U.PORTO

FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

MESTRADO INTEGRADO EM MEDICINA

2018/2019

João Marcelo de Almeida Cabral

Light therapy in hospital infection control:

advantages, drawbacks and pitfalls.

Fototerapia no controlo da infeção hospitalar:

vantagens, desvantagens e perigos.

março, 2019

FMUP

João Marcelo de Almeida Cabral

Light therapy in hospital infection control: advantages, drawbacks and pitfalls.

Mestrado Integrado em Medicina

Área: Microbiologia

Tipologia:Monografia

Trabalho efetuado sob a Orientação de:

Professor Doutor Acácio Rodrigues

Trabalho organizado de acordo com as normas da revista:

Antibiotics

março, 2019

U.PORTO
FMUP FACULDADE DE MEDICINA UNIVERSIDADE DO PORTO

Projeto de Opção do 6º ano - DECLARAÇÃO DE INTEGRIDADE

Eu, João Marcelo de Almeida Cabral, abaixo assinado, nº mecanográfico 14744649, estudante do 6º ano do Ciclo de Estudos Integrado em Medicina, na Faculdade de Medicina da Universidade do Porto, declaro ter atuado com absoluta integridade na elaboração deste projeto de opção.

Neste sentido, confirmo que **NÃO** incorri em plágio (ato pelo qual um indivíduo, mesmo por omissão, assume a autoria de um determinado trabalho intelectual, ou partes dele). Mais declaro que todas as frases que retirei de trabalhos anteriores pertencentes a outros autores, foram referenciadas, ou redigidas com novas palavras, tendo colocado, neste caso, a citação da fonte bibliográfica.

Faculdade de Medicina da Universidade do Porto, 22/3/2019

Assinatura conforme cartão de identificação:

João Cabral

_____

Projecto de Opção do 6º ano – DECLARAÇÃO DE REPRODUÇÃO

NOME

João Marcelo de Almeida Cabral

NÚMERO DE ESTUDANTE	E-MAIL
201304384	jmacabralmail.com

DESIGNAÇÃO DA ÁREA DO PROJECTO

Microbiologia

TÍTULO MONOGRAFIA

Light therapy in hospital infection control: advantages, drawbacks and pitfalls.

ORIENTADOR

Professor doutor Acácio Rodrigues

COORIENTADOR (se aplicável)

ASSINALE APENAS UMA DAS OPÇÕES:

É AUTORIZADA A REPRODUÇÃO INTEGRAL DESTE TRABALHO APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☐
É AUTORIZADA A REPRODUÇÃO PARCIAL DESTE TRABALHO (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) APENAS PARA EFEITOS DE INVESTIGAÇÃO, MEDIANTE DECLARAÇÃO ESCRITA DO INTERESSADO, QUE A TAL SE COMPROMETE.	☒
DE ACORDO COM A LEGISLAÇÃO EM VIGOR, (INDICAR, CASO TAL SEJA NECESSÁRIO, Nº MÁXIMO DE PÁGINAS, ILUSTRAÇÕES, GRÁFICOS, ETC.) NÃO É PERMITIDA A REPRODUÇÃO DE QUALQUER PARTE DESTE TRABALHO.	☐

Faculdade de Medicina da Universidade do Porto, 22/3/2019

Assinatura conforme cartão de identificação: João Cabral

U.PORTO

FMUP FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

Gostava de dedicar este trabalho, em primeiro lugar, aos meu pais e às minhas irmãs, pelo apoio incansável e imensurável com que nunca me faltaram.

Em especial, agradeço à minha mãe toda a paciência e perseverança que me permitiram atingir mais um objetivo.

À minha namorada Filipa, por todo o amor e pelo porto seguro que foi durante estes 6 anos.

Aos meus amigos, por fazerem estes 6 ano difíceis parecerem fáceis.

À Faculdade de Medicina da Universidade do Porto, por ter sido a minha segunda casa.

Ao Professor Doutor Acácio, por toda a ajuda ao longo deste trabalho.

Title

Light therapy in hospital infection control: advantages, drawbacks and pitfalls.

Index

Abbreviatures

Abstract/Resumo

1. Introduction

2. Hospital-acquired infections (HAIs)

    2.1 Reservoirs and transmission

    2.2 Challenges and costs

3. Prevention and control of hospital-acquired infections

    3.1 Surveillance methods

    3.2 Infection Control programs

    3.3 Limitations of current methods and new perspectives

4. Photodynamic therapy as an alternative approach for the control of colonization/infection in hospital settings and facilities

    4.1 Antimicrobial photodynamic therapy

    4.2 Mechanisms of action (type I e type II)

    4.3 Photosensitizers

    4.4 Light conditions

    4.5 Microbial targets

    4.6 Clinical and in vitro effectiveness of photodynamic therapy

    4..7 Effectiveness of photodynamic therapy in healthcare settings

    4..8 Advantages and drawbacks of photodynamic therapy relatively to conventional antibiotics

5. Blue light microbial photoinactivation

5..1 Mechanism of action of Blue Light

5..2 Endogenous photosensitizers of microorganisms

5.3 Effectiveness of blue light in the inactivation of microorganisms

5.4 Advantages and drawbacks of antimicrobial blue light relatively to antimicrobial photodynamic therapy and UV light

5.5 HINS

6. Conclusion

7. References

Attachments

Abbreviatures

aBL	Antimicrobial Blue Light
aPDT	Antimicrobial Photodynamic Therapy
CDC	Center for Disease Control
ECDC	European Centre for Disease and prevention Control
EDS	Environmental Decontamination System
HAI	Hospital Acquired Infection
HINS	High Intensity Narrow Spectrum light
HTS	High Touch Surfaces
ICU	Intensive Care Unit
MDRO	Multi Drug Resistant Organism
MRSA	Methicillin-resistant *Staphylococcus aureus*
NISS	Nosocomial Infection Surveillance System
PDT	Photodynamic Therapy
PS	Photosensitizer
ROS	Reactive Oxygen Species
UV	Ultraviolet
WHO	World Health Organization

Abstract

Hospital acquired infections (HAIs) are a serious problem that potentially affects millions of patients whenever in contact with hospital settings. Worsening the panorama is the emergence of antimicrobial resistance, by most microorganisms implicated in HAIs. Therefore, the improvement of the actual surveillance methods and the discovery of alternative approaches, with novel modes of action, is vital to overcome the threats created by the emergence of such resistances. Light therapy modalities represent a viable and effective alternative to the conventional antimicrobial treatment and can be preponderant in the control of HAIs, even against the (MDROs). This review will initially focus on the actual state of HAIs and MDROs, and which methods are currently available to fight them, followed by the exploration of antimicrobial light therapy and antimicrobial blue light therapy as alternative approaches to control microorganisms involved in HAIs. The advantages and drawbacks of blue light therapies relatively to conventional antimicrobial drugs and the potential applications of light therapy to destroy microorganisms on the healthcare settings will be also discussed.

Keywords: Hospital acquired infection; Multi drug resistant organisms; Infection control; Photodynamic therapy; Photosensitizer; Porphyrins; Blue light therapy.

Resumo

As infeções adquiridas em contexto hospitalar são um problema grave que afeta milhões de doentes quando estes recorrem aos hospitais. A agravar ainda mais este panorama, verifica-se o contínuo aparecimento de resistências microbianas, por microrganismos implicados nas HAIs, às principais classes de antimicrobianos. Assim sendo, a melhoria dos atuais métodos de vigilância e a descoberta de novas abordagens, com novos modos de ação, são aspetos vitais para enfrentar as ameaças criadas pelo aparecimento destas resistências. As terapias de luz representam uma viável e eficaz alternativa em relação ao tratamento antibacteriano convencional e poderão ser preponderantes no controlo das HAIs, mesmo aquelas causadas por MDROs. Esta revisão aborda inicialmente o estado atual das HAIs e dos MDRO, explorando os métodos que estão atualmente disponíveis para os combater. Em seguida, serão abordadas as terapias antimicrobianas de luz como abordagens alternativas para controlar microrganismos envolvidos em HAIs. As vantagens e desvantagens das terapias de luz em relação aos fármacos antimicrobianos convencionais e as possíveis aplicações fototerapia para destruir microrganismos no ambiente de saúde também serão discutidas.

Palavras-chave: Infeção adquirida em contexto hospitalar; Organismos multirresistentes; Controlo de infeção; Terapia fotodinâmica; Fotossensibilizador; Porfirinas; Terapia de luz azul.

antibiotics

MDPI

Review

Light therapy in hospital infection control: advantages, drawbacks and pitfalls.

João Cabral [1] and Acácio Rodrigues [2, *]

[1] Faculdade de Medicina da Universidade do Porto; jmacabral@gmail.com
[2] Faculdade de Medicina da Universidade do Porto; agr@med.up.pt
Correspondence: jmacabral@gmail.com

Received: date; Accepted: date; Published: date

Abstract: Hospital acquired infections (HAIs) are a serious problem that potentially affects millions of patients whenever in contact with hospital settings. Worsening the panorama is the emergence of antimicrobial resistance, by most microorganisms implicated in HAIs. Therefore, the improvement of the actual surveillance methods and the discovery of alternative approaches, with novel modes of action, is vital to overcome the threats created by the emergence of such resistances. Light therapy modalities represent a viable and effective alternative to the conventional antimicrobial treatment and can be preponderant in the control of HAIs, even against the multidrug resistant organisms MDROs. This review will initially focus on the actual state of HAIs and MDROs, and which methods are currently available to fight them, followed by the exploration of antimicrobial light therapy and antimicrobial blue light therapy as alternative approaches to control microorganisms involved in HAIs. The advantages and drawbacks of blue light therapies relatively to conventional antimicrobial drugs and the potential applications of light therapy to destroy microorganisms on the healthcare settings will be also discussed.

Keywords: Hospital acquired infection; Multi drug resistant organisms; Infection control; Photodynamic therapy; Photosensitizer; Porphyrins; Blue light therapy.

1. Introduction

Hospital acquired infections (HAIs) have become a recurrent transversal problem in every healthcare system. Its better understanding started in 1847 by the hand of Ignaz Semmelweis, known as "the father of infection control", who discovered that hand washing could lower the rates of infection in obstetric clinics. His legacy was later continued by Pasteur, Koch and Lister [1,2]. At present, HAIs have become a major problem, responsible for millions of deaths and huge costs for health systems, especially if the causing agent is a multidrug resistant organism (MDRO). Since most HAIs are potentially preventable, it is urgent to find alternatives to overcome this problem [3]. Due to the advances of science and technology, innovative ways to combat HAIs are arising, which can drastically reduce morbidity and mortality.

The Center of Disease Control (CDC) define a HAI as "a localized or systemic condition resulting from an adverse reaction to the presence of an infectious agent(s) or its toxin(s), that was present 48 h or more after the hospital admission. There must be no evidence that the infection was present or incubating at the time of admission to the acute care setting [4,5]". The origin of the

infection can be either endogenous or exogeneous. Endogenous infections, the most commons, originate from the microorganisms that normally inhabit certain human body niches, such as the gastrointestinal tract, the mouth and the skin. Exogeneous infections results from external sources, which can be widely varied, for example the hospital staff, visitors, medical devices or other patients [4]. A wide variety of agents can contribute to HAIs, some of the most common in Europe are listed in Table 1.

There has been an enormous advance in the variety of antimicrobial agents available over the last decades, which provided clinicians with a previously unavailable wide array of therapeutic options. However, given the increasing use of these drugs, especially in hospital settings, the development of microbial resistance has become an emerging problem, representing tremendous costs and being responsible for very high morbidity and mortality. Microorganisms, such as bacteria, fungi, viruses and parasites, can replicate very rapidly and acquire genetic traits, capable of promoting their survival in the presence of an antimicrobial agent; such an organism can quickly become predominant among a microbial population, by positive selection [6]. In addition to a wide array of mechanisms adopted by microorganisms to defend themselves against external aggression, many distinct factors may also contribute to the constant growing of antimicrobial resistance.

Examples of such factors are: 1. the inadequate or excessive prescription of antibiotics; 2. the overcrowding of patients and understaffing in hospitals; 3. the generalized use of antibiotics in cattle rations; 4. the facilitated transmission of microorganisms among populations, animal and/or human 5. the increasing globalization and the expansion of poverty, mostly among third world countries.

Drug resistance is extremely costly not only for health services, but also for the patient, who is unable to obtain the maximum therapeutic benefit, and also for the society, where resistant microorganisms can spread. It was estimated that, if nothing is done in the meantime, by 2050, MDRO will kill 10 million people every year, a figure which will outweigh the death caused by cancer. According to this assumption, the cost of MDRO in terms of lost global production between 2015 and 2050 would be 100 trillion USD, if no action is to be taken (O'Neill, 2016) [7].

HAIs caused by MDROs represent a serious health problem. In fact, not only the treatment of infectious diseases would be affected, but also several common clinical procedures such as cesarean sections, organ transplants and chemotherapy, that strictly depend on the use of antibiotics to prevent infections may be at risk [6]. Therefore, there is an urgent need to develop innovative and effective measures to combat pathogenic resistant microorganisms, often refractory to conventional treatment, as well as limiting the development and spreading of antimicrobial resistant microorganisms, particularly at niches/reservoirs like walls, floors and other hospital surfaces.

Further aggravating the panorama, pharmaceutical companies are withdrawing from the market. In fact, since the development of new antimicrobial agents is highly costly and resistances can arise in a few years, companies cannot profit as much as they used to and have focused in other more profitable markets [8].

Table 1 – Most common agents in HAIs in Europe in 2011-2012 according to ECDC [8]

Agent Involved	**Percentage of HAIs (%)**
Escherichia coli	15.9
Staphylococcus aureus	12.3
Enterococcus spp.	9.6
Pseudomonas aeruginosa	8.9
Klebsiella spp.	8.7
Coagulase-negative staphylococci	7.5
Candida spp.	6.1
Clostridium difficile	5.4

Alternative approaches to avoid the progression of HAIs must be pursued since millions of human lives are at risk. Furthermore, the prevention of HAIs and MDROs should be focused on non-antibiotic methods, with modes of action that interact with multiple targets, especially because even newly introduced antibiotics can become rapidly ineffective due to adaptive pressures exerted by other previous antibiotics [9] [10].

Antimicrobial blue light (aBL) is at present attracting increasing attention due to its intrinsic antimicrobial effect without the addition of exogenous PS [11] [12]. The use of aBL reduces the possibility of potential harmful effects on eukaryotic cells, thus reducing the possibility of human tissue damage. Moreover, the aBL impact in non-pathogenic microorganisms may be somewhat reduced when compared with that of antimicrobial photodynamic therapy (aPDT). Given these characteristics, there is an increasing interest in exploring the potential application of aBL in the sanitization of healthcare facilities, for instance in the disinfection of hospital wards, patient rooms and operating theaters with patients admitted. High Intensity Narrow Spectrum light (HINS), which takes advantage of a wave length of 405 nm, can be used by installing special lighting systems in the selected room. This type of sanitization can be more effective when compared to the usual manual cleaning, which by being highly worker dependent has a lot of variability [13].

2. Hospital Acquired-Infections

2.1 Reservoirs and transmission

Hospitals are, on a daily basis, confronted with multiple patients, possibly infected with pathogenic microorganisms, often MDRO, which represents a major challenge in terms of maintaining its facilities secured and disinfected.

The reservoirs of such pathogens not only involve medical indwelling devices (MIDs) (such as intravenous catheters), but also many distinct surfaces of the hospital [14]. The most common reservoirs involve patient's own microbiota and the hands and nostrils of healthcare personnel [15]. However, it is well known that contaminated surfaces also play a central role in the spreading of infections, like MRSA and Vancomycin Resistant *Enterococcus* (VRE), where they can survive for weeks in inorganic surfaces, if not submitted to a correct disinfection [16,17]. The most commonly infected surfaces are called High Touch Surfaces (HTS), that can be divided in patient care items, such as blood pressure cuffs, stethoscopes and thermometer; and environmental surfaces, that are further divided in medical equipment (for example monitor touch screen, controls and cables, supply carts) and patient room (bed rails/controls, bedside table/handles, chairs, telephone and TV remote, light switches, door and closet knobs/plates, toilet seat, flush handle and bedpan cleaner, sinks and soap dispensers, trash can, for instance) [15]. Recent studies even demonstrated that physician's mobile phones and boots are possible carriers of MRSA [18,19].

In fact, Carling et al demonstrated, that on average, a patient admitted into a room previously occupied by an infected or colonized person, has a 73% higher risk of acquiring the same pathogen of the last patient when compared to individuals not occupying such rooms. Furthermore it was demonstrated that only 40% of near patient surfaces were cleaned according to the hospital policies [20]. The same authors also reported that with the right education and monitorization of the cleaning staff, it was possible to improve the cleaned surfaces of the patient room from 49% to 82% [21].

Thus, as it will be discussed ahead, it is of utmost importance the methods chosen to disinfect healthcare facilities. Ideally, they should be worker independent, continuously active and assure the most complete surface disinfection.

The transmission of pathogens among healthcare settings can occur in a wide variety of ways. Microorganisms can be transmitted from their source to a new host through direct or indirect contact, in the air and/or water, or by vectors, being the indirect transmission the most common [22,23]. Transmission through vectors is more prevalent in tropical countries, where insects can transport the pathogens. Airborne transmission results from pathogen-laden droplets expelled from infected patients into the air when he sneezes, coughs, speaks or simply breathes, but can also result from problems in hospital ventilation systems [24]. The same situation applies for hospital water systems that can be colonized by microorganisms and be a source for waterborne infections, being

Legionella infections a well-known example of these situation [25]. Transmission by direct contact between patients is more uncommon. Indirect contact, as previously mentioned, is the most common route of infection, which consists on the transfer by a health care worker contaminated with organisms from their own body, or from other patient, to the patient whom he is taking care of.

2.2 Challenges and costs

There is an increasing interest in a more comprehensive understanding of hospital acquired infections and how to control them. Even though a lot of progress has been made, there are still many fields to improve and new challenges are always arising [26]. Not only patients' lives are seriously at risk, but it also involves extremely high costs to the health care system, being HAIs the most common complication of hospitalized patients [27]. HAIs lead to prolonged hospital stays, to the increase of microbial drug resistance, to a massive additional financial burden and, ultimately, to unacceptable deaths [28].

Despite all the efforts developed to combat these infections, the World Health Organization (WHO) estimates that the prevalence of HAIs in developed countries varies within the range of 3.5-12% and in developing countries within the range of 5.7-19.1%. Such infections are responsible for a minimum of 37000 deaths annually in Europe; of the 1.7 million HAIs estimated to occur annually in the USA, 99000 result in death [28,29]. The most recent studies in Europe report a prevalence of 6.0% of patients with at least one HAI in acute care hospitals in 2011 [30].

In terms of costs, it is also a terrific scenario since WHO estimates a 7 billion euros loss in Europe each year related to HAIs, likewise in the USA the estimated value is of 6.5 billion dollars [28]. To better understand the magnitude of these numbers, the direct medical cost of preventable HAIs is comparable to the annual costs in USA of stroke ($6.7 billion), diabetes mellitus with complications ($4.5 billion), and chronic obstructive lung disease ($4.2 billion) [31].

