difficult-to-debride wounds [210] Necrotic pressure ulcer Recurrent venous leg ulceration [211]
Medihoney® Antibacterial Honey DSC	100% sterilized MGMH	Deep, sinus, necrotic, infected surgical and malodorous wounds	Creates an antibacterial environment Debridement on sloughy and necrotic tissue, removes malodor Provides a moist environment	[212]	Wound healing [213] Prevention of catheter-associated infections in hemodialyzed patients [214]

Table 12. Cont.

Product	Description	Indications	Mechanism of Action	Refs.	Clinical Evidence
Medihoney® Apinate Dressing ^{DSC}	Calcium alginate dressing with 100% MGMH	Diabetic foot, leg, pressure ulcers, First- and second-degree partial-thickness burns, Donor sites, traumatic, surgical wounds.	Provides a moist environment Osmotic potential Draws fluid through the wound to the surface Low pH of 3.5–4.5.	[215]	Venous leg ulcers [216]
Medihoney® Barrier Cream ^{DSC}	Barrier cream with 30% MGMH	Protects skin damaged by irradiation treatment or in wet areas Prevents damage caused by shear and friction	Maintains skin moisture and pH.	[217]	Treatment for intertrigo in large skin folds [218]
Medihoney® Antibacterial Wound Gel ^{TM DSC}	Antibacterial wound gel ^{&}	Burns, cuts, grazes, and eczema wounds	Creates a moist, low-pH environment Cleans the wound by osmotic effect Reduces the risk of infection	[219]	Reduction in incidence of wound infection after microvascular free tissue reconstruction [220]
SurgihoneyRO TM MH	Antimicrobial wound gel ^{&&}	Infected, chronic wounds	Antimicrobial activity by controlled release of H ₂ O ₂ Promotes debridement and new tissue growth	[221]	Prevention of caesarean wound infection Prevention/eradication of bacterial colonies in dressing oncology long vascular lines; ulcers, surgical wounds, and trauma wounds [194,196,222] In vitro activity against biofilm-producing clinical bacterial isolates [189]

MGMH: medical-grade manuka honey; ^{AM}: Advances Medical manufacturer; ^{WM}: Welland Medicals Ltd. manufacturer; ^{AME}: Aspen Medical Europe Ltd. manufacturer; ^{MPI}: Medicareplus International manufacturer; ^{DSC}: Derma Science-Comvita manufacturer; ^{MH}: Matoke Holdings Ltd. manufacturer; #: pressure ulcers, leg ulcers, diabetic ulcers, surgical wounds, burns, graft sites, infected wounds, cavity wounds and sinuses; \$ pressure ulcers; superficial and partial-thickness burns; venous, arterial, and diabetic ulcers; ## contains 30% honey with vitamin C and E, as well as an acrylic polymer gel and water, with a polyurethane film backing; * pressure ulcers, venous and diabetic ulcers; ** cuts, abrasions, and donor sites; *** first- and second-degree; \$\$ made of 48% medical-grade honey, medical-grade hypoallergenic lanolin, oils, and vitamins; @ sterile honey from New Zealand. Non-adherent impregnated gauze; & 80% medical-grade manuka honey with natural waxes and oils; && utilizes bioengineered honey to deliver Reactive Oxygen[®] (ROTM).

3.3.2. Antibacterial Mechanisms of Honey

The antimicrobial activity of the majority of honeys is mainly due to its capability to generate high levels of hydrogen peroxide (H₂O₂) [10,21,190,223–226] by the oxidative action of the enzyme glucose oxidase (GOx) on glucose. Secreted into the nectar by bees during the preparation of honey, GOx oxidizes glucose to gluconic acid, thus producing H₂O₂ [10,226–230]. The enzyme presents no activity in raw honey due to a lack of free water. Therefore, to initiate the peroxide-dependent antimicrobial mechanism, the honey needs to be diluted. Other important antimicrobial features responsible for the non-peroxide activity of honey include low water content (osmotic effect), low pH (acidic environment), phenolic compounds, bee defensin-1 (Def-1), and methylglyoxal (MGO) (in *Leptospermum*-derived manuka honey). Table 13 summarizes the factors that confer honey its antibacterial effects.

Table 13. Components that confer honey antibacterial effects and healing properties.

Antibacterial Factors	Sources	Mechanism of Formation	Effects on Bacteria	Refs.
H ₂ O ₂	Glu	Oxidation of Glu deriving from sucrose captured by bees from flowers	OS DNA damage	[163]
Bee Def-1	Bee's hypopharyngeal gland	Innate immune response	Create pores in membrane Interferes with bacterial adhesion Alters the production of EPSs	[226]
Acidic pH (3.4–6.1)	GluLac GluA	By the enzymatic oxidation of Glu	Prevents bacterial growth	[163]
MGO *	Dihydroxyacetone	Heating	Alters bacteria fimbria and flagella	[231]
Osmotic pressure	Super concentration of sugars	N.A.	↓ Availability of free water molecules ↓ Bacterial growth	[232]
Polyphenols	Flowers as secondary metabolites	Flowers metabolism	Pro-oxidative properties Accelerate HO• formation Oxidative DNA breakage Non-enzymatic ↑ H ₂ O ₂	[233]

Glu = glucose; GluLac = gluconolactone; GluA = gluconic acid; EPSs = extracellular polymeric substances; Bee Def 1 = Bee defensin 1; MGO = methyl glyoxal; * exclusively in *Leptospermum* honeys (e.g., manuka); ↑ means increase, more; ↓ means decrease/reduction, less, minor.

Briefly, sucrose captured by bees from flowers is broken down via diastase and invertase enzymes into glucose and fructose. Glucose oxidase (Gluox), secreted by the bee's hypopharyngeal glands, in the presence of O₂ and sufficient H₂O, oxidizes glucose, forming gluconolactone/gluconic acid, which make honey acidic and H₂O₂ [234]. H₂O₂ is the most responsible for honey's antimicrobial activity, killing pathogens through DNA damage and being destructive to several cellular targets [234]. Interestingly, the antimicrobial effect of hydrogen peroxide in honey increases upon dilution, enabling the glucose oxidase enzyme to bind to glucose more readily, thus resulting in a continuous production of hydrogen peroxide [226]. Moreover, honey, predominantly due to gluconolactone/gluconic acid formation, is acidic with an average pH of 3.91 (ranges between 3.4 and 6.1), which makes it powerful against microbial strains, preventing their growth. Bee Def-1 is an antibacterial peptide originating in the bee's hypopharyngeal gland and identified in bee hemolymph (the bee blood system) [235]. Within the bee, it acts as an innate immune response, exhibiting activity against fungi, yeast, protozoa, and both Gram-positive and Gram-negative bacteria [200]. Importantly, bee Def-1 is mainly effective against Gram-positive bacteria, most notably *B. subtilis*, *S. aureus*, and *Paenibacillus larvae*, while it has limited effectiveness against MDR organisms [179]. Although the full mechanism of action for bee Def-1 has not been elucidated, defensin proteins from other species have been shown to create pores within the bacterial cell membrane, resulting in cell death [236]. Bee Def-1 demonstrated to play an important role in wound healing, through stimulation of MMP-9 secretions from keratinocytes [237]. It interferes with bacterial adhesion to surfaces, or in the early biofilm stage, by inhibiting the growth of attached cells and by altering the production of extracellular polymeric substances (EPS). MGO is generated in honey during storage by the non-enzymatic conversion of dihydroxyacetone, a saccharide found in high concentrations in the nectar of *Leptospermum* flowers [231]. The antimicrobial activity of MGO is attributed to alterations in bacterial fimbriae and flagella, which prohibit the bacterium's adherence and motility. Honey is a super-saturated solution of sugars. The strong interaction between these sugars and water molecules prevents the abundance of free water molecules (low water activity) available for microbes to grow [232]. Finally, the combination of different phenols acts as an enhancer of honey's antimicrobial efficacy. Produced as plant secondary metabolites, these bioactive compounds are transferred from the plant to the honey by

bees and have been identified as the major reason for the health-promoting properties of honey [233]. In alkaline conditions (pH 7.0–8.0), polyphenols can display pro-oxidative properties, inhibiting microbial growth by accelerating hydroxyl radical formation and oxidative strand breakage in DNA. They could also support the production of considerable amounts of H_2O_2 via a non-enzymatic pathway.

3.4. ROS Formation Induced by Antibacterial Hyperbaric Oxygen Therapy

Differently from antibacterial honey therapy, which is a topical treatment, antibacterial hyperbaric oxygen therapy (HBOT), which is part of hyperbaric medicine, is a systemic method to treat soft tissue infections [57]. Particularly, in a typical HBOT treatment, the patient (mono-place) or more than one patient (multi-place) inhale 100% O_2 for a specified time in a pressurized chamber [238]. From the lung, the inhaled oxygen is delivered to the whole body, where it induces the formation of ROS, which overwhelm the antioxidant defenses of the facultative anaerobic bacteria and aerobic bacteria, causing lipid peroxidation and membrane disruption, DNA injury, protein dysfunction, and death. A pressure greater than 1.4 atmosphere absolute (ATA) is necessary to have an effective antibacterial effect against some facultative anaerobic bacteria and aerobic bacteria [238]. In addition to being directly bactericidal via ROS formation, it has been reported that HBOT enhances the antimicrobial effects of the immune system, such as those of leukocytes in hypoxic wounds (Figure 5) [239].

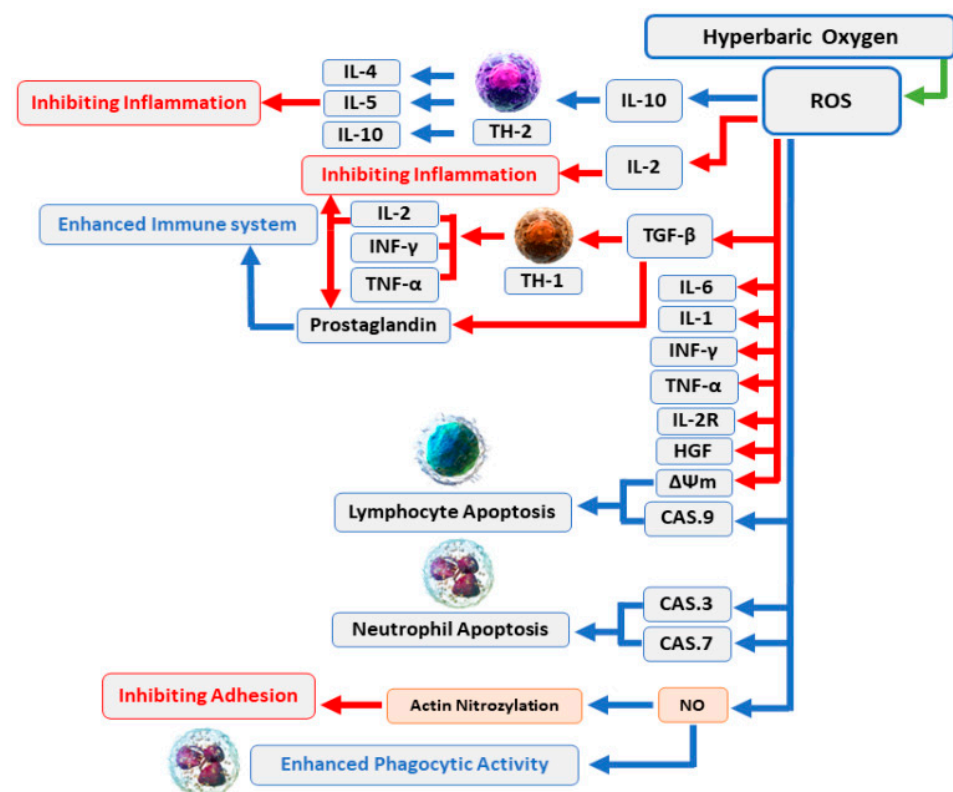


Figure 5. HBOT enhances the immune system’s antimicrobial effects: Increased O_2 levels during HBOT have a variety of biological effects, including suppression of proinflammatory mediators, transitory reduction in the CD4:CD8 T cell ratio, and stimulation of lymphocyte and neutrophil death through caspase-3-, caspase-7-, and caspase-9-dependent mechanisms. In general, these effects can boost the antibacterial processes of the immune system and infection recovery. Abbreviations: ROS, reactive oxygen species; IL, interleukin; INF, interferon; TNF, tumor necrosis factor; CAS, caspase; NO, nitric oxide. Licensee: MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/> accessed on 22 May 2024) [240].

Moreover, HBOT potentiates the antibacterial effects of some antibiotics, such as imipenem and tobramycin (Figure 6).



Figure 6. Events caused by hyperbaric oxygen therapy and the mechanisms by which its antibacterial effects derive.

Hyperoxia (98% O₂ at 2.8 absolute ATA—approximately 284.6 kPa) has been shown to enhance the effect of nitrofurantoin, sulfamethoxazole, trimethoprim, gentamicin, and tobramycin in *E. coli* strains (serotype 018 and ATCC 25922) [241]. Two works by Kolpen et al. report that the combination therapy of ciprofloxacin and HBOT may be potentially beneficial for the eradication of infections caused by the biofilm-forming *P. aeruginosa* [242,243]. Anyway, HBOT did not show any additive or synergistic effect with other antimicrobial agents, such as distamycin and rifampicin [57]. Very recently, it has been reported that wild strains of *Pseudomonads*, *Burkholderias*, and *Stenotrophomonads* stopped growing under hyperbaric conditions at a pressure of 2.8 ATA of 100% oxygen [241]. HBOT is used as a primary or alternative method for the treatment of infections such as diabetic foot infections, surgical site infections, gas gangrenes, osteomyelitis, and necrotizing compartments [240]. In addition to the bactericidal effects, HBOT suppresses the production of clostridia alpha toxin in gas gangrene diseases. HBOT has also demonstrated anti-inflammatory effects that may play a significant role in decreasing tissue damage and infection expansion. While generally safe, HBOT may have side effects such as ear discomfort, sinus pain, and temporary nearsightedness. Other contraindications include untreated pneumothorax (collapsed lung), certain medications, and claustrophobia. Although patients treated by HBOT need careful pre-examination and monitoring, when safety standards are strictly tracked, HBOT can be considered a suitable procedure to treat severe infections sustained by MDR bacteria with an acceptable rate of complication.

Clinical Application of HBOT in Infections

Several studies have evidenced that HBOT, either alone or as an accessory treatment, can be a valuable therapeutic option to cure patients with several diseases, including difficult-to-treat infections (Table 14).

Table 14. Diseases treatable with HBOT. Expressions bearing the * symbol concern the cause of the disease, while those bearing the ° symbol concern the circumstances that could provoke the disease.

Disease	Cause */Circumstances °	Refs.
CO poisoning	Several *,°	[244]
Acute anemia	Several *,°	[245]
Rheumatoid arthritis Cell hypoxia	Polarization of Th17 cells to T reg *	[246]
Inflammatory disorders (by ↓ inflammatory mediators) Microcirculatory disorder	Ischemic circumstances ° Compartment syndrome °	[238]
↓ Leucocyte chemotaxis and adhesion	Several *,°	[247]
↑ Proliferation of neutrophils	Several *,°	
Autoimmune syndrome Immune reactions to antigens Autoimmune symptoms.	Proteinuria ° Facial erythema ° Lymphadenopathy °	[248,249]
↓ Lymphocytes and leukocytes	Several *,°	[245]
↑ Mitochondrial Function	Several *,°	[250]
Chronic skin damage healing (by angiogenesis)	Several *,°	[251]
Recalcitrant infections	Necrotizing fasciitis ° Osteomyelitis ° Chronic soft tissue infections ° Infective endocarditis ° Acute or chronic wounds ° Diabetic foot ulcers °	[238,252–258]
Ocular disorders	Cystoid macular edema ° Scleral thinning ° Necrosis faced after pterygium surgery ° Nonhealing corneal edema ° Anterior segment ischemia ° Some blinding diseases °	[259,260]
Brain/cerebral injuries	Ischemic-reperfusion damage °	[261]
Cancer	Several *,°	[262]
Complications of radiotherapy	Radiation-induced skin necrosis °	[263]

↑ Means increase, improvement; ↓ means decrease/reduction.

Basically, HBOT strongly improves the levels of O₂ concentration in blood and the oxygen pressure both in blood and tissues (2000 mmHg and 500 mmHg, respectively), determining hyperoxia conditions, which provide beneficial effects in patients suffering from several diseases as reported in Table 14. HBOT was used to adjust immunology and maintain the durability of an allograft [264]. HBOT has demonstrated beneficial effects on the vascular endothelium, thus promoting angiogenesis and induced partial high tensions of O₂ in circulating plasma, thus stimulating O₂ dependent collagen matrix formation, which is an essential phase in wound healing [265]. On the other hand, HBOT effects on sepsis, urinary tract infections, and meningitis are not well known so far. Unequivocally, the most frequent clinical application of HBOT remains for several skin soft tissue infections and osteomyelitis infections, which are associated with hypoxia, caused by anaerobic infections due to antibiotic resistant bacteria [266,267]. Currently, HBOT is considered both alone and in combination with different antibacterial treatments as a relevant option for solving several acute or chronic diseases [260,262,268,269]. Although animal studies have shown the inhibitory effect of HBOT on inflammation and apoptosis after cerebral ischemia [270,271], clinical trials on humans have not shown any significant benefit. Anyway, it has been

indicated that HBOT can improve some neuropsychological and inflammatory outcomes, especially in stroke patients, within the first few hours [272,273]. Furthermore, studies on animals have shown that HBOT is associated with reduced blood-brain barrier breakdown, reduced cerebral edema, improved cerebral oxygenation, decreased intracranial pressure, reduced oxidative burden, reduced metabolic derangement, and increased neural regeneration [271,272,274]. The following Table 15 contains an overview of some clinical studies investigating the application of HBOT for different infections, while Table 16 collects the most relevant studies concerning, specifically, the clinical application of HBOT in surgical site infections (SSIs) categorized based on the type of SSI or surgery. Sternal wound infections following cardiac surgery, SSIs following neuromodulation or neuro-muscular surgery, and SSIs following male-to-female gender affirmation surgery (urogenital surgery) have been included.

Table 15. Overview of some clinical studies investigating the application of HBOT for different infections [238].

