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Treatment of oral fungal infections using antimicrobial photodynamic therapy: A systematic review and meta-analysis

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ABSTRACT

Part A is a research protocol which describes the background and proposed methodology of this systematic review. This section contains the definitions used for “photodynamic therapy”, “conventional antifungal medication” and “oral fungal infections”. It also provides details of quantitative and qualitative methods used to analyse the effectiveness of photodynamic therapy (PDT) and conventional antifungal medications in the treatment of oral fungal infections.

Part B is a literature review which expands on the protocol. It provides an in-depth description of the epidemiology and pathogenesis of oral fungal infections. It also discusses currently available and future treatment strategies and the potential and shortcomings thereof. The importance of this research is highlighted by contextualising PDT as an alternative treatment modality to conventional antifungal medications.

Part C presents the research as a journal manuscript according to the Photodiagnosis and Photodynamic Therapy Journal’s instructions for authors. The manuscript includes a brief introduction to the research followed by a summary of the methods and presentation of the results which are then discussed.

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PART A: PROTOCOL

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ABSTRACT

Oral fungal infections have a significant impact on the quality of life of those infected. Treatment of oral fungal infections conventionally make use of topical or systemic antifungal medications. However, antimicrobial resistance has limited the effectiveness of these medications. Thus, alternative therapies are required to treat these infections. Photodynamic therapy (PDT) has been proposed as an alternative treatment modality in the treatment of oral fungal infections.

The aim of this study is to determine whether PDT, compared with standard anti-fungal treatment modalities, is effective in the treatment of oral fungal infections.

A systematic review will be conducted using currently available human studies comparing PDT to conventional antifungal medication in the treatment of oral fungal infections. The search will include all studies up until September 2018 with no limitations on language. The outcomes of interest will be clinical improvement coupled with microbiological confirmation via direct microscopy or cell cultures. Study selection will follow the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines and study quality will be assessed by using the risk of bias tools of the Cochrane Collaboration. If sufficient data is available, a meta-analysis will be conducted. The effect size will be estimated and reported from continuous variables using mean difference and 95% confidence intervals. The weighting of each study will be calculated using the inverse of the variance. A random effects model will be used. The Cochrane test (P <0.1 cut-off for statistical significance) will be used to determine statistical homogeneity. The I^2test will be used to quantify heterogeneity. Sensitivity and subgroup analyses will be conducted as specified. An assessment of reporting bias will be done using funnel plots.

1. BACKGROUND

Fungal infections in humans are a growing public health concern and there is evidence of an increasing incidence and prevalence of fungal infections worldwide. [1-3] While accurate estimates of the burden of fungal infections are not commonly available, especially from developing countries,[1, 2, 4, 5] more than 300 million people suffer from a variety of fungal infections annually. [1] The increasing burden of fungal infections, in part, has been due to the ever-increasing population of immune-compromised individuals. This includes AIDS patients and individuals infected with HIV, established and uncontrolled diabetes mellitus, cancer and transplant patients, those who are seriously ill, malnourished, on immunosuppressive therapy and other medical conditions which affect the immune response. [3]

Fungal infections are gaining significance as a major cause of morbidity and mortality.[6] Invasive fungal infections are a serious cause for concern and carry a high risk of mortality.[2] Mucocutaneous fungal infections, although rarely fatal, are very common infections in humans.[2] Both types of fungal infections are commonly overlooked and receive insufficient research attention and investment. [7] Some of the most common mucocutaneous fungal infections in humans affect the oral cavity and are especially common in the critically ill and immune-compromised.[8] Oral Fungal infections are also common in neonates, babies and denture-wearers. The most common organisms involved in oral fungal infections are the *Candida* species.[2, 8] While, for the large part, not being life-threatening, oral fungal infections have a significant impact on an individual's quality of life due to oral discomfort, burning, pain, dysgeusia (altered taste) and reduced appetite.[9] In those living with HIV/AIDS, there is an increased possibility of oral fungal infections progressing to oropharyngeal fungal infections and those affected are particularly at risk of it fulminating into disseminated fungal infections if the immune system is highly suppressed. HIV/AIDS is associated with 10 million additional cases of oral candidiasis and 2 million cases of oesophageal fungal infections per annum.[10]

Treatment for mucocutaneous fungal infections includes nystatin and topical azoles as the first line treatment, followed by systemic antifungal medication.[11] However, it has become well-recognized internationally that fungi are rapidly gaining resistance to current medication [12-14] due to various reasons: (a) Multiple daily doses of triazoles, polyenes, and echinocandins are required due to limited bioavailability [8], (b) Sampling and lab testing is not always possible for monitoring progress [1, 13], therefore patients often do not finish their course of medication, (c) Unpleasant and dangerous side effects as well as drug interactions [9] especially important for patients who are chronically ill or HIV-positive and taking multiple medications concurrently. (d) Inherent properties of oral fungi that make them particularly difficult to treat [8] a recent study in South Africa and Cameroon found that 50% of the *Candida albicans* specimens sampled were resistant to azoles. [13] New drugs to treat fungal infections have not been developed since 2006 [12] and thus, alternate therapies are required to treat these minimally invasive fungal infections without propagating the rise in fungal antimicrobial resistance.[15] Recently, the use of photodynamic therapy has gained traction as an antifungal treatment modality.

Photodynamic therapy (PDT), also referred to as photodynamic antimicrobial chemotherapy (PACT), photoradiation therapy and photochemotherapy, comprises three components: a chemical photosensitizer (PS), the application of light and the presence of oxygen. Briefly, the PS is applied to the target tissue (either topically or systemically), light of an appropriate wavelength is then used to activate the PS generating highly reactive oxygen species (ROS), including the singlet oxygen, in the target tissue (Figure 1). This results in cytotoxicity of the target cells and elicits an acute inflammatory response in the surrounding tissues.[16, 17]