3. Prevention and control of hospital-acquired infections

3.1 Surveillance methods

The primary and most effective step towards the elimination of HAIs has always been its prevention. Ideally, the gold standard of surveillance would be a prospective, continuous and on-site system that covers the whole hospital. Unfortunately, such a programme rarely takes place. A surveillance method to be viable in hospitals must above all be financially equilibrated; this means that the costs saved by the prevention of HAIs through the method must outweigh its costs of implementation [32].

In Europe, the European Centre for Disease Prevention and Control (ECDC) is the entity responsible for the surveillance of nosocomial infections, being its objective to "ensure

standardisation of definitions, data collection and reporting procedures for hospitals participating in the national/regional surveillance of HAIs in ICUs across Europe, in order to contribute to the EU surveillance of HAIs, and to improve the quality of care in the ICU in a multicentre setting." There are 2 options for collecting data, patient-based (standard option) and unit-based (light option) [33]

In the United States, the Department of Health and Human Services, elaborated the "National Action Plan to Prevent Health Care-Associated Infections: Road Map to Elimination" to control and prevent HAIs. Its main line of action is "Demonstrable human and economic savings will be central to strengthening and sustaining our efforts. The HAI Action Plan has contributed to the significant progress in HAI prevention seen across the country and has brought about an enhanced level of federal collaboration" [34].

A study conducted in Austria in 2010 consisted in the introduction of a Nosocomial Infection Surveillance System (NISS), in 2 surgical and in 1 general intensive unit care (ICU), in order to improve safety and quality of treatment. After one year, it was demonstrated that the NISS was an effective method to improve patient's safety, allowed to generate data for comparative evaluation of infection rates and also was a helpful tool for the professionals, since it made possible to better appreciate the risks of medical procedures and to learn from previous data [35,36].

It is important to notice that, according to ECDC data from the first European-wide point prevalence survey, of all patients admitted to European hospitals in 2011-2012, 6% are infected with at least one HAI [37]. In the clinical practice, at least 20% of HAIs may be preventable by sustained and multifaceted infection prevention and control programmes, including surveillance of HAIs, which demonstrates the importance of the implementation of active policies [38].

3.2 Infection control programs

Due to the high morbidity and mortality of HAIs, associated with the increasingly emergence of MDRO, the implementation of infection control programs in all hospital facilities is crucial. This requires a multidisciplinar and multiprofessional approach, including all stakeholders related: physicians from diverse specialities (epidemiologists, infectiologists, surgeons, anaesthesiologists, microbiologists, etc.), nurses, pharmacists, cleaning employees, architects and hospital administrators.

A recent document of the Society for Healthcare Epidemiology of America (SHEA) exposed some key points to be included in infection control programs, like hand hygiene and asepsis; contact precautions and isolation; disinfection and sterilization of facilities and equipment; air handling and facility water supply management and control **[39].**

WHO has been strongly warning that hands are the most common vehicle for transmission of organisms and "hand hygiene" is the single most effective mean of preventing the horizontal

transmission of infections among hospital patients and health care personnel [40]. This also underlines the importance of standard contact precautions among healthcare workers and patients, reserving strict isolation measures to patients with highly transmissible and/or MDRO [41].

There are multiple approaches to avoid accumulation and transmission of hospital pathogens, being the conventional method, mostly used nowadays, the manual cleaning with detergents, performed by housekeepers, which obviously is worker dependent and has its specific concerns [13,42]. Although it is a cheaper technique in a short-term period, it can get much more expensive and put patients in risk in the long term.

In fact, manual routine cleaning techniques are often suboptimal: they are personnel dependent, which leads do diverse problems [43]; due to the different nature of surfaces being cleaned, some parts are left without proper cleaning, happening to be left, without cleaning at all 40 to 50% of surfaces that should be disinfected by the staff [20]; the disinfectants used can be inadequate for a certain kind of microorganism, can be contaminated and can also have an inappropriate over-dilution [44]; this results in a quick return to elevated levels of pathogens after a few hours of the cleaning, which is understandably ineffective.

Ventilation systems are widely used in hospitals; they can be natural or mechanical, being the last very commonly available in high/medium income countries. They help to renovate the air, diluting the particles in it, being able to reduce the infections by *Mycobacterium tuberculosis* for example. Not only tuberculosis is transmitted through the air, but also a wide and growing variety of other infectious organisms, which can travel in the air via droplets and infect other patients or surfaces if the ventilation is not effective [45]. It is very important to regularly monitor the functionality and microbiological quality of these systems in order to reduce the spread of airborne infections. The same consideration is valid for healthcare facility water supply. Waterborne infections, including *Legionella spp*., may be prevented by the implementation of firm hygiene practises and it is important to constantly revise the water management programs [46].

3.3 Limitations of current methods and new perspectives

The most commonly used methods for hospital cleaning, mostly involving manual routine cleaning techniques, are far from being perfect. In fact, studies demonstrated that there is a significant potential to decrease 10% to 70% of HAIs, depending on the conditions of each case [38].

Obviously, there is not a method without any flaws, but with the advances in science and technology, it is impossible to continue to ignore the potential of the new and innovative methods that are arising, which can lead to a more strict and effective prevention of HAIs. The ideal method should be highly germicidal, act continuously (since the contamination of hospital rooms is frequent [16]), non-harmful for humans, simple and cost-effective.

New methods such as self-disinfecting surfaces, hydrogen peroxide vapor, steam cleaning, ultraviolet light devices, aPDT, aBL and High Intensity Narrow Spectrum light (HINS)[13,47], are only a few in the vastness of methods that already exist and that are available to use daily in the hospital setting.

The main objective is not to stop using the conventional techniques, but to ally them with new methods, which can thus fill each other' gaps and reach the best disinfection rates.

4. Photodynamic therapy as an alternative approach in the control of colonization/infection in hospital settings and facilities

4.1 Antimicrobial Photodynamic Therapy (aPDT)

The use of light for the treatment of diseases goes back to antiquity [48]. Photoinactivation was first observed by Oscar Raab in the beginning of the XX century. While studying the effects of acridine orange dye, he found out that its application combined with the presence of light resulted in a lethal effect on microorganisms and that it was stronger than the isolated effect of orange acridine or light. In 1903, von Tappeiner and Jesionek found that oxygen was also essential for the process and later in 1907, Tappeiner and Jodlbauer, designated the process as a photodynamic effect, defining it as the destruction of biomolecules due to the dynamic interaction between light, a photosensitising agent and oxygen [49,50]. The first clinical use described was the topical application of eosin in basal cell carcinomas in 1907 [49]. However, it took another 70 years for the use of this therapeutic method, now called photodynamic therapy (PDT), to be recognized by medical science, namely in the treatment of cancer.

Today, PDT is a term commonly used to refer to the treatment of cancer by photochemotherapy. Since 1990, PDT has been successfully used in the treatment of various neoplasms, particularly at the skin, oral cavity, bronchial, oesophagus, bladder, head and neck [48]. It has also been applied in the field of ophthalmology, particularly in the treatment of age-related macular degeneration [51]

Due to the golden era of antibiotics, PDT was somewhat forgotten, but recently, with the unstoppable arising of MDROs, it was suggested as a promising solution for HAIs. PDT is extensively explored in the treatment of cancer, whereby studies on PS distribution, light exposure, light sources and endoscopy equipment previously used for cancer treatment, can also be applied to microbial photoinactivation (aPDT). In the treatment of cancer, the PS is usually injected into the bloodstream and accumulates in the tumour. However, to this day, aPDT has only been applied to localized infections rather than systemic. In the treatment of localized infections, the PS should be applied to the infected area by topical application, infiltration, injection or aerosol [52]. When aPDT is intended to inactivate microorganisms on surfaces, such as in healthcare settings, its application is much easier.

4.2 Mechanisms of action (type I e type II)

The effect of photodynamic inactivation results from a series of photophysical and photochemical events, which result from the PS excitation by light, which, through two distinct pathways (mechanisms of action type I and type II), lead to the production of ROS, which will oxidize the biomolecules [53].

In the dark, the PS is in the electronic ground state configuration. The absorption of a photon by the PS at a given wavelength causes its excitation to a new electric state which has a short lifetime (life time 10^{-9} to 10^{-6} seconds) [54]. The excited PS by emitting light returns to the ground state or undergo cross-system intersection and convert to an excited triplet state that has a superior lifetime (10^{-3} to 10 seconds) [54]. Once in this state, the PS has two paths to return to basal state by spin inversion, with light emission (phosphorescence) or by a non-radiative mode. Given that the triplet state has a prolonged lifetime, free radicals of oxygens are generated by type I reaction, or transfer energy directly to molecular oxygen by type II reaction, producing singlet oxygen (Figure 1). These reactions (type I and type II) give rise to lethal species, which causes irreparable oxidative damage in different vital cell targets [54-56].

The type I mechanism involves the abstract concept of the hydrogen atom or the transfer of electrons between the excited PS and a substrate, thus obtaining radical species [Table 2, equations (1) and (2)]. These free radical species interact with oxygen and create ROS, like the superoxide radical anion [Table 2, equation 3]. The superoxide radical is not very reactive in biological systems, but when protonated, can produce hydrogen peroxide and oxygen [Table 2, equations (4) and (5)] or the extremely reactive hydroxyl radicals [Table 2, equations (6) - (8)].

Type II mechanism is simpler, leading only to the production of singlet oxygen. In this way, the excited triplet state of the PS (3PS *) the excess of energy is transferred to the molecular oxygen ($^3O_2$), returning to its basal state (1PS) and producing singlet oxygen [Table 2, equation (9)]. Like radical species, $^1O_2$ is highly electrophilic and interact with various biomolecules, inactivating different microbes. [Table 2, equation (10)].

Both reactions originate ROS that instantly interact with biological components of the cell wall, like proteins, lipids, amino acid residues (cysteine, histidine, and tryptophan), nucleic acid bases (guanine and thymine) and also the pigments in certain cells [57,58]. Due to the high reactivity and short half-life of ROS, only molecules and structures close to the production area of singlet oxygen and free radical species are directly affected and destroyed [59]

The two types of mechanisms may occur simultaneously or separately, the predominance of each depends on the used PS, the substrate and the molecular oxygen concentration [54]. The predominant mechanism may be altered during the process [60].

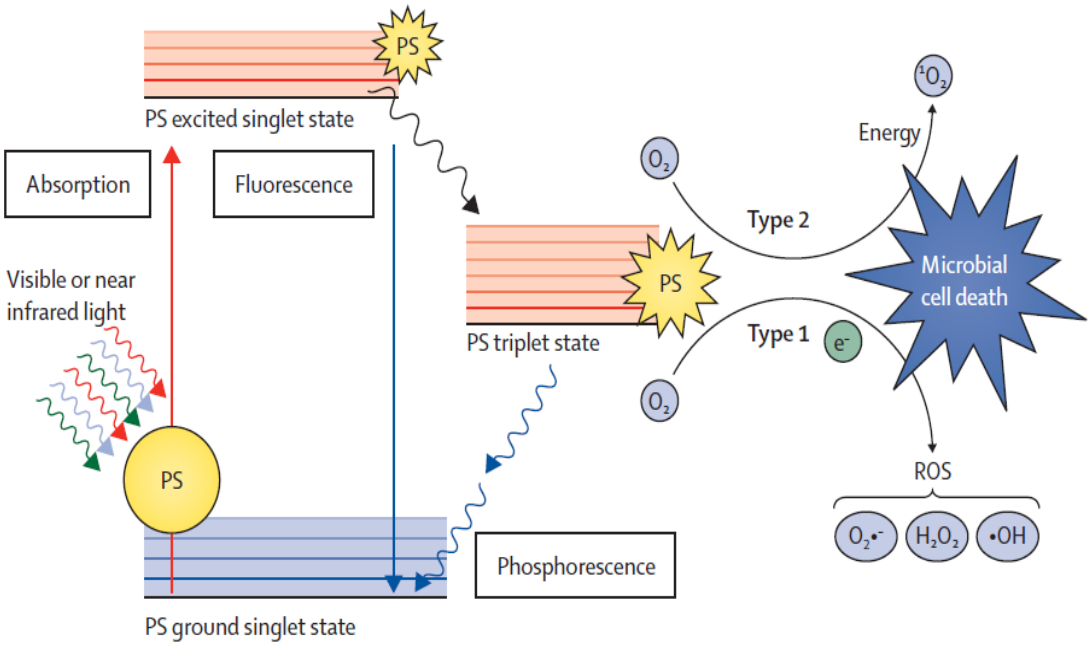


Figure 1 – Jablonski diagram showing photodynamic mechanism and respective pathways (Type 1/I and 2/II) (adapted from Wainwright, 1998 [61])

Table – 2 Photodynamic inactivation process, equations modified from [53]

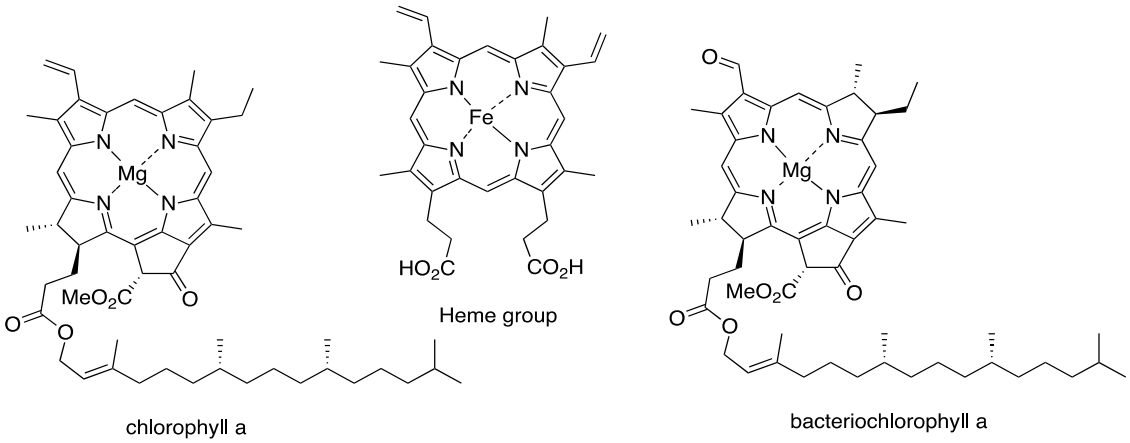
$SubstractH_2 + PS \rightarrow PSH\bullet + SubstractH\bullet$	(1)
$PS^* + Substract \rightarrow PS\bullet^- + Substract\bullet^+$ ou $PS^* + Substract \rightarrow PS\bullet^+ + Substract\bullet^-$	(2)
$PS\bullet^- + {}^3O_2 \rightarrow PS + O_2\bullet^-$	(3)
$O_2\bullet^- + H^+ \leftrightharpoons HOO\bullet$	(4)
$2\ HOO\bullet \rightarrow H_2O_2 + O_2$	(5)
$H_2O_2 + Fe^{2+} \rightarrow HO\bullet + OH^- + Fe^{3+}$	(6)
$BiomoleculeH + HO\bullet \rightarrow Biomolecule\bullet + H_2O$	(7)
$Biomolecule\bullet + {}^3O_2 \rightarrow Biomolecule\text{-}OO\bullet \rightarrow products$	(8)
$PS^* + {}^3O_2 \rightarrow PS + {}^1O_2$	(9)
$Biomolecules + {}^1O_2 \rightarrow$ oxidative products	(10)

4.3 Photosensitizers

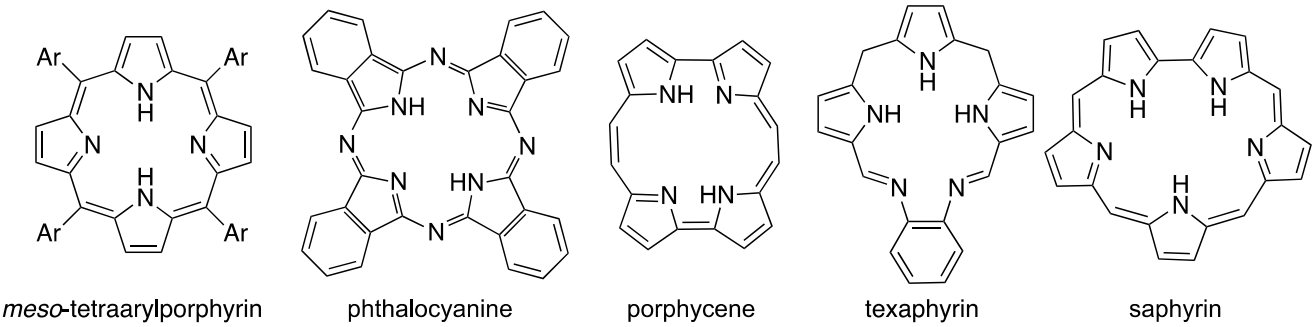
The photosensitizer (PS) has a special role in PDT, since it is the responsible for generating ROS, after being light activated [48,62,63]. The capacity of the PS to absorb light at a certain wavelength, relate to its structure and to the electronic absorption spectrum.

Most PS tested in aPDT are tetrapyrrol derivatives, known as porphyrins, even though some non-tetrapyrol derivatives have also been subject of studies. Porphyrins intervene in diverse vital functions, namely the respiration (heme group) and photosynthesis (chlorophyll and bacteriochlorophyll) (Figure 2). This class of PS was the first type of compounds to be used in PDT against tumors and to be allowed for clinical use (e.g. Photofrin®) [48,64]. Based on these macrocycles, several synthetic analogues have been developed, namely meso-tetraarylporphyrins, phthalocyanines, porphycans, texaphyrins and safirins, which exhibit very promising characteristics for use as PS (Figure 2) [64].