Infections	Study Population	Treatment Sessions *	Pressure **	Exposure Time (min)	Main Findings	Refs.
Burns	53	Based on outcomes	2.5	90	All patients survived	[275]
Burn	40	10	2.5	80	Faster healing, shorter hospitalization	[276]
Brain abscess	41	4–52	2.5–2.8	25–30	↓ Treatment failures, ↑ outcomes	[277]
SSIs	42	30	2.4	90	↓ Post-surgical deep infections in CSD	[278]
SSIs	32	Based on outcomes	2–3	90	Valuable AT for PO organ/space S-SSI	[279]
SSIs	6	28–106	2.5–2.8	75	AT for early PO-deep infections	[280]
NSTI	48		3	90	Not ↓ mortality rate, number of debridement, hospital stay, antibiotic use	[281]
NSTI	44	Based on outcomes	2.8	60	↑ Survival and limb salvage	[282]
NSTI	32		2.8	45	AT	[283]
NSTI	37		2.5	45	Doubtful advantage of using HBOT as an AT for NF in ↓ mortality and morbidity	[284]
DFIs	100	20 to 30	2–3	90	Useful AT for nonhealing DFIs	[285]
DFIs	42	Group1: <10 Group 2: >10	2.5	120	↓ Amputation rate	[258]
DFIs	94	40	2.5	85	Facilitates healing of chronic DFIs	[286]
DFIs	35	38 ± 8	2.2–2.5	90	↓ Amputations	[287]
DFIs	28	20	2.5	90	↑ Healing rate of nonischemic chronic DFIs	[288]
DFIs	36		2.5	90	Healing response in chronic DFIs	[289]
DFIs	38	40–60	2.5	90	↑ Healing rate ↓ Amputation rate	[290]
DFIs	18		2.4	90	AT when reconstructive surgery is not possible	[291]
Osteomyelitis	6	30	2.0–2.4	30	Effective following failure of primary therapy for osteomyelitis	[292]
Osteomyelitis	14		2.5	120	Effective and safe for chronic refractory osteomyelitis	[293]
Osteomyelitis	1		2	–	Early use of HBOT for a compromised host who develops recurrent osteomyelitis	[294]
Osteomyelitis	12	Based on outcomes	2.5	90	AT for patients who develop S-SSI and osteomyelitis after CTS	[295]

* Days; ** atmospheres absolute (ATA); DFIs = diabetic foot infections; HBOT = hyperbaric oxygen therapy; NSTI = necrotizing soft tissue infections; SSIs = surgical site infections; S-SSIs sternal surgical site infections; NF = neurofibromatosis; ↑ means increase, improvement, improved, high, higher; ↓ means decrease/reduction, low, lower, less; CTS = cardiothoracic surgery; CSD = complex spine deformity; PO = postoperative; AT = adjunctive treatment.

Table 16. Studies finalized to investigate the application of HBOT in the treatment of different surgical site infections (SSIs) [240].

Study	Surgery	SSI	Population ***/#	ATA Time (min)	Outcomes and Conclusion	Refs.
Case report	CTS	S-SSI	1	2.40 90	Rapid healing and epithelialization *	[296]
Retrospective	Sternotomy	S-SSI	55	2.50 90	S-SSI cured in all patients within an average of 8 weeks No in-hospital death ↑ Clinical outcome in patients with sterno-mediastinitis and post-sternotomy wound infection after CTS	[297]
Prospective trial	CTS	S-SSI	32; 14/18	2–3 90	<i>S. aureus</i> was the most common pathogen #, *** Infection duration was similar #, ***, ** Infection relapse rate was significantly ↓ in *** Intravenous antibiotic use duration ↓ in *** Total hospital stay ↓ in *** HBOT could be a valuable AT for treating PO organ/space S-SSI	[279]
Case report	CTS	S-SSI	1	2.50 90	Sternal wounds totally healed and epithelized in 9 weeks HBOT with TNP dressing is a good alternative method for patients who cannot tolerate or refuse any surgical reconstruction	[298]
Retrospective	NMSS	DWI	6	2.50 3 × 25	All infections resolved Wound healing in an average of 3 months Minor side effects of HBOT HBOT is a safe AT for early deep PO infections in the case of spinal implants in HR pediatric patients	[299]
Retrospective	CTS	S-SSI	12; 6/6	2.50 90	No treatment-related complications in *** Length of stay in ICU ↓ in *** Invasive and noninvasive positive pressure ventilation ↓ in *** Hospital mortality ↓ in *** HBOT may be used as a safe AT to ↑ clinical outcomes in patients with S-SSI and osteomyelitis after sternotomy and CTS	[295]
Retrospective	CABS	Mediastinitis	18	2.50 90	1 HBOT-unrelated death caused by sepsis HBOT was well-tolerated Favorable clinical outcomes using HBOT as an AT for treating mediastinitis patients after CABS	[300]
Retrospective	NMSS	DWI	42; 18/24	2.40 90	11.9% (5/42) Incidence of infection in both ***# 5.5% (1/18) Infection rate in *** 6.6% (4/24) Infection rate in # HBOT significantly ↓ PO infections in NMSS patients HBOT is a safe AT to prevent PO-deep infections in complex spine deformities in HR NM patients	[278]

Table 16. Cont.

Study	Surgery	SSI	Population ***/#	ATA Time (min)	Outcomes and Conclusion	Refs.
Retrospective	CTS	S-SSI	10	2.50 92	70% complete wound healing with fibrous scar formation in 4 weeks HBOT *** HBOT had 80% success as AT in DSWI *** No complications were observed	[301]
Retrospective	NM	HRI	14	2.0–2.8 75	86% of HRI was successfully treated without hardware removal *** Hardware was removed following HBOT failure in two infections Observed intrathecal pump malfunction caused by HBOT (2.8 bars) HBOT has potential as an AT in the treatment of HRI in NM Diminished need for hardware removal and treatment interruption	[302]
Retrospective	CTS	S-SSI	53		Readmitted with infected sternotomies discharged in 2–39 days Time for wound healing using NPWT alone was 21–42 days in # Time for wound healing using NPWT + HBOT was 28–42 days in *** HBOT was 5–35 days HBO treatments were 22.6 (+11.06) Restore time 21–98 days in *** (NPWT + HBOT) Multimodality therapy of incision and drainage using NPWT + HBOT + antibiotics is successful for treating complex deep sternal wound infections in the pediatric population after congenital heart surgery	[303]
Retrospective	MtF-GAS	SSI	33; 15/18	2.2–3.0/90	100% complete wound healing *** 94.4% complete wound healing # ↓ Duration of antibiotic therapy *** ↓ Perineal drain time *** ↓ Bladder catheter time *** ↓ Hospital stay *** HBOT as effective adjuvant treatment for SSIs in patients undergoing MtF GAS	[304]

* First reported case of HBOT use for the treatment of deep sternal (surgical site infections) SSI in a heart transplant recipient; S-SSI = sternal surgical site infections; ** 31.8 ± 7.6 vs. 29.3 ± 5.7 days, respectively, $p = 0.357$; ↑ means increase, improvement, improved, high, higher; ↓ means decrease/reduction, low, lower; MtF-GAS = male-to-female gender affirmation surgery; *** in the HBOT group; # not in the HBOT group, AT = adjuvant therapy; PO = postoperative; TNP = topical negative pressure; CTS = cardiothoracic surgery; CABS = coronary artery bypass surgery; DSWI = deep sternal wound infections; HRI = hardware-related infections; NMSS = neuromuscular sclerosis surgery; NM = neuromodulation; DWI = deep wound infections; ICU = intensive care unit; HR = high risk; NPWT = negative pressure wound care therapy.

4. Possible Novel Methods to Induce ROS Formation: Our Proposal

4.1. Environmental Persistent Free Radicals (EPFRs)

Environmentally persistent free radicals (EPFRs) are defined as long-living organic free radicals stabilized on or inside particles [305]. EPFRs are persistent because of the protection provided by the particles containing them and the presence of transition metals, thus having lifetimes that are exceptionally longer (from days to years) than other free radicals.

These surface-bound radicals are found in contaminated soil, tar balls, and cigarette smoke and primarily form during thermal processes such as pyrolysis and combustion of organic materials, waste incineration, and photoactivation [306]. In fact, the byproducts from these processes, such as phenols and aromatic polycyclic hydrocarbons, provide a breeding ground for EPFR generation. In fact, EPFRs are also found in the matrix of ultrafine and airborne fine particulate matter (PM), which is emitted into the environment by both natural and anthropogenic processes, including coal combustion emissions (16.8%), vehicular emissions (32.1%), industrial processes (11.7%), dust storms (27.2%), and nitrates (3.4%). PM is a mixture of organic species, inorganic species, solid, and liquid components of metals that have the tendency to form radicals with or without sunlight photoactivation [306,307]. The environmental factors affecting the EPFRs' formation, lifetime, and abundance include precursors, temperature, light irradiation, the presence of metals, temperature, pH, humidity, thermal processing time, and oxygen [308]. EPFRs are categorized as no decay, low decay, and fast decay radicals. The no-decay EPFRs are unpaired electrons delocalized over aromatic bonds entrapped inside PM. Phenoxy radicals are fast-decay EPFRs, while semiquinone radicals are subjected to slow decay [309]. EPFRs can produce reactive oxygen species, including hydroxyl radicals, which induce oxidative stress in living organisms, posing adverse environmental and human health effects. The atmospheric oxygen is the only sink for stable free radicals, converting them into particles and/or metal stabilized molecular species, thus decaying [306]. EPFR decay in the atmosphere depends on the reaction of EPFRs with molecular oxygen. By reacting with atmospheric oxygen, they generate high levels of reactive oxygen species (ROS) via electron transfer, such as hydroxyl radicals and superoxide anion radicals, thereby inducing cellular oxidative stress [23,24]. For this reason and their possible redox recycling, EPFRs are emerging as environmental pollutants with a ROS-dependent significant toxicity to organisms, including humans, plants, animals, and microorganisms. Anyway, recent research has also explored their potential for degrading organic environmental contaminants. By activating hydrogen peroxide or persulfate, EPFRs produce ROS species such as superoxide, singlet oxygen, or OH^- , thus inducing the degradation of environmental organic pollutants [306]. Collectively, although EPFRs are long-lived environmental pollutants harmful to the environment and living beings, their capacity to activate ROS generation, if properly controlled, can make them a wide range of tools for environmental remediation.

4.2. Biochar-Derived Persistent Free Radicals (PFRs)

Biochar (BC) is a carbonaceous material obtained by the pyrolysis of different vegetable and animal biomass feedstocks at 200–1000 °C in the limited presence or absence of O_2 . BC has demonstrated a broad prospective use in the treatment of environmental pollutants and in soil amendment. It has been used in photocatalytic and photothermal systems for photothermal conversion, to construct electrical and thermal devices, as well as 3D solar vapor-generation devices for water desalination [24]. All these potentials are due to its high surface area and rich pore structure, which determine its great physical absorptivity [23]. Additionally, they also depend on the chemical characteristics of BC, which in turn depend on the type of biomass used to produce BC, the original biomass chemical composition, and pyrolysis conditions [310,311]. Whereas, starting in 2014 (Figure 7), the presence of persistent free radicals (PFRs) in BC deriving from lignocellulosic biomasses, like the radicals previously detected in combustion-generated particulate matter (PMs), sediments, and contaminated soils, known as environmental persistent free radicals (EPFRs), has been reported.

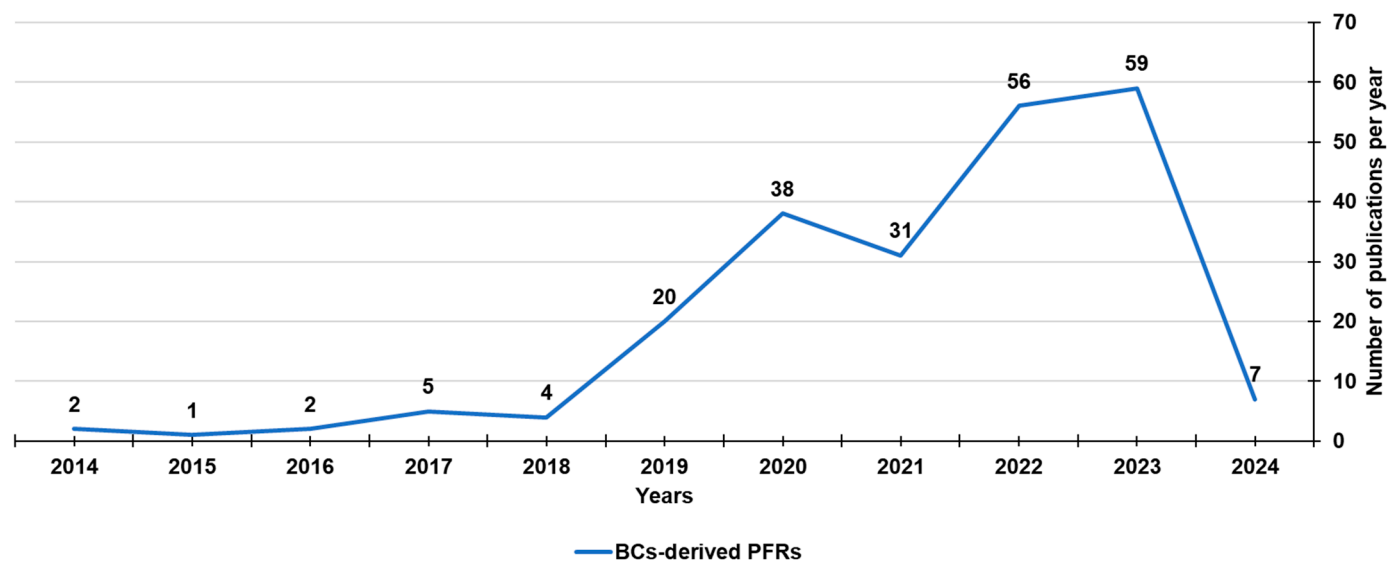


Figure 7. Number of publications on BCs-derived PFRs from 2014 according to the Scopus dataset (reviews and chapters in books included). The survey used the following keywords: persistent AND free AND radicals AND biochar [24].

As EPFRs, such reactive species can remain stable for months or years and play a crucial role in the capacity of BC to degrade different types of xenobiotics and pollutants by oxidative reactions via ROS formation. Unlike other free radicals, including ROS, PFRs are resonance-stabilized since they are bound to the external or internal surface of solid particles of BC [24]. If BC is conserved under vacuum, the lifetime of PFRs could be infinite (no decay radicals), while when air-exposed, they react with molecular oxygen in the air and decay over time, thus producing ROS. Similarly, in aqueous systems, PFRs act as transition metals such as Fe^{2+} , forming ROS as well [312–315]. PFRs are categorized into three classes, i.e., oxygen-centered PFRs (OCPFRs), carbon-centered PFRs (CCPFRs), and oxygenated carbon-centered radicals (CCPFRs-O). The possible presence of PFRs on a BC, their type, and their concentrations are significantly affected by pyrolysis conditions, biomass types, the elemental composition of pristine biomass, and the presence of external transition metals, as detailed in Table 17.

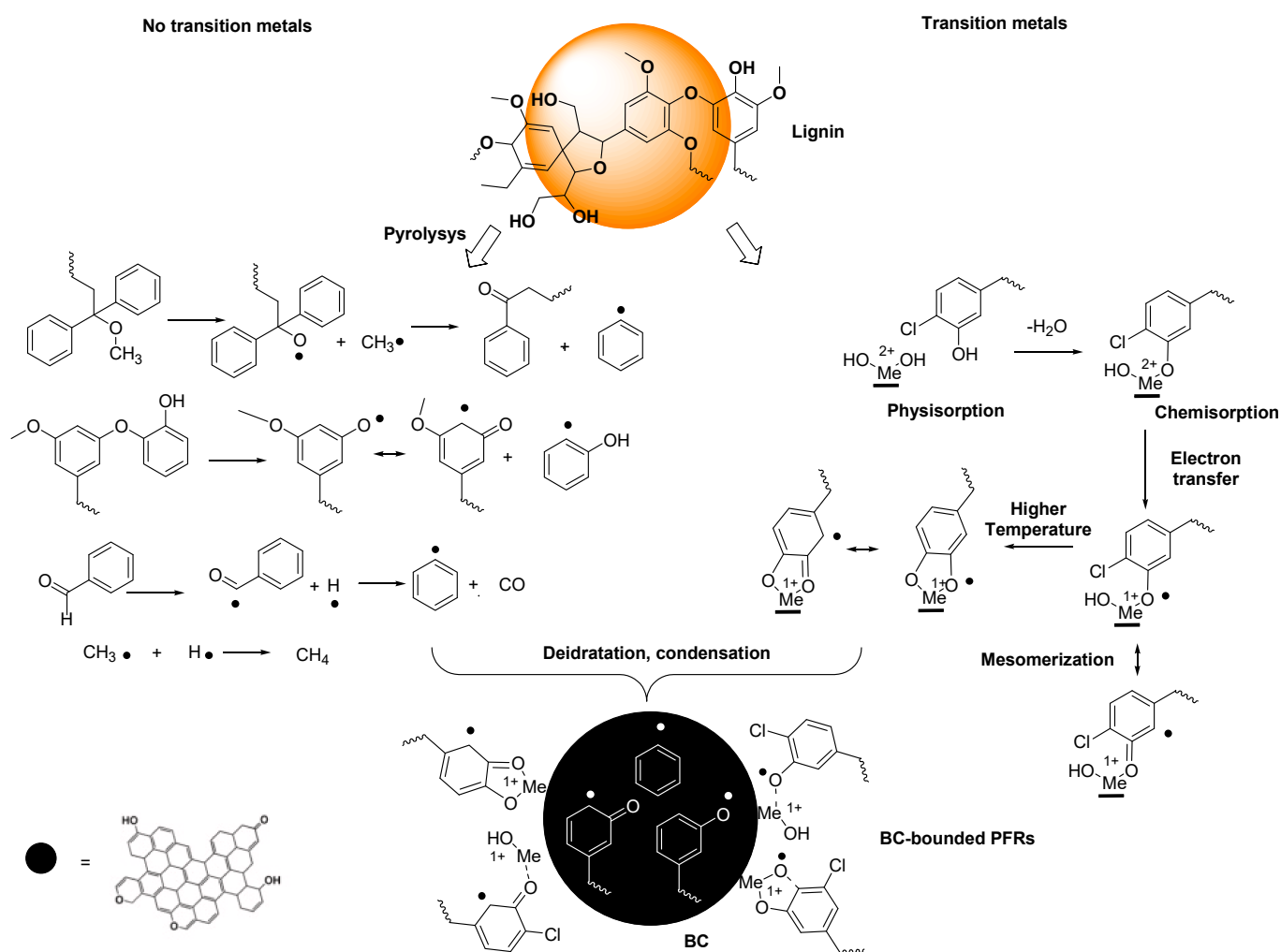
Table 17. Factors influencing PFR formation in BC.