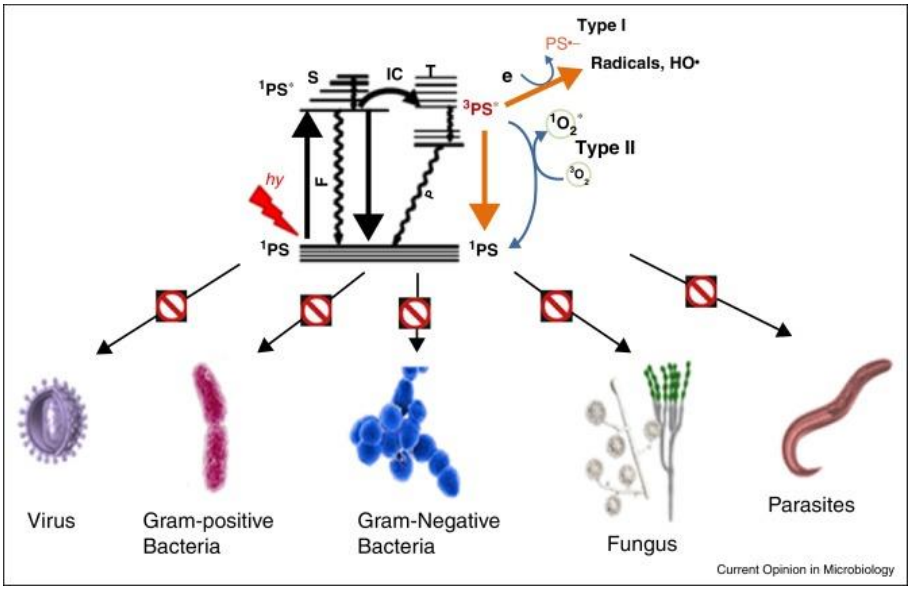

Figure 1 Jablonski Diagram illustrating the mechanism of action of Photodynamic therapy when used as antimicrobial therapy (Image used with permission of Hamblin[18])

Evidence of the clinical applications of PDT is growing, especially in the fields of oncology[16, 19] and dermatology.[20, 21] The American Society of Dermatological Surgery (ASDS) has recently published a consensus document recommending PDT as highly effective treatment modality for precancerous lesions, superficial nonmelanoma skin cancers, inflammatory acne vulgaris and others. [22]

The majority of studies on the treatment of microbial infections with PDT are in-vitro in nature. An earlier review in 2014 found PDT to be effective against *Candida* species in the experimental environment, with limited evidence for clinical efficacy [23]. More human studies are emerging, particularly from the oral health sector [24-27] with suggestions of PDT as a possible treatment for oral fungal infections.[24, 26, 27] However, recent studies have found PDT to be less effective in clinical and microbial resolution of specific oral fungal infections when compared with conventional antifungal medication.[25, 28] Given this equipoise, our proposed systematic review aims to review the available literature so as to provide the most current evidence on the use of PDT as a treatment option for oral fungal infections in humans.

2. THE REVIEW QUESTION

Is photodynamic therapy compared with standard anti-fungal treatment modalities, effective for the treatment of oral fungal infections in humans?

3. OBJECTIVES OF THE STUDY

3.1 Primary

The primary objective of the systematic review is to determine if photodynamic therapy (PDT), when compared with standard medication, is effective in the treatment of oral fungal infections in humans.

3.2 Secondary:

- What are the most effective treatment regimens of photodynamic therapy in the treatment of oral fungal infections? (Duration of treatment, number of visits and time between visits)
- Which photoactivators are the most effective in treatment of oral fungal infections?
- Which type of light delivery device and at which wavelength has been shown to be most effective for the treatment of oral fungal infections?

4. METHODS

This protocol has been compiled using the Preferred Reporting Items for Systematic reviews and Meta-Analyses for Protocols 2015 guidelines (PRISMA-P 2015)[29].

A literature search will be conducted to gather evidence for the systematic review. Studies will then be identified and screened for relevance and eligibility. Data will be extracted from selected studies and the studies will then be assessed for methodological quality and rigour. Evidence synthesis and statistical analysis will then be performed and a report will be written. The Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [30] will be conformed to. The protocol is registered with PROSPERO. PROSPERO registration at CRD42017076421.

4.1 *Inclusion Criteria of studies and participants*

Primary experimental and observational studies comparing the effect of treatment of oral fungal infections using photodynamic therapy (PDT) to treatment using systemic and topical antifungal treatment will be included. The search will be limited to human studies including adults over 18 years only. If participants have concomitant systemic conditions (e.g. HIV, diabetes mellitus), it will be required that the conditions be specified and accounted for in both control and treatment groups. The study designs will include but not be limited to randomized control trials, quasi-randomised control trials and cohort studies. The inclusion criteria has deliberately been left broad to attempt to find as many relevant articles which relate to the specified primary and secondary objectives as possible.

4.2 *Exclusion criteria*

All PDT must make use of a photosensitiser i.e. laser therapy alone will not be included. Treatment will need to be performed on the human subject. Case reports, case-series and letters to the editor will also be excluded.

4.3 *Target conditions*

Oral fungal infections are fungal infections affecting the oral mucosa. The most common type being oral candidiasis which is caused by commensal *Candida* species.[31] The most frequently isolated *Candida* species in oral lesions are *Candida albicans.* However, less common organisms may be the causative species, especially in immune compromised individuals. [31] These non-*albicans* species include *Candida parapsilosis, Candida glabrata, Candida tropicalis, Candida guillermondii* , *Candida krusei and Candida dubliniensis.* [32]

There are variations in the clinical presentation of candidal infections (Table 1). These can be broadly categorised into primary and secondary oral candidiasis.[32, 33] Primary oral candidiasis is confined to the oral and perioral tissues. Secondary oral candidiasis is oral candidiasis which is a manifestation of a systemic candidal infection. Furthermore, primary oral candidiasis can be subdivided into acute, chronic and *Candida*-associated lesions.[32, 33]

Pseudomembranous and erythematous oral candidiasis fall under acute primary candidiasis. There are four variations within chronic primary oral candidiasis i.e. hyperplastic, nodular, erythematous and plaque-like. *Candida*-associated lesions have a multifactorial aetiology and include denture stomatitis, angular cheilitis, median rhomboid glossitis and linear gingival erythema.[32, 33]

In this systematic review, all forms of oral fungal infections will be included as the "target condition".

Table 1: Classification of Oral Candidiasis as proposed by Samaranyake[32]

Primary Oral Candidiasis			**Secondary Oral Candidiasis**
Acute	**Chronic**	***Candida*-associated**	
Pseudomembranous	Hyperplastic	Denture stomatitis	Oral Candidiasis as a manifestation of systemic disease
Erythematous	Nodular Erythematous	Angular cheilitis Median rhomboid glossitis	
	Plaque-like	Linear gingival erythema	

4.4 Intervention

The use of any photodynamic therapy system which utilises a PS which is activated by a light delivery device, in the treatment of oral fungal infections on a human subject, will be included

4.5 Comparator

The comparator may be the use of topical or systemic anti-fungal medications.[31] Table 2 lists commonly used antifungal medication and dosages which are used for oral Candidiasis.

However, we will allow flexibility with the antifungal drugs used and dosages of the comparator as treatment regimens vary in different settings and for different patients. The treatment regimens will be noted and reported on. Clinical trials of new antifungal medications will not be included.

