Chapter

Antimicrobial Photodynamic Therapy of the Respiratory Tract: From the Proof of Principles to Clinical Application

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Abstract

Antimicrobial resistance (AMR) and its relevant health consequences have been explicitly framed as a shared global problem and are estimated to be one of the largest causes of death worldwide by 2050. Antimicrobial photodynamic therapy (aPDT) proposes an alternative treatment for localized infections in response to AMR's ever-growing problem. This technique combines molecular oxygen, a non-toxic photoactivatable photosensitizer (PS), and light of appropriate wavelength, leading to the formation of cytotoxic reactive oxygen species. Besides the ability to inactivate resistant pathogens via a non-selective approach (multiple targets), a relevant advantage of aPDT resides in the fact that no evidence of microorganism resistance has ever been reported to it. In this chapter, we address some efforts to use this technology to kill bacteria in the respiratory tract, from *in vitro* to clinical applications. We put forward three focuses: pharyngotonsillitis, pneumonia, and preventing secondary infections during the use of a photosensitizer-functionalized endotracheal tube. The results here presented offer a foundation for what may become a much larger clinical approach to treat respiratory tract infections.

Keywords: antimicrobial resistance, antimicrobial photodynamic therapy, photochemotherapy, infections of the respiratory tract, endotracheal tube

1. Introduction

The increasing use of antibiotics has an important impact on human health by introducing the emergence of resistant bacterial strains, both in humans treated in an indiscriminate manner, and in two other situations as worrying as, which are the presence of these molecules in drinking water and abusive use in agriculture. This has all resulted in the phenomenon of antimicrobial resistance (AMR) [1].

Each year worldwide, 700,000 deaths occur, approximately, due to diseases that had antimicrobial resistance as responsible for the deaths. By 2050, these deaths could reach the terrifying 10 million mark [1].

One of the biggest barriers to antibiotic-resistant infections is that they add significant costs to the any nation's already overburdened health system [2].

Thus, the paths have been opened for other ways to fight infections and photodynamic therapy (PDT) has stood out with the aim of inactivating not only bacteria, but also fungi, protozoa, and viruses. It is a promising technique, including the treatment of diseases that already have antimicrobial resistance.

In this chapter we will address the theme of advances in research involving microbiological control with photodynamic action, more specifically in the treatment or prevention of diseases of the respiratory tract.

1.1 Antimicrobial resistance

The bacteria have developed several mechanisms to fight against antibiotics action. An important molecular mechanism involves the horizontal transfer of genes from the efflux pumps when the organism acquires a gene that confers the ability to eliminate antibiotics from the intracellular environment [3]. A well-known example is the acquisition of the β-lactamase gene from antibiotic inactivating enzymes, which inactivates β-lactam antibiotics, such as penicillin and cephalosporins [3], where bacteria acquire the ability to inactivate antibiotics through an enzymatic mechanism.

Two interesting aspects are related to cell wall morphology and the ability of bacterial colonies to form biofilms, and interestingly, these aspects are directly related to the cell wall structure of Gram-positive bacteria. The cell-wall glycopolymers from Gram-positive bacteria present an essential role in host-cell adhesion, the first step towards forming a bacterial biofilm. In contrast to Gram-negative, Gram-positive bacteria have a thicker cell wall structure with multiple layers of peptidoglycan. In addition, many Gram-positive bacteria have protective surface structures, typically with glycopolymers bound to peptidoglycan or membrane lipids. These structures include glycopolymers of teicoic acids and branched mycobacterial polymers [4]. Infections caused by Gram-positive bacteria are important for human health and it is worrying that these bacteria are becoming increasingly resistant to existing antibiotics. The teicoic acids wall has multiple functional roles in Gram-positive bacteria including resistance to cationic antimicrobial peptides, such as the vancomycin, a glycopeptide antibiotic. Other cellular processes influenced by this wall include autolysis, cell division, the location of penicillin-binding protein, survival at higher temperatures, biofilm formation and epithelial cell adhesion [5].

Biofilms have an important impact on bacterial infections and also on bacterial resistance. Organisms structured in biofilms exhibit up to 1,000 times more resistance to antibiotics than planktonic cells.