Parameter	Influencing Factors	Specifications	Observations	Refs.
PFRs concentration	Biomass type	Cow manure, rice husk, and others (<500 °C)	≠ Concentrations	[316,317]
		Non-lignocellulosic biomass with ↓ H/C and O/C	↓ Concentration	[318]
		Lignocellulosic biomass	↑ Concentration	[316]
	Temperature	300 °C, 700 °C	≠ Concentrations	[319]
		Maximum concentration at 600 °C	≠ Concentrations	[320]
		Maximum concentration at 500–600 °C		
Transition metals	Adsorb onto biomass and transfer electrons from the polymer to the metal center during pyrolysis	↑ Concentration	[25]	
Type of PFRs	Temperature	200–300 °C	OCR	[320]
		400 °C	OCR + CCR	
		500–700 °C	CCR	

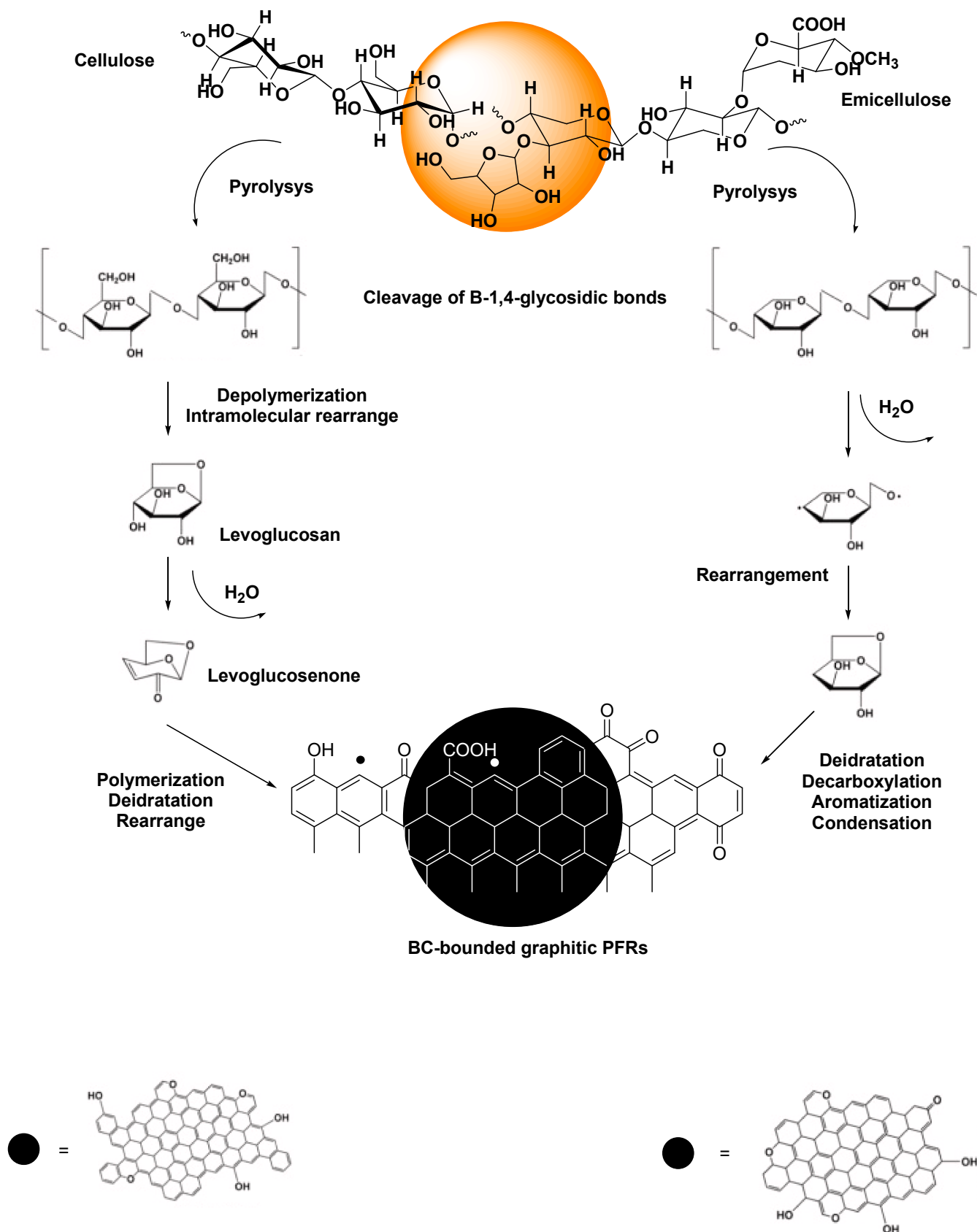
OCR = oxygen-centered radicals; CCR = carbon centered radicals [24]; ≠ means different; ↓ means reduced, decreased, or lower; ↑ means higher, improved, or increased.

4.2.1. Proposed Mechanisms for PFR Formation during Biomass Pyrolysis

The actual mechanism by which PFRs form during pyrolysis remains has not been fully clarified. However, transition metals capable of electron transfer and substituted aromatic molecules present in the lignin component of pristine biomass have been recognized to be essential for PFR formation. Anyway, high concentrations of PFRs have also been detected in products obtained by the pyrolysis of non-aromatic cellulose in the absence of transition metals [319]. Collectively, PFRs can form by different pathways, including or without the presence of transition metals, and once formed, PFRs could be either only surface-stabilized or surface-stabilized in metal-radical complexes [321]. Scheme 1 (concerning lignin) and Scheme 2 (concerning cellulose and emicellulose) report the possible chemical paths by which PFRs may form.



Scheme 1. Possible mechanisms leading to the formation of BC-bounded PFRs from lignin. The orange sphere represents biomass, while the black sphere represents BC, whose hypothetical structures depending on the pyrolysis condition have been shown at the bottom of the scheme [24].



Scheme 2. Possible mechanisms leading to the formation of BC-bounded graphitic PFRs from cellulose (left side) and emicellulose (right side). The orange sphere represents biomass, while the black sphere represents BC, whose hypothetical structures depending on the pyrolysis condition have been shown at the bottom of the scheme [24].

Since it is out of scope of this paper, a detailed discussion on the mechanisms reported in Schemes 1 and 2 has been avoided. Readers particularly interested can find major information in a very recent review [24].

4.2.2. Possible Activities of PFRs and Our Proposal

PFRs formed in BC during combustion of lignocellulosic biomasses, either in the presence or absence of external transition metals, could promote several beneficial reactions, such as PFR-mediated remediation and degradation of organic and inorganic pollutants by different actions and mechanisms, including oxidative and reductive processes. PFRs on BC can activate hydrogen peroxide (H_2O_2) or oxygen (O_2), as well as persulfate ($S_2O_8^{2-}$), to produce different radical and not radical oxygenated species (ROS) capable of efficiently degrading organic contaminants by oxidative mechanisms, as ROS generated by the previously reported antimicrobial therapies are bactericidal to pathogens inducing OS via ROS stimulation. Therefore, we thought that ROS induction using BC-derived PFRs, whose type, concentration, and reactivity can be tunable under pyrolysis conditions, could be a novel method to form ROS for a possibly more selective BC-based antibacterial oxidative therapy. In this regard, in a recent review on BC-derived PFRs, a random selection of the main experimental works regarding the applications of PFRs found in BCs conveyed in the last five years (2019–2023) has been reported. Among the reported PFR applications, three regarded their use as antibacterial agents (Table 18), thus supporting our idea.

Table 18. BC-derived PFRs and their applications as ROS-forming antibacterial agents reported in the years 2019–2023.

Biomass	Pyrolysis °C/Time	BC-Name	Active Radicals	Radical Mechanisms	Degraded Compound	Refs.
Anaerobic digestion sludge	400 °C 600 °C 800 °C 1000 °C	ADSBC 400 ADSBC 600 ADSBC 800 ADSBC 1000	$SO_4^{\bullet-}$ PFRs OH^{\bullet}	BC-mediated activation of PDS	Dyes, Estrogens Sulfonamides, <i>E. coli</i> Others	[30]
<i>Caragana korshinskii</i>	650 °C/3 h	ACB-K-gC3N	PFRs $h^+ \bullet OH^{\bullet}$ O_2^-	Electron photogeneration and PFR-mediated H_2O and O_2 activation	<i>S. aureus</i> , <i>E. coli</i> RhB, TC, NOR, CAP	[31]
Pinewood	600 °C	Ag ⁰ -PBC	PFRs $\bullet OH$ $\bullet O_2^-$	UV-light promoted excitation of the electron-hole pairs and subsequently, the production of ROS Enhanced ROS generation by PFRs	MB, <i>E. coli</i>	[32]

BCs = biochar; TC = tetracycline; RhB = rhodamine B; PDS = peroxydisulfate; CAP = chloramphenicol; MB = methylene blue; NOR = norfloxacin.

BC employed was derived from the pyrolysis of sludge, the *Caragana korshinskii* plant, and pinewood. In these processes, the electron transfer promoted by PFRs of diverse nature generated ROS such as $SO_4^{\bullet-}$, $\bullet OH$, $\bullet O_2^-$, and $\bullet O_2H$, which carried out the oxidative degradation of different organic pollutants, including drugs, dyes, antibiotics, and hormones, and showed antibacterial effects against *E. coli* and *S. aureus*.

5. Conclusions

Upon the colonization of the host cell during infection, the maintenance of redox homeostasis (RH) is a key process for bacterial survival and for escaping the oxidative stress physiologically generated by macrophages to oppose the development of the infection. Pathogens succeed in strictly controlling RH through a mechanism based on different redoxins and low-molecular-weight-thiol molecules. Therapeutic strategies based on the

capacity of different compounds or methods to cause ROS and RNS hyper-generation during phagocytosis to unbalance bacterial redox defenses and stop host cell colonization have a great potential to solve the increasing problem of antibiotic-resistant infections. ROS have demonstrated to be effective in inhibiting clinically important microbial pathogens by lipid peroxidation, thus damaging their membranes, harming DNA, and impairing protein functions. It has been observed that certain traditional antibiotics and alternative antimicrobials, including nanomaterials, as well as their combination, induce ROS as a secondary or main mechanism of antibacterial effect. Additionally, in the worrying scenario of an increasing global emergence of difficult-to-treat infections due to bacterial resistance to available antibiotics, old procedures that inhibit microbial growth by forming ROS, such as HBOT and medical honey, have been reinvigorated. Together with the more recent PDT, they are, in fact, currently successfully employed for the treatment or prevention of soft tissue infections and chronic ulcerations. The development of resistance to these methods has not been reported, but unfortunately, since ROS-mediated OS is destructive to eukaryotes as well their clinical application to treat systemic infections is at present impracticable. Further studies aimed at identifying novel delivery techniques for using ROS with superior selectivity for microbial pathogens are required. As our contribution to this challenge, we have now proposed BC-associated PFRs as a promising novel, low-cost, and eco-friendly method for ROS generation to be studied for the oxidative inhibition of MDR pathogens and as a potential treatment for a wide range of infections. Greater knowledge concerning the proper pyrolysis conditions employed to obtain the type of PFRs more suitable for this purpose and in optimized concentration could make this ROS-delivering method more selective for bacterial pathogens.

Author Contributions: The authors (S.A., G.C.S., A.M.S. and G.Z.) contributed equally to this work. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

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

References

1. Saliba, R.; Zahar, J.-R.; Dabar, G.; Riachy, M.; Karam-Sarkis, D.; Husni, R. Limiting the Spread of Multidrug-Resistant Bacteria in Low-to-Middle-Income Countries: One Size Does Not Fit All. *Pathogens* **2023**, *12*, 144. [[CrossRef](#)] [[PubMed](#)]
2. Bush, K.; Courvalin, P.; Dantas, G.; Davies, J.; Eisenstein, B.; Huovinen, P.; Jacoby, G.A.; Kishony, R.; Kreiswirth, B.N.; Kutter, E.; et al. Tackling Antibiotic Resistance. *Nat. Rev. Microbiol.* **2011**, *9*, 894–896. [[CrossRef](#)] [[PubMed](#)]
3. Franco, B.E.; Altagracia Martínez, M.; Sánchez Rodríguez, M.A.; Wertheimer, A.I. The Determinants of the Antibiotic Resistance Process. *Infect. Drug Resist.* **2009**, *2*, 1–11. [[PubMed](#)]
4. Dryden, M. Reactive Oxygen Species: A Novel Antimicrobial. *Int. J. Antimicrob. Agents* **2018**, *51*, 299–303. [[CrossRef](#)] [[PubMed](#)]
5. Davis, S.C.; Martinez, L.; Kirsner, R. The Diabetic Foot: The Importance of Biofilms and Wound Bed Preparation. *Curr. Diab Rep.* **2006**, *6*, 439–445. [[CrossRef](#)] [[PubMed](#)]
6. Alfei, S.; Caviglia, D. Prevention and Eradication of Biofilm by Dendrimers: A Possibility Still Little Explored. *Pharmaceutics* **2022**, *14*, 2016. [[CrossRef](#)] [[PubMed](#)]
7. Dryden, M.; Cooke, J.; Salib, R.; Holding, R.; Pender, S.L.F.; Brooks, J. Hot Topics in Reactive Oxygen Therapy: Antimicrobial and Immunological Mechanisms, Safety and Clinical Applications. *J. Glob. Antimicrob. Resist.* **2017**, *8*, 194–198. [[CrossRef](#)]
8. Dryden, M. Reactive Oxygen Therapy: A Novel Therapy in Soft Tissue Infection. *Curr. Opin. Infect. Dis.* **2017**, *30*, 143–149. [[CrossRef](#)] [[PubMed](#)]
9. Dryden, M.S.; Cooke, J.; Salib, R.J.; Holding, R.E.; Biggs, T.; Salamat, A.A.; Allan, R.N.; Newby, R.S.; Halstead, F.; Oppenheim, B.; et al. Reactive Oxygen: A Novel Antimicrobial Mechanism for Targeting Biofilm-Associated Infection. *J. Glob. Antimicrob. Resist.* **2017**, *8*, 186–191. [[CrossRef](#)]
10. Dunnill, C.; Patton, T.; Brennan, J.; Barrett, J.; Dryden, M.; Cooke, J.; Leaper, D.; Georgopoulos, N.T. Reactive Oxygen Species (ROS) and Wound Healing: The Functional Role of ROS and Emerging ROS-modulating Technologies for Augmentation of the Healing Process. *Int. Wound J.* **2017**, *14*, 89–96. [[CrossRef](#)]
11. Alfei, S.; Marengo, B.; Zuccari, G. Oxidative Stress, Antioxidant Capabilities, and Bioavailability: Ellagic Acid or Urolithins? *Antioxidants* **2020**, *9*, 707. [[CrossRef](#)]
12. Genestra, M. Oxy Radicals, Redox-Sensitive Signalling Cascades and Antioxidants. *Cell Signal.* **2007**, *19*, 1807–1819. [[CrossRef](#)] [[PubMed](#)]

13. Venkataraman, K.; Khurana, S.; Tai, T. Oxidative Stress in Aging-Matters of the Heart and Mind. *Int. J. Mol. Sci.* **2013**, *14*, 17897–17925. [[CrossRef](#)] [[PubMed](#)]
14. Marengo, B.; Nitti, M.; Furfaro, A.L.; Colla, R.; De Ciucis, C.; Marinari, U.M.; Pronzato, M.A.; Traverso, N.; Domenicotti, C. Redox Homeostasis and Cellular Antioxidant Systems: Crucial Players in Cancer Growth and Therapy. *Oxid. Med. Cell Longev.* **2016**, *2016*, 6235641. [[CrossRef](#)]
15. Marengo, B.; Raffaghello, L.; Pistoia, V.; Cottalasso, D.; Pronzato, M.A.; Marinari, U.M.; Domenicotti, C. Reactive Oxygen Species: Biological Stimuli of Neuroblastoma Cell Response. *Cancer Lett.* **2005**, *228*, 111–116. [[CrossRef](#)]
16. Ahmed, T.; Setzer, N.W.; Fazel Nabavi, S.; Erdogan Orhan, I.; Braidly, N.; Sobarzo-Sanchez, E.; Mohammad Nabavi, S. Insights into Effects of Ellagic Acid on the Nervous System: A Mini Review. *Curr. Pharm. Des.* **2016**, *22*, 1350–1360. [[CrossRef](#)] [[PubMed](#)]
17. Kaneto, H.; Katakami, N.; Matsuhisa, M.; Matsuoka, T. Role of Reactive Oxygen Species in the Progression of Type 2 Diabetes and Atherosclerosis. *Mediat. Inflamm.* **2010**, *2010*, 453892. [[CrossRef](#)]
18. Kim, S.Y.; Park, C.; Jang, H.-J.; Kim, B.; Bae, H.-W.; Chung, I.-Y.; Kim, E.S.; Cho, Y.-H. Antibacterial Strategies Inspired by the Oxidative Stress and Response Networks. *J. Microbiol.* **2019**, *57*, 203–212. [[CrossRef](#)]
19. Gaupp, R.; Ledala, N.; Somerville, G.A. Staphylococcal Response to Oxidative Stress. *Front. Cell Infect. Microbiol.* **2012**, *2*, 33. [[CrossRef](#)]
20. Hong, Y.; Zeng, J.; Wang, X.; Drlica, K.; Zhao, X. Post-Stress Bacterial Cell Death Mediated by Reactive Oxygen Species. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 10064–10071. [[CrossRef](#)] [[PubMed](#)]
21. Dryden, M.; Lockyer, G.; Saeed, K.; Cooke, J. Engineered Honey: In Vitro Antimicrobial Activity of a Novel Topical Wound Care Treatment. *J. Glob. Antimicrob. Resist.* **2014**, *2*, 168–172. [[CrossRef](#)]
22. Vaishampayan, A.; Grohmann, E. Antimicrobials Functioning through ROS-Mediated Mechanisms: Current Insights. *Microorganisms* **2021**, *10*, 61. [[CrossRef](#)] [[PubMed](#)]
23. Alfei, S.; Pandoli, O.G. Bamboo-Based Biochar: A Still Too Little-Studied Black Gold and Its Current Applications. *J. Xenobiot.* **2024**, *14*, 416–451. [[CrossRef](#)] [[PubMed](#)]
24. Alfei, S.; Pandoli, O.G. Biochar-Derived Persistent Free Radicals: A Plethora of Environmental Applications in a Light and Shadows Scenario. *Toxics* **2024**, *12*, 245. [[CrossRef](#)] [[PubMed](#)]
25. Beesley, L.; Moreno-Jiménez, E.; Gomez-Eyles, J.L.; Harris, E.; Robinson, B.; Sizmur, T. A Review of Biochars' Potential Role in the Remediation, Revegetation and Restoration of Contaminated Soils. *Environ. Pollut.* **2011**, *159*, 3269–3282. [[CrossRef](#)] [[PubMed](#)]
26. Jeffery, S.; Verheijen, F.G.A.; van der Velde, M.; Bastos, A.C. A Quantitative Review of the Effects of Biochar Application to Soils on Crop Productivity Using Meta-Analysis. *Agric. Ecosyst. Environ.* **2011**, *144*, 175–187. [[CrossRef](#)]
27. Kinney, T.J.; Masiello, C.A.; Dugan, B.; Hockaday, W.C.; Dean, M.R.; Zygourakis, K.; Barnes, R.T. Hydrologic Properties of Biochars Produced at Different Temperatures. *Biomass Bioenergy* **2012**, *41*, 34–43. [[CrossRef](#)]
28. Qin, Y.; Li, G.; Gao, Y.; Zhang, L.; Ok, Y.S.; An, T. Persistent Free Radicals in Carbon-Based Materials on Transformation of Refractory Organic Contaminants (ROCs) in Water: A Critical Review. *Water Res.* **2018**, *137*, 130–143. [[CrossRef](#)]
29. Vejerano, E.P.; Rao, G.; Khachatryan, L.; Cormier, S.A.; Lomnicki, S. Environmentally Persistent Free Radicals: Insights on a New Class of Pollutants. *Environ. Sci. Technol.* **2018**, *52*, 2468–2481. [[CrossRef](#)] [[PubMed](#)]
30. Chen, Y.; Duan, X.; Zhang, C.; Wang, S.; Ren, N.; Ho, S.-H. Graphitic Biochar Catalysts from Anaerobic Digestion Sludge for Nonradical Degradation of Micropollutants and Disinfection. *Chem. Eng. J.* **2020**, *384*, 123244. [[CrossRef](#)]
31. Wang, T.; Zheng, J.; Cai, J.; Liu, Q.; Zhang, X. Visible-Light-Driven Photocatalytic Degradation of Dye and Antibiotics by Activated Biochar Compositing with K⁺ Doped g-C₃N₄: Effects, Mechanisms, Actual Wastewater Treatment and Disinfection. *Sci. Total Environ.* **2022**, *839*, 155955. [[CrossRef](#)]
32. Shi, J.; Wang, J.; Liang, L.; Xu, Z.; Chen, Y.; Chen, S.; Xu, M.; Wang, X.; Wang, S. Carbothermal Synthesis of Biochar-Supported Metallic Silver for Enhanced Photocatalytic Removal of Methylene Blue and Antimicrobial Efficacy. *J. Hazard. Mater.* **2021**, *401*, 123382. [[CrossRef](#)]
33. Arshad, U.; Altaf, M.T.; Liaqat, W.; Ali, M.; Shah, M.N.; Jabran, M.; Ali, M.A. Biochar: Black Gold for Sustainable Agriculture and Fortification Against Plant Pathogens—A Review. *Gesunde Pflanz.* **2023**, *76*, 385–396. [[CrossRef](#)]
34. Liu, X.; Chen, Z.; Lu, S.; Shi, X.; Qu, F.; Cheng, D.; Wei, W.; Shon, H.K.; Ni, B.-J. Persistent Free Radicals on Biochar for Its Catalytic Capability: A Review. *Water Res.* **2024**, *250*, 120999. [[CrossRef](#)] [[PubMed](#)]
35. Fang, G.; Liu, C.; Gao, J.; Dionysiou, D.D.; Zhou, D. Manipulation of Persistent Free Radicals in Biochar to Activate Persulfate for Contaminant Degradation. *Environ. Sci. Technol.* **2015**, *49*, 5645–5653. [[CrossRef](#)] [[PubMed](#)]
36. Odinga, E.S.; Waigi, M.G.; Gudda, F.O.; Wang, J.; Yang, B.; Hu, X.; Li, S.; Gao, Y. Occurrence, Formation, Environmental Fate and Risks of Environmentally Persistent Free Radicals in Biochars. *Environ. Int.* **2020**, *134*, 105172. [[CrossRef](#)]
37. Andrés, C.M.C.; Pérez de la Lastra, J.M.; Juan, C.A.; Plou, F.J.; Pérez-Lebeña, E. Hypochlorous Acid Chemistry in Mammalian Cells—Influence on Infection and Role in Various Pathologies. *Int. J. Mol. Sci.* **2022**, *23*, 10735. [[CrossRef](#)]
38. Adams, L.; Franco, M.C.; Estevez, A.G. Reactive Nitrogen Species in Cellular Signaling. *Exp. Biol. Med.* **2015**, *240*, 711–717. [[CrossRef](#)] [[PubMed](#)]
39. Salisbury, D.; Bronas, U. Reactive Oxygen and Nitrogen Species. *Nurs. Res.* **2015**, *64*, 53–66. [[CrossRef](#)]
40. Schieber, M.; Chandel, N.S. ROS Function in Redox Signaling and Oxidative Stress. *Curr. Biol.* **2014**, *24*, R453–R462. [[CrossRef](#)]
41. Frijhoff, J.; Winyard, P.G.; Zarkovic, N.; Davies, S.S.; Stocker, R.; Cheng, D.; Knight, A.R.; Taylor, E.L.; Oettrich, J.; Ruskovska, T.; et al. Clinical Relevance of Biomarkers of Oxidative Stress. *Antioxid. Redox Signal.* **2015**, *23*, 1144–1170. [[CrossRef](#)]