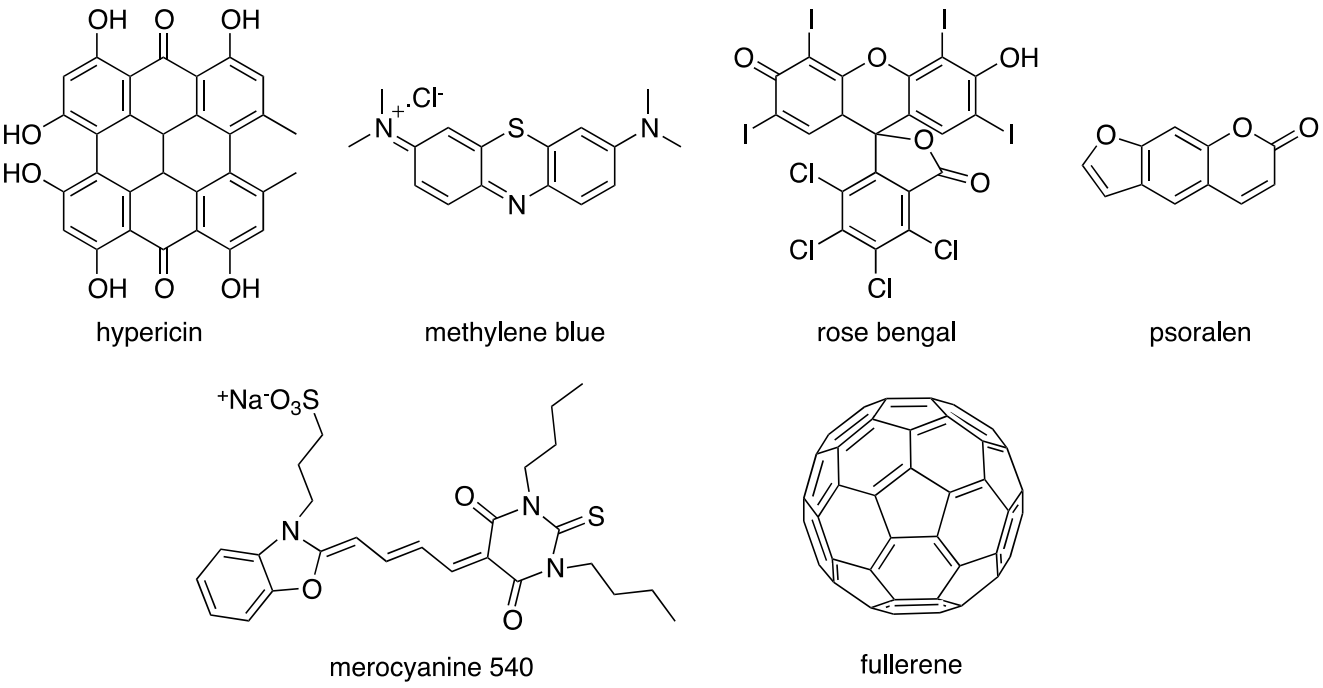
According to the literature, a good PS should display several characteristics to be used in aPDT [65-67]: (1) high chemical purity and simple synthesis; (2) photostability, so that it can be used in aPDT without being quickly degraded; (3) solubility (the PS does not aggregate or precipitate; if the PS aggregates, it is no longer available to bind to microorganisms and, consequently, there is a decrease of its function); (4) positive charge, mainly for the inactivation of Gram-negative bacteria. Photoinactivation is more effective with positively charged PS, because it promotes a tighter electrostatic interaction with the negative charges at the surface of bacteria; (5) amphiphilic properties; some studies showed that a PS with amphiphilic character exhibits more affinity to the microorganisms.



Tetrapyrrolic macrocycles with natural occurrence



Synthetic pyrrolic macrocycles used as photosensitizers



Non-tetrapyrrolic photosensitizers

Figure 2. Structure of some natural and synthetic pyrrolic macrocycles and non-pyrrolic photosensitizers used in aPDT modified from [53]

4.4 Light conditions

The characteristics of incident light, irradiance source and total light dose assume a vital role in the performance of aPDT and must be taken in account for the development of effective protocols.

The experimental results show that for the same PS and microorganism, the photodynamic efficiency depends on the light source used. The photodynamic inactivation requires a light source to activate the PS (visible light or near the visible) at wavelengths where the PS absorbs it efficiently [64]. The light source for aPDT should exhibit suitable spectral characteristics, preferably coincident or close to the maximum absorption wavelength of the PS to promote photodynamic effect by generating enough ROS.

In the inactivation of microorganisms, a wide variety of coherent and non-coherent light sources have been tested, ranging from the laser to tungsten filament lamps. In the aPDT, low power light is used and microbial inactivation can be achieved even with powers in the order of milliwatts [64].

The wavelength of light required for the induction of aPDT further depends on the structure and the absorption spectrum of the PS. For example, for porphyrin derivatives irradiated with white light, the efficacy of microbial therapy appears to decrease with increasing wavelength. For these PS, blue light (400-480 nm) is more effective at microbial inactivation than green (480-550 nm) or red (600-700 nm) light. The blue light of shorter wavelength (400-450 nm) is more phototoxic compared to that of blue light longer wavelength. Although red light is not as effective as blue light, it can be more effective treating infections, because it penetrates better into animal tissues [64].

The light dose used in aPDT also influences microbial inactivation in a time and irradiance-dependent manner. The law of reciprocity states that a given biological effect is directly proportional to the total energy dose. According to the literature, it can be assumed that the principle of reciprocity is only valid within a given dose range, which should be defined individually for each reaction. In addition, the responses of microorganisms and tissues to electromagnetic radiation involve a sequence of biological reactions that alter the linear dose - time relationship. On the other hand, PS molecules alone can induce different cellular and molecular responses to radiation [68]. Consequently, for aPDT, knowledge of the limits of this law is crucial for understanding the pathophysiology of photoinhibition.

The few studies on the influence of light parameters currently available in the literature, suggest that exposure to light with high irradiance in a short period of time, can give different results in terms of microbial inactivation than those obtained with exposure to low irradiance light over a long period of time, even if the total dose of light is the same. In general, the aPDT is most effective with a low irradiance light and a longer treatment time [69]. Gábor et al also demonstrated a higher inactivation rate of *E. coli* and *E. hirae* when a total dose of white light (irradiance 0.08-0.25 W/cm^2)

was received for a longer period [70]. For a T4-type phage with a high light dose (216 J cm^{-2}), the PDI rate was higher when lower radiances were used, namely 150, 300 and 600 W m^{-2} (Costa et al., 2010) [69].

Conventional lamps, also designated as non-coherent light sources, were the first to be used in PDT assays, because they were cheap, accessible and easy to use. However, they lack on other features, like the ability to control the light dose applied. At present, to overcome such limitations, lasers, also known as coherent light sources, started to be used in aPDT, becoming widely used due to their ability to produce a monochromatic light (with an exact wavelength) and to control light dose [48,62,63,65,71]. Other important factor is to match the wavelength with the chosen PS, to maximize the yield of produced ROS [62,63]. Regarding the influence of the tissue, it is important to mention that the travel direction of light is also affected by the inhomogeneity of the cells, namely the presence of organelles, macromolecules and the interstitial layers in fungi.

For environmental applications, such as healthcare settings disinfection, the use of wide wavelength potent light source in aPDT is a clever choice, because it can be used for different PS, making the process cheaper and easy to implement.

4.5 Microbial targets

The process of aPDT is obviously highly dependent on its targets and it is essential to understand them to maximize the effectiveness of the process. Different studies state that the key target of aPDT is influenced by the proper chemical structure of the PS, by the target microorganism and by the PS photoinactivation mechanism. [64,72].

Despite the multi-target nature of aPDT, the major microbial targets are proteins and lipids of the outer structures of the microorganisms (e.g. cytoplasmic membrane, cell wall, capsid and viral envelope) rather than nucleic acids.

Proteins

Cell membrane proteins of bacteria, fungi and protozoa and virus capsid proteins are considered the main targets of photoinactivation, mainly for their preponderant role in the cells, for their abundance and for their easiness to bind to the porphyrins. The oxidation of proteins leads to the formation of protein peroxides and carbonyl compounds; formation of cross-links and aggregates; changes of molecular conformation of proteins and inactivation of enzymes and loss of proteins [57,64,72].

Lipids

ROS can cause both direct and indirect oxidative modification of the lipids. Lipid hydroperoxides are intermediates in the peroxidation process, formed by its interaction with singlet

oxygen, which can modify the affected cell components, being the oxidation extended to the surrounding environment [73,74].

Nucleic acids

Nucleic acids are not a preponderant target of photoinactivation, even though they can strongly bind to PS. This is due to the photodynamic inactivation being a multi-target process that mainly affects the external constituents of the microorganisms and since the ROS are not generated in the nucleus, the limited lifespan and their outer location restricts its area of action.

4.6 *In vitro* and clinical effectiveness of photodynamic therapy

Although aPDT is effective against bacteria, viruses, fungi and parasites, its inactivation efficiency varies according to the microorganism. In general, bacteria and viruses are more easily inactivated than fungi and parasites. Spores of bacteria and fungi, particularly endospores, and parasite eggs and cysts are more resilient to inactivation than the corresponding vegetative cells [64].

Currently there is no routine application of aPDT for treatment of microbial infections, apart from the use of dihematoporphyrin ether and delta-aminolevulinic acid (ALA) in the treatment of skin infections by *Propionibacterium acnes*, local *papilloma virus* infections and cutaneous *leishmaniosis*.

Nonetheless, aPDT with methylene blue under visible light and psoralen and rivoflavin under UV light, are already approved for plasma disinfection in a few countries [75]. However, having into account the effective microbial inactivation in these laboratorial assays as well as in the few clinical trials already conducted, other applications of aPDT can be forecasted, such as the disinfection of infected skin, wound treatment, oral cavity and soft-tissue infections, treatment of abscesses and environmental disinfection.

Bacteria

Gram-positive bacteria are more easily inactivated than Gram-negative bacteria. The difference in the sensitivity of the two groups is related to their different cell wall composition. Most Gram-positive bacteria have a cell wall consisting of several layers of peptidoglycans, negatively charged, exhibiting a relatively high degree of porosity. Macromolecules having a molecular weight of 30,000-60,000 (e.g., glycopeptides and polysaccharides) can easily pass through this structure. Consequently, most PS can go through their membranes, since its molecular weight generally is situated between 1,500-1,800 Da [76]. On the contrary, Gram-negative bacteria display in the cell wall, an additional highly organized outer membrane, which are external to the peptidoglycan layer. The asymmetrical nature of the outer membrane is due to the distribution of its phospholipids, proteins, lipoproteins and negatively charged lipopolysaccharides (LPS) [77] which does not allow

the passage of various molecules to its interior. However, hydrophilic molecules of 600-700 Da can diffuse through the porins [78].

Gram positive bacteria can be efficiently inactivated by neutral and anionic PS, since the diverse PS can effortlessly go through their high permeable cell wall. Nevertheless, these PS are not effective against Gram negative bacteria [79], unless they are co-administered with external membrane disrupting agents, for instance CaCl2, EDTA and polymyxin B, which can lead to electrostatic repulsion, destabilizing the cell wall [56,80]. Gram negative bacteria can be directly and effectively inactivated by cationic PS, since these PS are able to bind to the negatively charged components of the outer membrane and allow a more effective interaction [79].

According to the literature, there are also differences in susceptibility to aPDT within each of the two bacterial groups, Gram positive [64,81] and Gram negative [64,82,83]. These variances observed are also due the differences in the cell wall of each bacteria [81]. Gram-negative bacteria have variances in their layers of peptidoglycan and lipidic outer membranes. As for the typical Gram-positive bacteria there is not a significant difference in their structure/composition.

Viruses

In the group of viruses, several studies suggest that lipid-enveloped ones are more susceptible to PDT [52,64,72]. However, there is still scarce information regarding the susceptibility of viruses with different types of nucleic acids (single- and double-stranded DNA and RNA). A recent study, revealed that the efficacy of PDI varied with the nucleic acid type of the phage viruses tested, since the RNA viruses were more susceptible to light inactivation than those of DNA; in fact, RNA viruses were much more sensitive to aPDT (concentration of PS needed 10 times smaller and inactivation 4 times faster) [72]. Although all the DNA and RNA viruses tested did not have envelope and their capsids were simple and without lipids, it is known that DNA phage capsids exhibit a higher diversity of proteins [84] than those of RNA [85]. Therefore, the difference between RNA and DNA phages is not only be attributed to their nucleic acid type, but also to the composition of their capsids.

The clinical application of aPDT to inactivate viruses has been successful. Neutral red/proflavine was effectively used to treat herpesvirus genital infection without relevant side effects [86]. Porphyrins were shown to be effective against herpesvirus, influenza virus and Papillomavirus [87,88]. aPDT is already approved to sterilize plasma; different viruses such as hepatitis viruses, parvoviruses, the West Nile virus and HIV, have been effectively inactivated by methylene blue [89,90].

Fungi and Parasites

Unlike bacteria and viruses, fungi and parasites are compartmented cells and, consequently, whenever the cell wall and membranes are damaged by the ROS, the PS enter to its interior. Similarly

to bacteria, fungi also have a cell wall, which is more permeable to external substances than Gram negative bacteria wall, but less than Gram positive [91]. As ROS are highly reactive and have short lifetime, the localization of the PS into the cell is very important, since the organelles located nearby to the PS have the highest probability of being affected.

Since fungi and parasite cells are larger when compared to bacteria and viruses, the amount of ROS needed to kill such a larger cell is much higher than that necessary to kill a bacterial cell or a viral particle [92]. On the other hand, the eukaryotic cell structure makes aPDT effect more difficult to work for these microorganisms than for bacteria and viruses. Even though, effective inactivation of fungi and parasites has already been observed [93,94]. In fact, to attain the effective inactivation of fungi and parasites, it is necessary to adjust the aPDT conditions, namely increase the PS concentration and the light dose [95]. Notably, *Candida* species are effectively inactivated by aPDT, but they are not as susceptible to PDT as several prokaryotic bacteria, including *Staphylococcus aureus* or *Streptococcus mutans* [96].

Likewise, it was observed that aPDT is effective for inactivating parasites, but also requiring higher PS concentration and higher light doses than those required for bacteria and viruses. aPDT with different PS have been tested for the inactivation of *Leishmania sp*. [97,98] and *Plasmodium falciparum* [99]. Cysts of *Colpoda inflate* and eggs of helminths like *Ascaris lumbricoides* and *Taenia sp* were also successfully photo inactivated [100].

4.7 Effectiveness of photodynamic therapy in healthcare settings/facilities

The potentialities of aPDT to eliminate microorganisms, even MDROs, surpasses the treatment of human infections, with a particular focus on the healthcare facilities. High doses of light can be used to destroy effectively microorganisms on surfaces. Moreover, higher concentrations of PS can be applied, which can even be supported in membranes/films, allowing its recovery and recycling, making this approach durable, sustainable, economic and environmentally friendly. In fact, recently a lot of developments were performed to renew the way that aPDT and its PS can be used. New methods have been tested to allow the immobilization of PS in diverse supports, which permits its use in the disinfection of materials and surfaces [101,102].

According to the literature, photoactive compounds/materials can potentially be used to prevent/eliminate microbial colonization in healthcare settings. Among these materials stand out nanoparticles, silica, chitosan and cellulose biopolymers, liposomes, nanogels and carbon photoactive compounds, which seem to be promising for disinfection/sterilization of polymeric materials (sodium chloride bags and tubing, gloves catheters, syringes and hemodialysis filters); protective clothes, masks and bedclothes; walls, floors and instruments, as well as hand disinfection of healthcare providers and even air disinfection of healthcare facilities [101-105].

Disinfection/sterilization of walls, floors, instruments

Healthcare surfaces are constantly contaminated with many microorganisms, including MDRO, which perpetuates the transmission of HAIs. Since surfaces are an important reservoir of pathogenic microorganisms, control of surface contamination with effective approaches such as aPDT can prevent its recontamination.

A surface coating of cellulose impregnated with toluidine blue O was contaminated with Gram positive and Gram-negative microorganisms. After 24 h of irradiation with white light, a 4 and 5 log reduction of *S. aureus* and *E. coli*, respectively, was observed. The authors suggested that this material has the potential to be used as wall paint to reduce the spread of nosocomial infection [106].

Disinfection/sterilization of polymeric materials

The colonization of polymeric materials, such as implants and catheters, by microorganisms is frequently associated with the development of antimicrobial/disinfectant resistant biofilm-related infections, which may lead not only to patient infection, but also to damage of the implant/catheter surface [107].

Antimicrobial materials based on polysiloxane (a polymer used in catheters) and incorporating methylene blue and gold nanoparticles were evaluated against *Escherichia coli* and methicillin-resistant *Staphylococcus aureus* (MRSA). There was a reduction of *E. coli* by 1.0 log after 5 min of irradiation with a red light (660 nm, 250 mW) and of *MRSA* by 3.5 log reduction, without affecting the mechanical properties of the photoactive material [108,109].

Other photoactive materials, based on polymers (polyurethane, silicone) used in catheters and in hospital touch surfaces (screen protectors for telephones and tablets, covers, keyboards and hand dryers), embedded with MB, toluidine blue O (TBO), crystal violet (CV) and gold nanoparticles, were also effective to inactivate MRSA, *S. epidermidis*, *Saccharomyces cerevisiae*, *E. coli*, *bacteriophage MS2*, the fungus-like organism *Pythium ultimum* and the filamentous fungus *Botrytis cinerea* [109-116].

Disinfection/sterilization of protective clothes, dressings and bedclothes

Another strategy to combat HAIs is the use of antimicrobial textile products, such as gowns, beddings, dressings and bedclothes. A stable, durable and washable fabric of cellulose coated with a first layer with ε-polylysine and a second layer with a zinc phthalocyanine PS, exhibit a potent antimicrobial activity against both Gram negative and Gram-positive bacteria. The survival of *E. coli* and *S. aureus* decreased by 99% and 98%, respectively, after aPDT with this photoactive material. Besides, this fabric was also effective to inactivate a drug resistant bacterial strain [117].

4.8 Advantages and drawbacks of photodynamic therapy relatively to conventional antimicrobials

aPDT shows many strengths when compared to conventional antimicrobial therapy [61,65,118]: 1. Multitarget and broad-spectrum activity. Unlike conventional antimicrobials, photo antimicrobials can inactivate bacteria (both Gram positive and Gram negative), viruses, fungi and parasites. 2. Appropriate to empiric treatment. When there is a lack of diagnosis and the microorganism causing the infection is not identified, this broad-spectrum and non-specific therapy could be a realistic alternative. 3. Less probability of resistance development. Since aPDT is not specific and involves *in situ* production of ROS that can affect several biomolecular sites, this therapy bypasses the usual mechanisms of resistance. Unfortunately, microorganisms can easily develop resistance against many of the available conventional antimicrobials, due to their single mode of action, which constitutes a major advantage of aPDT comparing to conventional antimicrobials. 4. Effectiveness against MDR microorganisms. The efficacy of aPDT against MDR microbial strains is similar to that of sensible ones. This efficacy is independent of the spectrum of resistance to conventional antimicrobials by the pathogen. 5. Rapid lethal effects. Although conventional treatments take hours or even days and repeated doses to induce effects, PDT exhibits a rapid killing effect. It is estimated that a single PS molecule can generate 10.000 molecules of singlet oxygen. 6. Safety and nontoxicity. At the normally used concentrations in aPDT (μM range), photo antimicrobials are harmless to the tissues, either excited by light or not; they inactivate effectively the microorganisms at very low concentrations. 7. Easy to implement. This therapy only requires a light source, the presence of molecular oxygen and a suitable PS. 8. Low risk to induce mutagenic effects.

Although the broad spectrum of aPDT activity could be useful for empirical treatment, the lack of selectivity to microorganisms can be also regarded as a disadvantage when the treatment is applied to treat human infections. However, this drawback can be bypassed, by using delivery approaches to achieve the inactivation of the pathogenic microorganism without the compromise of the human microbiome. Different drug delivery systems have been tested for aPDT, such as antibodies and liposomes, and more recently new biomaterials, with promising results [102,119,120]. The advances in biotechnology allowed the development of new drug delivery systems of PS with superior therapeutic properties and less toxic effects and also encouraged the use of new materials. Biocompatible polymers are a good example of these new biomaterials, which have valuable biological properties[102].