Table 2: Antifungal drugs used for oral Candidiasis in Adults (Adapted from Pienaar et al)[34]

Drug	Form	Dosage
Topical		
Amphotericin-B	Lozenge	10 000 iu dissolved slowly in the mouth 3-4 times a day for a minimum of 2 weeks
Nystatin	Cream	Apply to affected area twice a day
	Oral suspension	20ml 4 times a day; continue to use for several days post clinical resolution
	Pastille	Dissolve tablet in mouth 5 times a day
Clotrimazole	Solution	5ml 3-4 times a day for 2 weeks minimum
	Cream	Apply to affected area 2-3 times a day for 3-4 weeks
Miconazole	Oral gel	Apply to the affected area 3-4 times a day
	Cream	Apply twice a day and continue to use 10-14 days after lesions heal
Chlorhexidine gluconate	0,2% Mouthwash	10ml to be swirled in the mouth for 1 timed minute and then spat out
Gentian Violet	0,5% Aqueous solution	Paint on affected area(s) of mouth three times daily
Systemic		
Fluconazole	150mg Capsules	150mg stat or one 150mg capsule once a day for 2-3 weeks
Ketaconazole	200mg Tablets	One to two tablets twice a day with food for 2 weeks
Itraconazole	100mg Capsules	one capsule per day taken immediately after meals for 2 weeks
Posaconazole	Oral suspension	100 mg (2.5 mL) PO BID on Day 1, then 100 mg PO once a day for 13 days

4.6 Primary outcome measures

The primary objective is to determine if PDT is as effective in the treatment of oral fungal infections compared with standard antifungal medication. The effectiveness of therapy can be determined via clinical assessment coupled with microbiological confirmation via direct microscopy or cell cultures.

The presence or absence of *Candida* hyphae can be assessed and a change from hyphae present to absent would indicate improvement. Effectiveness can be quantified by determining fungal load via

Candida colony forming units per millilitre (CFU/mL)[26] with decreased fungal load indicating improvement in the condition. The speciation of *Candida* can be determined via colony characteristics, microscopic morphology, physiological or biomechanical characteristics, sero-diagnostic and molecular tests.[35] Speciation provides insights into the relative effectiveness of the treatment on different fungal strains. In addition, changes in *Candida* morphology can be assessed.[36] Different morphological forms, such as germ-tubes, pseudo-hyphae and mycelia, suggest changes in the virulence of the fungi.[37] A change from more virulent hyphae to less virulent yeasts and pseudo-hyphae also show improvement in the infection with the demonstration of pseudo-hyphae and yeast cells being an important method of distinguishing between normal colonisation and infection.[35]

4.7 *Secondary outcome measures*

The secondary objectives relate to parameters of the PDT. The various light delivery devices, wavelengths used, photosensitisers and treatment regimens will be compared to determine which is most effective in antifungal ability. The same measures of effectiveness will be used as to determine the primary objective.

4.8 *Time Frame and Language*

No restrictions will be placed on date or language in the electronic database search.

4.9 *Information Sources and Search Strategy*

An electronic search of several databases and journals will be conducted using a planned search strategy (Table 3). The following databases will be searched: The Cochrane Library, BioMed, EBSCOhost, PubMed/MEDLINE, EMBASE, ISI Web of Science, Clinicaltrials.gov, ProQuest and WorldCat.

Each electronic database will require a tailored keyword/ MeSH term search. The results of the search will be documented, reported and compared between databases.

Where articles are found to be relevant, the reference lists will be hand-searched to backward track for additional relevant articles. Forward tracking will be conducted using the search term "photodynamic therapy oral fungal infections" with Google Scholar, ISI Citation Indices, and Web of Science ISI proceedings (conference proceedings).

Unpublished and ongoing studies will also be sought from online registers. These include NIH Health Services Research Projects in Progress, the ISRCTN registry, The UK Trials Gateway, the WHO International Clinical Trials Platform (ICTRP). Where studies are found to be potentially relevant and full-text articles cannot be found, the authors will be contacted to gain more information and determine if the study should be included.

Table: 3 Proposed Search Strategy for photodynamic therapy for oral fungal infections

Database	**PubMed**	
Date	**4/11/2017**	
Limits	**None**	
No	**Search**	**Result**
1	PHOTOTHERAPY[MESH]	34250
2	PHOTODYNAMIC THERAP*[Title/Abstract]	15548
3	#1 OR #2	38967
4	CANDID*[Title/Abstract]	314251
5	FUNGAL INFECT*[Title/Abstract]	18417
6	MYCOSES[MESH]	116485
7	DENTURE STOMATITIS[Other Term]	61
8	#4 OR #5 OR #6 OR#7	411274
9	(ORAL[Title/Abstract]) OR DENTAL[Title/Abstract]	682360
10	#8 AND #9	20147
11	#3 AND #10	90

5. DATA COLLECTION AND ANALYSIS

5.1 Study Selection

Articles will be screened in two stages i.e. initially by title and abstract screening and then full-text screening. Two reviewers will conduct the screening and data extraction independently and any failure of consensus will be resolved by a senior third party. Reasons for exclusion of studies will be provided in the report.

5.2 Data Extraction and Management

Data extraction will be conducted by each reviewer independently. An electronic data extraction form will be custom-made for this review, piloted and amended as required. The data collection form will include the following information:

- Author(s), year of publication, journal
- Methods
    - study design, study duration
    - allocation sequence concealment, blinding, other concerns about bias
- Study setting
- Study population
- Participants
    - recruitment procedures
    - baseline characteristics, demographics
    - medical conditions, current medication used, previous medication used
    - dental prosthesis
- Target conditions
    - Type of oral fungal infection, species involved
    - History of infection, previous treatments for condition
    - Method of determining type and presence of infection

- Interventions:
    - Photodynamic therapy (PDT): type of light delivery device, wavelength, photosensitizer, number of applications, time period between applications, length of time of application
    - Any adjunctive treatments
- Comparator
    - Medication type, dosage and duration of treatment
    - Adjunctive treatment
- Outcome
    - Clinical parameters- how clinical change was determined, whether there is a clinical change
    - Microbial parameters- Which clinical and laboratory procedures were utilise

      Speciation, fungal load, fungal morphology
- Presence of missing/ unavailable data
- Results
    - Comparison of before-after fungal loads, fungal species and fungal morphology
    - Assessment of clinical change via scales (e.g. Newton scale for denture stomatitis) or qualitatively via descriptive means

5.3 Study Quality and Risk of Bias Assessment

Each reviewer will conduct an assessment of study quality and the risk of bias of each included study. This will be performed using the risk of bias tools of the Cochrane Collaboration. [38] These assessments will be summarized in a "summary of findings" table and included in the report. In order to determine the extent of the risk of bias for the body of evidence, the Grading of Recommendations Assessment, Development and Evaluation (GRADE) will be used.[39]

5.4 *Statistical Analysis and Data Synthesis*

Where it is possible to extract quantitative data from the studies, these results will be captured into Review Manager (RevMan) statistical software and pooled where appropriate to conduct a meta-analysis. The effect size will be estimated and reported from continuous variables using mean difference and 95% confidence intervals. The weighting of each study will be calculated using the inverse of the variance. We expect the studies will differ in the mixes of participants and in the implementation of interventions, with different effect sizes underlying different studies; thus, a random-effects model will be used for analysis [40]. This will provide a more conservative estimate of treatment effects. Where there are insufficient studies, a narrative report of the results will be done.