42. Barreiro, E. Role of Protein Carbonylation in Skeletal Muscle Mass Loss Associated with Chronic Conditions. *Proteomes* **2016**, *4*, 18. [[CrossRef](#)] [[PubMed](#)]
43. Trpkovic, A.; Resanovic, I.; Stanimirovic, J.; Radak, D.; Mousa, S.A.; Cenic-Milosevic, D.; Jevremovic, D.; Isenovic, E.R. Oxidized Low-Density Lipoprotein as a Biomarker of Cardiovascular Diseases. *Crit. Rev. Clin. Lab. Sci.* **2015**, *52*, 70–85. [[CrossRef](#)] [[PubMed](#)]
44. Reynaert, N.L.; Gopal, P.; Rutten, E.P.A.; Wouters, E.F.M.; Schalkwijk, C.G. Advanced Glycation End Products and Their Receptor in Age-Related, Non-Communicable Chronic Inflammatory Diseases; Overview of Clinical Evidence and Potential Contributions to Disease. *Int. J. Biochem. Cell Biol.* **2016**, *81*, 403–418. [[CrossRef](#)] [[PubMed](#)]
45. Jacob, K.D.; Noren Hooten, N.; Trzeciak, A.R.; Evans, M.K. Markers of Oxidant Stress That Are Clinically Relevant in Aging and Age-Related Disease. *Mech. Ageing Dev.* **2013**, *134*, 139–157. [[CrossRef](#)] [[PubMed](#)]
46. Morikawa, K.; Ushijima, Y.; Ohniwa, R.L.; Miyakoshi, M.; Takeyasu, K. What Happens in the Staphylococcal Nucleoid under Oxidative Stress? *Microorganisms* **2019**, *7*, 631. [[CrossRef](#)] [[PubMed](#)]
47. Doukyu, N.; Taguchi, K. Involvement of Catalase and Superoxide Dismutase in Hydrophobic Organic Solvent Tolerance of *Escherichia coli*. *AMB Express* **2021**, *11*, 97. [[CrossRef](#)] [[PubMed](#)]
48. Chiang, S.M.; Schellhorn, H.E. Regulators of Oxidative Stress Response Genes in *Escherichia Coli* and Their Functional Conservation in Bacteria. *Arch. Biochem. Biophys.* **2012**, *525*, 161–169. [[CrossRef](#)]
49. Battesti, A.; Majdalani, N.; Gottesman, S. The RpoS-Mediated General Stress Response in *Escherichia coli*. *Annu. Rev. Microbiol.* **2011**, *65*, 189–213. [[CrossRef](#)]
50. Nguyen, G.T.; Green, E.R.; Meccas, J. Neutrophils to the ROScUE: Mechanisms of NADPH Oxidase Activation and Bacterial Resistance. *Front. Cell Infect. Microbiol.* **2017**, *7*, 373. [[CrossRef](#)]
51. Li, H.; Zhou, X.; Huang, Y.; Liao, B.; Cheng, L.; Ren, B. Reactive Oxygen Species in Pathogen Clearance: The Killing Mechanisms, the Adaption Response, and the Side Effects. *Front. Microbiol.* **2021**, *11*, 622534. [[CrossRef](#)]
52. Li, C.; Zhu, L.; Pan, D.; Li, S.; Xiao, H.; Zhang, Z.; Shen, X.; Wang, Y.; Long, M. Siderophore-Mediated Iron Acquisition Enhances Resistance to Oxidative and Aromatic Compound Stress in *Cupriavidus necator* JMP134. *Appl. Environ. Microbiol.* **2019**, *85*, e01938-18. [[CrossRef](#)] [[PubMed](#)]
53. Peralta, D.R.; Adler, C.; Corbalán, N.S.; Paz García, E.C.; Pomares, M.F.; Vincent, P.A. Enterobactin as Part of the Oxidative Stress Response Repertoire. *PLoS ONE* **2016**, *11*, e0157799. [[CrossRef](#)] [[PubMed](#)]
54. Vaishampayan, A.; de Jong, A.; Wight, D.J.; Kok, J.; Grohmann, E. A Novel Antimicrobial Coating Represses Biofilm and Virulence-Related Genes in Methicillin-Resistant *Staphylococcus aureus*. *Front. Microbiol.* **2018**, *9*, 221. [[CrossRef](#)] [[PubMed](#)]
55. Wang, T.; El Meouche, I.; Dunlop, M.J. Bacterial Persistence Induced by Salicylate via Reactive Oxygen Species. *Sci. Rep.* **2017**, *7*, 43839. [[CrossRef](#)] [[PubMed](#)]
56. Grant, S.S.; Hung, D.T. Persistent Bacterial Infections, Antibiotic Tolerance, and the Oxidative Stress Response. *Virulence* **2013**, *4*, 273–283. [[CrossRef](#)]
57. Memar, M.Y.; Ghotaslou, R.; Samiei, M.; Adibkia, K. Antimicrobial Use of Reactive Oxygen Therapy: Current Insights. *Infect. Drug Resist.* **2018**, *11*, 567–576. [[CrossRef](#)]
58. Sampson, T.R.; Liu, X.; Schroeder, M.R.; Kraft, C.S.; Burd, E.M.; Weiss, D.S. Rapid Killing of *Acinetobacter baumannii* by Polymyxins Is Mediated by a Hydroxyl Radical Death Pathway. *Antimicrob. Agents Chemother.* **2012**, *56*, 5642–5649. [[CrossRef](#)]
59. Arriaga-Alba, M.; Rivera-Sánchez, R.; Parra-Cervantes, G.; Barro-Moreno, F.; Flores-Paz, R.; García-Jiménez, E. Antimutagenesis of β -Carotene to Mutations Induced by Quinolone on *Salmonella typhimurium*. *Arch. Med. Res.* **2000**, *31*, 156–161. [[CrossRef](#)]
60. Wang, X.; Zhao, X. Contribution of Oxidative Damage to Antimicrobial Lethality. *Antimicrob. Agents Chemother.* **2009**, *53*, 1395–1402. [[CrossRef](#)]
61. Rasouly, A.; Nudler, E. Reactive Oxygen Species as the Long Arm of Bactericidal Antibiotics. *Proc. Natl. Acad. Sci. USA* **2019**, *116*, 9696–9698. [[CrossRef](#)] [[PubMed](#)]
62. Drlica, K.; Zhao, X. Bacterial death from treatment with fluoroquinolones and other lethal stressors. *Expert Rev. Anti-Infect. Ther.* **2021**, *19*, 601–618. [[CrossRef](#)] [[PubMed](#)]
63. Dong, C.; Zhou, J.; Wang, P.; Li, T.; Zhao, Y.; Ren, X.; Lu, J.; Wang, J.; Holmgren, A.; Zou, L. Topical Therapeutic Efficacy of Ebselen Against Multidrug-Resistant *Staphylococcus aureus* LT-1 Targeting Thioredoxin Reductase. *Front. Microbiol.* **2020**, *10*, 3016. [[CrossRef](#)] [[PubMed](#)]
64. Mourenza, Á.; Gil, J.A.; Mateos, L.M.; Letek, M. Oxidative Stress-Generating Antimicrobials, a Novel Strategy to Overcome Antibacterial Resistance. *Antioxidants* **2020**, *9*, 361. [[CrossRef](#)] [[PubMed](#)]
65. Díaz-García, D.; Ardiles, P.; Prashar, S.; Rodríguez-Diéguez, A.; Páez, P.; Gómez-Ruiz, S. Preparation and Study of the Antibacterial Applications and Oxidative Stress Induction of Copper Maleamate-Functionalized Mesoporous Silica Nanoparticles. *Pharmaceutics* **2019**, *11*, 30. [[CrossRef](#)] [[PubMed](#)]
66. Gao, F.; Shao, T.; Yu, Y.; Xiong, Y.; Yang, L. Surface-Bound Reactive Oxygen Species Generating Nanozymes for Selective Antibacterial Action. *Nat. Commun.* **2021**, *12*, 745. [[CrossRef](#)] [[PubMed](#)]
67. Linzner, N.; Antelmann, H. The Antimicrobial Activity of the AGXX[®] Surface Coating Requires a Small Particle Size to Efficiently Kill *Staphylococcus aureus*. *Front. Microbiol.* **2021**, *12*, 731564. [[CrossRef](#)]
68. Clauss-Lendzian, E.; Vaishampayan, A.; de Jong, A.; Landau, U.; Meyer, C.; Kok, J.; Grohmann, E. Stress Response of a Clinical Enterococcus Faecalis Isolate Subjected to a Novel Antimicrobial Surface Coating. *Microbiol. Res.* **2018**, *207*, 53–64. [[CrossRef](#)]

69. Van Loi, V.; Busche, T.; Preuß, T.; Kalinowski, J.; Bernhardt, J.; Antelmann, H. The AGXX[®] Antimicrobial Coating Causes a Thiol-Specific Oxidative Stress Response and Protein S-Bacillithiolation in *Staphylococcus aureus*. *Front. Microbiol.* **2018**, *9*, 03037. [CrossRef]
70. Van Loi, V.; Huyen, N.T.T.; Busche, T.; Tung, Q.N.; Gruhlke, M.C.H.; Kalinowski, J.; Bernhardt, J.; Slusarenko, A.J.; Antelmann, H. *Staphylococcus aureus* Responds to Allicin by Global S-Thioallylation—Role of the Brx/BSH/YpdA Pathway and the Disulfide Reductase MerA to Overcome Allicin Stress. *Free Radic. Biol. Med.* **2019**, *139*, 55–69. [CrossRef]
71. Paulander, W.; Wang, Y.; Folkesson, A.; Charbon, G.; Løbner-Olesen, A.; Ingmer, H. Bactericidal Antibiotics Increase Hydroxyphenyl Fluorescein Signal by Altering Cell Morphology. *PLoS ONE* **2014**, *9*, e92231. [CrossRef]
72. Kohanski, M.A.; Dwyer, D.J.; Hayete, B.; Lawrence, C.A.; Collins, J.J. A Common Mechanism of Cellular Death Induced by Bactericidal Antibiotics. *Cell* **2007**, *130*, 797–810. [CrossRef] [PubMed]
73. Chua, N.G.; Zhou, Y.P.; Tan, T.T.; Lingegowda, P.B.; Lee, W.; Lim, T.P.; Teo, J.; Cai, Y.; Kwa, A.L. Polymyxin B with Dual Carbapenem Combination Therapy against Carbapenemase-Producing *Klebsiella pneumoniae*. *J. Infect.* **2015**, *70*, 309–311. [CrossRef]
74. Alfei, S.; Schito, A.M. Positively Charged Polymers as Promising Devices against Multidrug Resistant Gram-Negative Bacteria: A Review. *Polymers* **2020**, *12*, 1195. [CrossRef]
75. Ezraty, B.; Vergnes, A.; Banzhaf, M.; Duverger, Y.; Huguenot, A.; Brochado, A.R.; Su, S.-Y.; Espinosa, L.; Loiseau, L.; Py, B.; et al. Fe-S Cluster Biosynthesis Controls Uptake of Aminoglycosides in a ROS-Less Death Pathway. *Science* **2013**, *340*, 1583–1587. [CrossRef]
76. Keren, I.; Wu, Y.; Inocencio, J.; Mulcahy, L.R.; Lewis, K. Killing by Bactericidal Antibiotics Does Not Depend on Reactive Oxygen Species. *Science* **2013**, *339*, 1213–1216. [CrossRef]
77. Liu, Y.; Imlay, J.A. Cell Death from Antibiotics Without the Involvement of Reactive Oxygen Species. *Science* **2013**, *339*, 1210–1213. [CrossRef] [PubMed]
78. Yu, Z.; Li, Q.; Wang, J.; Yu, Y.; Wang, Y.; Zhou, Q.; Li, P. Reactive Oxygen Species-Related Nanoparticle Toxicity in the Biomedical Field. *Nanoscale Res. Lett.* **2020**, *15*, 115. [CrossRef] [PubMed]
79. Guo, B.; Liu, G.; Hu, C.; Lei, B.; Liu, Y. The Structural Characteristics and Mechanisms of Antimicrobial Carbon Dots: A Mini Review. *Mater. Adv.* **2022**, *3*, 7726–7741. [CrossRef]
80. Abu Rabe, D.I.; Al Awak, M.M.; Yang, F.; Okonjo, P.A.; Dong, X.; Teisl, L.R.; Wang, P.; Tang, Y.; Pan, N.; Sun, Y.-P.; et al. The Dominant Role of Surface Functionalization in Carbon Dots' Photo-Activated Antibacterial Activity. *Int. J. Nanomed.* **2019**, *14*, 2655–2665. [CrossRef] [PubMed]
81. Bing, W.; Sun, H.; Yan, Z.; Ren, J.; Qu, X. Programmed Bacteria Death Induced by Carbon Dots with Different Surface Charge. *Small* **2016**, *12*, 4713–4718. [CrossRef]
82. van Loosdrecht, M.C.; Lyklema, J.; Norde, W.; Schraa, G.; Zehnder, A.J. The Role of Bacterial Cell Wall Hydrophobicity in Adhesion. *Appl. Environ. Microbiol.* **1987**, *53*, 1893–1897. [CrossRef]
83. Sattarahmady, N.; Rezaie-Yazdi, M.; Tondro, G.H.; Akbari, N. Bactericidal Laser Ablation of Carbon Dots: An in Vitro Study on Wild-Type and Antibiotic-Resistant *Staphylococcus aureus*. *J. Photochem. Photobiol. B* **2017**, *166*, 323–332. [CrossRef] [PubMed]
84. Wang, L.; Li, Y.; Wang, Y.; Kong, W.; Lu, Q.; Liu, X.; Zhang, D.; Qu, L. Chlorine-Doped Graphene Quantum Dots with Enhanced Anti- and Pro-Oxidant Properties. *ACS Appl. Mater. Interfaces* **2019**, *11*, 21822–21829. [CrossRef] [PubMed]
85. AGXX—Innovative Antimicrobial Technology—Heraeus. Available online: [https://www.heraeus.com/en/hpm/hmp_products_solutions/antimicrobial_technology/about_agxx/agxx_1.html#:~:text=AGXX%20is%20a%20new%20highly%20efficient%20antimicrobial%20technology,species%20\(ROS\)%20in%20the%20presence%20of%20air%20humidity](https://www.heraeus.com/en/hpm/hmp_products_solutions/antimicrobial_technology/about_agxx/agxx_1.html#:~:text=AGXX%20is%20a%20new%20highly%20efficient%20antimicrobial%20technology,species%20(ROS)%20in%20the%20presence%20of%20air%20humidity) (accessed on 22 May 2024).
86. Cieplik, F.; Deng, D.; Crielaard, W.; Buchalla, W.; Hellwig, E.; Al-Ahmad, A.; Maisch, T. Antimicrobial Photodynamic Therapy—What We Know and What We Don't. *Crit. Rev. Microbiol.* **2018**, *44*, 571–589. [CrossRef] [PubMed]
87. Mitton, D.; Ackroyd, R. A Brief Overview of Photodynamic Therapy in Europe. *Photodiagnosis Photodyn. Ther.* **2008**, *5*, 103–111. [CrossRef]
88. Dolmans, D.E.J.G.J.; Fukumura, D.; Jain, R.K. Photodynamic Therapy for Cancer. *Nat. Rev. Cancer* **2003**, *3*, 380–387. [CrossRef] [PubMed]
89. Ackroyd, R.; Kelty, C.; Brown, N.; Reed, M. The History of Photodetection and Photodynamic Therapy. *Photochem. Photobiol.* **2001**, *74*, 656–669. [CrossRef]
90. Wainwright, M. Dyes, Flies, and Sunny Skies: Photodynamic Therapy and Neglected Tropical Diseases. *Color. Technol.* **2017**, *133*, 3–14. [CrossRef]
91. Wainwright, M.; Maisch, T.; Nonell, S.; Plaetzer, K.; Almeida, A.; Tegos, G.P.; Hamblin, M.R. Photoantimicrobials—Are We Afraid of the Light? *Lancet Infect. Dis.* **2017**, *17*, e49–e55. [CrossRef]
92. Correia, J.H.; Rodrigues, J.A.; Pimenta, S.; Dong, T.; Yang, Z. Photodynamic Therapy Review: Principles, Photosensitizers, Applications, and Future Directions. *Pharmaceutics* **2021**, *13*, 1332. [CrossRef]
93. Lutkus, L.V.; Rickenbach, S.S.; McCormick, T.M. Singlet Oxygen Quantum Yields Determined by Oxygen Consumption. *J. Photochem. Photobiol. A Chem.* **2019**, *378*, 131–135. [CrossRef]
94. Liu, Y.; Qin, R.; Zaat, S.A.J.; Breukink, E.; Heger, M. Antibacterial Photodynamic Therapy: Overview of a Promising Approach to Fight Antibiotic-Resistant Bacterial Infections. *J. Clin. Transl. Res.* **2015**, *1*, 140–167.