The valid use of aPDT to control human microbial infections can be achieved using the free form of the PS. However, this approach is far from appropriate for application to disinfect medical devices, such as catheters and surfaces, where residual traces of PS would certainly be not acceptable. Free PS might not only introduce residual traces of sensitizer but would also make this technology more expensive. The immobilization of efficient PS in insoluble supports can be an interesting

approach to inactivate pathogenic microorganisms present in surfaces. In fact, some research groups developed on solid supports with immobilized PS and tested their efficacy in the inactivation of different microorganisms [102,121]. Moreover, the immobilization of the PS on solid supports avoids its release with the environment, but also allows its recovery and readjust, making the aPDT approach cost-effective and environmentally friendly.

5. Blue light microbial photoinactivation

5.1. Mechanism of action of blue light

As mentioned before, antimicrobial blue light (aBL) is a specific type of light therapy. The principle of aBL is the same as for aPDT, using a wavelength of visible light comprised between 400-470 nm; contrarily to conventional aPDT, dispenses the use of exogeneous PS [122]. Although the mechanism is not yet fully understood, the mostly accepted explanation is that the aBL activates naturally occurring endogenous PS of pathogens, leading to the formation of ROS. These ROS, as mentioned before for aPDT, through oxidation, result in cytotoxicity by reacting with proteins, lipids and nucleic acids, of microbial cells, leading to cell death [123,124]. The blue light inactivation effect on microorganisms is oxygen dependent. Thus, the increase of the quantity of oxygen, provides a superior action of ROS, decreasing the dose of light required to inactivate pathogens [125].

5.2 Endogenous photosensitizers of microorganisms

Although only a few studies are yet available regarding the topic of endogenous photosensitizers, it is known that aBL exerts its actions mainly by iron-free porphyrins (Table 3). These iron-free porphyrins have two possible origins, they are either synthetized by bacteria as a by-product of heme biosynthesis or they arise as residuum of porphyrins that had their heme taken by the bacteria [126].

Studies demonstrated that the aBL oxidation effect was due to the presence of coproporphyrin III and/or uroporphyrin III within *P. aeruginosa* cells [11,131]. The same research group also described the presence of protoporphyrin IX not only in *P. aeruginosa,* but also in *A. baumannii* [126]. As for *S. aureus* and *C. albicans*, uroporphyrin and coproporphyrin, and flavins, respectively, were the almost exclusively produced photosensitisers [130,132,133]. The inactivation of *H. pylori* was also found to be related with coproporphyrin and protoporphyrin IX [123,134].

Table 3 - Endogenous porphyrin of bacteria

Agent	Most important endogenous porphyrin	References
A. baumannii	Coproporphyrin III	Zhang et al, 2014 [127]
C. albicans	Flavins	Tielker et al, 2009 [128]
E. coli	Protoporphyrin IX	Kwon et al, 2003 [129]
H. pylori	Coproporphyrin; Protoporphyrin IX	Hamblin et al, 2005 [123]
P. aeruginosa	Coproporphyrin III; Uroporphyrin III; Protoporphyrin IX	Amin et al, 2016 [131]
S. aureus	Uroporphyrin; Coproporphyrin	Hobbs et al, 2017 [130]

5.3 Effectiveness of blue light in the inactivation of microorganisms

An indispensable characteristic of a microorganism to be inactivated by aBL is the presence of photosensitisers. Interestingly, recent studies demonstrated the presence of endogenous photosensitizing chromophores in several microbial strains, which are commonly found in hospital environments, such as *S. aureus*, MRSA, *P. aeruginosa*, *Klebsiella pneumoniae*, *Clostridium difficile*, *Streptococcus pyogenes*, *Mycobacterium spp*, *Salmonella, H. pylori, A. baumannii* and *C. albicans* [11,123,124,126,127,133,135-138]. Notably, Gram positive bacteria are supposed to be usually more sensible to aBL than Gram negative bacteria [12].

As mentioned before, one of the biggest flaws of antibiotics is the emergence of resistance after some time of use, leading to failure to treat MDRO infections. Inversely, aBL, similarly to aPDT, is effective against a wide variety of pathogens, regardless of their classic drug resistance profile [126,127,135,139-141]. Several researchers tried to understand the potential of the arising of resistance to aBL. Guffey et al discovered that *S. aureus* can develop resistance if the light therapy is not correctly used [142]. However, it was found that resistance to aBL is very unlikely to occur, similarly to other light therapy modalities [141,143,144].

Numerous studies recognised the effectiveness of aBL to inactivate pathogenic bacteria. Recently, Huang *et al* were able to decrease MDR *E. coli* colony forming units (CFU) by 4-5 log10 [145]. Dai *et al* inactivated 4.75 log10 CFU of MRSA [135]. Fila *et al* successfully reduced *P. aeruginosa*, wild type and MDR, by 5.2 and 8 log10 CFU [146]. Halstead *et al* demonstrated that all the 34 different planktonic phase bacteria, specific tested *in vitro*, including *K. pneumoniae* and *E. faecium*, were susceptible to aBL, with 71% of them suffering a ≥5 log10 CFU decrease after 15-30 minutes of exposure [147]. Bacterial biofilms also suffered significant decreases [147]; Wang *et al* confirmed relevant antimicrobial activity of aBL towards Gram negative pathogens in biofilms, reducing 3.59 log10 CFU of *A. baumannii* biofilms and 3.02 log10 CFU of *P. aeruginosa* [126]. Moorhead *et al* were able to inactivate *C. difficile* vegetative cells and spores by 3 log10 CFU, even though the inactivation of spores required 10 times more dose of light [148]. Zhang *et al* inactivated 1.75 log10 CFU of *C. albicans* following a single exposure to light [133]. Wang *et al* performed a review in 2017 about the capacity of aBL to inactivate pathogens; they verified that more than 47 different pathogens, possible agents of HAIs, were successfully inactivated by aBL [122].

Therefore, aBL stands out as an effective method of disinfection against a great variety of major agents responsible for HAIs, even MDRO. Thus, it is easily understandable the tremendous potential of aBL for disinfection of hospital facilities, without the addition of any external agent, contrarily to conventional aPDT light therapy.

5.4 Advantages and drawbacks of antimicrobial blue light relatively to aPDT and UV light

As previously mentioned, several light therapy modalities, such as aBL, ultraviolet light irradiation (UV) and aPDT, exhibit a significant potential for the disinfection of hospital settings. Nevertheless, each one has its proper characteristics, advantages and drawbacks (Table 4).

aBL in comparison to UV light is equally effective against wild type and MDRO (even though it requires higher light doses to achieve the same results), but is much less harmful to human cells, such as fibroblasts, Langerhans cells and keratinocytes [133,147,149-154]. Thus, it does not constitute a risk for skin cancer, once it does not cause DNA damage or early photo-ageing [150].

Comparing to aPDT, aBL has the advantage of not requiring the addition of exogenous photosensitizers to inactivate pathogens, which facilitates and simplifies its use in healthcare facilities [153].

Interestingly, it was discovered that aBL was able to improve skin hydration [155], to facilitate wound healing [156] and bone regeneration in mice [157].

Unfortunately, aBL is not a perfect technique; it also has its drawbacks. The rapid increase of ROS leads to its interaction with retinal photoreceptor cells, causing oxidative stress and,

consequently, severe eye damage. Even though, the use of eye-protectors and eye antioxidants can prevent the oxidative damage [158].

Table 4 - Light therapies comparison

	UV therapy	**aBL**	**aPDT**
Damage of self-cells	High, risk of cancer	Reduced, risk of eye damage	Negligible
Resistance development	Microorganisms may adapt to UV irradiation by developing several repair mechanisms	Improvable	Improvable
Effectiveness	Permanent inactivation of microorganisms is impossible	High, even against MDRO	High, even against MDRO
Multitarget capacity	No, only nucleic acid	Yes, lipids, proteins and nucleic acids	Yes, lipids, proteins and nucleic acids
Response time	Slow/medium lethal effects	Quick lethal effects	Quick lethal effects

5.5 High Intensity Narrow Spectrum light

High Intensity Narrow Spectrum light (HINS-light) is a concretization of aBL in the disinfection of healthcare settings, using an inactivating blue light with a wavelength of 405 nm, which was proven to be the most effective [138].

This approach, as previously mentioned, displays numerous advantages, an innovative procedure would be the use of continuous irradiation in clinical areas, even in the presence of staff and patients, since the light used is harmless. This would provide an incessant control of environmental agents, even of MDROs, a fact that could substantially improve the actual paradigm of HAI control, sparing many human lives and considerable financial resources.

Maclean et al conducted several studies in this area; one of these studies, demonstrated the *in vitro* effectiveness of a 405 nm LED light to inhibit several bacteria accountable for HAIs. HINS light was able to reduce Gram-positive species as *S. aureus*, MRSA, *S. epidermidis*, *C. perfringens* and *S. pyogenes* by around 5 log10 CFU with low light dose (around 40 J/cm^2). Notably, *E. faecalis* was not so susceptible; to achieve a reduction of 2.6 log10 CFU, it required a higher light dose (216 J/cm2). Gram negative bacteria were also less susceptible; higher light doses were necessary (around 180 J/cm2) to reduce approximately 4 log10 CFU [138].

The same group conducted a study in a Scottish hospital, using a 2 HINS environmental decontamination system (HINS-light EDS) placed in an isolation room. Firstly, it was tested empty; the HINS-light EDS working for 24 hours reduced in 92% *S. aureus* contamination levels. Secondly, the system was tested while a patient with MRSA was admitted and the levels of the pathogen were reduced by 65%. Finally, a room was occupied by a patient with MRSA and the MRSA concentration was determined before, during and after the use of HINS-light. With the use of HINS-light, MRSA levels were decreased by 50%, but shortly after the light being turned off, *S. aureus* levels recovered by 98%, which corroborates the need of a continuous treatment to effectively reduce the environmental burden [159]. Other studies demonstrated that this technology is also efficient to disinfect areas frequented by outpatients as well as intensive care units [160,161].

In fact, a diversity of other species showed to be susceptible to HINS-light, namely *L. monocytogenes* [162], *C. difficile* [148,163], *P. aeruginosa* [146], *A. baumannii, K. pneumoniae, P. vulgaris, E. coli, S. enteriditis, S. sonnei, Serratia spp, Aspergillus niger, C. albicans* and *Saccharomyces cerevisiae [164].*

As previously mentioned, aBL and, in particular HINS, exhibits a wide range of other potential applications, such as the very effective disinfection of orthopedic osteosynthetic biomaterials [165].

Since HINS-light EDS integrates the spectrum of aBL, it shares its advantages: the continuous disinfection of air and surface treatment; effectiveness against a wide variety of pathogens; few installing and maintenance requirements; no need for staff training; no compliance problems with staff and patients; low financial costs [164].

Since such a therapeutic approach is recent, the number of studies performed to evaluate its effectiveness and applicability *in vivo* is still very limited; therefore, more field work will be required to better understand the particularities of this therapy.

6. Conclusion

HAIs are a serious threat to our modern healthcare systems, carrying not only huge morbidity and mortality, but also tremendous financial costs. Further worsening the panorama, MDROs are increasing in hospital facilities, which allied to the economical disinterest of pharmaceutical companies in producing new antimicrobials, diminishes the capacity of conventional antimicrobials to treat HAIs. Therefore, new options are required, especially in the context of HAIs, to avoid the total incapacity of treating MDROs, which would be a catastrophe, since even the most banal infection could lead to death due to the absence of a valid antimicrobial therapy.

A crucial pillar of action against HAIs should be its prevention with effective surveillance programs and ideally continuous disinfection of hospital settings. Unfortunately, current conventional methods, such as manual cleaning with detergents, are usually incapable of doing so.

Hospital surfaces colonization constitute the reservoir to the maintenance and propagation of HAIs. As mentioned before, current methods lack of effectiveness in eliminating pathogens. It is mandatory to counteract this trend; fortunately, blue light therapy modalities constitute a relevant and continuously acting solution to this problem.

Thus, blue light therapy modalities represent promising approaches in the combat of HAIs. They are capable of effectively eliminate even the most dangerous MDROs without significant adverse effects to patients and materials, and development of photo resistance. HINS-light EDS may be effectively used for the continuous control of colonization/infection in hospital settings.

Therefore, it is imperative to continue to explore these new promising techniques in order to transpose easily its application to the hospital facilities, including day-care centers and nursing homes.

Acknowledgments: The authors thank the Faculty of Medicine of Porto for the opportunity and support in the development of this work.

Author Contributions: João Cabral wrote the paper. Acácio Rodrigues revised the paper.

Funding: This research received no external funding.

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

7. References

1. Best, M.; Neuhauser, D. Ignaz Semmelweis and the birth of infection control. *Qual Saf Health Care* **2004**, *13*, 233-234, doi:10.1136/qhc.13.3.233.
2. Kampf, G.; Degenhardt, S.; Lackner, S.; Jesse, K.; von Baum, H.; Ostermeyer, C. Poorly processed reusable surface disinfection tissue dispensers may be a source of infection. *BMC Infect Dis* **2014**, *14*, 37, doi:10.1186/1471-2334-14-37.
3. Kasper, D.L., Fauci, A. S., Hauser, S. L., Longo, D. L. 1., Jameson, J. L., & Loscalzo, J. *Harrison's principles of internal medicine*, 19 ed.; New York: McGraw Hill Education: 2015.
4. Horan, T.C.; Andrus, M.; Dudeck, M.A. CDC/NHSN surveillance definition of health care-associated infection and criteria for specific types of infections in the acute care setting. *Am J Infect Control* **2008**, *36*, 309-332, doi:10.1016/j.ajic.2008.03.002.
5. Cardoso, T.; Almeida, M.; Carratala, J.; Aragao, I.; Costa-Pereira, A.; Sarmento, A.E.; Azevedo, L. Microbiology of healthcare-associated infections and the definition accuracy to predict infection by potentially drug resistant pathogens: a systematic review. *BMC Infect Dis* **2015**, *15*, 565, doi:10.1186/s12879-015-1304-2.
6. Organization, W.H. Antimicrobial resistance. Availabe online: http://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance (accessed on 21/08/2018).
7. O’NEILL, J. *TACKLING DRUG-RESISTANT INFECTIONS GLOBALLY: FINAL REPORT AND RECOMMENDATIONS*; 2016.
8. Cookson, B. Clinical significance of emergence of bacterial antimicrobial resistance in the hospital environment. *J Appl Microbiol* **2005**, *99*, 989-996, doi:10.1111/j.1365-2672.2005.02693.x.
9. Chambers, H.F.; Deleo, F.R. Waves of resistance: Staphylococcus aureus in the antibiotic era. *Nat Rev Microbiol* **2009**, *7*, 629-641, doi:10.1038/nrmicro2200.
10. Harkins, C.P.; Pichon, B.; Doumith, M.; Parkhill, J.; Westh, H.; Tomasz, A.; de Lencastre, H.; Bentley, S.D.; Kearns, A.M.; Holden, M.T.G. Methicillin-resistant Staphylococcus aureus emerged long before the introduction of methicillin into clinical practice. *Genome Biol* **2017**, *18*, 130, doi:10.1186/s13059-017-1252-9.
11. Dai, T.; Gupta, A.; Huang, Y.Y.; Yin, R.; Murray, C.K.; Vrahas, M.S.; Sherwood, M.E.; Tegos, G.P.; Hamblin, M.R. Blue light rescues mice from potentially fatal Pseudomonas aeruginosa burn infection: efficacy, safety, and mechanism of action. *Antimicrob Agents Chemother* **2013**, *57*, 1238-1245, doi:10.1128/AAC.01652-12.
12. Maclean, M.; McKenzie, K.; Anderson, J.G.; Gettinby, G.; MacGregor, S.J. 405 nm light technology for the inactivation of pathogens and its potential role for environmental disinfection and infection control. *J Hosp Infect* **2014**, *88*, 1-11, doi:10.1016/j.jhin.2014.06.004.
13. Boyce, J.M. Modern technologies for improving cleaning and disinfection of environmental surfaces in hospitals. *Antimicrob Resist Infect Control* **2016**, *5*, 10, doi:10.1186/s13756-016-0111-x.
14. Adlhart, C.; Verran, J.; Azevedo, N.F.; Olmez, H.; Keinanen-Toivola, M.M.; Gouveia, I.; Melo, L.F.; Crijns, F. Surface modifications for antimicrobial effects in the healthcare setting: a critical overview. *J Hosp Infect* **2018**, *99*, 239-249, doi:10.1016/j.jhin.2018.01.018.
15. Cobrado, L.; Silva-Dias, A.; Azevedo, M.M.; Rodrigues, A.G. High-touch surfaces: microbial neighbours at hand. *Eur J Clin Microbiol Infect Dis* **2017**, *36*, 2053-2062, doi:10.1007/s10096-017-3042-4.