5.5 *Investigation of heterogeneity*

The Cochrane test (P <0.1 cut-off for statistical significance) will be used to determine statistical homogeneity. The I^2test will be used to quantify heterogeneity. The effect of patient characteristics such as age, smoking status, denture wearing and comorbidities will be investigated to determine the effect on the test's performance. The effect of the photodynamic therapy on various strains of fungi will be compared where possible. This will be achieved by including covariates into fitted models if sufficient data is available. Where insufficient data exists for quantitative analysis, results will be summarised narratively.

5.6 *Sensitivity Analysis*

In the sensitivity analysis, we assess whether the findings are robust to the decisions made in the process of obtaining them.[41] It is not always possible to pre-specify all factors requiring sensitivity analyses as the need to do these may become more apparent as idiosyncrasies of the studies are identified.[41]

Where possible, there will be an attempt to retrieve any pertinent missing information from the study authors. The effect of missing data will be assessed by testing various assumptions. This includes data from studies that could not be extracted and included in the review and studies which have missing participant data, including loss to follow up.

5.7 *Subgroup Analysis*

If there are sufficient data, we will use the different treatment parameters to conduct subgroup analyses. This includes a comparison of different light delivery devices and wavelengths. Different photosensitisers will be assessed as well as different treatment regimens i.e. duration of application, frequency of applications and time between applications. The various antifungal medications used will be compared. Furthermore, the effect of PDT on different fungal strains. Also the effect of comorbidities/ predisposing medical conditions such as HIV, diabetes mellitus, and dental prosthesis use.

5.8 *Assessment of Reporting Bias*

In order to assess risk of publication bias, we will make use of funnel plots to examine asymmetry, provided there are 10 or more studies included. When we find evidence of small study effects we will

consider publication bias as a possible explanation. A sensitivity analysis will be undertaken if plots suggest treatment effects may not be from a symmetric distribution.

6. DISCUSSION

With the emergence of antimicrobial resistance and the growing burden of disease due to fungal infections, alternative treatment modalities is needed to mitigate this growing problem. One such treatment which has garnered attention in various spheres including oncology, the treatment of bacterial infections and various dermatological conditions, is photodynamic therapy. [42]

The body of research around photodynamic therapy (PDT) is growing and several laboratory studies have shown that PDT is effective as an antifungal treatment. [43-46] There is, however, less consensus from the limited human studies available on PDT and oral fungal infections. Furthermore, there is even greater discordance regarding the best photoactivators, light sources, and wave-lengths which are both effective against the oral fungal infections and safe for use in humans.[23, 47]

If PDT is found to be a suitable treatment alternative, it has the potential to be a breakthrough in preventing over-use of currently available antimicrobial medication in the treatment of superficial oral fungal infections. This may reduce the rate of antimicrobial resistance in fungi and improve the quality of life of those affected with the condition. PDT has shown the potential to be effective against certain drug-resistant strains of micro-organisms[18, 47-49] and there is emerging evidence that microorganisms may have a reduced ability to become resistant to PDT.[48]

In addition, PDT was previously thought to be the forte of expensive laser devices, however, any source that is capable of emitting adsorbable energy within a particular range of wavelengths which is able to activate the photosensitisers and penetrate tissue as required, can be used for PDT. [50] In recent years, there has been an interest in developing cost-effective light delivery systems that can be used in low-resource settings - this includes light-emitting diode (LED) battery-operated devices [50-53] and the use of modified cell phones.[54] The previous focus has been oncology but there is potential for these devices to be adapted for other uses including the treatment of oral infections.

Therefore, it is important that the evidence be frequently evaluated to assess whether PDT is an effective treatment option and whether it requires further research investment. The systematic review by Javed *et al*[23] found that there is insufficient evidence to show the clinical effectiveness of PDT and the heterogeneity between studies was too great to conduct a meta-analysis. This systematic review will attempt to determine if there has been sufficient progress in research to more thoroughly fulfill the objectives of the review.

7. CONFLICT OF INTEREST

None to declare

8. FUNDING

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PART B: LITERATURE REVIEW

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1. *Oral fungal infections*

Fungi are free-living, eukaryotic organisms that occur as yeasts (round fungi), moulds (filamentous fungi), or a combination thereof (dimorphic fungi). [1] Various fungal infections (mycoses) affect the oral cavity, some superficial causing little problems to the affected individual, whereas others may be deep and disseminate. The latter may lead to serious morbidity and even mortality. [2]

The incidence of both superficial and invasive fungal infections are increasing. This is largely attributed to the increased prevalence of immune compromised individuals.[3, 4] Telles *et al*[2] provided a list of superficial and deep mycoses (fungal infections) effecting the oral and maxillofacial region (Table 1). Oral candidiasis is the most common oral fungal infection and is the focus of this review.

Table 1: Superficial and Deep Oral and Maxillofacial Fungal infections [2]

Superficial Mycoses		**Deep Mycoses**	
Candidiasis		Subcutaneous	
Hyperplastic	Stomatitis	Sporotrichiosis	Entomophthoromycosis
Erythematous	Median Rhomboid Glossitis	Lobomycosis	Chromomycosis
Angular Cheilitis	Cutaneous	Rhinosporidiosis	
Pseudomembranous	Pneumonia		
Deep Systemic Mycoses			
Histoplasmosis	Blastomycosis	Coccidioidomycosis	Paracoccidiodomycosis
cryptococcosis			
Deep Opportunistic			
Aspergillosis	Mucormycosis	Geotrichosis	Trichosporan
Penicillosis	Basisdiomycosis	Cephalosporiomycosis	Paecilomycosis
Alternariosis	Cercosporomycosis	Fusariomycosis	

2. *Candida Species*

Candida is a genus comprising more than 150 species of yeast [1], many of which are part of the commensal microbiota in humans. [5] At least 17 *Candida* species are known to cause disease in

humans. These species include: *Candida albicans, Candida glabrata, Candida dubliniensis, Candida guilliermondii, Candida krusei, Candida lusitaniae, Candida parapsilosis* and *Candida tropicalis.* [1, 6, 7] *Candida albicans* is responsible for the majority of mycoses in humans, although other species are increasingly being isolated. [6]