1.2 Mechanisms of antibiotic resistance

Pathogenic bacteria resistant to antibiotics are prevalent in different populations of the environment such as from the soil and water containing encode genes with resistance mechanisms [6], which can be mobilized for new hosts, including humans [7] and, depending on genic expression, may result in significant public health problems [8]. If the microbial mutations are for its benefice, such as antibiotic resistance, they are predominant in the species and transmitted for subsequent generations, making the bacteria predominant antibiotic-resistant [9]. The mechanisms of an antimicrobial resistance may be intrinsic to the microorganisms or even acquired through the transmission of the genetic material or by mutation (which may occur during replication) during the bacterial evolution, whether induced or spontaneous, by mutation mechanisms in a chromosome or transfer genes loci,

which can encode inactivate enzymes in antibiotics or even reducing their permeability in cells [10]. The bacterial mutations that can occur are replacement (transition and transversion); deletion (macrodelection and microdeletion); insertion (macroinsertion and microinsection) and inversion, with exchange of pyrimidine or purine, removal of nucleotides, the inclusion of nucleotides, and removal or insertion of DNA, respectively.

Strains resistant to antibiotics can be transmitted between patients in healthcare units, often through healthcare professionals' contaminated hands, medical-surgical equipment, or inanimate objects from the hospital environment [11]. This type of spread is generally clonal, involving the transmission of a single resistant strain. Outbreaks caused by the clonal spread of an antibiotic-resistant organism have been commonly reported in *S. aureus* MRSA strains [12]. Patients' transmission can be clonal in multiple species of strains with different prevalence according to the geographic region [13].

1.3 The worldwide impact of antimicrobial resistance

Infectious diseases are a major cause of human deaths. According to the World Health Organization (WHO), on the top ten global causes of death (2016), chronic obstructive pulmonary disease and lower respiratory infections are occupying the third and fourth places, respectively, behind ischemic heart disease and stroke [14]. It is relevant to note that infectious diseases outperform all types of cancer in terms of mortality, according to WHO data. Figures reported in 2016 indicate that there were 3.190 million deaths due to respiratory infections, with a mortality rate of 43/100,000. Analyzing again the top ten global causes of death but now, in low-income countries (2016), lower respiratory infections were among the leading causes of death across all income groups [14].

It is essential to discover and invest in the development of new antibiotic molecules, following the growing global need. But just as importantly, research into new non-antibiotic approaches for the prevention and protection against infectious diseases is needed and should be encouraged and a high priority research and development project [15].

In the US, the Centers for Disease Control and Prevention (CDC) estimated that antibiotic-resistant infections are responsible for $20 billion a year in additional health care costs, and $35 billion a due to loss of productivity [16]. Thus, a deeper understanding of the mechanisms of resistance to antibiotics is relevant in terms of human health, that is, it saves human lives, but it also reduces an important economic burden for public and private health systems.

Penicillin, discovered by Fleming in 1928, was first tested for the treatment of infectious diseases in the 1930s and became a widespread drug in the 1940s. β-lactam antibiotics, the group to which penicillin belongs, are effectively drugs of choice for the treatment of community-based respiratory diseases, for example, which are usually caused by Gram-positive bacteria, such as *Staphylococcus* and *Streptococcus*.

The introduction of new antibiotics in clinical use was quickly followed by the clinical observation of resistant strains and the time between clinical use and resistance has become shorter and shorter. For example, sulfonamides were introduced for clinical use in 1930 and resistant strains appeared in the 1940s. Vancomycin was introduced in 1956 and resistant strains were first reported in 1988. However, for newer antibiotics, such as daptomycin, fidaxomycin and linezolid, resistance was observed in the same year in which clinical use began [17].

2. Antimicrobial photodynamic therapy (aPDT)

The mechanisms of aPDT are basically the same of PDT for tumors, based on the combined action of three elements: a photosensitizer (PS), a light source at appropriate wavelength to excite the PS and molecular oxygen (O_2) in the target tissue.