16. Weber, D.J.; Anderson, D.; Rutala, W.A. The role of the surface environment in healthcare-associated infections. *Curr Opin Infect Dis* **2013**, *26*, 338-344, doi:10.1097/QCO.0b013e3283630f04.
17. Kramer, A.; Schwebke, I.; Kampf, G. How long do nosocomial pathogens persist on inanimate surfaces? A systematic review. *BMC Infect Dis* **2006**, *6*, 130, doi:10.1186/1471-2334-6-130.
18. Smibert, O.C.; Aung, A.K.; Woolnough, E.; Carter, G.P.; Schultz, M.B.; Howden, B.P.; Seemann, T.; Spelman, D.; McGloughlin, S.; Peleg, A.Y. Mobile phones and computer keyboards: unlikely reservoirs of multidrug-resistant organisms in the tertiary intensive care unit. *J Hosp Infect* **2018**, *99*, 295-298, doi:10.1016/j.jhin.2018.02.013.
19. Clesham, K.; Ryan, P.R.; Murphy, C.G. Assessment of theatre shoe contamination in an orthopaedic theatre. *J Hosp Infect* **2018**, *99*, 299-302, doi:10.1016/j.jhin.2018.03.009.
20. Carling, P.C.; Bartley, J.M. Evaluating hygienic cleaning in health care settings: what you do not know can harm your patients. *Am J Infect Control* **2010**, *38*, S41-50, doi:10.1016/j.ajic.2010.03.004.
21. Carling, P.C.; Parry, M.F.; Bruno-Murtha, L.A.; Dick, B. Improving environmental hygiene in 27 intensive care units to decrease multidrug-resistant bacterial transmission. *Crit Care Med* **2010**, *38*, 1054-1059, doi:10.1097/CCM.0b013e3181cdf705.
22. Chander, Y.; Rai, R. Hospital Acquired Infection. *Medical Journal Armed Forces India* **1998**, *54*, 179-181, doi:10.1016/s0377-1237(17)30535-x.
23. Organization, W.H. Hospital hygiene and infection control. Health, W.S., Ed.
24. Qian, H.; Zheng, X. Ventilation control for airborne transmission of human exhaled bio-aerosols in buildings. *J Thorac Dis* **2018**, *10*, S2295-S2304, doi:10.21037/jtd.2018.01.24.
25. Decker, B.K.; Palmore, T.N. The role of water in healthcare-associated infections. *Curr Opin Infect Dis* **2013**, *26*, 345-351, doi:10.1097/QCO.0b013e3283630adf.
26. Calfee, D.P. Crisis in hospital-acquired, healthcare-associated infections. *Annu Rev Med* **2012**, *63*, 359-371, doi:10.1146/annurev-med-081210-144458.
27. Sydnor, E.R.; Perl, T.M. Hospital epidemiology and infection control in acute-care settings. *Clin Microbiol Rev* **2011**, *24*, 141-173, doi:10.1128/CMR.00027-10.
28. Organization, W.H. Health care-associated infections FACT SHEET. Organization, W.H., Ed. 2017.
29. Klevens, R.M.; Edwards, J.R.; Richards, C.L., Jr.; Horan, T.C.; Gaynes, R.P.; Pollock, D.A.; Cardo, D.M. Estimating health care-associated infections and deaths in U.S. hospitals, 2002. *Public Health Rep* **2007**, *122*, 160-166, doi:10.1177/003335490712200205.
30. Control, E.C.f.D.P.a. Point prevalence survey of healthcare associated infections and antimicrobial use in European acute care hospitals 2011-2012. 2013.
31. R., S. The direct medical costs of healthcare-associated infections in U.S. hospitals and the benefits of prevention. Prevention, C.f.D.C.a., Ed. 2009.
32. Surveillance of nosocomial infections in Europe: which methodologies? *Clinical Microbiology and Infection* **1999**, *5*, doi:10.1111/j.1469-0691.1999.tb00734.x.
33. Control, E.C.f.D.P.a. Surveillance of healthcare-associated infections and prevention indicators in European intensive care units. Control, E.C.f.D.P.a., Ed. Stockholm, 2017; 10.2900/833186.
34. Services, U.S.D.o.H.H. National Action Plan to Prevent Health Care-Associated Infections: Road Map to Elimination. Services, U.S.D.o.H.H., Ed. 2013.
35. F. G. Linck-Velarde, H.M., G. Bernhardt, W. Schwab-Ganster, M. Kucher, G., Feierl, A. H. Niemetz, K. Vander, A. Bogiatzis. Implementation of a region wide computerized

surveillance program for nosocomial postoperative wound infections in surgical units of Styria. Graz, M.U.o., Ed. 2011.
36. B. F. Höfler, B.H., F. G. Linck-Velarde, A. H. Niemetz, A. Bogiatzis, H. Metzler, H. Mischinger. Nosocomial Infection Surveillance System - Intensive Care Units (NISS-ITS). Graz, M.U.o., Ed. 2011.
37. Control, E.C.f.D.P.a. Healthcare-associated infections in European hospitals. 2015.
38. Harbarth, S.; Sax, H.; Gastmeier, P. The preventable proportion of nosocomial infections: an overview of published reports. *Journal of Hospital Infection* **2003**, *54*, 258-266, doi:10.1016/s0195-6701(03)00150-6.
39. Kaye, K.S.; Anderson, D.J.; Cook, E.; Huang, S.S.; Siegel, J.D.; Zuckerman, J.M.; Talbot, T.R. Guidance for infection prevention and healthcare epidemiology programs: healthcare epidemiologist skills and competencies. *Infect Control Hosp Epidemiol* **2015**, *36*, 369-380, doi:10.1017/ice.2014.79.
40. WHO. Guidelines on hand hygiene in health care: A summary. **2014**.
41. Landelle, C.; Pagani, L.; Harbarth, S. Is patient isolation the single most important measure to prevent the spread of multidrug-resistant pathogens? *Virulence* **2013**, *4*, 163-171, doi:10.4161/viru.22641.
42. Boyce, J.M.; Havill, N.L.; Lipka, A.; Havill, H.; Rizvani, R. Variations in hospital daily cleaning practices. *Infect Control Hosp Epidemiol* **2010**, *31*, 99-101, doi:10.1086/649225.
43. Fernando, S.A.; Gray, T.J.; Gottlieb, T. Healthcare-acquired infections: prevention strategies. *Intern Med J* **2017**, *47*, 1341-1351, doi:10.1111/imj.13642.
44. Boyce, J.M.; Sullivan, L.; Booker, A.; Baker, J. Quaternary Ammonium Disinfectant Issues Encountered in an Environmental Services Department. *Infect Control Hosp Epidemiol* **2016**, *37*, 340-342, doi:10.1017/ice.2015.299.
45. Organization, W.H. Natural Ventilation for Infection Control in Health-Care Settings. 2009.
46. Decker, B.K.; Palmore, T.N. Hospital water and opportunities for infection prevention. *Curr Infect Dis Rep* **2014**, *16*, 432, doi:10.1007/s11908-014-0432-y.
47. Dancer, S.J. Controlling hospital-acquired infection: focus on the role of the environment and new technologies for decontamination. *Clin Microbiol Rev* **2014**, *27*, 665-690, doi:10.1128/CMR.00020-14.
48. Ackroyd, R.; Kelty, C.; Brown, N.; Reed, M. The history of photodetection and photodynamic therapy. *Photochem Photobiol* **2001**, *74*, 656-669.
49. von Tappeiner, H., & Jesionek, A. Therapeutische versuche mit fluoreszierenden stoffen. In *Münch Med Wochenschr*, 1903; Vol. 47, pp. 2042-2044.
50. von Tappeiner, H., & Jodlbauer, A. Die Sensibilisierende Wirkung Fluorescierender Substanzen: Gesammelte Untersuchungen. In *Über die Photodynamische Erscheinung*, GW Vogel: Leipzig, Germany, 1907; Vol. 42, pp. 13-28.
51. Wormald, R.; Evans, J.; Smeeth, L.; Henshaw, K. Photodynamic therapy for neovascular age-related macular degeneration. *Cochrane Database Syst Rev* **2007**, 10.1002/14651858.CD002030.pub3, CD002030, doi:10.1002/14651858.CD002030.pub3.
52. Hamblin, M.R.; Hasan, T. Photodynamic therapy: a new antimicrobial approach to infectious disease? *Photochem Photobiol Sci* **2004**, *3*, 436-450, doi:10.1039/b311900a.
53. Costa, L.; Faustino, M.A.F.; Neves, M.G.P.M.S.; Cunha, Â.; Almeida, A. Photodynamic Inactivation of Mammalian Viruses and Bacteriophages. *Viruses* **2012**, *4*, 1034.
54. Dalla Via, L.; Marciani Magno, S. Photochemotherapy in the treatment of cancer. *Curr Med Chem* **2001**, *8*, 1405-1418.

55. DeRosa, M.C.; Crutchley, R.J. Photosensitized singlet oxygen and its applications. *Coordination Chemistry Reviews* **2002**, *233-234*, 351-371, doi:https://doi.org/10.1016/S0010-8545(02)00034-6.
56. Jori, G.; Brown, S.B. Photosensitized inactivation of microorganisms. *Photochem Photobiol Sci* **2004**, *3*, 403-405, doi:10.1039/b311904c.
57. Alves, E.; Faustino, M.A.; Neves, M.G.; Cunha, A.; Tome, J.; Almeida, A. An insight on bacterial cellular targets of photodynamic inactivation. *Future Med Chem* **2014**, *6*, 141-164, doi:10.4155/fmc.13.211.
58. Almeida, A.; Faustino, M.A.; Tome, J.P. Photodynamic inactivation of bacteria: finding the effective targets. *Future Med Chem* **2015**, *7*, 1221-1224, doi:10.4155/fmc.15.59.
59. Bonnett, R. *Chemical aspects of photodynamic therapy*; Gordon and Breach Science Publishers: Amsterdam, The Netherlands, 2000.
60. Choe, E.; Min, D.B. Chemistry and reactions of reactive oxygen species in foods. *Crit Rev Food Sci Nutr* **2006**, *46*, 1-22, doi:10.1080/10408390500455474.
61. Wainwright, M. Photodynamic antimicrobial chemotherapy (PACT). *J Antimicrob Chemother* **1998**, *42*, 13-28.
62. Hamblin, M.; Mroz, P. *Advances in Photodynamic Therapy: Basic, Translational, and Clinical*; 2008.
63. Castano, A.P.; Demidova, T.N.; Hamblin, M.R. Mechanisms in photodynamic therapy: part one-photosensitizers, photochemistry and cellular localization. *Photodiagnosis Photodyn Ther* **2004**, *1*, 279-293, doi:10.1016/S1572-1000(05)00007-4.
64. Almeida A, C.A., Faustino MAF, Tomé AC, Neves MGPMS Porphyrins as antimibrobial photosensitizing agents. In: Photodynamic Inactivation of Microbial Pathogens: Medical and Environmental Applications. Royal Society of Chemistry, Cambridge: 2011; pp. 83-160.
65. Wainwright, M.; Maisch, T.; Nonell, S.; Plaetzer, K.; Almeida, A.; Tegos, G.P.; Hamblin, M.R. Photoantimicrobials–are we afraid of the light? *The Lancet Infectious Diseases* **2017**, *17*, e49-e55, doi:10.1016/s1473-3099(16)30268-7.
66. Costa, L.; Alves, E.; Carvalho, C.M.; Tome, J.P.; Faustino, M.A.; Neves, M.G.; Tome, A.C.; Cavaleiro, J.A.; Cunha, A.; Almeida, A. Sewage bacteriophage photoinactivation by cationic porphyrins: a study of charge effect. *Photochem Photobiol Sci* **2008**, *7*, 415-422, doi:10.1039/b712749a.
67. Alves, E.; Costa, L.; Carvalho, C.M.; Tome, J.P.; Faustino, M.A.; Neves, M.G.; Tome, A.C.; Cavaleiro, J.A.; Cunha, A.; Almeida, A. Charge effect on the photoinactivation of Gram-negative and Gram-positive bacteria by cationic meso-substituted porphyrins. *BMC Microbiol* **2009**, *9*, 70, doi:10.1186/1471-2180-9-70.
68. Schindl, A.; Rosado-Schlosser, B.; Trautinger, F. [Reciprocity regulation in photobiology. An overview]. *Hautarzt* **2001**, *52*, 779-785.
69. Costa, L.; Carvalho, C.M.; Faustino, M.A.; Neves, M.G.; Tome, J.P.; Tome, A.C.; Cavaleiro, J.A.; Cunha, A.; Almeida, A. Sewage bacteriophage inactivation by cationic porphyrins: influence of light parameters. *Photochem Photobiol Sci* **2010**, *9*, 1126-1133, doi:10.1039/c0pp00051e.
70. Gabor, F.; Szolnoki, J.; Toth, K.; Fekete, A.; Maillard, P.; Csik, G. Photoinduced inactivation of T7 phage sensitized by symmetrically and asymmetrically substituted tetraphenyl porphyrin: comparison of efficiency and mechanism of action. *Photochem Photobiol* **2001**, *73*, 304-311.
71. Sperandio, F.F.; Huang, Y.Y.; Hamblin, M.R. Antimicrobial photodynamic therapy to kill Gram-negative bacteria. *Recent Pat Antiinfect Drug Discov* **2013**, *8*, 108-120.
72. Costa, L.; Tome, J.P.; Neves, M.G.; Tome, A.C.; Cavaleiro, J.A.; Cunha, A.; Faustino, M.A.; Almeida, A. Susceptibility of non-enveloped DNA- and RNA-type viruses to

photodynamic inactivation. *Photochem Photobiol Sci* **2012**, *11*, 1520-1523, doi:10.1039/c2pp25156f.
73. Girotti, A.W. Photosensitized oxidation of membrane lipids: reaction pathways, cytotoxic effects, and cytoprotective mechanisms. *J Photochem Photobiol B* **2001**, *63*, 103-113.
74. Stark, G. Functional consequences of oxidative membrane damage. *J Membr Biol* **2005**, *205*, 1-16, doi:10.1007/s00232-005-0753-8.
75. Marciel, L.; Teles, L.; Moreira, B.; Pacheco, M.; Lourenco, L.M.; Neves, M.G.; Tome, J.P.; Faustino, M.A.; Almeida, A. An effective and potentially safe blood disinfection protocol using tetrapyrrolic photosensitizers. *Future Med Chem* **2017**, *9*, 365-379, doi:10.4155/fmc-2016-0217.
76. Jori, G.; Fabris, C.; Soncin, M.; Ferro, S.; Coppellotti, O.; Dei, D.; Fantetti, L.; Chiti, G.; Roncucci, G. Photodynamic therapy in the treatment of microbial infections: basic principles and perspective applications. *Lasers Surg Med* **2006**, *38*, 468-481, doi:10.1002/lsm.20361.
77. Maisch, T.; Szeimies, R.M.; Jori, G.; Abels, C. Antibacterial photodynamic therapy in dermatology. *Photochem Photobiol Sci* **2004**, *3*, 907-917, doi:10.1039/b407622b.
78. Nikaido, H. Prevention of drug access to bacterial targets: permeability barriers and active efflux. *Science* **1994**, *264*, 382-388.
79. Hamblin, M.R.; O'Donnell, D.A.; Murthy, N.; Rajagopalan, K.; Michaud, N.; Sherwood, M.E.; Hasan, T. Polycationic photosensitizer conjugates: effects of chain length and Gram classification on the photodynamic inactivation of bacteria. *J Antimicrob Chemother* **2002**, *49*, 941-951.
80. Ehrenberg, B.; Gross, E.; Nitzan, Y.; Malik, Z. Electric depolarization of photosensitized cells: lipid vs. protein alterations. *Biochim Biophys Acta* **1993**, *1151*, 257-264.
81. Grinholc, M.; Szramka, B.; Kurlenda, J.; Graczyk, A.; Bielawski, K.P. Bactericidal effect of photodynamic inactivation against methicillin-resistant and methicillin-susceptible Staphylococcus aureus is strain-dependent. *J Photochem Photobiol B* **2008**, *90*, 57-63, doi:10.1016/j.jphotobiol.2007.11.002.
82. Nitzan, Y.; Arielly, H.; Maayan, M.C.; Rozenszajn, A. Gram negative bacteria isolated from blood cultures in a general hospital. *The new microbiologica : official journal of the Italian Society for Medical, Odontoiatric, and Clinical Microbiology (SIMMOC)* **1994**, *17*, 111-122.
83. Arrojado, C.; Pereira, C.; Tome, J.P.; Faustino, M.A.; Neves, M.G.; Tome, A.C.; Cavaleiro, J.A.; Cunha, A.; Calado, R.; Gomes, N.C., et al. Applicability of photodynamic antimicrobial chemotherapy as an alternative to inactivate fish pathogenic bacteria in aquaculture systems. *Photochem Photobiol Sci* **2011**, *10*, 1691-1700, doi:10.1039/c1pp05129f.
84. Mesyanzhinov, V.V.; Leiman, P.G.; Kostyuchenko, V.A.; Kurochkina, L.P.; Miroshnikov, K.A.; Sykilinda, N.N.; Shneider, M.M. Molecular architecture of bacteriophage T4. *Biochemistry (Mosc)* **2004**, *69*, 1190-1202.
85. Cho, M.; Chung, H.; Choi, W.; Yoon, J. Different inactivation behaviors of MS-2 phage and Escherichia coli in TiO2 photocatalytic disinfection. *Appl Environ Microbiol* **2005**, *71*, 270-275, doi:10.1128/AEM.71.1.270-275.2005.
86. Moore, C.; Wallis, C.; Melnick, J.L.; Kuns, M.D. Photodynamic treatment of herpes keratitis. *Infect Immun* **1972**, *5*, 169-171.
87. Abramson, A.L.; Hirschfield, L.S.; Shikowitz, M.J.; Barrezueta, N.X. The pathologic effects of photodynamic therapy on the larynx. Experimental study. *Arch Otolaryngol Head Neck Surg* **1988**, *114*, 33-39.

88. Perlin, M.; Mao, J.C.; Otis, E.R.; Shipkowitz, N.L.; Duff, R.G. Photodynamic inactivation of influenza and herpes viruses by hematoporphyrin. *Antiviral Res* **1987**, *7*, 43-51.
89. Mohr, H.; Knuver-Hopf, J.; Gravemann, U.; Redecker-Klein, A.; Muller, T.H. West Nile virus in plasma is highly sensitive to methylene blue-light treatment. *Transfusion* **2004**, *44*, 886-890, doi:10.1111/j.1537-2995.2004.03424.x.
90. Wainwright, M. The emerging chemistry of blood product disinfection. *Chem Soc Rev* **2002**, *31*, 128-136.
91. St Denis, T.G.; Dai, T.; Izikson, L.; Astrakas, C.; Anderson, R.R.; Hamblin, M.R.; Tegos, G.P. All you need is light: antimicrobial photoinactivation as an evolving and emerging discovery strategy against infectious disease. *Virulence* **2011**, *2*, 509-520, doi:10.4161/viru.2.6.17889.
92. Demidova, T.N.; Hamblin, M.R. Effect of cell-photosensitizer binding and cell density on microbial photoinactivation. *Antimicrob Agents Chemother* **2005**, *49*, 2329-2335, doi:10.1128/AAC.49.6.2329-2335.2005.
93. Calzavara-Pinton, P.; Rossi, M.T.; Sala, R.; Venturini, M. Photodynamic antifungal chemotherapy. *Photochem Photobiol* **2012**, *88*, 512-522, doi:10.1111/j.1751-1097.2012.01107.x.
94. Smijs, T.G.; Pavel, S. The susceptibility of dermatophytes to photodynamic treatment with special focus on Trichophyton rubrum. *Photochem Photobiol* **2011**, *87*, 2-13, doi:10.1111/j.1751-1097.2010.00848.x.
95. Donnelly, R.F.; McCarron, P.A.; Tunney, M.M.; David Woolfson, A. Potential of photodynamic therapy in treatment of fungal infections of the mouth. Design and characterisation of a mucoadhesive patch containing toluidine blue O. *J Photochem Photobiol B* **2007**, *86*, 59-69, doi:10.1016/j.jphotobiol.2006.07.011.
96. Pereira, C.A.; Romeiro, R.L.; Costa, A.C.; Machado, A.K.; Junqueira, J.C.; Jorge, A.O. Susceptibility of Candida albicans, Staphylococcus aureus, and Streptococcus mutans biofilms to photodynamic inactivation: an in vitro study. *Lasers Med Sci* **2011**, *26*, 341-348, doi:10.1007/s10103-010-0852-3.
97. Morgenthaler, J.B.; Peters, S.J.; Cedeno, D.L.; Constantino, M.H.; Edwards, K.A.; Kamowski, E.M.; Passini, J.C.; Butkus, B.E.; Young, A.M.; Lash, T.D., et al. Carbaporphyrin ketals as potential agents for a new photodynamic therapy treatment of leishmaniasis. *Bioorg Med Chem* **2008**, *16*, 7033-7038, doi:10.1016/j.bmc.2008.05.037.
98. van der Snoek, E.M.; Robinson, D.J.; van Hellemond, J.J.; Neumann, H.A. A review of photodynamic therapy in cutaneous leishmaniasis. *J Eur Acad Dermatol Venereol* **2008**, *22*, 918-922, doi:10.1111/j.1468-3083.2008.02805.x.
99. Grellier, P.; Santus, R.; Mouray, E.; Agmon, V.; Maziere, J.C.; Rigomier, D.; Dagan, A.; Gatt, S.; Schrevel, J. Photosensitized inactivation of Plasmodium falciparum- and Babesia divergens-infected erythrocytes in whole blood by lipophilic pheophorbide derivatives. *Vox Sang* **1997**, *72*, 211-220.
100. Alouini, Z.; Jemli, M. Destruction of helminth eggs by photosensitized porphyrin. *J Environ Monit* **2001**, *3*, 548-551.
101. Alves, E.; Esteves, A.C.; Correia, A.; Cunha, A.; Faustino, M.A.; Neves, M.G.; Almeida, A. Protein profiles of Escherichia coli and Staphylococcus warneri are altered by photosensitization with cationic porphyrins. *Photochem Photobiol Sci* **2015**, *14*, 1169-1178, doi:10.1039/c4pp00194j.
102. M, Q.M.; C, J.D.; MG, P.M.S.N.; Almeida, A.; MA, F.F. Revisiting Current Photoactive Materials for Antimicrobial Photodynamic Therapy. *Molecules* **2018**, *23*, doi:10.3390/molecules23102424.