Candida species share similar characteristics. They are able to metabolize glucose in aerobic and anaerobic environments and require fixed carbon environmental sources for growth.[1] Candidal growth is also sensitive to temperature changes.[1] One of the most important features of *C. albicans* is its ability to exist in different morphological forms. It is either found as a unicellular budding yeast (blastospore), or in filamentous forms such as hyphae (mycelial) and pseudohyphae.[8] The morphological plasticity is an important virulence factor of *C. albicans* as the hyphal form is important for epithelial invasion and in the process of causing infection. [8] Another important virulence factor is the ability of *Candida* to adhere to cell walls, which is enabled by components of the fungal cell wall.[1]

3. *Predisposing factors to oral fungal infections*

Candida is usually a benign commensal organism causing no ill-effect in healthy individuals. However, an imbalance in the normal flora can lead to an overgrowth of these organisms and result in a fungal infection. [1] The most common fungal infections are of a superficial nature, affecting the skin and nails as well as the mucosa of the oral cavity and genital tracts.[4] Typically, this can occur via several mechanisms: (1) breakdown of the skin or mucosal barrier, (2) compromised host defences, (3) external factors affecting the host or (e.g. medication use), (4) a combination of the aforementioned.[2]

Both systemic and local factors can increase ones' likeliness to develop oral candidiasis. Oral mycoses is more common in neonates and infants due to their underdeveloped immune systems. Similarly, those with systemic conditions or on medication negatively affecting the immune response, are also at an increased risk. This includes those who are malnourished, those with diabetes, leukaemia and other malignancies, blood dyscrasias, transplant patients, patients undergoing chemo- or radiation

therapy and those on chronic steroid medications. [4, 9] One of the most prominent diseases directly effecting the immune response is HIV/AIDS. HIV/AIDS was estimated to contribute 10 million cases of oral candidiasis and 2 million cases of oesophageal mycoses per annum. [4, 10] The increasing use of highly active antiretroviral therapy (HAART) has reduced the incidence of opportunistic infections, including oral candidiasis. [11, 12]

Furthermore, there are several local factors which predispose to oral mycoses development: poor oral hygiene and denture wearing [13]; hyposalivation and a decreased pH of saliva. [14] Smoking is also associated with oral candidiasis, although though the exact mechanism of the pathogenesis remains unclear.[15]

4. *Pathogenesis of oral candidiasis*

There is a complex interplay between the host, fungi and the oral microenvironment. [16] For oral candidiasis to occur the fungi has to overcome the host defences and this is facilitated (or inhibited) by the oral microenvironment. For *C. albicans* to transform from a commensal organism to pathogenic, the fungi first needs to ***colonize*** the host tissue.[17] Adhesion is essential for colonization. Usually *C. albicans* is found in mixed biofilms within the oral cavity. Biofilms are beneficial to the fungi as they assist in colonization and survival within hostile environments (physical and chemical). In addition, biofilms contribute to the development of drug resistance.[17] In its commensal form, *C. albicans* usually exists as budding yeasts. However, *C. albicans* hyphae are considered to be superior in adhesion ability and are thus the more virulent morphological form. Adhesion stimulates hypha formation which enhances adhesion.[17, 18] Attachment is facilitated by adhesins on the fungal cell surface as well as glycoproteins such as glucose and mannose. [16, 18]

Secondly, the fungi need to ***invade*** the host tissues. *C. albicans* uses two mechanisms to invade host cells: induced endocytosis and active penetration.[17, 18] Invasion is facilitated by the presence of germinal tubes and phospholipase C.[16] Certain components of the fungal cell wall are capable of

modifying host defences by either inhibiting phagocytosis or antigen presentation, thus interfering with the immune response of the host.[16]

Lastly, the hallmark of *C. albicans* pathogenicity is ***tissue damage***. Tissue damage occurs either directly, due to invasion of the microorganism into the host's cell, or indirectly due to the host's immune response. Filamentation and active penetration is essential for epithelial damage.[17] Furthermore, maintenance of hyphal elongation after invasion is also required to damage cells. Indirectly, *C. albicans* causes damage to cells by initiating cell death pathways leading to apoptosis. It has been found that in more established infections, anti-apoptotic signalling pathways are inhibited and a majority of tissue damage occurs due to necrosis and not apoptosis. [17]

5. *Clinical spectrum and classification of oral fungal infections*

There are variations in the clinical presentation of candidal infections (Table 2). These can be broadly categorised into primary and secondary oral candidiasis. [19, 20] Primary oral candidiasis is confined to the oral and perioral tissues. Secondary oral candidiasis is a manifestation of a systemic candidal infection. Furthermore, primary oral candidiasis can be subdivided into acute, chronic and *Candida*-associated lesions. [19, 20]

Table 2: Classification of Oral Candidiasis as proposed by Samaranyake[19]

Primary Oral Candidiasis			Secondary Oral Candidiasis
Acute	**Chronic**	***Candida*-associated**	
Pseudomembranous	Hyperplastic	Denture stomatitis	Oral Candidiasis as a manifestation of systemic disease
Erythematous	Nodular	Angular cheilitis	
	Erythematous	Median rhomboid glossitis	
	Plaque-like	Linear gingival erythema	

Pseudomembranous and erythematous oral candidiasis falls under acute primary candidiasis. Pseudomembranous candidiasis is frequently referred to as "thrush". It is commonly seen in neonates, those using corticosteroids or immune suppressive medication and the immune compromised.[6] Pseudomembranous candidiasis appears as white plaques which can be removed to reveal erythematous mucosa. Erythematous candidiasis is often associated with broad spectrum antibiotic use [16] and presents as red, atrophic areas anywhere in the oral cavity, but most commonly the tongue and palate.

There are four variations within chronic primary oral candidiasis i.e. hyperplastic, nodular, erythematous and plaque-like. *Candida*-associated lesions have a multifactorial aetiology and include denture stomatitis, angular cheilitis, median rhomboid glossitis and linear gingival erythema.[19, 20] Patients may exhibit a combination of the aforementioned infections. Denture wearers frequently have both erythematous denture stomatitis and angular cheilitis.[6]

Angular cheilitis presents as fissures or cracks at the labial commissures. It is associated with vitamin and/ or iron deficiency as well as bacterial infections. [6, 16] It is also commonly found in those with vertical facial dimension loss due to tooth wear, tooth loss and dentures which have insufficient vertical dimensions. [6, 16] This leads to accumulation of saliva at the commissures and colonization of microorganisms including *Candida* species. Denture stomatitis is usually found in patients with poorly fitting or older dentures, especially when concomitant with poor oral and/ or denture hygiene. Barbeau *et al*[21] found the greatest risk factors to be nocturnal denture-wearing and smoking.[21] Denture stomatitis is commonly classified clinically according to Newton's classification[22]: Type I: petechiae dispersed on the palatal mucosa in contact with the denture; Type II: macular erythema with no hyperplasia; and Type III: generalized erythema with hyperplasia.