The photodynamic process inactivating microorganisms occurs through the action of reactive oxygen species (ROS) that destroy vital constituents of fungi, bacteria, viruses and protozoa. In 1933 Jablonski published his article explaining the electronic states of a molecule and the transitions between them [18]. In this famous "Jablonski's diagram", we understand how a photosensitizer in the singlet ground state, moves to the excited singlet state after absorbing photons from a light source. And through the process named "intersystem crossing", a spin inversion occurs and then, this molecule goes to the excited triplet state, giving it conditions to transfer energy (type II mechanism) or electron (type I mechanism) to O_2, generating ROS.

For antimicrobial purposes, the photodynamic action will take place within the cells or at the extracellular matrix of the microbial biofilm where the photosensitizer molecules are present, the main sites being the outer membrane or cell wall, membrane lipids and lipopolysaccharides. The singlet oxygen produced has a very small radius of action, less than 0.02 μm, so the damage produced by PDT will only be in the presence of the photosensitizer and under photoactivation. As a result, cell death is caused by cell wall or membrane lysis and/or inactivation of proteins or enzymes essential for microbial metabolism [19].

3. aPDT of respiratory tract diseases

3.1 Pharyngotonsillitis

Sore throat is a frequent complaint in outpatient medical consultations and emergencies. Acute pharyngotonsillitis represents a significant source of social disorders in the child population, such as missed classes repeated use of antimicrobials, and can cause complications such as peritonsillar or retropharyngeal abscess, otitis, sinusitis, pneumonia, rheumatic fever, and post-streptococcal glomerulonephritis [20]. Bacterial infections of the respiratory system can be located in the pharynx (pharyngotonsillitis). Viruses cause around 90% of pharyngitis, and 10% are caused by bacteria that have the vast majority associated with *Streptococcus pyogenes* or Beta-hemolytics of Lancefield group A (EBHGA) [21] however, other bacteria can cause pharyngotonsillitis such as *Streptococcus mutans and Streptococcus pyogenes, Staphylococcus aureus, Moraxella catarrhalis, Haemophilus influenzae, Prevotella* sp.*, Bacteroids fragilis, and Fusobacterium* sp.

The diagnosis of EBHGA infection should preferably be confirmed microbiologically by rapid antigens detection tests and through oropharyngeal secretion culture. The gold standard for diagnosing oropharyngeal infections by EBHGA is culture [22], which should be done before starting treatment with antibiotics [23]. Clinical samples should be seeded on blood agar plates, which allows a preliminary screening of β-hemolytic colonies. Subsequent confirmation of suspected colonies such as EBHGA can be obtained by several laboratory tests, which are easily and quickly performed and which are still widely applied in clinical microbiology, despite the increasing use of automatic identification systems. EBHGA can be an oropharyngeal colonizing agent and thus, the microbiological investigation must be guided by clinical and epidemiological factors: patient's age, clinical signs and symptoms, season, and personal exposure to EBHGA [24].

According to the World Health Organization (WHO), approximately 600 million new pharyngotonsillitis cases due to EBHGA occur annually, and of these, 500 thousand may progress with rheumatic fever and about 300 thousand with rheumatic carditis [25]. In developing countries, the occurrence is three times higher than in developed countries. The preliminary diagnosis and treatment of tonsillitis and pharyngitis is a common cause of inappropriate use of antibiotics.

Penicillin is the drug of choice for *S. pyogenes* infections' empirical treatment, despite more than 60 years of use. S. pyogenes remains sensitive to penicillin, and resistance tests for penicillins or other beta-lactams approved for its treatment are unnecessary. However, more than 10% of patients report an allergy to penicillin, which leads to the use of cephalosporins, clindamycin, or macrolides as alternative treatments [26]. As rates of resistance to macrolides among isolated *S. pyogenes* have been increasing in North America and Europe, resistance tests for these substances may be indicated. Sore throat is a symptom that leading people to seek medical attention, and although it spontaneously remits, primary care doctors usually prescribe antibiotics for it. In a systematic review, Spinks and collaborators concluded that antibiotics confer relative benefits in the treatment of sore throat. However, the absolute benefits are modest [27].