103. Alvarez, M.G.; Gomez, M.L.; Mora, S.J.; Milanesio, M.E.; Durantini, E.N. Photodynamic inactivation of Candida albicans using bridged polysilsesquioxane films doped with porphyrin. *Bioorg Med Chem* **2012**, *20*, 4032-4039, doi:10.1016/j.bmc.2012.05.012.
104. Bozja, J.; Sherrill, J.; Michielsen, S.; Stojiljkovic, I. Porphyrin-based, light-activated antimicrobial materials. *Journal of Polymer Science Part A: Polymer Chemistry* **2003**, *41*, 2297-2303, doi:doi:10.1002/pola.10773.
105. Krouit, M.; Granet, R.; Krausz, P. Photobactericidal films from porphyrins grafted to alkylated cellulose – synthesis and bactericidal properties. *European Polymer Journal* **2009**, *45*, 1250-1259, doi:https://doi.org/10.1016/j.eurpolymj.2008.11.036.
106. Wilson, M. Light-activated antimicrobial coating for the continuous disinfection of surfaces. *Infect Control Hosp Epidemiol* **2003**, *24*, 782-784, doi:10.1086/502136.
107. Dortbudak, O.; Haas, R.; Bernhart, T.; Mailath-Pokorny, G. Lethal photosensitization for decontamination of implant surfaces in the treatment of peri-implantitis. *Clin Oral Implants Res* **2001**, *12*, 104-108.
108. Perni, S.; Piccirillo, C.; Pratten, J.; Prokopovich, P.; Chrzanowski, W.; Parkin, I.P.; Wilson, M. The antimicrobial properties of light-activated polymers containing methylene blue and gold nanoparticles. *Biomaterials* **2009**, *30*, 89-93, doi:10.1016/j.biomaterials.2008.09.020.
109. Perni, S.; Prokopovich, P.; Parkin, I.P.; Wilson, M.; Pratten, J. Prevention of biofilm accumulation on a light-activated antimicrobial catheter material. *Journal of Materials Chemistry* **2010**, *20*, 8668-8673, doi:10.1039/C0JM01891K.
110. Bovis, M.J.; Noimark, S.; Woodhams, J.H.; Kay, C.W.M.; Weiner, J.; Peveler, W.J.; Correia, A.; Wilson, M.; Allan, E.; Parkin, I.P., et al. Photosensitisation studies of silicone polymer doped with methylene blue and nanogold for antimicrobial applications. *RSC Advances* **2015**, *5*, 54830-54842, doi:10.1039/C5RA09045H.
111. Macdonald, T.J.; Wu, K.; Sehmi, S.K.; Noimark, S.; Peveler, W.J.; du Toit, H.; Voelcker, N.H.; Allan, E.; MacRobert, A.J.; Gavriilidis, A., et al. Thiol-Capped Gold Nanoparticles Swell-Encapsulated into Polyurethane as Powerful Antibacterial Surfaces Under Dark and Light Conditions. *Sci Rep* **2016**, *6*, 39272, doi:10.1038/srep39272.
112. Noimark, S.; Allan, E.; Parkin, I.P. Light-activated antimicrobial surfaces with enhanced efficacy induced by a dark-activated mechanism. *Chemical Science* **2014**, *5*, 2216-2223, doi:10.1039/C3SC53186D.
113. Noimark, S.; Bovis, M.; MacRobert, A.J.; Correia, A.; Allan, E.; Wilson, M.; Parkin, I.P. Photobactericidal polymers; the incorporation of crystal violet and nanogold into medical grade silicone. *RSC Advances* **2013**, *3*, 18383-18394, doi:10.1039/C3RA42629G.
114. Noimark, S.; Dunnill, C.W.; Kay, C.W.M.; Perni, S.; Prokopovich, P.; Ismail, S.; Wilson, M.; Parkin, I.P. Incorporation of methylene blue and nanogold into polyvinyl chloride catheters; a new approach for light-activated disinfection of surfaces. *Journal of Materials Chemistry* **2012**, *22*, 15388-15396, doi:10.1039/C2JM31987J.
115. Walker, T.; Canales, M.; Noimark, S.; Page, K.; Parkin, I.; Faull, J.; Bhatti, M.; Ciric, L. A Light-Activated Antimicrobial Surface Is Active Against Bacterial, Viral and Fungal Organisms. *Sci Rep* **2017**, *7*, 15298, doi:10.1038/s41598-017-15565-5.
116. Page, K.; Correia, A.; Wilson, M.; Allan, E.; Parkin, I.P. Light-activated antibacterial screen protectors for mobile telephones and tablet computers. *Journal of Photochemistry and Photobiology A: Chemistry* **2015**, *296*, 19-24, doi:https://doi.org/10.1016/j.jphotochem.2014.08.011.
117. Chen, J.; Wang, W.; Hu, P.; Wang, D.; Lin, F.; Xue, J.; Chen, Z.; Iqbal, Z.; Huang, M. Dual antimicrobial actions on modified fabric leads to inactivation of drug-resistant

bacteria. *Dyes and Pigments* **2017**, *140*, 236-243, doi:https://doi.org/10.1016/j.dyepig.2017.01.032.
118. Almeida, A.; Cunha, A.; Gomes, N.C.; Alves, E.; Costa, L.; Faustino, M.A. Phage therapy and photodynamic therapy: low environmental impact approaches to inactivate microorganisms in fish farming plants. *Mar Drugs* **2009**, *7*, 268-313, doi:10.3390/md7030268.
119. Bourre, L.; Giuntini, F.; Eggleston, I.M.; Mosse, C.A.; Macrobert, A.J.; Wilson, M. Effective photoinactivation of Gram-positive and Gram-negative bacterial strains using an HIV-1 Tat peptide-porphyrin conjugate. *Photochem Photobiol Sci* **2010**, *9*, 1613-1620, doi:10.1039/c0pp00146e.
120. Yin, R.; Agrawal, T.; Khan, U.; Gupta, G.K.; Rai, V.; Huang, Y.Y.; Hamblin, M.R. Antimicrobial photodynamic inactivation in nanomedicine: small light strides against bad bugs. *Nanomedicine (Lond)* **2015**, *10*, 2379-2404, doi:10.2217/nnm.15.67.
121. Alves, E.; Rodrigues, J.M.M.; Faustino, M.A.F.; Neves, M.G.P.M.S.; Cavaleiro, J.A.S.; Lin, Z.; Cunha, Â.; Nadais, M.H.; Tomé, J.P.C.; Almeida, A. A new insight on nanomagnet–porphyrin hybrids for photodynamic inactivation of microorganisms. *Dyes and Pigments* **2014**, *110*, 80-88, doi:https://doi.org/10.1016/j.dyepig.2014.05.016.
122. Wang, Y.; Wang, Y.; Wang, Y.; Murray, C.K.; Hamblin, M.R.; Hooper, D.C.; Dai, T. Antimicrobial blue light inactivation of pathogenic microbes: State of the art. *Drug Resist Updat* **2017**, *33-35*, 1-22, doi:10.1016/j.drup.2017.10.002.
123. Hamblin, M.R.; Viveiros, J.; Yang, C.; Ahmadi, A.; Ganz, R.A.; Tolkoff, M.J. Helicobacter pylori accumulates photoactive porphyrins and is killed by visible light. *Antimicrob Agents Chemother* **2005**, *49*, 2822-2827, doi:10.1128/AAC.49.7.2822-2827.2005.
124. Kim, M.J.; Yuk, H.G. Antibacterial Mechanism of 405-Nanometer Light-Emitting Diode against Salmonella at Refrigeration Temperature. *Appl Environ Microbiol* **2017**, *83*, doi:10.1128/AEM.02582-16.
125. Maclean, M.; Macgregor, S.J.; Anderson, J.G.; Woolsey, G.A. The role of oxygen in the visible-light inactivation of Staphylococcus aureus. *J Photochem Photobiol B* **2008**, *92*, 180-184, doi:10.1016/j.jphotobiol.2008.06.006.
126. Wang, Y.; Wu, X.; Chen, J.; Amin, R.; Lu, M.; Bhayana, B.; Zhao, J.; Murray, C.K.; Hamblin, M.R.; Hooper, D.C., et al. Antimicrobial Blue Light Inactivation of Gram-Negative Pathogens in Biofilms: In Vitro and In Vivo Studies. *J Infect Dis* **2016**, *213*, 1380-1387, doi:10.1093/infdis/jiw070.
127. Zhang, Y.; Zhu, Y.; Gupta, A.; Huang, Y.; Murray, C.K.; Vrahas, M.S.; Sherwood, M.E.; Baer, D.G.; Hamblin, M.R.; Dai, T. Antimicrobial blue light therapy for multidrug-resistant Acinetobacter baumannii infection in a mouse burn model: implications for prophylaxis and treatment of combat-related wound infections. *J Infect Dis* **2014**, *209*, 1963-1971, doi:10.1093/infdis/jit842.
128. Tielker, D.; Eichhof, I.; Jaeger, K.E.; Ernst, J.F. Flavin mononucleotide-based fluorescent protein as an oxygen-independent reporter in Candida albicans and Saccharomyces cerevisiae. *Eukaryotic cell* **2009**, *8*, 913-915, doi:10.1128/ec.00394-08.
129. Kwon, S.J.; de Boer, A.L.; Petri, R.; Schmidt-Dannert, C. High-level production of porphyrins in metabolically engineered Escherichia coli: systematic extension of a pathway assembled from overexpressed genes involved in heme biosynthesis. *Appl Environ Microbiol* **2003**, *69*, 4875-4883.
130. Hobbs, C.; Reid, J.D.; Shepherd, M. The coproporphyrin ferrochelatase of Staphylococcus aureus: mechanistic insights into a regulatory iron-binding site. *The Biochemical journal* **2017**, *474*, 3513-3522, doi:10.1042/bcj20170362.

131. Amin, R.M.; Bhayana, B.; Hamblin, M.R.; Dai, T. Antimicrobial blue light inactivation of Pseudomonas aeruginosa by photo-excitation of endogenous porphyrins: In vitro and in vivo studies. *Lasers Surg Med* **2016**, *48*, 562-568, doi:10.1002/lsm.22474.
132. Fotinos, N.; Convert, M.; Piffaretti, J.-C.; Gurny, R.; Lange, N. Effects on Gram-Negative and Gram-Positive Bacteria Mediated by 5-Aminolevulinic Acid and 5-Aminolevulinic Acid Derivatives. *Antimicrobial Agents and Chemotherapy* **2008**, *52*, 1366-1373, doi:10.1128/AAC.01372-07.
133. Zhang, Y.; Zhu, Y.; Chen, J.; Wang, Y.; Sherwood, M.E.; Murray, C.K.; Vrahas, M.S.; Hooper, D.C.; Hamblin, M.R.; Dai, T. Antimicrobial blue light inactivation of Candida albicans: In vitro and in vivo studies. *Virulence* **2016**, *7*, 536-545, doi:10.1080/21505594.2016.1155015.
134. Ashkenazi, H.; Malik, Z.; Harth, Y.; Nitzan, Y. Eradication of Propionibacterium acnes by its endogenic porphyrins after illumination with high intensity blue light. *FEMS Immunol Med Microbiol* **2003**, *35*, 17-24.
135. Dai, T.; Gupta, A.; Huang, Y.Y.; Sherwood, M.E.; Murray, C.K.; Vrahas, M.S.; Kielian, T.; Hamblin, M.R. Blue light eliminates community-acquired methicillin-resistant Staphylococcus aureus in infected mouse skin abrasions. *Photomed Laser Surg* **2013**, *31*, 531-538, doi:10.1089/pho.2012.3365.
136. Davies, A.; Pottage, T.; Bennett, A.; Walker, J. Gaseous and air decontamination technologies for Clostridium difficile in the healthcare environment. *J Hosp Infect* **2011**, *77*, 199-203, doi:10.1016/j.jhin.2010.08.012.
137. Murdoch, L.E.; Maclean, M.; Endarko, E.; MacGregor, S.J.; Anderson, J.G. Bactericidal effects of 405 nm light exposure demonstrated by inactivation of Escherichia, Salmonella, Shigella, Listeria, and Mycobacterium species in liquid suspensions and on exposed surfaces. *ScientificWorldJournal* **2012**, *2012*, 137805, doi:10.1100/2012/137805.
138. Maclean, M.; MacGregor, S.J.; Anderson, J.G.; Woolsey, G. Inactivation of bacterial pathogens following exposure to light from a 405-nanometer light-emitting diode array. *Appl Environ Microbiol* **2009**, *75*, 1932-1937, doi:10.1128/AEM.01892-08.
139. Enwemeka, C.S.; Williams, D.; Enwemeka, S.K.; Hollosi, S.; Yens, D. Blue 470-nm light kills methicillin-resistant Staphylococcus aureus (MRSA) in vitro. *Photomed Laser Surg* **2009**, *27*, 221-226, doi:10.1089/pho.2008.2413.
140. Enwemeka, C.S.; Williams, D.; Hollosi, S.; Yens, D.; Enwemeka, S.K. Visible 405 nm SLD light photo-destroys methicillin-resistant Staphylococcus aureus (MRSA) in vitro. *Lasers Surg Med* **2008**, *40*, 734-737, doi:10.1002/lsm.20724.
141. Dai, T. The antimicrobial effect of blue light: What are behind? *Virulence* **2017**, *8*, 649-652, doi:10.1080/21505594.2016.1276691.
142. Guffey, J.S., Payne, W., Martin, K., Dodson, C. Delaying the onset of resistance formation: effect of manipulating dose, wavelength, and rate of energy delivery of 405-, 464-, and 850-nanometer light for Staphylococcus aureus. *Wounds* **2014**, *26*, 95‑100.
143. Tomb, R.M.; Maclean, M.; Coia, J.E.; MacGregor, S.J.; Anderson, J.G. Assessment of the potential for resistance to antimicrobial violet-blue light in Staphylococcus aureus. *Antimicrob Resist Infect Control* **2017**, *6*, 100, doi:10.1186/s13756-017-0261-5.
144. Yin, R.; Dai, T.; Avci, P.; Jorge, A.E.; de Melo, W.C.; Vecchio, D.; Huang, Y.Y.; Gupta, A.; Hamblin, M.R. Light based anti-infectives: ultraviolet C irradiation, photodynamic therapy, blue light, and beyond. *Curr Opin Pharmacol* **2013**, *13*, 731-762, doi:10.1016/j.coph.2013.08.009.
145. Huang, S.T.; Wu, C.Y.; Lee, N.Y.; Cheng, C.W.; Yang, M.J.; Hung, Y.A.; Wong, T.W.; Liang, J.Y. Effects of 462 nm Light-Emitting Diode on the Inactivation of Escherichia coli and a

Multidrug-Resistant by Tetracycline Photoreaction. *J Clin Med* **2018**, *7*, doi:10.3390/jcm7090278.

146. Fila, G.; Kawiak, A.; Grinholc, M.S. Blue light treatment of Pseudomonas aeruginosa: Strong bactericidal activity, synergism with antibiotics and inactivation of virulence factors. *Virulence* **2017**, *8*, 938-958, doi:10.1080/21505594.2016.1250995.
147. Halstead, F.D.; Thwaite, J.E.; Burt, R.; Laws, T.R.; Raguse, M.; Moeller, R.; Webber, M.A.; Oppenheim, B.A. Antibacterial Activity of Blue Light against Nosocomial Wound Pathogens Growing Planktonically and as Mature Biofilms. *Appl Environ Microbiol* **2016**, *82*, 4006-4016, doi:10.1128/AEM.00756-16.
148. Moorhead, S.a.M., Michelle and Coia, John and MacGregor,; Scott. Inactivation of C. difficile by 405 nm HINS-light. In Proceedings of 8th Annual Scottish Environmental and Clean Technology Conference, Scotland, 26/06/2014.
149. McDonald, R.; Macgregor, S.J.; Anderson, J.G.; Maclean, M.; Grant, M.H. Effect of 405-nm high-intensity narrow-spectrum light on fibroblast-populated collagen lattices: an in vitro model of wound healing. *J Biomed Opt* **2011**, *16*, 048003, doi:10.1117/1.3561903.
150. Kleinpenning MM, S.T., Frunt MH, van Erp PE, van de Kerkhof; PC, G.R. Clinical and histological effects of blue light on normal skin. *Photodermatol Photoimmunol Photomed* **2010**, *26*, 16‐21, doi:10.1111/j.1600-0781.2009.00474.x.
151. Ramaswamy, P.; Powers, J.G.; Bhawan, J.; Polyak, I.; Gilchrest, B.A. Effective blue light photodynamic therapy does not affect cutaneous langerhans cell number or oxidatively damage DNA. *Dermatol Surg* **2014**, *40*, 979-987, doi:10.1097/01.DSS.0000452624.01889.8a.
152. Liebmann, J.; Born, M.; Kolb-Bachofen, V. Blue-light irradiation regulates proliferation and differentiation in human skin cells. *J Invest Dermatol* **2010**, *130*, 259-269, doi:10.1038/jid.2009.194.
153. Dai, T.; Gupta, A.; Murray, C.K.; Vrahas, M.S.; Tegos, G.P.; Hamblin, M.R. Blue light for infectious diseases: Propionibacterium acnes, Helicobacter pylori, and beyond? *Drug Resist Updat* **2012**, *15*, 223-236, doi:10.1016/j.drup.2012.07.001.
154. Pfaff, S.; Liebmann, J.; Born, M.; Merk, H.F.; von Felbert, V. Prospective Randomized Long-Term Study on the Efficacy and Safety of UV-Free Blue Light for Treating Mild Psoriasis Vulgaris. *Dermatology* **2015**, *231*, 24-34, doi:10.1159/000430495.
155. F C Menezes, P.; Requena, M.; Lizarelli, R.F.Z.; Bagnato, V. *Blue LED irradiation to hydration of skin*; 2015; Vol. 9531.
156. Adamskaya, N.; Dungel, P.; Mittermayr, R.; Hartinger, J.; Feichtinger, G.; Wassermann, K.; Redl, H.; van Griensven, M. Light therapy by blue LED improves wound healing in an excision model in rats. *Injury* **2011**, *42*, 917-921, doi:10.1016/j.injury.2010.03.023.
157. Dereci, O.; Sindel, A.; Serap Toru, H.; Yuce, E.; Ay, S.; Tozoglu, S. The Comparison of the Efficacy of Blue Light-Emitting Diode Light and 980-nm Low-Level Laser Light on Bone Regeneration. *J Craniofac Surg* **2016**, *27*, 2185-2189, doi:10.1097/SCS.0000000000003068.
158. Kuse, Y.; Ogawa, K.; Tsuruma, K.; Shimazawa, M.; Hara, H. Damage of photoreceptor-derived cells in culture induced by light emitting diode-derived blue light. *Scientific Reports* **2014**, *4*, 5223, doi:10.1038/srep05223

https://www.nature.com/articles/srep05223#supplementary-information.