95. Wainwright, M. Photoantimicrobials and PACT: What's in an Abbreviation? *Photochem. Photobiol. Sci.* **2019**, *18*, 12–14. [[CrossRef](#)] [[PubMed](#)]
96. Yan, E.; Kwek, G.; Qing, N.S.; Linges, S.; Xing, B. Antimicrobial Photodynamic Therapy for the Remote Eradication of Bacteria. *Chempluschem* **2023**, *88*, e202300009. [[CrossRef](#)] [[PubMed](#)]
97. Hamblin, M.R.; Abrahamse, H. Oxygen-Independent Antimicrobial Photoinactivation: Type III Photochemical Mechanism? *Antibiotics* **2020**, *9*, 53. [[CrossRef](#)] [[PubMed](#)]
98. Ghorbani, J.; Rahban, D.; Aghamiri, S.; Teymouri, A.; Bahador, A. Photosensitizers in Antibacterial Photodynamic Therapy: An Overview. *Laser Ther.* **2018**, *27*, 293–302. [[CrossRef](#)]
99. Youf, R.; Müller, M.; Balasini, A.; Thétiot, F.; Müller, M.; Hascoët, A.; Jonas, U.; Schönherr, H.; Lemerrier, G.; Montier, T.; et al. Antimicrobial Photodynamic Therapy: Latest Developments with a Focus on Combinatory Strategies. *Pharmaceutics* **2021**, *13*, 1995. [[CrossRef](#)] [[PubMed](#)]
100. Lan, M.; Zhao, S.; Liu, W.; Lee, C.; Zhang, W.; Wang, P. Photosensitizers for Photodynamic Therapy. *Adv. Healthc. Mater.* **2019**, *8*, e1900132. [[CrossRef](#)] [[PubMed](#)]
101. Jiang, Z.; Shao, J.; Yang, T.; Wang, J.; Jia, L. Pharmaceutical Development, Composition and Quantitative Analysis of Phthalocyanine as the Photosensitizer for Cancer Photodynamic Therapy. *J. Pharm. Biomed. Anal.* **2014**, *87*, 98–104. [[CrossRef](#)]
102. Amalraj, A.; Pius, A.; Gopi, S.; Gopi, S. Biological Activities of Curcuminoids, Other Biomolecules from Turmeric and Their Derivatives—A Review. *J. Tradit. Complement. Med.* **2017**, *7*, 205–233. [[CrossRef](#)]
103. Pröhl, M.; Schubert, U.S.; Weigand, W.; Gottschaldt, M. Metal Complexes of Curcumin and Curcumin Derivatives for Molecular Imaging and Anticancer Therapy. *Coord. Chem. Rev.* **2016**, *307*, 32–41. [[CrossRef](#)]
104. Yamakoshi, Y.; Umezawa, N.; Ryu, A.; Arakane, K.; Miyata, N.; Goda, Y.; Masumizu, T.; Nagano, T. Active Oxygen Species Generated from Photoexcited Fullerene (C₆₀) as Potential Medicines: O²⁻-*versus ¹O₂. *J. Am. Chem. Soc.* **2003**, *125*, 12803–12809. [[CrossRef](#)] [[PubMed](#)]
105. Fontana, C.R.; Abernethy, A.D.; Som, S.; Ruggiero, K.; Doucette, S.; Marcantonio, R.C.; Boussios, C.I.; Kent, R.; Goodson, J.M.; Tanner, A.C.R.; et al. The Antibacterial Effect of Photodynamic Therapy in Dental Plaque-derived Biofilms. *J. Periodontal Res.* **2009**, *44*, 751–759. [[CrossRef](#)] [[PubMed](#)]
106. Zanin, I.C.J.; Lobo, M.M.; Rodrigues, L.K.A.; Pimenta, L.A.F.; Höfling, J.F.; Gonçalves, R.B. Photosensitization of in vitro Biofilms by Toluidine Blue O Combined with a Light-emitting Diode. *Eur. J. Oral. Sci.* **2006**, *114*, 64–69. [[CrossRef](#)] [[PubMed](#)]
107. Fekrazad, R.; Zare, H.; Vand, S.M.S. Photodynamic Therapy Effect on Cell Growth Inhibition Induced by Radachlorin and Toluidine Blue O on *Staphylococcus Aureus* and *Escherichia Coli*: An in Vitro Study. *Photodiagnosis Photodyn. Ther.* **2016**, *15*, 213–217. [[CrossRef](#)] [[PubMed](#)]
108. Voos, A.C.; Kranz, S.; Tonndorf-Martini, S.; Voelpel, A.; Sigusch, H.; Staudte, H.; Albrecht, V.; Sigusch, B.W. Photodynamic Antimicrobial Effect of Safranin O on an Ex Vivo Periodontal Biofilm. *Lasers Surg. Med.* **2014**, *46*, 235–243. [[CrossRef](#)] [[PubMed](#)]
109. Collins, T.L.; Markus, E.A.; Hassett, D.J.; Robinson, J.B. The Effect of a Cationic Porphyrin on *Pseudomonas Aeruginosa* Biofilms. *Curr. Microbiol.* **2010**, *61*, 411–416. [[CrossRef](#)] [[PubMed](#)]
110. Di Poto, A.; Sbarra, M.S.; Provenza, G.; Visai, L.; Speziale, P. The Effect of Photodynamic Treatment Combined with Antibiotic Action or Host Defence Mechanisms on *Staphylococcus Aureus* Biofilms. *Biomaterials* **2009**, *30*, 3158–3166. [[CrossRef](#)]
111. Cieplik, F.; Späth, A.; Regensburger, J.; Gollmer, A.; Tabenski, L.; Hiller, K.-A.; Bäuml, W.; Maisch, T.; Schmalz, G. Photodynamic Biofilm Inactivation by SAPYR—An Exclusive Singlet Oxygen Photosensitizer. *Free Radic. Biol. Med.* **2013**, *65*, 477–487. [[CrossRef](#)]
112. Malá, Z.; Žárská, L.; Bajgar, R.; Bogdanová, K.; Kolář, M.; Panáček, A.; Binder, S.; Kolářová, H. The Application of Antimicrobial Photodynamic Inactivation on Methicillin-Resistant *S. Aureus* and ESBL-Producing *K. Pneumoniae* Using Porphyrin Photosensitizer in Combination with Silver Nanoparticles. *Photodiagnosis Photodyn. Ther.* **2021**, *33*, 102140. [[CrossRef](#)]
113. Board-Davies, E.L.; Rhys-Williams, W.; Hynes, D.; Williams, D.; Farnell, D.J.J.; Love, W. Antibacterial and Antibiofilm Potency of XF Drugs, Impact of Photodynamic Activation and Synergy with Antibiotics. *Front. Cell Infect. Microbiol.* **2022**, *12*, 904465. [[CrossRef](#)] [[PubMed](#)]
114. Karygianni, L.; Ruf, S.; Follo, M.; Hellwig, E.; Bucher, M.; Anderson, A.C.; Vach, K.; Al-Ahmad, A. Novel Broad-Spectrum Antimicrobial Photoinactivation of In Situ Oral Biofilms by Visible Light plus Water-Filtered Infrared A. *Appl. Environ. Microbiol.* **2014**, *80*, 7324–7336. [[CrossRef](#)] [[PubMed](#)]
115. Mesquita, M.Q.; Menezes, J.C.J.M.D.S.; Neves, M.G.P.M.S.; Tomé, A.C.; Cavaleiro, J.A.S.; Cunha, Â.; Almeida, A.; Hackbarth, S.; Röder, B.; Faustino, M.A.F. Photodynamic Inactivation of Bioluminescent *Escherichia Coli* by Neutral and Cationic Pyrrolidine-Fused Chlorins and Isobacteriochlorins. *Bioorg. Med. Chem. Lett.* **2014**, *24*, 808–812. [[CrossRef](#)] [[PubMed](#)]
116. Souza, B.M.N.; Pinto, J.G.; Pereira, A.H.C.; Miñán, A.G.; Ferreira-Strixino, J. Efficiency of Antimicrobial Photodynamic Therapy with Photodithazine[®] on MSSA and MRSA Strains. *Antibiotics* **2021**, *10*, 869. [[CrossRef](#)] [[PubMed](#)]
117. Bertoloni, G.; Rossi, F.; Valduga, G.; Jori, G.; Ali, H.; van Lier, J.E. Photosensitizing Activity of Water- and Lipid-Soluble Phthalocyanines on Prokaryotic and Eukaryotic Microbial Cells. *Microbios* **1992**, *71*, 33–46. [[PubMed](#)]
118. Fan, B.; Peng, W.; Zhang, Y.; Liu, P.; Shen, J. ROS Conversion Promotes the Bactericidal Efficiency of Eosin Y Based Photodynamic Therapy. *Biomater. Sci.* **2023**, *11*, 4930–4937. [[CrossRef](#)] [[PubMed](#)]
119. Gonçalves, M.L.L.; Sobral, A.P.T.; Gallo, J.M.A.S.; Gimenez, T.; Ferri, E.P.; Ianello, S.; de Motta, B.P.; Motta, L.J.; Horliana, A.C.R.T.; Santos, E.M.; et al. Antimicrobial Photodynamic Therapy with Erythrosine and Blue Light on Dental Biofilm Bacteria: Study Protocol for Randomised Clinical Trial. *BMJ Open* **2023**, *13*, e075084. [[CrossRef](#)] [[PubMed](#)]

120. Shrestha, A.; Kishen, A. Polycationic Chitosan-Conjugated Photosensitizer for Antibacterial Photodynamic Therapy [†]. *Photochem. Photobiol.* **2012**, *88*, 577–583. [[CrossRef](#)] [[PubMed](#)]
121. Spesia, M.B.; Milanesio, M.E.; Durantini, E.N. Synthesis, Properties and Photodynamic Inactivation of *Escherichia Coli* by Novel Cationic Fullerene C60 Derivatives. *Eur. J. Med. Chem.* **2008**, *43*, 853–861. [[CrossRef](#)]
122. Wang, M.; Huang, L.; Sharma, S.K.; Jeon, S.; Thota, S.; Sperandio, F.F.; Nayka, S.; Chang, J.; Hamblin, M.R.; Chiang, L.Y. Synthesis and Photodynamic Effect of New Highly Photostable Decacationically Armed [60]- and [70]Fullerene Decaiodide Monoadducts To Target Pathogenic Bacteria and Cancer Cells. *J. Med. Chem.* **2012**, *55*, 4274–4285. [[CrossRef](#)]
123. Cieplik, F.; Pummer, A.; Regensburger, J.; Hiller, K.-A.; Späth, A.; Tabenski, L.; Buchalla, W.; Maisch, T. The Impact of Absorbed Photons on Antimicrobial Photodynamic Efficacy. *Front. Microbiol.* **2015**, *6*, 706. [[CrossRef](#)] [[PubMed](#)]
124. Maisch, T.; Eichner, A.; Späth, A.; Gollmer, A.; König, B.; Regensburger, J.; Bäuml, W. Fast and Effective Photodynamic Inactivation of Multiresistant Bacteria by Cationic Riboflavin Derivatives. *PLoS ONE* **2014**, *9*, e111792. [[CrossRef](#)] [[PubMed](#)]
125. Najafi, S.; Khayamzadeh, M.; Paknejad, M.; Poursepanj, G.; Kharazi Fard, M.J.; Bahador, A. An In Vitro Comparison of Antimicrobial Effects of Curcumin-Based Photodynamic Therapy and Chlorhexidine, on Aggregatibacter Actinomycetemcomitans. *J. Lasers Med. Sci.* **2016**, *7*, 21–25. [[CrossRef](#)] [[PubMed](#)]
126. Dahl, T.A.; McGowan, W.M.; Shand, M.A.; Srinivasan, V.S. Photokilling of Bacteria by the Natural Dye Curcumin. *Arch. Microbiol.* **1989**, *151*, 183–185. [[CrossRef](#)] [[PubMed](#)]
127. García, I.; Ballesta, S.; Gilaberte, Y.; Rezusta, A.; Pascual, Á. Antimicrobial Photodynamic Activity of Hypericin against Methicillin-Susceptible and Resistant *Staphylococcus aureus* Biofilms. *Future Microbiol.* **2015**, *10*, 347–356. [[CrossRef](#)] [[PubMed](#)]
128. Yow, C.M.N.; Tang, H.M.; Chu, E.S.M.; Huang, Z. Hypericin-mediated Photodynamic Antimicrobial Effect on Clinically Isolated Pathogens [†]. *Photochem. Photobiol.* **2012**, *88*, 626–632. [[CrossRef](#)] [[PubMed](#)]
129. Morimoto, K.; Ozawa, T.; Awazu, K.; Ito, N.; Honda, N.; Matsumoto, S.; Tsuruta, D. Photodynamic Therapy Using Systemic Administration of 5-Aminolevulinic Acid and a 410-Nm Wavelength Light-Emitting Diode for Methicillin-Resistant *Staphylococcus Aureus*-Infected Ulcers in Mice. *PLoS ONE* **2014**, *9*, e105173. [[CrossRef](#)] [[PubMed](#)]
130. Wang, P.; Wang, B.; Zhang, L.; Liu, X.; Shi, L.; Kang, X.; Lei, X.; Chen, K.; Chen, Z.; Li, C.; et al. Clinical Practice Guidelines for 5-Aminolevulinic Acid Photodynamic Therapy for Acne Vulgaris in China. *Photodiagnosis Photodyn. Ther.* **2023**, *41*, 103261. [[CrossRef](#)]
131. Polat, E.; Kang, K. Natural Photosensitizers in Antimicrobial Photodynamic Therapy. *Biomedicines* **2021**, *9*, 584. [[CrossRef](#)]
132. Abrahamse, H.; Hamblin, M.R. New Photosensitizers for Photodynamic Therapy. *Biochem. J.* **2016**, *473*, 347–364. [[CrossRef](#)]
133. Gunaydin, G.; Gedik, M.E.; Ayan, S. Photodynamic Therapy—Current Limitations and Novel Approaches. *Front. Chem.* **2021**, *9*, 691697. [[CrossRef](#)] [[PubMed](#)]
134. Clément, S.; Winum, J.-Y. Photodynamic Therapy Alone or in Combination to Counteract Bacterial Infections. *Expert. Opin. Ther. Pat.* **2024**, *34*, 401–414. [[CrossRef](#)] [[PubMed](#)]
135. Embleton, M.L. Selective Lethal Photosensitization of Methicillin-Resistant *Staphylococcus Aureus* Using an IgG-Tin (IV) Chlorin E6 Conjugate. *J. Antimicrob. Chemother.* **2002**, *50*, 857–864. [[CrossRef](#)] [[PubMed](#)]
136. Dosselli, R.; Gobbo, M.; Bolognini, E.; Campestrini, S.; Reddi, E. Porphyrin–Apidaecin Conjugate as a New Broad Spectrum Antibacterial Agent. *ACS Med. Chem. Lett.* **2010**, *1*, 35–38. [[CrossRef](#)] [[PubMed](#)]
137. Sperandio, F.; Huang, Y.-Y.; Hamblin, M. Antimicrobial Photodynamic Therapy to Kill Gram-Negative Bacteria. *Recent. Pat. Antiinfect. Drug Discov.* **2013**, *8*, 108–120. [[CrossRef](#)] [[PubMed](#)]
138. Rice, D.R.; Gan, H.; Smith, B.D. Bacterial Imaging and Photodynamic Inactivation Using Zinc(II)-Dipicolylamine BODIPY Conjugates. *Photochem. Photobiol. Sci.* **2015**, *14*, 1271–1281. [[CrossRef](#)] [[PubMed](#)]
139. Soto-Moreno, A.; Montero-Vilchez, T.; Diaz-Calvillo, P.; Molina-Leyva, A.; Arias-Santiago, S. The Impact of Photodynamic Therapy on Skin Homeostasis in Patients with Actinic Keratosis: A Prospective Observational Study. *Skin. Res. Technol.* **2023**, *29*, e13493. [[CrossRef](#)] [[PubMed](#)]
140. Rapačka-Zdończyk, A.; Woźniak, A.; Michalska, K.; Pierański, M.; Ogonowska, P.; Grinholc, M.; Nakonieczna, J. Factors Determining the Susceptibility of Bacteria to Antibacterial Photodynamic Inactivation. *Front. Med.* **2021**, *8*, 642609. [[CrossRef](#)] [[PubMed](#)]
141. Klausen, M.; Ucuncu, M.; Bradley, M. Design of Photosensitizing Agents for Targeted Antimicrobial Photodynamic Therapy. *Molecules* **2020**, *25*, 5239. [[CrossRef](#)]
142. Zhou, W.; Jiang, X.; Zhen, X. Development of Organic Photosensitizers for Antimicrobial Photodynamic Therapy. *Biomater. Sci.* **2023**, *11*, 5108–5128. [[CrossRef](#)]
143. Badran, Z.; Rahman, B.; De Bonfils, P.; Nun, P.; Coeffard, V.; Verron, E. Antibacterial Nanophotosensitizers in Photodynamic Therapy: An Update. *Drug Discov. Today* **2023**, *28*, 103493. [[CrossRef](#)]
144. Piksa, M.; Lian, C.; Samuel, I.C.; Pawlik, K.J.; Samuel, I.D.W.; Matczyszyn, K. The Role of the Light Source in Antimicrobial Photodynamic Therapy. *Chem. Soc. Rev.* **2023**, *52*, 1697–1722. [[CrossRef](#)]
145. Usui, Y. Determination of quantum yield of singlet oxygen formation by photosensitization. *Chem. Lett.* **1973**, *2*, 743–744. [[CrossRef](#)]
146. Wiegell, S.R.; Skødt, V.; Wulf, H.C. Daylight-mediated Photodynamic Therapy of Basal Cell Carcinomas—An Explorative Study. *J. Eur. Acad. Dermatol. Venereol.* **2014**, *28*, 169–175. [[CrossRef](#)]