The research carried out at Santa Casa Hospital of São Carlos city (São Paulo, Brazil) by the CEPOF - Optics and Photonics Research Center" from University of São Paulo - São Carlos is composed of a clinical trial - "Turmeric and LED in the treatment of sore throat" with objectives as assessing the therapeutic efficacy of PDT with curcumin as an adjunct in the treatment of acute pharyngotonsillitis in adults in the municipality of São Carlos [28]. The photosensitizer used in this study was curcumin (0.75 mg/ml), using two minutes of illumination with a blue light (LED) at 450 nm. The clinical trial is randomized and controlled with adults aged 18 to 45 years diagnosed with acute pharyngotonsillitis. Participants are undergoing a rapid test for the detection of group A beta-hemolytic streptococcus (EBHGA). Participants with streptococcal pharyngotonsillitis are divided into Antibiotic therapy comparison groups in conjunction with photodynamic therapy; and Antibiotic Therapy Group in conjunction with a photodynamic therapy placebo, and the therapeutic response will be evaluated in terms of clinical symptoms (sore throat and fever) and microbiological response, mainly considering the presence of EBHGA in the clinical response.

3.2 Designing antimicrobial-coating for endotracheal tube to prevent ventilator-associated respiratory tract infections

Mechanical ventilation (endotracheal intubation) is an effective intervention performed for breathing support in patients admitted in the intensive care unit, but it is also identified as one of the highest risk factors for developing ventilator-associated pneumonia (VAP) [29]. VAP is a type of nosocomial infection that results in a higher mortality (increase from 20–75%) and morbidity rate, prolonged lengths of hospitalization, and also increased hospitalization costs ($10,000 to $25,000) [30–32]. Furthermore, each year, approximately 50 million patients in the intensive care unit are intubated with an endotracheal tube (ETT) for breathing support worldwide [33].

Most cases of VAP are caused by the aspiration of infected (bacteria and/or virus) secretions from the oropharynx, although a small number of cases can result from direct bloodstream infection [34]. Moreover, there is a growing concern associated with the ETT as the primary target related to VAP by biofilm formation on its surface [35]. Biofilms are characterized by its resistance to commercial antibiotics that favor resistant microorganisms' proliferation and make them inaccessible to antimicrobials [36].

Regarding VAP occur by ETT, aspiration occurs when there is distal migration of microorganisms present in the secretions accumulated above the ETT cuff. Moreover, biofilm is formed and attached in the lumen of ETT facilitating the transfer to the sterile bronchial tree [37], as presented in **Figure 1**.

Currently, there are methods used to prevent VAP based on its pathogenesis such as prevent aspiration of secretions and bacterial colonization of aerodigestive tract. Lastly, strategies include measures to minimize the risk of contaminated equipment but these methods show some practical limitations. In this regard, the development of strategies and new medical devices to avoid VAP is urgently need.

New medical devices based on the development of antimicrobial coated for ETT surface should be considered if they have been able to prevent VAP in well-designed clinical studies and be cost-effective [38]. Along the years, different strategies and antimicrobial coated for ETT surface (e.g. metal/antiseptics, metal/zeolites/d--tyrosine, nanorough/fructose, antimicrobial peptides, antibiotics/antiseptics, photo-based therapy, micropatterned surfaces, nanorough surfaces, and hydrophobic/hydrophilic) have been evaluated aiming to prevent the biofilm formation and VAP [38] (**Figure 2**).

These antimicrobial coated are functionalized on ETT surface via covalently or ionic bonding or creating a matrix on a polymer (e.g. polyvinyl chloride (PVC)) depending on the molecular structure of both antimicrobial and type of polymer-based ETT and the presence of additives on ETT constitution [39].

As a selected example, in 2020, the Optics and Photonics Research Center from University of São Paulo developed a photo-based antimicrobial coating for ETT *via* functionalization of a natural product (curcumin) photosensitizer on PVC-based ETT surface [40] (**Figure 3**).