159. Maclean, M.; Macgregor, S.J.; Anderson, J.G.; Woolsey, G.A.; Coia, J.E.; Hamilton, K.; Taggart, I.; Watson, S.B.; Thakker, B.; Gettinby, G. Environmental decontamination of a hospital isolation room using high-intensity narrow-spectrum light. *J Hosp Infect* **2010**, *76*, 247-251, doi:10.1016/j.jhin.2010.07.010.

160. Bache, S.E.; Maclean, M.; MacGregor, S.J.; Anderson, J.G.; Gettinby, G.; Coia, J.E.; Taggart, I. Clinical studies of the High-Intensity Narrow-Spectrum light Environmental Decontamination System (HINS-light EDS), for continuous disinfection in the burn unit inpatient and outpatient settings. *Burns* **2012**, *38*, 69-76, doi:10.1016/j.burns.2011.03.008.
161. Maclean, M.; Booth, M.; Anderson, J.; MacGregor, S.; Woolsey, G.; Coia, J.; Hamilton, K.; Gettinby, G. Continuous decontamination of an intensive care isolation room during patient occupancy using 405 nm light technology. *Journal of Infection Prevention* **2013**, *14*, 176-181, doi:10.1177/1757177413483646.
162. Endarko, E.; Maclean, M.; Timoshkin, I.V.; MacGregor, S.J.; Anderson, J.G. High-intensity 405 nm light inactivation of Listeria monocytogenes. *Photochem Photobiol* **2012**, *88*, 1280-1286, doi:10.1111/j.1751-1097.2012.01173.x.
163. Moorhead, S.; Maclean, M.; Coia, J.E.; MacGregor, S.J.; Anderson, J.G. Synergistic efficacy of 405 nm light and chlorinated disinfectants for the enhanced decontamination of Clostridium difficile spores. *Anaerobe* **2016**, *37*, 72-77, doi:10.1016/j.anaerobe.2015.12.006.
164. Michelle Maclean, S.M., John Anderson, Gerry Woolsey. HINS-light Environmental Decontamination System: A new method for pathogen control in the clinical envionment. In Proceedings of Infection Prevention Society 2010, Glasgow, 27/10/2010.
165. Ramakrishnan, P.; Maclean, M.; MacGregor, S.J.; Anderson, J.G.; Grant, M.H. Differential sensitivity of osteoblasts and bacterial pathogens to 405-nm light highlighting potential for decontamination applications in orthopedic surgery. *J Biomed Opt* **2014**, *19*, 105001, doi:10.1117/1.JBO.19.10.105001.

U.PORTO

FMUP FACULDADE DE MEDICINA
UNIVERSIDADE DO PORTO

Attachments

João Marcelo de Almeida Cabral

Light therapy in hospital infection control:
advantages, drawbacks and pitfalls.

Mestrado Integrado em Medicina

FMUP

Submission Checklist

Please.

1. read the Aims & Scope to gain an overview and assess if your manuscript is suitable for this journal;
2. use the Microsoft Word template or LaTeX template to prepare your manuscript;
3. make sure that issues about publication ethics, research ethics, copyright, authorship, figure formats, dataand references format have been appropriately considered;
4. ensure that all authors have approved the content of the submitted manuscript.

Manuscript Submission Overview

Types of Publications

Antibiotics has no restrictions on the length of manuscripts, provided that the text is concise and comprehensive. Full experimental details must be provided so that the results can be reproduced. *Antibiotics*requires that authors publish all experimental controls and make full datasets available where possible (see the guidelines on Supplementary Materials and references to unpublished data).

Manuscripts submitted to *Antibiotics* should neither been published before nor be under consideration for publication in another journal. The main article types are as follows:

- *Articles:* Original research manuscripts. The journal considers all original research manuscripts provided that the work reports scientifically sound experiments and provides a substantial amount of new information. Authors should not unnecessarily divide their work into several related manuscripts, although Short *Communications* of preliminary, but significant, results will be considered. Quality and impact of the study will be considered during peer review.
- *Reviews:* These provide concise and precise updates on the latest progress made in a given area of research. Systematic reviews should follow the PRISMA guidelines.
- *Case reports:* Case reports present detailed information on the symptoms, signs, diagnosis, treatment (including all types of interventions), and outcomes of an individual patient. Case reports usually describe new or uncommon conditions that serve to enhance medical care or highlight diagnostic approaches.

Submission Process

Manuscripts for *Antibiotics* should be submitted online at susy.mdpi.com The submitting author, who is generally the corresponding author, is responsible for the manuscript during the submission and peer-review process. The submitting author must ensure that all eligible co-authors have been included in the author list (read the criteria to qualify for authorship) and that they have all read and approved the submitted version of the manuscript. To submit your manuscript, register and log in to the submission website. Once you have registered, go to the submission form for *Antibiotics*. All co-authors can see the manuscript details in the submission system, if they register and log in using the e-mail address provided during manuscript submission.

Accepted File Formats

Authors must use the Microsoft Word template or LaTeX template to prepare their manuscript. Using the template file will substantially shorten the time to complete copy-editing and publication of accepted manuscripts. The total amount of data for all files must not exceed 120 MB. If this is a problem, please contact the editorial office antibiotics@mdpi.com. Accepted file formats are:

- *Microsoft Word:* Manuscripts prepared in Microsoft Word must be converted into a single file before submission. When preparing manuscripts in Microsoft Word, the *Antibiotics* Microsoft Word template file must be used. Please insert your graphics (schemes, figures, *etc.*) in the main text after the paragraph of its first citation.
- *LaTeX:* Manuscripts prepared in LaTeX must be collated into one ZIP folder (include all source files and images, so that the Editorial Office can recompile the submitted PDF). When preparing manuscripts in LaTeX, please use the *Antibiotics* LaTeX template files. You can now also use the online application writeLaTeX to submit articles directly to *Antibiotics*. The MDPI LaTeX template file should be selected from the writeLaTeX template gallery.
- *Supplementary files:* May be any format, but it is recommended that you use common, non-proprietary formats where possible (see below for further details).

Cover Letter

A cover letter must be included with each manuscript submission. It should be concise and explain why the content of the paper is significant, placing the findings in the context of existing work and why it fits the scope of the journal. Confirm that neither the manuscript nor any parts of its content are currently under consideration or published in another journal. Any prior submissions of the manuscript to MDPI journals must be acknowledged. The names of proposed and excluded reviewers should be provided in the submission system, not in the cover letter.

Note for Authors Funded by the National Institutes of Health (NIH)

This journal automatically deposits papers to PubMed Central after publication of an issue. Authors do not need to separately submit their papers through the NIH Manuscript Submission System (NIHMS, http://nihms.nih.gov/).

Manuscript Preparation

General Considerations

- **Research manuscripts** should comprise:
    - Front matter: Title, Author list, Affiliations, Abstract, Keywords
    - Research manuscript sections: Introduction, Results, Discussion, Materials and Methods, Conclusions (optional).
    - Back matter: Supplementary Materials, Acknowledgments, Author Contributions, Conflicts of Interest, References.
- **Review manuscripts** should comprise the front matter, literature review sections and the back matter. The template file can also be used to prepare the front and back matter

of your review manuscript. It is not necessary to follow the remaining structure. Structured reviews and meta-analyses should use the same structure as research articles and ensure they conform to the PRISMA guidelines.

- **Case reports** should include a succinct introduction about the general medical condition or relevant symptoms that will be discussed in the case report; the case presentation including all of the relevant de-identified demographic and descriptive information about the patient(s), and a description of the symptoms, diagnosis, treatment, and outcome; a discussion providing context and any necessary explanation of specific treatment decisions; a conclusion briefly outlining the take-home message and the lessons learned.
- **Graphical abstract:** Authors are encouraged to provide a graphical abstract as a self-explanatory image to appear alongside with the text abstract in the Table of Contents. Figures should be a high quality image in any common image format. Note that images displayed online will be up to 11 by 9 cm on screen and the figure should be clear at this size.
- **Abbreviations** should be defined in parentheses the first time they appear in the abstract, main text, and in figure or table captions and used consistently thereafter.
- **SI Units** (International System of Units) should be used. Imperial, US customary and other units should be converted to SI units whenever possible
- **Accession numbers** of RNA, DNA and protein sequences used in the manuscript should be provided in the Materials and Methods section. Also see the section on Deposition of Sequences and of Expression Data.
- **Equations:** If you are using Word, please use either the Microsoft Equation Editor or the MathType add-on. Equations should be editable by the editorial office and not appear in a picture format.
- **Research Data and supplementary materials:** Note that publication of your manuscript implies that you must make all materials, data, and protocols associated with the publication available to readers. Disclose at the submission stage any restrictions on the availability of materials or information. Read the information about Supplementary Materials and Data Deposit for additional guidelines.
- **Preregistration:** Where authors have preregistered studies or analysis plans, links to the preregistration must be provided in the manuscript.
- **Guidelines and standards:** MDPI follows standards and guidelines for certain types of research. See https://www.mdpi.com/editorial_process for further information.

Front Matter

These sections should appear in all manuscript types

- **Title:** The title of your manuscript should be concise, specific and relevant. It should identify if the study reports (human or animal) trial data, or is a systematic review, meta-analysis or replication study. When gene or protein names are included, the abbreviated name rather than full name should be used.
- **Author List and Affiliations:** Authors' full first and last names must be provided. The initials of any middle names can be added. The PubMed/MEDLINE standard format is used for affiliations: complete address information including city, zip code, state/province, country, and all email addresses. At least one author should be designated as corresponding author, and his or her email address and other details

should be included at the end of the affiliation section. Please read the criteria to qualify for authorship.

- **Abstract:** The abstract should be a total of about 200 words maximum. The abstract should be a single paragraph and should follow the style of structured abstracts, but without headings: 1) Background: Place the question addressed in a broad context and highlight the purpose of the study; 2) Methods: Describe briefly the main methods or treatments applied. Include any relevant preregistration numbers, and species and strains of any animals used. 3) Results: Summarize the article's main findings; and 4) Conclusion: Indicate the main conclusions or interpretations. The abstract should be an objective representation of the article: it must not contain results which are not presented and substantiated in the main text and should not exaggerate the main conclusions.
- **Keywords:** Three to ten pertinent keywords need to be added after the abstract. We recommend that the keywords are specific to the article, yet reasonably common within the subject discipline.

Research Manuscript Sections

- **Introduction:** The introduction should briefly place the study in a broad context and highlight why it is important. It should define the purpose of the work and its significance, including specific hypotheses being tested. The current state of the research field should be reviewed carefully and key publications cited. Please highlight controversial and diverging hypotheses when necessary. Finally, briefly mention the main aim of the work and highlight the main conclusions. Keep the introduction comprehensible to scientists working outside the topic of the paper.
- **Results:** Provide a concise and precise description of the experimental results, their interpretation as well as the experimental conclusions that can be drawn.
- **Discussion:** Authors should discuss the results and how they can be interpreted in perspective of previous studies and of the working hypotheses. The findings and their implications should be discussed in the broadest context possible and limitations of the work highlighted. Future research directions may also be mentioned. This section may be combined with Results.
- **Materials and Methods:** They should be described with sufficient detail to allow others to replicate and build on published results. New methods and protocols should be described in detail while well-established methods can be briefly described and appropriately cited. Give the name and version of any software used and make clear whether computer code used is available. Include any pre-registration codes.
- **Conclusions:** This section is not mandatory, but can be added to the manuscript if the discussion is unusually long or complex.
- **Patents:** This section is not mandatory, but may be added if there are patents resulting from the work reported in this manuscript.

Back Matter

- **Supplementary Materials:** Describe any supplementary material published online alongside the manuscript (figure, tables, video, spreadsheets, etc.). Please indicate the name and title of each element as follows Figure S1: title, Table S1: title, etc.
- **Acknowledgments:** All sources of funding of the study should be disclosed. Clearly indicate grants that you have received in support of your research work and if you received funds to cover publication costs. Note that some funders will not refund article

processing charges (APC) if the funder and grant number are not clearly and correctly identified in the paper. Funding information can be entered separately into the submission system by the authors during submission of their manuscript. Such funding information, if available, will be deposited to FundRef if the manuscript is finally published.

- **Author Contributions:** Each author is expected to have made substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data; or the creation of new software used in the work; or have drafted the work or substantively revised it; AND has approved the submitted version (and version substantially edited by journal staff that involves the author's contribution to the study); AND agrees to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature.
  For research articles with several authors, a short paragraph specifying their individual contributions must be provided. The following statements should be used "Conceptualization, X.X. and Y.Y.; Methodology, X.X.; Software, X.X.; Validation, X.X., Y.Y. and Z.Z.; Formal Analysis, X.X.; Investigation, X.X.; Resources, X.X.; Data Curation, X.X.; Writing – Original Draft Preparation, X.X.; Writing – Review & Editing, X.X.; Visualization, X.X.; Supervision, X.X.; Project Administration, X.X.; Funding Acquisition, Y.Y.", please turn to the CRediT taxonomy for the term explanation. For more background on CRediT, see here. "**Authorship must include and be limited to those who have contributed substantially to the work. Please read the section concerning the criteria to qualify for authorship carefully**".
- **Conflicts of Interest:** Authors must identify and declare any personal circumstances or interest that may be perceived as inappropriately influencing the representation or interpretation of reported research results. If there is no conflict of interest, please state "The authors declare no conflict of interest." Any role of the funding sponsors in the choice of research project; design of the study; in the collection, analyses or interpretation of data; in the writing of the manuscript; or in the decision to publish the results must be declared in this section. *Antibiotics* does not publish studies funded by the tobacco industry. Any projects funded by pharmaceutical or food industries must pay special attention to the full declaration of funder involvement. If there is no role, please state "The sponsors had no role in the design, execution, interpretation, or writing of the study".
- **References:** References must be numbered in order of appearance in the text (including table captions and figure legends) and listed individually at the end of the manuscript. We recommend preparing the references with a bibliography software package, such as EndNote, ReferenceManager or Zotero to avoid typing mistakes and duplicated references. We encourage citations to data, computer code and other citable research material. If available online, you may use reference style 9. below.
- Citations and References in Supplementary files are permitted provided that they also appear in the main text and in the reference list.

In the text, reference numbers should be placed in square brackets [ ], and placed before the punctuation; for example [1], [1–3] or [1,3]. For embedded citations in the text with pagination, use both parentheses and brackets to indicate the reference number and page numbers; for example [5] (p. 10). or [6] (pp. 101–105).

The reference list should include the full title, as recommended by the ACS style guide. Style files for Endnoteand Zotero are available.

References should be described as follows, depending on the type of work:

- Journal Articles:

1. Author 1, A.B.; Author 2, C.D. Title of the article. *Abbreviated Journal Name* **Year**, *Volume*, page range. Available online: URL (accessed on Day Month Year).

- Books and Book Chapters:

2. Author 1, A.; Author 2, B. *Book Title*, 3rd ed.; Publisher: Publisher Location, Country, Year; pp. 154–196.
3. Author 1, A.; Author 2, B. Title of the chapter. In *Book Title*, 2nd ed.; Editor 1, A., Editor 2, B., Eds.; Publisher: Publisher Location, Country, Year; Volume 3, pp. 154–196.

- Unpublished work, submitted work, personal communication:

4. Author 1, A.B.; Author 2, C. Title of Unpublished Work. status (unpublished; manuscript in preparation).
5. Author 1, A.B.; Author 2, C. Title of Unpublished Work. *Abbreviated Journal Name* stage of publication (under review; accepted; in press).
6. Author 1, A.B. (University, City, State, Country); Author 2, C. (Institute, City, State, Country). Personal communication, Year.

- Conference Proceedings:

7. Author 1, A.B.; Author 2, C.D.; Author 3, E.F. Title of Presentation. In *Title of the Collected Work* (if available), Proceedings of the Name of the Conference, Location of Conference, Country, Date of Conference; Editor 1, Editor 2, Eds. (if available); Publisher: City, Country, Year (if available); Abstract Number (optional), Pagination (optional).

- Thesis:

8. Author 1, A.B. Title of Thesis. Level of Thesis, Degree-Granting University, Location of University, Date of Completion.

- Websites:

9. Title of Site. Available online: URL (accessed on Day Month Year).

Unlike published works, websites may change over time or disappear, so we encourage you create an archive of the cited website using a service such as WebCite. Archived websites should be cited using the link provided as follows:

10. Title of Site. URL (archived on Day Month Year).

See the Reference List and Citations Guide for more detailed information.

Preparing Figures, Schemes and Tables

- File for Figures and schemes must be provided during submission in a single zip archive and at a sufficiently high resolution (minimum 1000 pixels width/height, or a resolution of 300 dpi or higher). Common formats are accepted, however, TIFF, JPEG, EPS and PDF are preferred.
- *Antibiotics* can publish multimedia files in articles or as supplementary materials. Please contact the editorial office for further information.
- All Figures, Schemes and Tables should be inserted into the main text close to their first citation and must be numbered following their number of appearance (Figure 1, Scheme I, Figure 2, Scheme II, Table 1, *etc.*).
- All Figures, Schemes and Tables should have a short explanatory title and caption.

- All table columns should have an explanatory heading. To facilitate the copy-editing of larger tables, smaller fonts may be used, but no less than 8 pt. in size. Authors should use the Table option of Microsoft Word to create tables.
- Authors are encouraged to prepare figures and schemes in color (RGB at 8-bit per channel). There is no additional cost for publishing full color graphics.