6. *Diagnosis of oral fungal infection*

The presence of *Candida* species is not sufficient for a diagnosis of candidiasis as several species of *Candida* are commensals in humans. Thus, the first step of diagnosing oral fungal infections is the identification of the lesions.[16] Knowledge of the clinical presentation of oral fungal infections and that of similar looking lesions is important in providing a differential diagnosis.[9] The clinical diagnosis of oral fungal infections is usually sufficient for the general practitioner to initiate management.[9] However, there are several instances where a definitive diagnosis is necessary: the clinical diagnosis is inconclusive, the prescribed treatments are not curative, the lesions are recurring, there is a high risk of the infection spreading to deeper tissues, the host's immune response is compromised, species require identification for sensitivity testing or research purposes.[16] A definitive diagnosis is confirmed by microbiological studies.

First, a sample needs to be acquired via swabbing, an imprint culture, oral rinses, collection of whole saliva and incisional/excisional biopsy.[9, 23] Thereafter, identification of the yeasts can be made based on four criteria: morphological, biochemical, immunological and genetic.[16]

7. *Management of oral fungal infections*

The treatment of oral fungal infections consists of four parts: (1) diagnosis, (2) identifying and correcting the predisposing factors or underlying disease where possible, (3) evaluating the type of infection, and (4) prescribing appropriate antifungal therapies.[13]

After the initial suspicion or identification of oral fungal infections, a thorough history taking and clinical examination is required. This will assist to identify any local/systemic predisposing factors which may contribute to the development of the infection.[24] Local predisposing factors including hyposalivation and dental prosthesis require assessment. The patient's dental and denture hygiene needs to be evaluated and the patient educated about its care. Nocturnal and continuous denture wearing should be strongly discouraged as it is found to be a major risk factor. [25-27] Disinfection of the dentures and oral cavity should be encouraged.[24, 28] Emami *et al*[28] conducted a meta- analyses of randomised control trials comparing different methods of treatment for denture stomatitis. It was found that disinfection of the dentures using chlorhexidine or hexitidine has a similar efficacy to the use of antifungal medication. Disinfection of dentures could prevent overuse and reduce associated side effects of antifungal medications. [28, 29] However these studies reported recurrence of disease, had small sample sizes, many were methodologically flawed, making the recommendations less reliable.

Once local factors have been assessed, it is necessary to look at systemic influences as oral fungal infections rarely occur in the absence of compromising factors. [9] Underlying deficiencies and medical conditions (as mentioned previously) should be excluded or patients should be treated or referred accordingly.

Following the initial phases of management mentioned above, if resolution of the infection does not occur, pharmacological intervention is then required. Treatment of oral fungal infections differs for every case as it depends on the nature of the lesion/ infection, the medical history of the patient, including concomitant medication, and also factors effecting patient compliance to therapy. There are

primarily four classes of antifungal medication available for the treatment of oral fungal infections i.e. (1) azoles, (2) polyenes, (3) echinocandins, and (4) pyrimidines.[2, 30] Both systemic and topical formulations are available.

Azoles inhibit ergosterol in the cell membranes by inhibiting cytochrome P450 (CYP)-dependent 14α-demethylation.[13, 30, 31] CYP is needed in several human metabolic pathways including the metabolism of many drugs, thus it has many drug interactions. There are two subclasses of azoles: imidazoles and triazoles. The imidazole group includes miconazole, clotrimazole and ketoconazole. Clotrimazole and miconzazole are available in topical and systemic forms and are thus frequently used for oral fungal infections (Table 3). Miconazole is additionally available as a buccal-adhesive tablet which is used in the treatment or oropharyngeal candidiasis.[32] Ketoconazole is only available in systemic form. The use of these medications in systemic form should be reserved for more serious fungal infections due to the side effects and possible drug interactions.[30, 33] The triazole subclass includes fluconazole, itraconazole, voriconazole and posaconazole. These drugs have an improved solubility and specificity for fungal enzymes which makes them less toxic to humans than the imidazoles[30], however hepatotoxicity is still a concern.[33] Re *et al*[34], reported incidents of liver injury to be relatively low amongst oral azole users, however those with pre-existing liver disease were at a much greater risk than those without liver injury. [34] These drugs are rarely used for uncomplicated oral fungal infections.

There are only three commercially available polyenes. These are nystatin, amphotericin B and natamycin.[2] Polyenes bind to ergosterol resulting in membrane-selective permeability which causes the cell contents to leak out, thus causing cell death.[30] Topical nystatin has been used as the first choice pharmacotherapy for oral fungal infections for years.[13] Nystatin is not absorbed systemically and therefore there appears to be less toxic, however gastrointestinal side effects are common if taken systemically, thus topical treatment is the usual route of application. Systemic Amphotericin B use should be limited to serious fungal infections as the drug has a high potential for toxicity and adverse

drug effects.[2] Even so, it is considered the gold standard for fulminating *Candida* infections. Resistance to Amphotericin B has so far found to be rare. Non-systemic applications of amphotericin B are now available which lacks the toxic effects associated with the systemic version.[2]

Table 3: Antifungal drugs used for oral Candidiasis in Adults (Adapted from Pienaar et al[35])

Drug	Form	Dosage
Topical		
Amphotericin-B	Lozenge	10 000 iu dissolved slowly in the mouth 3-4 times a day for a minimum of 2 weeks
Nystatin	Cream	Apply to affected area twice a day
	Oral suspension	20ml 4 times a day; continue to use for several days post clinical resolution
	Pastille	Dissolve tablet in mouth 5 times a day
Clotrimazole	Solution	5ml 3-4 times a day for 2 weeks minimum
	Cream	Apply to affected area 2-3 times a day for 3-4 weeks
Miconazole	Oral gel	Apply to the affected area 3-4 times a day
	Cream	Apply twice a day and continue to use 10-14 days after lesions heal
Chlorhexidine gluconate	0,2% Mouthwash	10ml to be swirled in the mouth for 1 timed minute and then spat out
Gentian Violet	0,5% Aqueous solution	Paint on affected area(s) of mouth three times daily
Systemic		
Fluconazole	150mg Capsules	150mg stat or one 150mg capsule once a day for 2-3 weeks
Ketoconazole	200mg Tablets	One to two tablets twice a day with food for 2 weeks
Itraconazole	100mg Capsules	One capsule per day taken immediately after meals for 2 weeks
Posaconazole	Oral suspension	100 mg (2.5 mL) PO BID on Day 1, then 100 mg PO once a day for 13 days

Echinocandins interfere with the synthesis of cell walls and include caspofungin, micafungin and anidulafungin. These drugs are all limited to parenteral form and are not used for uncomplicated oral candidiasis. Capsofungin may be used to treat invasive aspergillosis, disseminated candidiasis and rarely candidiasis refractory to fluconazole.[30] Lastly, pyrimidines such as flucytosine is an antimetabolite that inhibits fungal protein uracil in fungal RNA with 5- fluorouracil.[30] It is usually used in conjunction with another antifungal agent in the treatment of severe *Candida* or *Cryptococcal* infections.