147. Al-Mutairi, R.; Tovmasyan, A.; Batinic-Haberle, I.; Benov, L. Sublethal Photodynamic Treatment Does Not Lead to Development of Resistance. *Front. Microbiol.* **2018**, *9*, 1699. [[CrossRef](#)]
148. Nakonieczna, J.; Wozniak, A.; Pieranski, M.; Rapacka-Zdonczyk, A.; Ogonowska, P.; Grinholc, M. Photoinactivation of ESKAPE Pathogens: Overview of Novel Therapeutic Strategy. *Future Med. Chem.* **2019**, *11*, 443–461. [[CrossRef](#)]
149. Songca, S.P.; Adjei, Y. Applications of Antimicrobial Photodynamic Therapy against Bacterial Biofilms. *Int. J. Mol. Sci.* **2022**, *23*, 3209. [[CrossRef](#)]
150. Ribeiro, M.; Gomes, I.B.; Saavedra, M.J.; Simões, M. Photodynamic Therapy and Combinatory Treatments for the Control of Biofilm-Associated Infections. *Lett. Appl. Microbiol.* **2022**, *75*, 548–564. [[CrossRef](#)]
151. Barolet, D.; Boucher, A. Radiant near Infrared Light Emitting Diode Exposure as Skin Preparation to Enhance Photodynamic Therapy Inflammatory Type Acne Treatment Outcome. *Lasers Surg. Med.* **2010**, *42*, 171–178. [[CrossRef](#)]
152. Sakamoto, F.H.; Torezan, L.; Anderson, R.R. Photodynamic Therapy for Acne Vulgaris: A Critical Review from Basics to Clinical Practice. *J. Am. Acad. Dermatol.* **2010**, *63*, 195–211. [[CrossRef](#)]
153. Boen, M.; Brownell, J.; Patel, P.; Tsoukas, M.M. The Role of Photodynamic Therapy in Acne: An Evidence-Based Review. *Am. J. Clin. Dermatol.* **2017**, *18*, 311–321. [[CrossRef](#)] [[PubMed](#)]
154. Wojewoda, K.; Gillstedt, M.; Tovi, J.; Salah, L.; Wennberg Larkö, A.-M.; Sjöholm, A.; Sandberg, C. Optimizing Treatment of Acne with Photodynamic Therapy (PDT) to Achieve Long-Term Remission and Reduce Side Effects. A Prospective Randomized Controlled Trial. *J. Photochem. Photobiol. B* **2021**, *223*, 112299. [[CrossRef](#)]
155. Ning, X.; He, G.; Zeng, W.; Xia, Y. The Photosensitizer-Based Therapies Enhance the Repairing of Skin Wounds. *Front. Med.* **2022**, *9*, 915548. [[CrossRef](#)]
156. Brown, S. Clinical Antimicrobial Photodynamic Therapy: Phase II Studies in Chronic Wounds. *J. Natl. Compr. Cancer Netw.* **2012**, *10*, S-80–S-83. [[CrossRef](#)] [[PubMed](#)]
157. Pérez-Laguna, V.; Gilaberte, Y.; Millán-Lou, M.I.; Agut, M.; Nonell, S.; Rezusta, A.; Hamblin, M.R. A Combination of Photodynamic Therapy and Antimicrobial Compounds to Treat Skin and Mucosal Infections: A Systematic Review. *Photochem. Photobiol. Sci.* **2019**, *18*, 1020–1029. [[CrossRef](#)]
158. Prazmo, E.; Mielczarek, A.; Kwaśny, M.; Łapiński, M. Photodynamic Therapy as a Promising Method Used in the Treatment of Oral Diseases. *Adv. Clin. Exp. Med.* **2016**, *25*, 799–807. [[CrossRef](#)] [[PubMed](#)]
159. Dragana, R.; Jelena, M.; Jovan, M.; Biljana, N.; Dejan, M. Antibacterial Efficiency of Adjuvant Photodynamic Therapy and High-Power Diode Laser in the Treatment of Young Permanent Teeth with Chronic Periapical Periodontitis. A Prospective Clinical Study. *Photodiagnosis Photodyn. Ther.* **2023**, *41*, 103129. [[CrossRef](#)]
160. Vohra, F.; Akram, Z.; Safii, S.H.; Vaithilingam, R.D.; Ghanem, A.; Sergis, K.; Javed, F. Role of Antimicrobial Photodynamic Therapy in the Treatment of Aggressive Periodontitis: A Systematic Review. *Photodiagnosis Photodyn. Ther.* **2016**, *13*, 139–147. [[CrossRef](#)] [[PubMed](#)]
161. Bechara Andere, N.M.R.; dos Santos, N.C.C.; Araujo, C.F.; Mathias, I.F.; Rossato, A.; de Marco, A.C.; Santamaria, M.; Jardini, M.A.N.; Santamaria, M.P. Evaluation of the Local Effect of Nonsurgical Periodontal Treatment with and without Systemic Antibiotic and Photodynamic Therapy in Generalized Aggressive Periodontitis. A Randomized Clinical Trial. *Photodiagnosis Photodyn. Ther.* **2018**, *24*, 115–120. [[CrossRef](#)]
162. Ohba, S.; Sato, M.; Noda, S.; Yamamoto, H.; Egahira, K.; Asahina, I. Assessment of Safety and Efficacy of Antimicrobial Photodynamic Therapy for Peri-Implant Disease. *Photodiagnosis Photodyn. Ther.* **2020**, *31*, 101936. [[CrossRef](#)]
163. Yupanqui Mieleles, J.; Vyas, C.; Aslan, E.; Humphreys, G.; Diver, C.; Bartolo, P. Honey: An Advanced Antimicrobial and Wound Healing Biomaterial for Tissue Engineering Applications. *Pharmaceutics* **2022**, *14*, 1663. [[CrossRef](#)] [[PubMed](#)]
164. Deng, J.; Liu, R.; Lu, Q.; Hao, P.; Xu, A.; Zhang, J.; Tan, J. Biochemical Properties, Antibacterial and Cellular Antioxidant Activities of Buckwheat Honey in Comparison to Manuka Honey. *Food Chem.* **2018**, *252*, 243–249. [[CrossRef](#)] [[PubMed](#)]
165. Girma, A.; Seo, W.; She, R.C. Antibacterial Activity of Varying UMF-Graded Manuka Honeys. *PLoS ONE* **2019**, *14*, e0224495. [[CrossRef](#)]
166. Cokcetin, N.; Williams, S.; Blair, S.; Carter, D.; Brooks, P.; Harry, L. *Active Australian Leptospermum Honey: New Sources and Their Bioactivity*; Agrifutures Australia: Canberra, Australia, 2019.
167. Brudzynski, K. Effect of Hydrogen Peroxide on Antibacterial Activities of Canadian Honeys. *Can. J. Microbiol.* **2006**, *52*, 1228–1237. [[CrossRef](#)] [[PubMed](#)]
168. Alvarez-Suarez, J.M.; Tulipani, S.; Díaz, D.; Estevez, Y.; Romandini, S.; Giampieri, F.; Damiani, E.; Astolfi, P.; Bompadre, S.; Battino, M. Antioxidant and Antimicrobial Capacity of Several Monofloral Cuban Honeys and Their Correlation with Color, Polyphenol Content and Other Chemical Compounds. *Food Chem. Toxicol.* **2010**, *48*, 2490–2499. [[CrossRef](#)]
169. Sherlock, O.; Dolan, A.; Athman, R.; Power, A.; Gethin, G.; Cowman, S.; Humphreys, H. Comparison of the Antimicrobial Activity of Ulmo Honey from Chile and Manuka Honey against Methicillin-Resistant *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. *BMC Complement. Altern. Med.* **2010**, *10*, 47. [[CrossRef](#)]
170. Isla, M.I.; Craig, A.; Ordoñez, R.; Zampini, C.; Sayago, J.; Bedascarrasbure, E.; Alvarez, A.; Salomón, V.; Maldonado, L. Physico Chemical and Bioactive Properties of Honeys from Northwestern Argentina. *LWT Food Sci. Technol.* **2011**, *44*, 1922–1930. [[CrossRef](#)]
171. Fyfe, L.; Okoro, P.; Paterson, E.; Coyle, S.; McDougall, G.J. Compositional Analysis of Scottish Honeys with Antimicrobial Activity against Antibiotic-Resistant Bacteria Reveals Novel Antimicrobial Components. *LWT Food Sci. Technol.* **2017**, *79*, 52–59. [[CrossRef](#)]

172. Escuredo, O.; Silva, L.R.; Valentão, P.; Seijo, M.C.; Andrade, P.B. Assessing Rubus Honey Value: Pollen and Phenolic Compounds Content and Antibacterial Capacity. *Food Chem.* **2012**, *130*, 671–678. [[CrossRef](#)]
173. Matzen, R.D.; Zinck Leth-Espensen, J.; Jansson, T.; Nielsen, D.S.; Lund, M.N.; Matzen, S. The Antibacterial Effect In Vitro of Honey Derived from Various Danish Flora. *Dermatol. Res. Pract.* **2018**, *2018*, 7021713. [[CrossRef](#)]
174. Bucekova, M.; Jardekova, L.; Juricova, V.; Bugarova, V.; Di Marco, G.; Gismondi, A.; Leonardi, D.; Farkasovska, J.; Godocikova, J.; Laho, M.; et al. Antibacterial Activity of Different Blossom Honeys: New Findings. *Molecules* **2019**, *24*, 1573. [[CrossRef](#)] [[PubMed](#)]
175. Ahmed, G. Hegazi Antimicrobial Activity of Different Egyptian Honeys as Comparison of Saudi Arabia Honey. *Res. J. Microbiol.* **2011**, *6*, 488–495.
176. Laallam, H.; Boughediri, L.; Bissati, S.; Menasria, T.; Mouzaoui, M.S.; Hadjadj, S.; Hammoudi, R.; Chenchouni, H. Modeling the Synergistic Antibacterial Effects of Honey Characteristics of Different Botanical Origins from the Sahara Desert of Algeria. *Front. Microbiol.* **2015**, *6*, 1239. [[CrossRef](#)]
177. John-Isa, J.F.; Adebolu, T.T.; Oyetayo, V.O. Antibacterial Effects of Honey in Nigeria on Selected Diarrhoeagenic Bacteria. *South Asian J. Res. Microbiol.* **2019**, *3*, 1–11. [[CrossRef](#)]
178. ElBorai, A. Antibacterial and Antioxidant Activities of Different Varieties of Locally Produced Egyptian Honey. *Egypt. J. Bot.* **2018**, *58*, 97–107. [[CrossRef](#)]
179. Kwakman, P.H.S.; Zaat, S.A.J. Antibacterial Components of Honey. *IUBMB Life* **2012**, *64*, 48–55. [[CrossRef](#)]
180. Moore, O.A.; Smith, L.A.; Campbell, F.; Seers, K.; McQuay, H.J.; Moore, R.A. Systematic Review of the Use of Honey as a Wound Dressing. *BMC Complement. Altern. Med.* **2001**, *1*, 2. [[CrossRef](#)] [[PubMed](#)]
181. Jull, A.B.; Rodgers, A.; Walker, N. Honey as a Topical Treatment for Wounds. In *Cochrane Database of Systematic Reviews*; Jull, A.B., Ed.; John Wiley & Sons, Ltd.: Chichester, UK, 2008.
182. Johnson, D.W.; van Eps, C.; Mudge, D.W.; Wiggins, K.J.; Armstrong, K.; Hawley, C.M.; Campbell, S.B.; Isbel, N.M.; Nimmo, G.R.; Gibbs, H. Randomized, Controlled Trial of Topical Exit-Site Application of Honey (Medihoney) versus Mupirocin for the Prevention of Catheter-Associated Infections in Hemodialysis Patients. *J. Am. Soc. Nephrol.* **2005**, *16*, 1456–1462. [[CrossRef](#)]
183. Love, N.R.; Chen, Y.; Ishibashi, S.; Kritsiligkou, P.; Lea, R.; Koh, Y.; Gallop, J.L.; Dorey, K.; Amaya, E. Amputation-Induced Reactive Oxygen Species Are Required for Successful Xenopus Tadpole Tail Regeneration. *Nat. Cell Biol.* **2013**, *15*, 222–228. [[CrossRef](#)]
184. Hixon, K.R.; Klein, R.C.; Eberlin, C.T.; Linder, H.R.; Ona, W.J.; Gonzalez, H.; Sell, S.A. A Critical Review and Perspective of Honey in Tissue Engineering and Clinical Wound Healing. *Adv. Wound Care* **2019**, *8*, 403–415. [[CrossRef](#)]
185. Ding, Y.; Li, W.; Zhang, F.; Liu, Z.; Zanzanzadeh Ezazi, N.; Liu, D.; Santos, H.A. Electrospun Fibrous Architectures for Drug Delivery, Tissue Engineering and Cancer Therapy. *Adv. Funct. Mater.* **2019**, *29*, 1802852. [[CrossRef](#)]
186. Li, J.; Mooney, D.J. Designing Hydrogels for Controlled Drug Delivery. *Nat. Rev. Mater.* **2016**, *1*, 16071. [[CrossRef](#)] [[PubMed](#)]
187. Rossi, M.; Marrazzo, P. The Potential of Honeybee Products for Biomaterial Applications. *Biomimetics* **2021**, *6*, 6. [[CrossRef](#)] [[PubMed](#)]
188. Minden-Birkenmaier, B.; Bowlin, G. Honey-Based Templates in Wound Healing and Tissue Engineering. *Bioengineering* **2018**, *5*, 46. [[CrossRef](#)] [[PubMed](#)]
189. Halstead, F.D.; Webber, M.A.; Rauf, M.; Burt, R.; Dryden, M.; Oppenheim, B.A. In Vitro Activity of an Engineered Honey, Medical-Grade Honeys, and Antimicrobial Wound Dressings against Biofilm-Producing Clinical Bacterial Isolates. *J. Wound Care* **2016**, *25*, 93–102. [[CrossRef](#)] [[PubMed](#)]
190. Cooke, J.; Dryden, M.; Patton, T.; Brennan, J.; Barrett, J. The Antimicrobial Activity of Prototype Modified Honeys That Generate Reactive Oxygen Species (ROS) Hydrogen Peroxide. *BMC Res. Notes* **2015**, *8*, 20. [[CrossRef](#)] [[PubMed](#)]
191. Wilkinson, J.M.; Cavanagh, H.M.A. Antibacterial Activity of 13 Honeys Against *Escherichia coli* and *Pseudomonas aeruginosa*. *J. Med. Food* **2005**, *8*, 100–103. [[CrossRef](#)] [[PubMed](#)]
192. Mullai, V.; Menon, T. Bactericidal Activity of Different Types of Honey against Clinical and Environmental Isolates of *Pseudomonas aeruginosa*. *J. Altern. Complement. Med.* **2007**, *13*, 439–442. [[CrossRef](#)] [[PubMed](#)]
193. Percival, S.L.; Hill, K.E.; Williams, D.W.; Hooper, S.J.; Thomas, D.W.; Costerton, J.W. A Review of the Scientific Evidence for Biofilms in Wounds. *Wound Repair. Regen.* **2012**, *20*, 647–657. [[CrossRef](#)]
194. Dryden, M.; Dickinson, A.; Brooks, J.; Hudgell, L.; Saeed, K.; Cutting, K.F. A Multi-Centre Clinical Evaluation of Reactive Oxygen Topical Wound Gel in 114 Wounds. *J. Wound Care* **2016**, *25*, 140–146. [[CrossRef](#)]
195. Dryden, M.; Milward, G.; Saeed, K. Infection Prevention in Wounds with Surgihoney. *J. Hosp. Infect.* **2014**, *88*, 121–122. [[CrossRef](#)]
196. Dryden, M.; Tawse, C.; Adams, J.; Howard, A.; Saeed, K.; Cooke, J. The Use of Surgihoney to Prevent or Eradicate Bacterial Colonisation in Dressing Oncology Long Vascular Lines. *J. Wound Care* **2014**, *23*, 338–341. [[CrossRef](#)]
197. Nair, H.K.R.; Tatavilis, N.; Pospíšilová, I.; Kučerová, J.; Cremers, N.A.J. Medical-Grade Honey Kills Antibiotic-Resistant Bacteria and Prevents Amputation in Diabetics with Infected Ulcers: A Prospective Case Series. *Antibiotics* **2020**, *9*, 529. [[CrossRef](#)]
198. Hermanns, R.; Mateescu, C.; Thrasyvoulou, A.; Tananaki, C.; Wagener, F.A.D.T.G.; Cremers, N.A.J. Defining the Standards for Medical Grade Honey. *J. Apic. Res.* **2020**, *59*, 125–135. [[CrossRef](#)]
199. Kwakman, P.H.S.; te Velde, A.A.; Boer, L.; Speijer, D.; Christina Vandenbroucke-Grauls, M.J.; Zaat, S.A.J. How Honey Kills Bacteria. *FASEB J.* **2010**, *24*, 2576–2582. [[CrossRef](#)]
200. Kwakman, P.H.S.; te Velde, A.A.; de Boer, L.; Vandenbroucke-Grauls, C.M.J.E.; Zaat, S.A.J. Two Major Medicinal Honeys Have Different Mechanisms of Bactericidal Activity. *PLoS ONE* **2011**, *6*, e17709. [[CrossRef](#)] [[PubMed](#)]