This therapeutic approach is based on the photoactivation of curcumin-functionalized endotracheal tube using an optical fiber followed by the production of reactive oxygen species and $^1O_2$ able to destroy biofilm and preventing its formation in the lumen of ETT. In this regard, the authors observed a photoelimination of bacteria biofilm such as *E. coli* (72%), *S. aureus* (95%), and *P. aeruginosa* (73%) previously formed on the ETT surface using a light dose of 50 J/cm^2. Moreover,

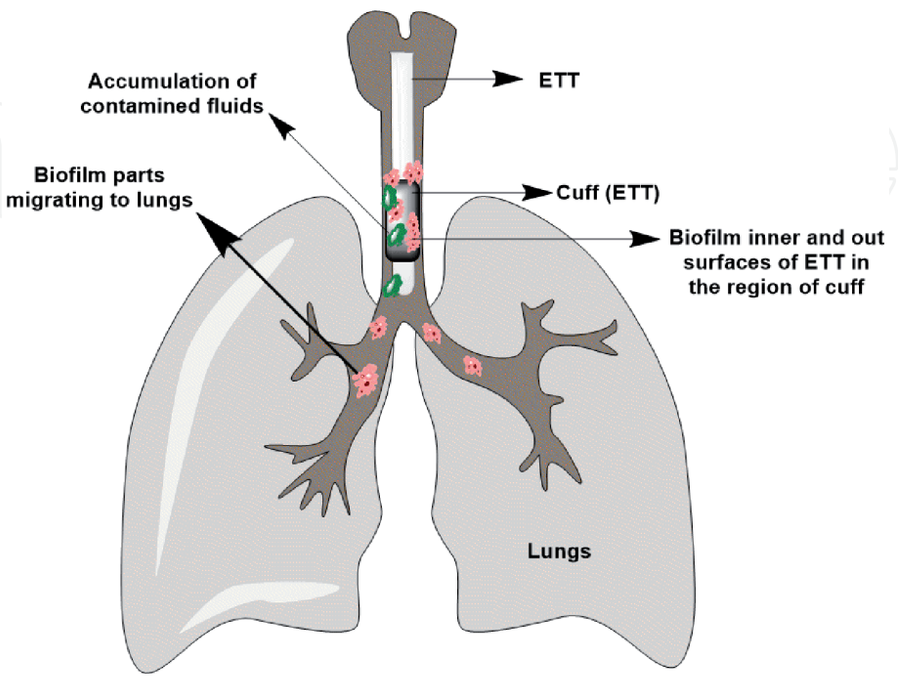

Figure 1.
Pathogenesis of ventilator-associated pneumonia (VAP). Copyright (2020) National Academy of Sciences.

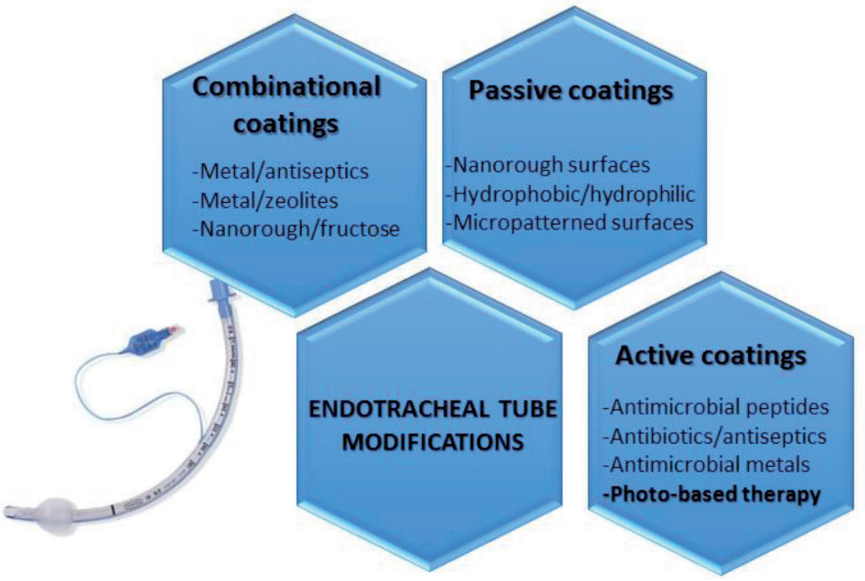

Figure 2.
Antimicrobial coatings for ETT.