Despite the currently available antifungal therapies, several problems exist with the treatment of fungal infections. As mentioned, inherent properties of fungi, especially within biofilms, make them

particularly difficult to treat.[36] In addition triazoles, polyenes, and echinocandins require multiple daily dosis due to their limited bioavailability.[36] This, together with the adverse drug effects and drug interactions- especially important for patients who are chronically ill or and those taking multiple medications concurrently- adversely affect patient compliance with medication.[37] These medications are particularly ineffective in those with compromised immunity as natural immunity is essential for combatting these infections.[38] Also, sampling and lab testing is not always possible for monitoring the progress of treatment.[39, 40] These factors have contributed to the increasing incidence of drug resistant fungi currently experienced.[3, 39, 41]

8. *Antifungal resistance*

In 2014, the WHO report on the global surveillance on antibiotic resistance stated that "*antibiotic resistance is no longer a prediction for the future; it is happening right now, across the world, and is putting at risk the ability to treat common infections in the community and hospitals. Without urgent, coordinated action, the world is heading towards a post-antibiotic era, in which common infections and minor injuries, which have been treatable for decades, can once again kill*". [42] Antifungal resistance is an equally urgent matter which receives less attention and funding.[41]

A recent study in South Africa and Cameroon found that as much as 50% of the *Candida albicans* specimens sampled were resistant to azoles.[39] This increase in resistant strains of fungi requires urgent attention, research and investment to understand the mechanisms and find suitable therapies.[5, 41] According to Parente-Rocha *et al*[38], fungi utilize several mechanisms to promote resistance. These include the mutation of drug targets, overexpression of the targeted protein, expression of the efflux system, degradation of antifungal agents and pleiotropic drug responses.[38]

In spite of the rising prevalence of fungi resistant to antifungal medication, there has been no new antifungal drug classes approved for human use since 2006. [41] However, there is ongoing research into various strategies which may be used to overcome fungal resistance. Research to identify antifungal properties in novel bioactive compounds is under way.[38] Also, bioinformatics analyses are being used

to search genomic databases for peptide sequences which have the physical-chemical characteristics of antifungal drugs. In addition, promising research in the search of vaccines against fungal infections is been investigated.[38] Nevertheless, since these drugs and vaccines are still currently in the pipeline, alternative therapies are required to treat fungal infections without propagating the rise in fungal resistance.[43] One such treatment which has been proposed as an antifungal therapy is photodynamic therapy.

9. *Introduction to Photodynamic therapy (PDT)*

PDT is a treatment modality that utilises light of a certain wavelength to activate a photosensitizing agent (PS). This reaction takes place in the presence of oxygen. The treatment results in the formation of reactive oxygen species and other reactive molecules which causes damage to the cell and cell death.[44, 45]

The PS is administered to the patient either topically or systemically. Several modes of administration exist depending on the nature of the PS and the site of the lesion. These include intravenous, par enteral, nasal, pulmonary, topical and targeted delivery.[46, 47] The PS then requires time to accumulate within the target cells. Thereafter, an energy source of an appropriate wavelength is used to activate the PS for a certain amount of time to produce the desired effect - cell damage and/ or death.[48]

10. *Current uses of PDT*

Evidence of the clinical applications of PDT is rapidly growing, especially in the fields of oncology[49, 50] and dermatology. [51, 52] A review by van Straten *et al*[53] assessed current studies about the clinical use of PDT in oncology and PDT has been found to be successful in all stages of cancer therapies i.e. pre-, intra-, and post-operatively and when used in combination treatment with other therapies.[53] It also appears to be well tolerated by patients, with less adverse effects than what is associated with chemo- and radiation therapy. However, it should be noted that many of the studies employ small sample sizes and thus lack adequate statistical power. It was also found that in spite of the mounting evidence in its favour, as well as 30 years since regulatory approval, PDT is still very much underutilised clinically

in favour of radiation and chemotherapy.[53] Nevertheless, more clinical and preclinical research is being done to overcome these challenges as well as to find and improve PSs for these purposes.[53]

In addition to oncology, the adoption of PDT into "standard care" within dermatology has also grown. This, in part, is due to the ease of application of PDT to the skin.[52] The American Society of Dermatological Surgery (ASDS) has recently published a consensus document recommending PDT as a "highly effective treatment modality" for precancerous lesions, superficial nonmelanoma skin cancers, inflammatory acne vulgaris and others.[54] Furthermore the applications of PDT is expanding with PDT now being looked towards as a potential antimicrobial therapy.

Photodynamic antimicrobial therapy (PACT) is a relatively new approach for the treatment of pathogens infecting tissues.[55] There are several advantages to the use of PACT as opposed to conventional antibiotics: Studies evaluating PDT on antibiotic resistant pathogens have found PDT to be equally or more effective than antimicrobials on resistant strains of microbes.[56] In addition, no resistance to PDT has been found thus far and due to the mechanism of action of PDT, which differs from conventional drug therapy. It is hypothesized that it is unlikely that resistance would develop in the same way it has with conventional antimicrobial drugs.[55, 56] However, it should be noted that some microbes have been found to be inherently less susceptible to PDT than others.[56] PACT has been found to be effective against biofilms which, as mentioned earlier, are notoriously difficult to treat.[57, 58] PDT has the added benefit of rapid action which is in contrast to systemic antimicrobials which require time and high concentrations to reach biologically active levels and these levels may lead to toxicity in the host.[59]

PACT is also relatively selective resulting in less damage to host tissues. Hamblin *et al*[59] found that PACT was most effective when the PS has a significantly cationic charge as microbial cells have a more negative charge than mammalian cells, thereby enhancing the selective binding of the PS. Furthermore, the microbial cells bind to the PS more quickly than the mammalian cells, reducing pre-

irradiation exposure time. [59] More research is being conducted to find other selective carriers which include liposomes, nanoparticles and microspheres.[45, 60]

One area of focus of research is the use of PDT for the treatment of periodontitis. Periodontitis is an infection of the tooth supporting tissues caused by a multitude of microorganisms. Results of studies vary drastically from PACT being ineffective [61-63] to effective.[64-66] However, one of the major issues regarding studies using PDT is that there is no standardised or ideal treatment regimens and parameters which makes comparing studies very difficult. Also, studies use different outcome measures and utilise different time points.