201. Advancis Medical Activon[®] Manuka Honey. Available online: <https://uk.advancismedical.com/products/activon-manuka-honey> (accessed on 5 May 2024).
202. Rafter, L.; Reynolds, T.; Collier, M.; Rafter, M.; West, M. *A Clinical Evaluation of Algivon[®] Plus Manuka Honey Dressings for Chronic Wounds*; Wounds UK: London, UK, 2017; Volume 13, pp. 132–140.
203. Edwards, J. *A Prospective Evaluation of the Use of Honey Dressings to Manage Burn Wounds*; Wounds UK: London, UK, 2013; Volume 9, pp. 102–106.
204. Cryer, S. *The Use of Manuka Honey to Facilitate the Autoamputation of Fingertip Necrosis*; Wounds UK: London, UK, 2016; Volume 12, pp. 66–70.
205. Welland Medical Aurum with Manuka Honey. Available online: https://wellandmedical.com/brand_type/aurum/ (accessed on 6 May 2024).
206. Martin-Skurr, K. Case Study: Pyoderma Gangrenosum and the Effects of Manuka Honey. Available online: https://wellandmedical.com/wp-content/uploads/2019/02/case-study_nz-pyoderma-gangrenosum-manuka-honey.pdf (accessed on 6 May 2024).
207. L-Mesitran. Products. Available online: <https://l-mesitran.com/eu/en/products> (accessed on 6 May 2024).
208. Boggust, A. *Evaluation of L-Mesitran[®] Dressings in the Treatment of Minor Burns and Scalds at a Paediatric Emergency Department*; Wounds UK: London, UK, 2013; Volume 9, pp. 114–117.
209. Stephen-Haynes, J.; Callaghan, R. *Properties of Honey: Its Mode of Action and Clinical Outcomes*; Wounds UK: London, UK, 2011; Volume 7, pp. 50–57.
210. Callaghan, R. *Treating Difficult-to-Debride Wounds Using a Manuka Honey Dressing: A Case Study Evaluation*; Wounds UK: London, UK, 2014; Volume 10, pp. 104–109.
211. Bradbury, S.; Callaghan, R.; Ivins, N. *Manuka Dress Made Easy*; Wounds UK: London, UK, 2014; Volume 10, pp. 1–6.
212. Derma Science Europe Antibacterial Dressing Medihoney[®]: Antibacterial Medical Honey. Available online: https://media.supplychain.nhs.uk/media/documents/ely302/marketing/48251_ely302_3.pdf.pdf (accessed on 6 May 2024).
213. Robson, V.; Dodd, S.; Thomas, S. Standardized Antibacterial Honey (MedihoneyTM) with Standard Therapy in Wound Care: Randomized Clinical Trial. *J. Adv. Nurs.* **2009**, *65*, 565–575. [[CrossRef](#)] [[PubMed](#)]
214. Johnson, D.W.; Clark, C.; Isbel, N.M.; Hawley, C.M.; Beller, E.; Cass, A.; de Zoysa, J.; McTaggart, S.; Playford, G.; Rosser, B.; et al. The HoneyPot Study Protocol: A Randomized Controlled Trial of Exit-Site Application of Medihoney Antibacterial Wound Gel for the Prevention of Catheter-Associated Infections in Peritoneal Dialysis Patients. *Perit. Dial. Int.* **2009**, *29*, 303–309. [[CrossRef](#)]
215. Kestrel Health Information MEDIHONEY[®] Calcium Alginate Dressing. Available online: <https://www.woundsource.com/product/medihoney-calcium-alginate-dressing> (accessed on 6 May 2024).
216. Jull, A.; Walker, N.; Parag, V.; Molan, P.; Rodgers, A. Randomized Clinical Trial of Honey-Impregnated Dressings for Venous Leg Ulcers. *Br. J. Surg.* **2008**, *95*, 175–182. [[CrossRef](#)]
217. Derma Sciences Europe Antibacterial Dressing Medihoney[®]: Barrier Cream. Available online: https://media.supplychain.nhs.uk/media/documents/ely289/marketing/48248_ely289.pdf.pdf (accessed on 6 May 2024).
218. Nijhuis, W.; Houwing, R.; Van der Zwet, W.; Jansman, F. A Randomised Trial of Honey Barrier Cream versus Zinc Oxide Ointment. *Br. J. Nurs.* **2012**, *21*, S10–S13. [[CrossRef](#)]
219. Comvita Antibacterial Wound GelTM 25g. Available online: <https://www.comvita.co.uk/product/antibacterial-wound-gel%E2%84%A2-25g/6011> (accessed on 6 May 2024).
220. Robson, V.; Yorke, J.; Sen, R.A.; Lowe, D.; Rogers, S.N. Randomised Controlled Feasibility Trial on the Use of Medical Grade Honey Following Microvascular Free Tissue Transfer to Reduce the Incidence of Wound Infection. *Br. J. Oral. Maxillofac. Surg.* **2012**, *50*, 321–327. [[CrossRef](#)] [[PubMed](#)]
221. Matoke Holdings Surgihoney RO. Available online: <https://www.surgihoneyro.com/> (accessed on 6 May 2024).
222. Dryden, M.; Goddard, C.; Madadi, A.; Heard, M.; Saeed, K.; Cooke, J. Using Antimicrobial Surgihoney to Prevent Caesarean Wound Infection. *Br. J. Midwifery* **2014**, *22*, 111–115. [[CrossRef](#)]
223. Molan, P. Why Honey Is Effective as a Medicine. *Bee World* **2001**, *82*, 22–40. [[CrossRef](#)]
224. Molan, P.C. The Antibacterial Activity of Honey. *Bee World* **1992**, *73*, 5–28. [[CrossRef](#)]
225. Mandal, M.D.; Mandal, S. Honey: Its Medicinal Property and Antibacterial Activity. *Asian Pac. J. Trop. Biomed.* **2011**, *1*, 154–160. [[CrossRef](#)]
226. Nolan, V.C.; Harrison, J.; Cox, J.A.G. Dissecting the Antimicrobial Composition of Honey. *Antibiotics* **2019**, *8*, 251. [[CrossRef](#)] [[PubMed](#)]
227. Bucekova, M.; Valachova, I.; Kohutova, L.; Prochazka, E.; Klaudivny, J.; Majtan, J. Honeybee Glucose Oxidase—Its Expression in Honeybee Workers and Comparative Analyses of Its Content and H₂O₂-Mediated Antibacterial Activity in Natural Honeys. *Naturwissenschaften* **2014**, *101*, 661–670. [[CrossRef](#)] [[PubMed](#)]
228. Cebrero, G.; Sanhueza, O.; Pezoa, M.; Báez, M.E.; Martínez, J.; Báez, M.; Fuentes, E. Relationship among the Minor Constituents, Antibacterial Activity and Geographical Origin of Honey: A Multifactor Perspective. *Food Chem.* **2020**, *315*, 126296. [[CrossRef](#)]
229. Wong, C.M.; Wong, K.H.; Chen, X.D. Glucose Oxidase: Natural Occurrence, Function, Properties and Industrial Applications. *Appl. Microbiol. Biotechnol.* **2008**, *78*, 927–938. [[CrossRef](#)]

230. Kuś, P.M.; Szweda, P.; Jerković, I.; Tuberoso, C.I.G. Activity of Polish Unifloral Honeys against Pathogenic Bacteria and Its Correlation with Colour, Phenolic Content, Antioxidant Capacity and Other Parameters. *Lett. Appl. Microbiol.* **2016**, *62*, 269–276. [[CrossRef](#)]
231. Johnston, M.; McBride, M.; Dahiya, D.; Owusu-Apenten, R.; Singh Nigam, P. Antibacterial Activity of Manuka Honey and Its Components: An Overview. *AIMS Microbiol.* **2018**, *4*, 655–664. [[CrossRef](#)] [[PubMed](#)]
232. Albaridi, N.A. Antibacterial Potency of Honey. *Int. J. Microbiol.* **2019**, *2019*, 2464507. [[CrossRef](#)] [[PubMed](#)]
233. Güneş, M.E.; Şahin, S.; Demir, C.; Borum, E.; Tosunoğlu, A. Determination of Phenolic Compounds Profile in Chestnut and Floral Honeys and Their Antioxidant and Antimicrobial Activities. *J. Food Biochem.* **2017**, *41*, e12345. [[CrossRef](#)]
234. Ball, D.W. The Chemical Composition of Honey. *J. Chem. Educ.* **2007**, *84*, 1643. [[CrossRef](#)]
235. McLoone, P.; Warnock, M.; Fyfe, L. Honey: A Realistic Antimicrobial for Disorders of the Skin. *J. Microbiol. Immunol. Infect.* **2016**, *49*, 161–167. [[CrossRef](#)] [[PubMed](#)]
236. Ganz, T. Defensins: Antimicrobial Peptides of Innate Immunity. *Nat. Rev. Immunol.* **2003**, *3*, 710–720. [[CrossRef](#)] [[PubMed](#)]
237. Bucekova, M.; Sojka, M.; Valachova, I.; Martinotti, S.; Ranzato, E.; Szep, Z.; Majtan, V.; Klaudiny, J.; Majtan, J. Bee-Derived Antibacterial Peptide, Defensin-1, Promotes Wound Re-Epithelialisation In Vitro and In Vivo. *Sci. Rep.* **2017**, *7*, 7340. [[CrossRef](#)] [[PubMed](#)]
238. Memar, M.Y.; Yekani, M.; Alizadeh, N.; Baghi, H.B. Hyperbaric Oxygen Therapy: Antimicrobial Mechanisms and Clinical Application for Infections. *Biomed. Pharmacother.* **2019**, *109*, 440–447. [[CrossRef](#)]
239. Çimşit, M.; Uzun, G.; Yıldız, Ş. Hyperbaric Oxygen Therapy as an Anti-Infective Agent. *Expert. Rev. Anti Infect. Ther.* **2009**, *7*, 1015–1026. [[CrossRef](#)]
240. Zhou, D.; Fu, D.; Yan, L.; Xie, L. The Role of Hyperbaric Oxygen Therapy in the Treatment of Surgical Site Infections: A Narrative Review. *Medicina* **2023**, *59*, 762. [[CrossRef](#)]
241. Chmelař, D.; Rozložník, M.; Hájek, M.; Pospíšilová, N.; Kuzma, J. Effect of Hyperbaric Oxygen on the Growth and Susceptibility of Facultatively Anaerobic Bacteria and Bacteria with Oxidative Metabolism to Selected Antibiotics. *Folia Microbiol.* **2024**, *69*, 101–108. [[CrossRef](#)] [[PubMed](#)]
242. Kolpen, M.; Mousavi, N.; Sams, T.; Bjarnsholt, T.; Ciofu, O.; Moser, C.; Kühn, M.; Høiby, N.; Jensen, P.Ø. Reinforcement of the Bactericidal Effect of Ciprofloxacin on *Pseudomonas Aeruginosa* Biofilm by Hyperbaric Oxygen Treatment. *Int. J. Antimicrob. Agents* **2016**, *47*, 163–167. [[CrossRef](#)] [[PubMed](#)]
243. Kolpen, M.; Lerche, C.J.; Kragh, K.N.; Sams, T.; Koren, K.; Jensen, A.S.; Line, L.; Bjarnsholt, T.; Ciofu, O.; Moser, C.; et al. Hyperbaric Oxygen Sensitizes Anoxic *Pseudomonas Aeruginosa* Biofilm to Ciprofloxacin. *Antimicrob. Agents Chemother.* **2017**, *61*, e01024-17. [[CrossRef](#)] [[PubMed](#)]
244. Weaver, L.K. Hyperbaric Oxygen Therapy for Carbon Monoxide Poisoning. *Undersea Hyperb. Med.* **2014**, *41*, 339–354. [[PubMed](#)]
245. Ortega, M.A.; Fraile-Martinez, O.; García-Montero, C.; Callejón-Peláez, E.; Sáez, M.A.; Álvarez-Mon, M.A.; García-Honduvilla, N.; Monserrat, J.; Álvarez-Mon, M.; Bujan, J.; et al. A General Overview on the Hyperbaric Oxygen Therapy: Applications, Mechanisms and Translational Opportunities. *Medicina* **2021**, *57*, 864. [[CrossRef](#)] [[PubMed](#)]
246. Harnanik, T.; Soeroso, J.; Suryokusumo, M.G.; Juliandhy, T. Effects of Hyperbaric Oxygen on T Helper 17/Regulatory T Polarization in Antigen and Collagen-Induced Arthritis: Hypoxia-Inducible Factor-1 α as a Target. *Oman Med. J.* **2020**, *35*, e90. [[CrossRef](#)] [[PubMed](#)]
247. Baiula, M.; Greco, R.; Ferrazzano, L.; Caligiana, A.; Hoxha, K.; Bandini, D.; Longobardi, P.; Spampinato, S.; Tolomelli, A. Integrin-Mediated Adhesive Properties of Neutrophils Are Reduced by Hyperbaric Oxygen Therapy in Patients with Chronic Non-Healing Wound. *PLoS ONE* **2020**, *15*, e0237746. [[CrossRef](#)] [[PubMed](#)]
248. Xu, X.; Yi, H.; Kato, M.; Suzuki, H.; Kobayashi, S.; Takahashi, H.; Nakashima, I. Differential Sensitivities to Hyperbaric Oxygen of Lymphocyte Subpopulations of Normal and Autoimmune Mice. *Immunol. Lett.* **1997**, *59*, 79–84. [[CrossRef](#)] [[PubMed](#)]
249. Saito, K.; Tanaka, Y.; Ota, T.; Eto, S.; Yamashita, U. Suppressive Effect of Hyperbaric Oxygenation on Immune Responses of Normal and Autoimmune Mice. *Clin. Exp. Immunol.* **2008**, *86*, 322–327. [[CrossRef](#)]
250. Schottlender, N.; Gottfried, I.; Ashery, U. Hyperbaric Oxygen Treatment: Effects on Mitochondrial Function and Oxidative Stress. *Biomolecules* **2021**, *11*, 1827. [[CrossRef](#)]
251. Růžička, J.; Dejmek, J.; Bolek, L.; Beneš, J.; Kuncová, J. Hyperbaric Oxygen Influences Chronic Wound Healing—A Cellular Level Review. *Physiol. Res.* **2021**, *70*, S261–S273. [[CrossRef](#)] [[PubMed](#)]
252. Baethge, C.; Goldbeck-Wood, S.; Mertens, S. SANRA—A Scale for the Quality Assessment of Narrative Review Articles. *Res. Integr. Peer Rev.* **2019**, *4*, 5. [[CrossRef](#)]
253. Lerche, C.J.; Schwartz, F.; Pries-Heje, M.M.; Fosbøl, E.L.; Iversen, K.; Jensen, P.Ø.; Høiby, N.; Hyldegaard, O.; Bundgaard, H.; Moser, C. Potential Advances of Adjunctive Hyperbaric Oxygen Therapy in Infective Endocarditis. *Front. Cell Infect. Microbiol.* **2022**, *12*, 805964. [[CrossRef](#)] [[PubMed](#)]
254. Löndahl, M.; Boulton, A.J.M. Hyperbaric Oxygen Therapy in Diabetic Foot Ulceration: Useless or Useful? A Battle. *Diabetes Metab. Res. Rev.* **2020**, *36*, e3233. [[CrossRef](#)]
255. Brouwer, R.J.; Laliou, R.C.; Hoencamp, R.; van Hulst, R.A.; Ubbink, D.T. A Systematic Review and Meta-Analysis of Hyperbaric Oxygen Therapy for Diabetic Foot Ulcers with Arterial Insufficiency. *J. Vasc. Surg.* **2020**, *71*, 682–692.e1. [[CrossRef](#)] [[PubMed](#)]
256. Laliou, R.C.; Brouwer, R.J.; Ubbink, D.T.; Hoencamp, R.; Bol Raap, R.; van Hulst, R.A. Hyperbaric Oxygen Therapy for Nonischemic Diabetic Ulcers: A Systematic Review. *Wound Repair. Regen.* **2020**, *28*, 266–275. [[CrossRef](#)] [[PubMed](#)]