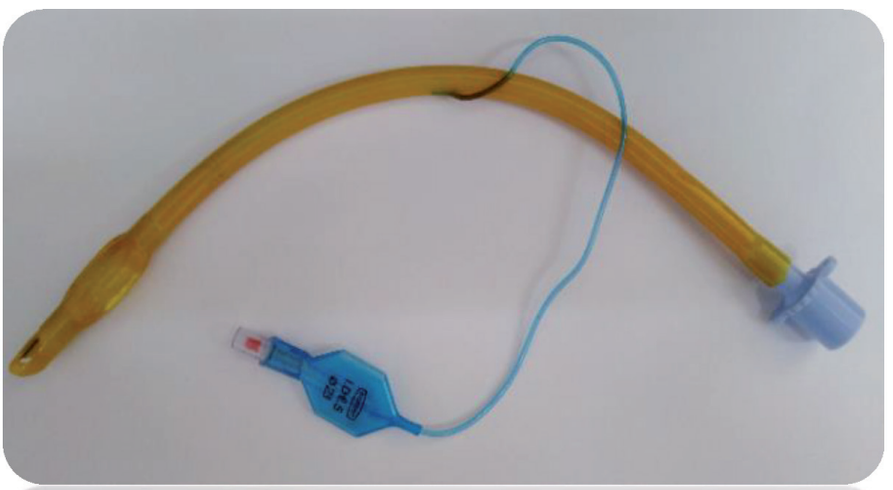

Figure 3.
Curcumin-functionalized endotracheal tube. Copyright (2020) National Academy of Sciences.

a prevention on formation of *S. aureus* bacteria biofilm in the lumen of curcumin-functionalized endotracheal tube was observed when it was under illumination (at 450 nm, 35 mW/cm^2) [39]. Furthermore, no degradation and leaching for curcumin-functionalized endotracheal tube under different pH values (2.0, 4.5, 7.0, 8.0, and 10.0) were observed. These results pave the way for developing of photosensitizers-functionalized ETT and photodynamic action to combat hospital-acquired infections like VAP [40].

Overall, the development and application of antimicrobials coatings for ETT have shown great promise and continue to progress. Significant results are being obtained with a wide family of the antimicrobial coating, including photosensitizers. From perspective, these *in vitro* methodologies developed so far could be applied in *ex vivo* and *in vivo* tests to evaluate and optimize these antimicrobial medical devices to be applied in clinical trials. In sum, this approach possesses excellent potential to reduce the number of deaths worldwide and decrease healthcare costs.

3.3 Lower respiratory tract infections and current treatment challenges

Lower respiratory infections are the fourth-largest cause of death worldwide and the main cause of death in low-income countries [14]. The most frequent lower respiratory infections are acute bronchitis and bronchiolitis, influenza, and pneumonia [41]. In Brazil, pneumonia is the number one cause of hospitalization [42]. It is also the main worldwide cause of death of children younger than 5 years old [43]. Although the number of hospitalizations has decreased over the past decades, the in-hospital mortality increased, mainly explained by the aging of the population and the occurrence of pneumonia cases that are more difficult to treat [42].

The European Respiratory Society defines pneumonia as an acute illness of the lower respiratory tract that includes cough and at least one other symptom: new focal chest signs, new lung shadowing shown by radiography, otherwise unexplained fever for more than 4 days, or otherwise unexplained tachypnea/dyspnea [41]. Community Acquired Pneumonia (CAP) is contracted from contact with the infection in day-to-day life [41]. It is predominantly bacterial in origin, being *Streptococcus pneumoniae* its most prevalent pathogen [44]. Other important agents are *Haemophilus influenza*, *Pseudomonas aeruginosa* [44, 45]. Also, about 30% of cases are coinfections with viruses [46]. However, in the vast majority of CAP cases, there is no investigation of the etiological agent [42]. In such situations, the treatment is based on the most prevalent microorganisms of that locality [42].