Supplementary Materials, Data Deposit and Software Source Code

Data Availability

In order to maintain the integrity, transparency and reproducibility of research records, authors must make their experimental and research data openly available either by depositing into data repositories or by publishing the data and files as supplementary information in this journal.

Computer Code and Software

For work where novel computer code was developed, authors should release the code either by depositing in a recognized, public repository or uploading as supplementary information to the publication. The name and version of all software used should be clearly indicated.

Supplementary Material

Additional data and files can be uploaded as "Supplementary Files" during the manuscript submission process. The supplementary files will also be available to the referees as part of the peer-review process. Any file format is acceptable, however we recommend that common, non-proprietary formats are used where possible.

Unpublished Data

Restrictions on data availability should be noted during submission and in the manuscript. "Data not shown" should be avoided: authors are encouraged to publish all observations related to the submitted manuscript as Supplementary Material. "Unpublished data" intended for publication in a manuscript that is either planned, "in preparation" or "submitted" but not yet accepted, should be cited in the text and a reference should be added in the References section. "Personal Communication" should also be cited in the text and reference added in the References section. (see also the MDPI reference list and citations style guide).

Remote Hosting and Large Data Sets

Data may be deposited with specialized service providers or institutional/subject repositories, preferably those that use the DataCite mechanism. Large data sets and files greater than 60 MB must be deposited in this way. For a list of other repositories specialized in scientific and experimental data, please consult databib.org or re3data.org. The data repository name, link to the data set (URL) and accession number, doi or handle number of the data set must be provided in the paper. The journal Data also accepts submissions of data set papers.

Deposition of Sequences and of Expression Data

New sequence information must be deposited to the appropriate database prior to submission of the manuscript. Accession numbers provided by the database should be included in the submitted manuscript. Manuscripts will not be published until the accession number is provided.

- *New nucleic acid sequences* must be deposited in one of the following databases: GenBank, EMBL, or DDBJ. Sequences should be submitted to only one database.
- *New high throughput sequencing (HTS) datasets* (RNA-seq, ChIP-Seq, degradome analysis, …) must be deposited either in the GEO database or in the NCBI's Sequence Read Archive.
- *New microarray data* must be deposited either in the GEO or the ArrayExpress databases.The "Minimal Information About a Microarray Experiment" (MIAME) guidelines published by the Microarray Gene Expression Data Society must be followed.
- *New protein sequences* obtained by protein sequencing must be submitted to UniProt (submission tool SPIN).

All sequence names and the accession numbers provided by the databases should be provided in the Materials and Methods section of the article.

References in Supplementary Files

Citations and References in Supplementary files are permitted provided that they also appear in the reference list of the main text.

Research and Publication Ethics

Research Ethics

Research Involving Human Subjects

When reporting on research that involves human subjects, human material, human tissues, or human data, authors must declare that the investigations were carried out following the rules of the Declaration of Helsinki of 1975 (https://www.wma.net/what-we-do/medical-ethics/declaration-of-helsinki/), revised in 2013. According to point 23 of this declaration, an approval from an ethics committee should have been obtained before undertaking the research. At a minimum, a statement including the project identification code, date of approval and name of the ethics committee or institutional review board should be cited in the Methods Section of the article. Data relating to individual participants must be described in detail, but private information identifying participants need not be included unless the identifiable materials are of relevance to the research (for example, photographs of participants' faces that show a particular symptom). Editors reserve the right to reject any submission that does not meet these requirements.

Example of an ethical statement: "All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by the Ethics Committee of XXX (Project identification code)."

A written informed consent for publication must be obtained from participating patients who can be identified (including by the patients themselves). Patients' initials or other personal identifiers must not appear in any images. For manuscripts that include any case details, personal information, and/or images of patients, authors must obtain signed informed consent from patients (or their relatives/guardians) before submitting to an MDPI journal. Patient details must be anonymized as far as possible, e.g., do not mention specific age, ethnicity, or occupation where they are not relevant to the conclusions.

A template permission form is available to download. A blank version of the form used to obtain permission (without the patient names or signature) must be uploaded with your submission.

You may refer to our sample form and provide an appropriate form after consulting with your affiliated institution. Alternatively, you may provide a detailed justification of why informed consent is not necessary. For the purposes of publishing in MDPI journals, a consent, permission, or release form should include unlimited permission for publication in all formats (including print, electronic, and online), in sublicensed and reprinted versions (including translations and derived works), and in other works and products under open access license. To respect patients' and any other individual's privacy, please do not send signed forms. The journal reserves the right to ask authors to provide signed forms if necessary.

Ethical Guidelines for the Use of Animals in Research

The editors will require that the benefits potentially derived from any research causing harm to animals are significant in relation to any cost endured by animals, and that procedures followed are unlikely to cause offense to the majority of readers. Authors should particularly ensure that their research complies with the commonly-accepted '3Rs':

- Replacement of animals by alternatives wherever possible,
- Reduction in number of animals used, and
- Refinement of experimental conditions and procedures to minimize the harm to animals.

Any experimental work must also have been conducted in accordance with relevant national legislation on the use of animals for research. For further guidance authors should refer to the Code of Practice for the Housing and Care of Animals Used in Scientific Procedures [1].

Manuscripts containing original descriptions of research conducted in experimental animals must contain details of approval by a properly constituted research ethics committee. As a minimum, the project identification code, date of approval and name of the ethics committee or institutional review board should be cited in the Methods section.

Antibiotics endorses the ARRIVE guidelines (www.nc3rs.org.uk/ARRIVE) for reporting experiments using live animals. Authors and reviewers can use the ARRIVE guidelines as a checklist, which can be found at www.nc3rs.org.uk/ARRIVEchecklist.

1. Home Office. Animals (Scientific Procedures) Act 1986. Code of Practice for the Housing and Care of Animals Used in Scientific Procedures. Available online: http://www.official-documents.gov.uk/document/hc8889/hc01/0107/0107.pdf.

Research Involving Cell Lines

Methods sections for submissions reporting on research with cell lines should state the origin of any cell lines. For established cell lines the provenance should be stated and references must also be given to either a published paper or to a commercial source. If previously unpublished *de novo* cell lines were used, including those gifted from another laboratory, details of institutional review board or ethics committee approval must be given, and confirmation of written informed consent must be provided if the line is of human origin.

An example of Ethical Statements:

The HCT116 cell line was obtained from XXXX. The $MLH1^{+}$ cell line was provided by XXXXX, Ltd. The DLD-1 cell line was obtained from Dr. XXXX. The DR-GFP and SA-GFP reporter plasmids were obtained from Dr. XXX and the Rad51K133A expression vector was obtained from Dr. XXXX.

Research Involving Plants

Experimental research on plants (either cultivated or wild) including collection of plant material, must comply with institutional, national, or international guidelines. We recommend that authors comply with the Convention on Biological Diversity and the Convention on the Trade in Endangered Species of Wild Fauna and Flora.

For each submitted manuscript supporting genetic information and origin must be provided. For research manuscripts involving rare and non-model plants (other than, e.g., *Arabidopsis thaliana, Nicotiana benthamiana, Oriza sativa*, or many other typical model plants), voucher specimens must be deposited in an accessible herbarium or museum. Vouchers may be requested for review by future investigators to verify the identity of the material used in the study (especially if taxonomic rearrangements occur in the future). They should include details of the populations sampled on the site of collection (GPS coordinates), date of collection, and document the part(s) used in the study where appropriate. For rare, threatened or endangered species this can be waived but it is necessary for the author to describe this in the cover letter.

Editors reserve the rights to reject any submission that does not meet these requirements.

An example of Ethical Statements:

Torenia fournieri plants were used in this study. White-flowered Crown White (CrW) and violet-flowered Crown Violet (CrV) cultivars selected from 'Crown Mix' (XXX Company, City, Country) were kindly provided by Dr. XXX (XXX Institute, City, Country).

Arabidopis mutant lines (SALKxxxx, SAILxxxx,…) were kindly provided by Dr. XXX , institute, city, country).

Publication Ethics Statement

Antibiotics is a member of the Committee on Publication Ethics (COPE). We fully adhere to its Code of Conduct and to its Best Practice Guidelines.

The editors of this journal enforce a rigorous peer-review process together with strict ethical policies and standards to ensure to add high quality scientific works to the field of scholarly publication. Unfortunately, cases of plagiarism, data falsification, image manipulation, inappropriate authorship credit, and the like, do arise. The editors of *Antibiotics* take such publishing ethics issues very seriously and are trained to proceed in such cases with a zero tolerance policy.

Authors wishing to publish their papers in *Antibiotics* must abide to the following:

- Any facts that might be perceived as a possible conflict of interest of the author(s) must be disclosed in the paper prior to submission.
- Authors should accurately present their research findings and include an objective discussion of the significance of their findings.
- Data and methods used in the research need to be presented in sufficient detail in the paper, so that other researchers can replicate the work.
- Raw data should preferably be publicly deposited by the authors before submission of their manuscript. Authors need to at least have the raw data readily available for presentation to the referees and the editors of the journal, if requested. Authors need to ensure appropriate measures are taken so that raw data is retained in full for a reasonable time after publication.
- Simultaneous submission of manuscripts to more than one journal is not tolerated.
- Republishing content that is not novel is not tolerated (for example, an English translation of a paper that is already published in another language will not be accepted).
- If errors and inaccuracies are found by the authors after publication of their paper, they need to be promptly communicated to the editors of this journal so that appropriate actions can be taken. Please refer to our policy regarding publication of publishing addenda and corrections.
- Your manuscript should not contain any information that has already been published. If you include already published figures or images, please obtain the necessary permission from the copyright holder to publish under the CC-BY license. For further information, see the Rights and Permissions page.
- Plagiarism, data fabrication and image manipulation are not tolerated.
    - **Plagiarism is not acceptable** in *Antibiotics* submissions.

      Plagiarism includes copying text, ideas, images, or data from another source, even from your own publications, without giving any credit to the original source.

      Reuse of text that is copied from another source must be between quotes and the original source must be cited. If a study's design or the manuscript's structure or language has been inspired by previous works, these works must be explicitly cited.

      If plagiarism is detected during the peer review process, the manuscript may be rejected. If plagiarism is detected after publication, we may publish a correction or retract the paper.

- **Image files must not be manipulated or adjusted in any way** that could lead to misinterpretation of the information provided by the original image.

  Irregular manipulation includes: 1) introduction, enhancement, moving, or removing features from the original image; 2) grouping of images that should obviously be presented separately (e.g., from different parts of the same gel, or from different gels); or 3) modifying the contrast, brightness or color balance to obscure, eliminate or enhance some information.

  If irregular image manipulation is identified and confirmed during the peer review process, we may reject the manuscript. If irregular image manipulation is identified and confirmed after publication, we may correct or retract the paper.

Our in-house editors will investigate any allegations of publication misconduct and may contact the authors' institutions or funders if necessary. If evidence of misconduct is found, appropriate action will be taken to correct or retract the publication. Authors are expected to comply with the best ethical publication practices when publishing with MDPI.

Reviewer Suggestions

During the submission process, please suggest three potential reviewers with the appropriate expertise to review the manuscript. The editors will not necessarily approach these referees. Please provide detailed contact information (address, homepage, phone, e-mail address). The proposed referees should neither be current collaborators of the co-authors nor have published with any of the co-authors of the manuscript within the last five years. Proposed reviewers should be from different institutions to the authors. You may identify appropriate Editorial Board members of the journal as potential reviewers. You may suggest reviewers from among the authors that you frequently cite in your paper.

English Corrections

To facilitate proper peer-reviewing of your manuscript, it is essential that it is submitted in grammatically correct English. Advice on some specific language points can be found.

If you are not a native English speaker, we recommend that you have your manuscript professionally edited before submission or read by a native English-speaking colleague. This can be carried out by MDPI's English editing service. Professional editing will enable reviewers and future readers to more easily read and assess the content of submitted manuscripts. All accepted manuscripts undergo language editing, however **an additional fee will be charged** to authors if very extensive English corrections must be made by the Editorial Office: pricing is according to the service.

Preprints and Conference Papers

Antibiotics accepts articles that have previously been made available as preprints provided that they have not undergone peer review. A preprint is a draft version of a paper made available online before submission to a journal.

MDPI operates *Preprints*, a preprint server to which submitted papers can be uploaded directly after completing journal submission. Note that *Preprints* operates independently of the journal and posting a preprint does not affect the peer review process. Check the *Preprints* instructions for authors for further information.

Expanded and high quality conference papers can be considered as articles if they fulfil the following requirements: (1) the paper should be expanded to the size of a research article; (2) the conference paper should be cited and noted on the first page of the paper; (3) if the authors do not hold the copyright of the published conference paper, authors should seek the appropriate permission from the copyright holder; (4) authors are asked to disclose that it is conference paper in their cover letter and include a statement on what has been changed compared to the original conference paper. *Antibiotics* does not publish pilot studies or studies with inadequate statistical power.

Qualification for Authorship

Each author is expected to have made substantial contributions to the conception or design of the work; acquisition, analysis, or interpretation of data; the creation of new software used in the work; and/or writing or substantively revising the manuscript. In addition, all authors must have approved the submitted version (and any substantially modified version that involves the author's contribution to the study); AND agrees to be personally accountable for the author's own contributions and for ensuring that questions related to the accuracy or integrity of any part of the work, even those in which the author was not personally involved, are appropriately investigated, resolved, and documented in the literature. Note that acquisition of funding, collection of data, or general supervision of the research group do not, by themselves, justify authorship. Those who contributed to the work but do not qualify for authorship should be listed in the acknowledgements.

More detailed guidance on authorship is given by the International Council of Medical Journal Editors (ICMJE).The journal also adheres to the standards of the Committee on Publication Ethics (COPE) that "all authors should agree to be listed and should approve the submitted and accepted versions of the publication. Any change to the author list should be approved by all authors including any who have been removed from the list. The corresponding author should act as a point of contact between the editor and the other authors and should keep co-authors informed and involve them in major decisions about the publication (e.g. answering reviewers' comments)." [1]. We reserve the right to request confirmation that all authors meet the authorship conditions.

1. Wager, E.; Kleinert, S. Responsible research publication: international standards for authors. A position statement developed at the 2nd World Conference on Research Integrity, Singapore, July 22-24, 2010. In Promoting Research Integrity in a Global

Environment; Mayer, T., Steneck, N., eds.; Imperial College Press / World Scientific Publishing: Singapore; Chapter 50, pp. 309-16.

Editorial Procedures and Peer-Review

Initial Checks

All submitted manuscripts received by the Editorial Office will be checked by a professional in-house *Managing Editor* to determine whether they are properly prepared and whether they follow the ethical policies of the journal, including those for human and animal experimentation. Manuscripts that do not fit the journal's ethics policy or do not meet the standards of the journal will be rejected before peer-review. Manuscripts that are not properly prepared will be returned to the authors for revision and resubmission. After these checks, the *Managing Editor* will consult the journals' *Editor-in-Chief* or *Associate Editors* to determine whether the manuscript fits the scope of the journal and whether it is scientifically sound. No judgment on the potential impact of the work will be made at this stage. Reject decisions at this stage will be verified by the *Editor-in-Chief*.

Peer-Review

Once a manuscript passes the initial checks, it will be assigned to at least two independent experts for peer-review. A single-blind review is applied, where authors' identities are known to reviewers. Peer review comments are confidential and will only be disclosed with the express agreement of the reviewer.

In the case of regular submissions, in-house assistant editors will invite experts, including recommendations by an academic editor. These experts may also include *Editorial Board members* and Guest Editors of the journal. Potential reviewers suggested by the authors may also be considered. Reviewers should not have published with any of the co-authors during the past five years and should not currently work or collaborate with any of the institutions of the co-authors of the submitted manuscript.

Optional Open Peer-Review

The journal operates optional open peer-review: *Authors are given the option for all review reports and editorial decisions to be published alongside their manuscript. In addition, reviewers can sign their review, i.e., identify themselves in the published review reports.* Authors can alter their choice for open review at any time before publication, however once the paper has been published changes will only be made at the discretion of the *Publisher* and *Editor-in-Chief*. We encourage authors to take advantage of this opportunity as proof of the rigorous process employed in publishing their research. To guarantee an impartial refereeing the names of referees will be revealed only if the referees agree to do so, and after a paper has been accepted for publication.

Editorial Decision and Revision

All the articles, reviews and communications published in MDPI journals go through the peer-review process and receive at least two reviews. The in-house editor will communicate the decision of the academic editor, which will be one of the following:

- *Accept after Minor Revisions*:
  The paper is in principle accepted after revision based on the reviewer's comments. Authors are given five days for minor revisions.
- *Reconsider after Major Revisions*:
  The acceptance of the manuscript would depend on the revisions. The author needs to provide a point by point response or provide a rebuttal if some of the reviewer's comments cannot be revised. Usually, only one round of major revisions is allowed. Authors will be asked to resubmit the revised paper within a suitable time frame, and the revised version will be returned to the reviewer for further comments.
- *Reject and Encourage Resubmission*:
  If additional experiments are needed to support the conclusions, the manuscript will be rejected and the authors will be encouraged to re-submit the paper once further experiments have been conducted.
- *Reject*:
  The article has serious flaws, and/or makes no original significant contribution. No offer of resubmission to the journal is provided.

All reviewer comments should be responded to in a point-by-point fashion. Where the authors disagree with a reviewer, they must provide a clear response.

Author Appeals

Authors may appeal a rejection by sending an e-mail to the Editorial Office of the journal. The appeal must provide a detailed justification, including point-by-point responses to the reviewers' and/or Editor's comments. The *Managing Editor* of the journal will forward the manuscript and related information (including the identities of the referees) to the Editor-in-Chief, Associate Editor, or Editorial Board member. The academic Editor being consulted will be asked to give an advisory recommendation on the manuscript and may recommend acceptance, further peer-review, or uphold the original rejection decision. A reject decision at this stage is final and cannot be reversed.

In the case of a special issue, the *Managing Editor* of the journal will forward the manuscript and related information (including the identities of the referees) to the *Editor-in-Chief* who will be asked to give an advisory recommendation on the manuscript and may recommend acceptance, further peer-review, or uphold the original rejection decision. A reject decision at this stage will be final and cannot be reversed.

Production and Publication

Once accepted, the manuscript will undergo professional copy-editing, English editing, proofreading by the authors, final corrections, pagination, and, publication on the www.mdpi.com website.

Clinical Trials Registration

Registration

Authors are strongly encouraged to pre-register clinical trials with an international clinical trials register or and to cite a reference to the registration in the Methods section. Suitable databases include clinicaltrials.gov, the EU Clinical Trials Register and those listed by the World Health Organisation International Clinical Trials Registry Platform.

CONSORT Statement

Antibiotics requires a completed CONSORT 2010 checklist and flow diagram as a condition of submission when reporting the results of a randomized trial. Templates for these can be found here or on the CONSORT website (http://www.consort-statement.org) which also describes several CONSORT checklist extensions for different designs and types of data beyond two group parallel trials. At minimum, your article should report the content addressed by each item of the checklist. Meeting these basic reporting requirements will greatly improve the value of your trial report and may enhance its chances for eventual publication.