257. Lerche, C.J.; Christophersen, L.J.; Kolpen, M.; Nielsen, P.R.; Trøstrup, H.; Thomsen, K.; Hyldegaard, O.; Bundgaard, H.; Jensen, P.Ø.; Høiby, N.; et al. Hyperbaric Oxygen Therapy Augments Tobramycin Efficacy in Experimental *Staphylococcus Aureus* Endocarditis. *Int. J. Antimicrob. Agents* **2017**, *50*, 406–412. [[CrossRef](#)]
258. Chen, C.-E.; Ko, J.-Y.; Fong, C.-Y.; Juhn, R.-J. Treatment of Diabetic Foot Infection with Hyperbaric Oxygen Therapy. *Foot Ankle Surg.* **2010**, *16*, 91–95. [[CrossRef](#)]
259. Lin, L.J.; Chen, T.X.; Wald, K.J.; Tooley, A.A.; Lisman, R.D.; Chiu, E.S. Hyperbaric Oxygen Therapy in Ophthalmic Practice: An Expert Opinion. *Expert. Rev. Ophthalmol.* **2020**, *15*, 119–126. [[CrossRef](#)]
260. Oguz, H.; Sobaci, G. The Use of Hyperbaric Oxygen Therapy in Ophthalmology. *Surv. Ophthalmol.* **2008**, *53*, 112–120. [[CrossRef](#)] [[PubMed](#)]
261. Ahmadi, F.; Khalatbary, A. A Review on the Neuroprotective Effects of Hyperbaric Oxygen Therapy. *Med. Gas. Res.* **2021**, *11*, 72. [[CrossRef](#)] [[PubMed](#)]
262. Moen, I.; Stuhr, L.E.B. Hyperbaric Oxygen Therapy and Cancer—A Review. *Target. Oncol.* **2012**, *7*, 233–242. [[CrossRef](#)] [[PubMed](#)]
263. Borab, Z.; Mirmanesh, M.D.; Gantz, M.; Cusano, A.; Pu, L.L.Q. Systematic Review of Hyperbaric Oxygen Therapy for the Treatment of Radiation-Induced Skin Necrosis. *J. Plast. Reconstr. Aesthetic Surg.* **2017**, *70*, 529–538. [[CrossRef](#)] [[PubMed](#)]
264. Bai, X.; Song, Z.; Zhou, Y.; Pan, S.; Wang, F.; Guo, Z.; Jiang, M.; Wang, G.; Kong, R.; Sun, B. The Apoptosis of Peripheral Blood Lymphocytes Promoted by Hyperbaric Oxygen Treatment Contributes to Attenuate the Severity of Early Stage Acute Pancreatitis in Rats. *Apoptosis* **2014**, *19*, 58–75. [[CrossRef](#)] [[PubMed](#)]
265. Godman, C.A.; Chheda, K.P.; Hightower, L.E.; Perdrizet, G.; Shin, D.-G.; Giardina, C. Hyperbaric Oxygen Induces a Cytoprotective and Angiogenic Response in Human Microvascular Endothelial Cells. *Cell Stress. Chaperones* **2010**, *15*, 431–442. [[CrossRef](#)] [[PubMed](#)]
266. Fielden, M.P.; Martinovic, E.; Ells, A.L. Hyperbaric Oxygen Therapy in the Treatment of Orbital Gas Gangrene. *J. Am. Assoc. Pediatr. Ophthalmol. Strabismus* **2002**, *6*, 252–254. [[CrossRef](#)] [[PubMed](#)]
267. Bumah, V.V.; Whelan, H.T.; Masson-Meyers, D.S.; Quirk, B.; Buchmann, E.; Enwemeka, C.S. The Bactericidal Effect of 470-Nm Light and Hyperbaric Oxygen on Methicillin-Resistant *Staphylococcus Aureus* (MRSA). *Lasers Med. Sci.* **2015**, *30*, 1153–1159. [[CrossRef](#)] [[PubMed](#)]
268. Mogami, H.; Hayakawa, T.; Kanai, N.; Kuroda, R.; Yamada, R.; Ikeda, T.; Katsurada, K.; Sugimoto, T. Clinical Application of Hyperbaric Oxygenation in the Treatment of Acute Cerebral Damage. *J. Neurosurg.* **1969**, *31*, 636–643. [[CrossRef](#)]
269. Edwards, M.L. Hyperbaric Oxygen Therapy. Part 2: Application in Disease. *J. Vet. Emerg. Crit. Care* **2010**, *20*, 289–297. [[CrossRef](#)]
270. Wang, R.-Y.; Yang, Y.-R.; Chang, H.-C. The SDF1-CXCR4 Axis Is Involved in the Hyperbaric Oxygen Therapy-Mediated Neuronal Cells Migration in Transient Brain Ischemic Rats. *Int. J. Mol. Sci.* **2022**, *23*, 1780. [[CrossRef](#)]
271. Helms, A.K.; Whelan, H.T.; Torbey, M.T. Hyperbaric Oxygen Therapy of Cerebral Ischemia. *Cerebrovasc. Dis.* **2005**, *20*, 417–426. [[CrossRef](#)] [[PubMed](#)]
272. Matchett, G.A.; Martin, R.D.; Zhang, J.H. Hyperbaric Oxygen Therapy and Cerebral Ischemia: Neuroprotective Mechanisms. *Neurol. Res.* **2009**, *31*, 114–121. [[CrossRef](#)] [[PubMed](#)]
273. Cevolani, D.; Di Donato, F.; Santarella, L.; Bertossi, S.; Cellnerini, M. Functional MRI (fMRI) Evaluation of Hyperbaric Oxygen Therapy (HBOT) Efficacy in Chronic Cerebral Stroke: A Small Retrospective Consecutive Case Series. *Int. J. Environ. Res. Public Health* **2020**, *18*, 190. [[CrossRef](#)] [[PubMed](#)]
274. Thiankhaw, K.; Chattipakorn, N.; Chattipakorn, S.C. The Effects of Hyperbaric Oxygen Therapy on the Brain with Middle Cerebral Artery Occlusion. *J. Cell Physiol.* **2021**, *236*, 1677–1694. [[CrossRef](#)]
275. Chiang, I.-H.; Chen, S.-G.; Huang, K.-L.; Chou, Y.-C.; Dai, N.-T.; Peng, C.-K. Adjunctive Hyperbaric Oxygen Therapy in Severe Burns: Experience in Taiwan Formosa Water Park Dust Explosion Disaster. *Burns* **2017**, *43*, 852–857. [[CrossRef](#)] [[PubMed](#)]
276. Misiuga, M.; Glik, J.; Kawecki, M.; Dziurzyńska, I.; Ples, M.; Łabuś, W.; Nowak, M. The Effect of Hyperbaric Oxygen Therapy on Burn Wounds Covered with Skin Allografts. *J. Orthop. Trauma Surg. Relat. Res.* **2016**, *1*, 17–27.
277. Bartek, J.; Jakola, A.S.; Skyrman, S.; Förander, P.; Alpkvist, P.; Schechtmann, G.; Glimåker, M.; Larsson, A.; Lind, F.; Mathiesen, T. Hyperbaric Oxygen Therapy in Spontaneous Brain Abscess Patients: A Population-Based Comparative Cohort Study. *Acta Neurochir.* **2016**, *158*, 1259–1267. [[CrossRef](#)] [[PubMed](#)]
278. Inanmaz, M.E.; Kose, K.C.; Isik, C.; Atmaca, H.; Basar, H. Can Hyperbaric Oxygen Be Used to Prevent Deep Infections in Neuro-Muscular Scoliosis Surgery? *BMC Surg.* **2014**, *14*, 85. [[CrossRef](#)] [[PubMed](#)]
279. Barili, F.; Polvani, G.; Topkara, V.K.; Dainese, L.; Cheema, F.H.; Roberto, M.; Naliato, M.; Parolari, A.; Alamanni, F.; Biglioli, P. Role of Hyperbaric Oxygen Therapy in the Treatment of Postoperative Organ/Space Sternal Surgical Site Infections. *World J. Surg.* **2007**, *31*, 1702–1706. [[CrossRef](#)]
280. Larsson, A.; Uusijärvi, J.; Lind, F.; Gustavsson, B.; Saraste, H. Hyperbaric Oxygen in the Treatment of Postoperative Infections in Paediatric Patients with Neuromuscular Spine Deformity. *Eur. Spine J.* **2011**, *20*, 2217–2222. [[CrossRef](#)]
281. George, M.E.; Rueth, N.M.; Skarda, D.E.; Chipman, J.G.; Quickel, R.R.; Beilman, G.J. Hyperbaric Oxygen Does Not Improve Outcome in Patients with Necrotizing Soft Tissue Infection. *Surg. Infect.* **2009**, *10*, 21–28. [[CrossRef](#)] [[PubMed](#)]
282. Wilkinson, D. Hyperbaric Oxygen Treatment and Survival from Necrotizing Soft Tissue Infection. *Arch. Surg.* **2004**, *139*, 1339. [[CrossRef](#)]
283. Massey, P.R.; Sakran, J.V.; Mills, A.M.; Sarani, B.; Aufhauser, D.D.; Sims, C.A.; Pascual, J.L.; Kelz, R.R.; Holena, D.N. Hyperbaric Oxygen Therapy in Necrotizing Soft Tissue Infections. *J. Surg. Res.* **2012**, *177*, 146–151. [[CrossRef](#)] [[PubMed](#)]

284. Shupak, A.; Oren, S.; Goldenberg, I.; Barzilai, A.; Moskuna, R.; Bursztein, S. Necrotizing Fasciitis: An Indication for Hyperbaric Oxygenation Therapy? *Surgery* **1995**, *118*, 873–878. [[CrossRef](#)] [[PubMed](#)]
285. Duzgun, A.P.; Satir, H.Z.; Ozozan, O.; Saylam, B.; Kulah, B.; Coskun, F. Effect of Hyperbaric Oxygen Therapy on Healing of Diabetic Foot Ulcers. *J. Foot Ankle Surg.* **2008**, *47*, 515–519. [[CrossRef](#)]
286. Löndahl, M.; Katzman, P.; Nilsson, A.; Hammarlund, C. Hyperbaric Oxygen Therapy Facilitates Healing of Chronic Foot Ulcers in Patients with Diabetes. *Diabetes Care* **2010**, *33*, 998–1003. [[CrossRef](#)]
287. Faglia, E.; Favales, F.; Aldeghi, A.; Calia, P.; Quarantiello, A.; Oriani, G.; Michael, M.; Campagnoli, P.; Morabito, A. Adjunctive Systemic Hyperbaric Oxygen Therapy in Treatment of Severe Prevalently Ischemic Diabetic Foot Ulcer: A Randomized Study. *Diabetes Care* **1996**, *19*, 1338–1343. [[CrossRef](#)]
288. Kessler, L.; Bilbault, P.; Ortéga, F.; Grasso, C.; Passemard, R.; Stephan, D.; Pinget, M.; Schneider, F. Hyperbaric Oxygenation Accelerates the Healing Rate of Nonischemic Chronic Diabetic Foot Ulcers. *Diabetes Care* **2003**, *26*, 2378–2382. [[CrossRef](#)]
289. Ma, L.; Li, P.; Shi, Z.; Hou, T.; Chen, X.; Du, J. A Prospective, Randomized, Controlled Study of Hyperbaric Oxygen Therapy: Effects on Healing and Oxidative Stress of Ulcer Tissue in Patients with a Diabetic Foot Ulcer. *Ostomy Wound Manag.* **2013**, *59*, 18–24.
290. Kalani, M.; Jörneskog, G.; Naderi, N.; Lind, F.; Brismar, K. Hyperbaric Oxygen (HBO) Therapy in Treatment of Diabetic Foot Ulcers. *J. Diabetes Complicat.* **2002**, *16*, 153–158. [[CrossRef](#)]
291. Abidia, A.; Laden, G.; Kuhan, G.; Johnson, B.F.; Wilkinson, A.R.; Renwick, P.M.; Masson, E.A.; McCollum, P.T. The Role of Hyperbaric Oxygen Therapy in Ischaemic Diabetic Lower Extremity Ulcers: A Double-Blind Randomised-Controlled Trial. *Eur. J. Vasc. Endovasc. Surg.* **2003**, *25*, 513–518. [[CrossRef](#)]
292. Ahmed, R.; Severson, M.A.; Traynelis, V.C. Role of Hyperbaric Oxygen Therapy in the Treatment of Bacterial Spinal Osteomyelitis. *J. Neurosurg. Spine* **2009**, *10*, 16–20. [[CrossRef](#)] [[PubMed](#)]
293. Chen, C.-E.; Shih, S.-T.; Fu, T.-H.; Wang, J.-W.; Wang, C.-J. Hyperbaric Oxygen Therapy in the Treatment of Chronic Refractory Osteomyelitis: A Preliminary Report. *Chang. Gung Med. J.* **2003**, *26*, 114–121. [[PubMed](#)]
294. Delasotta, L.A.; Hanflik, A.; Bicking, G.; Mannella, W.J. Hyperbaric Oxygen for Osteomyelitis in a Compromised Host. *Open Orthop. J.* **2013**, *7*, 114–117. [[CrossRef](#)] [[PubMed](#)]
295. Yu, W.-K.; Chen, Y.-W.; Shie, H.-G.; Lien, T.-C.; Kao, H.-K.; Wang, J.-H. Hyperbaric Oxygen Therapy as an Adjunctive Treatment for Sternal Infection and Osteomyelitis after Sternotomy and Cardiothoracic Surgery. *J. Cardiothorac. Surg.* **2011**, *6*, 141. [[CrossRef](#)] [[PubMed](#)]
296. Petzold, T.; Feindt, P.R.; Carl, U.M.; Gams, E. Hyperbaric Oxygen Therapy in Deep Sternal Wound Infection After Heart Transplantation. *Chest* **1999**, *115*, 1455–1458. [[CrossRef](#)] [[PubMed](#)]
297. Siondalski, P.; Keita, L.; Sićko, Z.; Zelechowski, P.; Jaworski, Ł.; Rogowski, J. [Surgical Treatment and Adjunct Hyperbaric Therapy to Improve Healing of Wound Infection Complications after Sterno-Mediastinitis]. *Pneumonol. Alergol. Pol.* **2003**, *71*, 12–16. [[PubMed](#)]
298. Sun, I.; Lee, S.; Chiu, C.; Lin, S.; Lai, C. Hyperbaric Oxygen Therapy with Topical Negative Pressure: An Alternative Treatment for the Refractory Sternal Wound Infection. *J. Card. Surg.* **2008**, *23*, 677–680. [[CrossRef](#)] [[PubMed](#)]
299. Dowdell, J.; Brochin, R.; Kim, J.; Overley, S.; Oren, J.; Freedman, B.; Cho, S. Postoperative Spine Infection: Diagnosis and Management. *Glob. Spine J.* **2018**, *8* (Suppl. 4), 375–435. [[CrossRef](#)]
300. do Egito, J.G.T.; Abboud, C.S.; de Oliveira, A.P.V.; Máximo, C.A.G.; Montenegro, C.M.; Amato, V.L.; Bammann, R.; Farsky, P.S. Evolução Clínica de Pacientes Com Mediastinite Pós-Cirurgia de Revascularização Miocárdica Submetidos à Oxigenoterapia Hiperbárica Como Terapia Adjuvante. *Einstein* **2013**, *11*, 345–349. [[CrossRef](#)]
301. Litwinowicz, R.; Bryndza, M.; Chrapusta, A.; Kobielska, E.; Kapelak, B.; Grudzień, G. Hyperbaric Oxygen Therapy as Additional Treatment in Deep Sternal Wound Infections—A Single Center’s Experience. *Pol. J. Cardio Thorac. Surg.* **2016**, *3*, 198–202. [[CrossRef](#)] [[PubMed](#)]
302. Bartek, J., Jr.; Skyrman, S.; Nekludov, M.; Mathiesen, T.; Lind, F.; Schechtmann, G. Hyperbaric Oxygen Therapy as Adjuvant Treatment for Hardware-Related Infections in Neuromodulation. *Stereotact. Funct. Neurosurg.* **2018**, *96*, 100–107. [[CrossRef](#)] [[PubMed](#)]
303. Copeland, H.; Newcombe, J.; Yamin, F.; Bhajri, K.; Mille, V.A.; Hasaniya, N.; Bailey, L.; Razzouk, A.J. Role of Negative Pressure Wound Care and Hyperbaric Oxygen Therapy for Sternal Wound Infections After Pediatric Cardiac Surgery. *World J. Pediatr. Congenit. Heart Surg.* **2018**, *9*, 440–445. [[CrossRef](#)] [[PubMed](#)]
304. Stizzo, M.; Manfredi, C.; Spirito, L.; Sciorio, C.; Romero Otero, J.; Martinez Salamanca, J.I.; Crocetto, F.; Verze, P.; Imbimbo, C.; Fusco, F.; et al. Hyperbaric Oxygen Therapy as Adjuvant Treatment for Surgical Site Infections after Male-to-female Gender Affirmation Surgery: A 10-year Experience. *Andrology* **2022**, *10*, 1310–1316. [[CrossRef](#)] [[PubMed](#)]
305. Pan, B.; Li, H.; Lang, D.; Xing, B. Environmentally Persistent Free Radicals: Occurrence, Formation Mechanisms and Implications. *Environ. Pollut.* **2019**, *248*, 320–331. [[CrossRef](#)] [[PubMed](#)]
306. Vinayak, A.; Mudgal, G.; Singh, G.B. *Environment Persistent Free Radicals: Long-Lived Particles*; Springer Nature: Cham, Switzerland, 2021; pp. 1–19.
307. Saravia, J.; Lee, G.I.; Lomnicki, S.; Dellinger, B.; Cormier, S.A. Particulate Matter Containing Environmentally Persistent Free Radicals and Adverse Infant Respiratory Health Effects: A Review. *J. Biochem. Mol. Toxicol.* **2013**, *27*, 56–68. [[CrossRef](#)] [[PubMed](#)]

308. Gao, P.; Yao, D.; Qian, Y.; Zhong, S.; Zhang, L.; Xue, G.; Jia, H. Factors Controlling the Formation of Persistent Free Radicals in Hydrochar during Hydrothermal Conversion of Rice Straw. *Environ. Chem. Lett.* **2018**, *16*, 1463–1468. [[CrossRef](#)]
309. Xu, Y.; Yang, L.; Wang, X.; Zheng, M.; Li, C.; Zhang, A.; Fu, J.; Yang, Y.; Qin, L.; Liu, X.; et al. Risk Evaluation of Environmentally Persistent Free Radicals in Airborne Particulate Matter and Influence of Atmospheric Factors. *Ecotoxicol. Environ. Saf.* **2020**, *196*, 110571. [[CrossRef](#)] [[PubMed](#)]
310. Zhang, K.; Sun, P.; Faye, M.C.A.S.; Zhang, Y. Characterization of Biochar Derived from Rice Husks and Its Potential in Chlorobenzene Degradation. *Carbon* **2018**, *130*, 730–740. [[CrossRef](#)]
311. Kumbhar, D.; Palliyarayil, A.; Reghu, D.; Shrunagar, D.; Umapathy, S.; Sil, S. Rapid Discrimination of Porous Bio-Carbon Derived from Nitrogen Rich Biomass Using Raman Spectroscopy and Artificial Intelligence Methods. *Carbon* **2021**, *178*, 792–802. [[CrossRef](#)]
312. Wu, C.; Fu, L.; Li, H.; Liu, X.; Wan, C. Using Biochar to Strengthen the Removal of Antibiotic Resistance Genes: Performance and Mechanism. *Sci. Total Environ.* **2022**, *816*, 151554. [[CrossRef](#)] [[PubMed](#)]
313. Huang, C.; Qin, F.; Zhang, C.; Huang, D.; Tang, L.; Yan, M.; Wang, W.; Song, B.; Qin, D.; Zhou, Y.; et al. Effects of Heterogeneous Metals on the Generation of Persistent Free Radicals as Critical Redox Sites in Iron-Containing Biochar for Persulfate Activation. *ACS EST Water* **2023**, *3*, 298–310. [[CrossRef](#)]
314. Zhou, B.; Liu, Q.; Shi, L.; Liu, Z. Electron Spin Resonance Studies of Coals and Coal Conversion Processes: A Review. *Fuel Process. Technol.* **2019**, *188*, 212–227. [[CrossRef](#)]
315. Ruan, X.; Sun, Y.; Du, W.; Tang, Y.; Liu, Q.; Zhang, Z.; Doherty, W.; Frost, R.L.; Qian, G.; Tsang, D.C.W. Formation, Characteristics, and Applications of Environmentally Persistent Free Radicals in Biochars: A Review. *Bioresour. Technol.* **2019**, *281*, 457–468. [[CrossRef](#)] [[PubMed](#)]
316. Jothirani, R.; Kumar, P.S.; Saravanan, A.; Narayan, A.S.; Dutta, A. Ultrasonic Modified Corn Pith for the Sequestration of Dye from Aqueous Solution. *J. Ind. Eng. Chem.* **2016**, *39*, 162–175. [[CrossRef](#)]
317. Suganya, S.; Kumar, P.S.; Saravanan, A.; Rajan, P.S.; Ravikumar, C. Computation of Adsorption Parameters for the Removal of Dye from Wastewater by Microwave Assisted Sawdust: Theoretical and Experimental Analysis. *Environ. Toxicol. Pharmacol.* **2017**, *50*, 45–57. [[CrossRef](#)]
318. Saravanan, A.; Kumar, P.S.; Renita, A.A. Hybrid Synthesis of Novel Material through Acid Modification Followed Ultrasonication to Improve Adsorption Capacity for Zinc Removal. *J. Clean. Prod.* **2018**, *172*, 92–105. [[CrossRef](#)]
319. Luo, Z.; Yao, B.; Yang, X.; Wang, L.; Xu, Z.; Yan, X.; Tian, L.; Zhou, H.; Zhou, Y. Novel Insights into the Adsorption of Organic Contaminants by Biochar: A Review. *Chemosphere* **2022**, *287*, 132113. [[CrossRef](#)] [[PubMed](#)]
320. Zhao, F.; Tang, L.; Jiang, H.; Mao, Y.; Song, W.; Chen, H. Prediction of Heavy Metals Adsorption by Hydrochars and Identification of Critical Factors Using Machine Learning Algorithms. *Bioresour. Technol.* **2023**, *383*, 129223. [[CrossRef](#)]
321. Chauhan, S.; Shafi, T.; Dubey, B.K.; Chowdhury, S. Biochar-Mediated Removal of Pharmaceutical Compounds from Aqueous Matrices via Adsorption. *Waste Dispos. Sustain. Energy* **2023**, *5*, 37–62. [[CrossRef](#)]

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