Hospital Acquired Pneumonia (HAP), also called nosocomial pneumonia, is the one that develops after at least 48 hours after the patients' admission [47]. Its reported mortality rate ranges from 20 to 50%, the highest among nosocomial infections [47]. As mentioned above, ventilator-associated pneumonia (VAP) is the one contracted at least 48–72 hours after endotracheal intubation [41]. The most relevant HAP and VAP agents are also bacteria, like *Staphylococcus aureus, Pseudomonas aeruginosa*, *Escherichia coli*, and *Klebsiella*, *Acinetobacter*, and *Enterobacter* species [48]. Knowledge of the etiological agents is essential in treating these infections since patients who receive the wrong initial therapy have a high risk of mortality and morbidity [47]. However, the delay in starting the treatment also leads to poor prognostic [47]. A significant concern in HAP and VAP cases is the present of methicillin-resistant *Staphylococcus aureus* (MRSA), which is associated with elevated mortality rates and treatment costs [49]. Traditionally, the first-choice drug for MRSA infections is vancomycin, which due to its low penetration in the lungs and high renal toxicity, leads to a failure rate the can reach 70% [49].

Even with new drugs like linezolid, tigecycline and ceftaroline, persists the difficulty in increasing the success rate of treatments and the worry with the development of resistance [49, 50]. Linezolid, for example, was approved for clinical use in 2000, and cases of resistance in patients were reported as early as 2002 [51]. In a study from 2014, the occurrence of non-susceptibility to this antibiotic remained relatively low, but several different resistance mechanisms had already been observed by then [51].

Another approach to hinder the burden of pneumonia is vaccination. Two types of vaccines are currently available for *S. pneumoniae*, the main agent in CAP: the pneumococcal polysaccharide vaccine (PPV) has been recommended for adults since the mid-1980's, but it lacks efficacy in neonates and infants [52]; the pneumococcal conjugate vaccines (PCVs), designed to overcome that, were first approved in 2000 [53]. However, pneumococcal vaccination faces two main challenges: first, each vaccine is only effective against the serotypes contained in it; second, the reduction of the said serotypes increases the colonization of other serotypes that are not covered by the vaccines, and of other pathogen species like *S. aureus* and *H. influenza* [52]. Thus, new vaccines need to be developed continuously, similarly to what happens to antibiotics [52].

In face of so many challenges, PDT using indocyanine green (ICG) and infrared light has been studied in the treatment of bacterial pneumonia. ICG is a water-soluble dye that emits fluorescence when exposed to infrared light [54]. Its absorption peak in human plasma is 805 nm [55]. It is desirable to have the light excitation at this range because it penetrates deeper into biological tissue, since it is less absorbed by water, melanin and hemoglobin [56].

In an *in vitro* study by Leite *et al*., the *in vitro* inactivation of *S. pneumoniae* was effective using concentrations of ICG as low as 5 μM when combined with a 780 nm laser device or 10 μM when using an 850 nm LED. In these conditions, the treatment was safe for RAW 264.7 macrophages, and seemed to enhance their ability to fight the bacteria [57]. Other studies have also investigated similar protocols for other relevant pneumonia pathogens. Topaloglu *et al.* found an effective *in vitro* killing of *S. aureus* using 84 J/cm^2 of light (809 nm) with 6 μg/mL of ICG, and of *P. aeruginosa* using 125 μg/mL ICG and 252 J/cm^2 [58]. Kassab *et al.* had similar results for *S. aureus*, and showed that the same protocol, with up to 200 J/cm^2 and 10 μM of ICG, was harmless to multiple mammalian cell lines [59].

The first *in vivo* investigation of the proposed protocol, performed by Geralde *et al.*, found a reduction in the bacterial burden and an increase in the survival rate of SKH-1 hairless mice infected with *S. pneumoniae* after a single PDI session using ICG 100 μM and 120 J/cm^2 of light at 780 nm, with a waiting interval of 3 minutes [60]. In this study, the light exposure did not seem to be harmful to the animals. Additionally, the ICG alone was no different form the control, suggesting that the activation with light was essential to the observed effects. It was then demonstrated that nebulization would be a viable delivery method for ICG to reach the lungs. ICG is compatible with air-jet nebulization in multiple concentrations, and it reaches and distributes in the lungs similarly as intranasal instillation [59, 61]. Additionally, mice exposed to pulmonary PDT using ICG and 216 J/cm^2 of light at 808 nm showed no clinical signs of toxicity or histological damage to the lungs, liver or stomach 7 days after the treatment [59]. Replicating such results in larger models and patients might be challenging due to the layers of biological tissue the light needs to go through to reach the target. Nonetheless, aPDT using ICG and infrared light shows good efficacy and safety in pre-clinical studies, and has great potential to become a treatment for lower respiratory infections.

4. Nanotechnology and future perspectives for aPDT

Antimicrobial Photodynamic Therapy is one of the main option that have been investigated against resistant bacteria. However, even with the use of some photosensitizers in the clinic, especially for tumor treatment and already approved by the FDA, some restrictions of these molecules, such as low solubility, little tissue penetration, low specificity and little accumulation in the target cells are some of the characteristics that hinders the greater use of this technique as the gold standard in various diseases [62] Nanotechnologies is one possibility to increase the efficacy of molecules with poor pharmacokinetics and pharmacodynamics properties, including PS [63].

Drug delivery is, therefore, one of the most challenges for aPDT [64]. For this reason, nanotechnology has been used in PDT as a possibility to increase its effect. Nano-systems can be stable (even under light), present good optical properties and high penetration in the tissue, as the skin (for topical application), have more specificity (with surface functionalization) and be more efficient in ROS production [62]. Nanomaterials can be used as PS itself or to load the PS (as carrier), opening several possibilities to conjugate nanotechnology with aPDT.

The nanoparticles used as drug carrier present some advantages in relation to traditional molecules, such as the transport in the blood circulation of hydrophobic substances, the incorporation of some antigen given them desired properties, the facility to enter in the target and yet, it is possible to control drug delivery [64]. Thus, several types of nanoparticles, with different sizes, shapes and functions, have been synthetized in the last years, including for aPDT [65]. They are classified according to their material: inorganic (as metal nanoparticles), organic (as liposomes) and nanocomposites, organic or inorganic [66].

Some nanomaterials have been explored under irradiation, showing photodynamic effect and have been applied in different tests. Gold and Silver nanoparticles, nanomaterials based in silica and silicon, quantum-dots, carbon-based materials and nanoparticles from organic molecules are examples of the materials already used in photodynamic therapy and its multimodal conjugation treatments in several application [62].

The nanosystems also enable the delivery of PS with desirable optical characteristics, such as the use of absorption by two photons or upconversion nanoparticles and can result in high penetration into the tissue. Thus, they can be activated from X-ray to infrared, reaching regions of the body that previously were not possible with traditional PDT. This prospect of applying nano PDT can make this technique extremely useful in the context of respiratory diseases, especially due to the current concern about infections caused by resistant bacteria, the pandemic of the coronavirus, or the next outbreaks that are yet to come [64].

However, it is still necessary to overcome the barrier between *in vitro* and *in vivo* studies to reach nanotechnology's clinical applications. Viral, fungal and bacterial infections characterize a global public health problem and, with the coronavirus pandemic, humanity saw the urgency to invest in new therapeutic possibilities, especially because new pandemics have been predicted. The advent of nanotechnology has helped to provide quick answers to urgent problems [63]. The scientific and clinical community's joint efforts and their integration into industry are needed to respond quickly to respiratory diseases [67].

APDT is increasingly becoming a viable option for upper and lower airway infections and nanosystems can help to break traditional PDT barriers. The search for highly efficient PS has been one of the main research lines when it comes to improving PDT. Many molecules synthesis methods have been explored, as well as the synthesis of nanoparticles, but they are usually complicated and, especially with nanoparticles, are difficult to apply for large-scale production. Thus, simpler synthesis methods with functionalization of these nanometric systems have been gaining relevance in the scientific community, since it is one of the challenges for the clinical implementation of nano-PDT [68]. It is also necessary to understand the parameters beyond the laboratory, such as dose, irradiation and clinical efficacy [69].

5. Conclusions

Increased resistance to antibiotics has a direct impact on humanity and is one of the most important public health problems worldwide. Especially in the respiratory tract (lower and upper), which involves pharyngotonsillitis, pneumonia and infections by endotracheal tube, new therapeutic possibilities are needed. APDT has been shown to be highly effective against the microorganisms that cause these diseases and several protocols with different photosensitizers and illumination devices have been developed to make aPDT a great therapeutic option. New molecules and nanotechnology have been developed to improve aPDT and break down barriers to clinical applications.

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Conflict of interest

The authors declare no conflict of interest.

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