Although there is more evidence on PDT and bacterial infections, a few studies have investigated the application of PDT to treat fungal infections. PDT has been used to treat onchomycosis, a fungal infection of the nails and toenails that is particularly difficult to manage due to the need for protracted courses of antibiotics, slow growth of the nail and poor penetration of topical antibiotics.[67] PDT in the management of onchomycosis overcomes the aforementioned problems and the side effects of treatment appear to be minimal.[67, 68]

Similarly, animal and human studies investigating PDT and oral fungal infections have shown positive results.[69] Mima *et al* [70]performed a case series treating denture stomatitis with PDT on 5 patients and found resolution of the denture stomatitis in 4 of the 5 patients [71]. It was suggested that smoking may have contributed to the poor outcome on the single patient. Abduljabbar *et al*[72] compared PDT in smokers versus non-smokers and found statistically higher *Candida albicans* counts in smokers. They also concluded that PDT is effective against oral fungal colonization [72]. Mima *et al*[73] followed up the case series with a randomised control trial of 40 patients comparing PDT to topical nystatin. PDT was found to be comparable in effectiveness to nystatin. However, recent studies have diverged from the previous findings.[74, 75] Maciel *et al*[74] evaluated a combination light laser therapy (LLT) and a single session of PDT in the treatment of denture stomatitis and found the treatment to be inferior to topical

miconazole therapy. This treatment regimen is unconventional as most studies thus far evaluate repeated applications of PDT clinically[71, 76, 77] and do not combine it with LLT.

11. Mechanism of action of PDT

PDT involves the absorption of a photon of light of a specific wavelength which leads to the excitation of the photosensitizer (PS) to its singlet electronic state.[59] Irradiation causes the transit of electrons to a different orbital, exciting the PS to form an unstable molecule with a short half-life i.e. the excited singlet state.[45] According to Hamblin[59], the singlet-state PS can then undergo an electronic transition to a longer- lived "triplet state"[59], the main mediator of the PD reaction.[45] This allows the triplet-state PS to react with ground state oxygen. There are 2 photochemical pathways whereby this reaction can take place: in the Type 1 pathway, electron transfer occurs to produce a superoxide radical and then hydroxyl radicals (HO.). The Type 2 pathway produces excited state singlet oxygen ($^1O_2$) via energy transfer. The hydroxyl radicals and the singlet oxygen are highly reactive oxygen species (ROS) which are capable of causing cellular component damage and cell death.[59, 78]

Figure 1 from Hamblin[79] is a Jablonski diagram which illustrates the photochemical reaction which occurs during PDT and their antimicrobial activity.

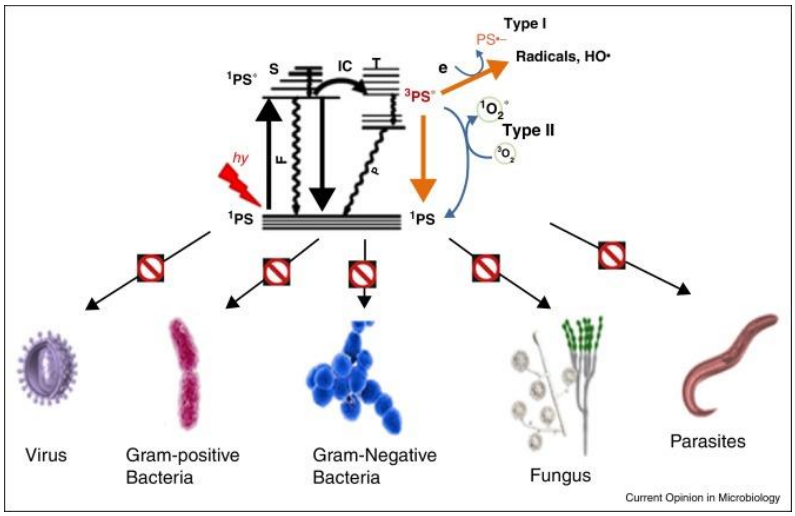

Figure 1 Jablonski diagram showing the photochemical reaction caused by photodynamic therapy (Hamblin[59])

There is no single pathway that leads to cell death after PDT. [80] The radicals, described above, cause a change in cell membrane and wall integrity, which allows a further influx of PS into the cells. Damage is caused in various ways. The radicals are capable of causing direct damage to the cell. Cytoplasmic leakage, damage to cytoplasmic organelles and nucleic acid cause death by apoptosis, necrosis and autophagy.[45] The cell death pathway is dependent on various factors including the dose of light, the concentration and type of PS, and their intracellular localization.[80] When PDT damage is strong and cell damage is beyond repair, apoptosis will occur. However, at higher doses necrosis can occur.[45, 80]

12. Photosensitisers

Photosensitizers (PS) are dyes which are able to absorb energy from light and transfer the energy to another molecule.[45, 81] The ideal photosensitizer has not yet been found and the requirements of the PS differs according to the target tissue, lesion and purpose of treatment. Anti-cancer PSs should be lipophilic with little or no overall charge and usually have a long wavelength absorption band for tissue penetration; whereas antimicrobial PS's usually have a pronounced cationic charge and requires only superficial penetration.[78, 79]

According to Abrahamse & Hamblin[79], the PS should ideally be a single, pure compound with low manufacturing costs and high chemical stability when stored. It should have a strong absorption peak in the near-infra-red spectral region (650nm- 800nm). PSs should have no dark toxicity (toxicity when not activated), but have the ability to accumulate in target tissues with a relatively rapid clearance from normal tissues to avoid damage to normal cells.[79]

With cancer therapy, most PSs are based on the tetrapyrrole backbone [79], such as porphyrins, chlorins, bacteriochlorins and phthalocyanines. Another category of PSs is synthetic dyes which includes Phenothiazine salts, Rose Bengal, Squaraines, BODIPY dyes, Phenalenones and transitional metal compounds. Phenothiazine salts, toluidine blue O (TBO) and methylene blue (MB) have been approved for human use and are effective against fungal cell membranes. Naturally occurring products

are also being studied. These include Hypericin (from St. John's Wort), Hypocrellin, Riboflavin and Curcumin.[45, 79, 81]

Recent years have seen rapid advances of targeted PDT and the use of nanotechnology in the treatment of cancer and PACT.[79] Targeted PDT enables the PSs to bind to specific molecules which have an affinity for the target cells or to specific receptors on the target cells. This includes monoclonal antibodies, antibody fragment, proteins, peptides, hormones and metabolites. Nanotechnology is being used in two ways in PDT. The first is as a nanoparticle PS delivery system which enables the PS to better localize in the target and it also improves the photochemical efficiency of the PS. The second method is that the nanoparticles are able to act as a PS itself and absorb light and emit ROS. Fullerenes, quantum dots and titanium dioxide [79] are examples thereof.

13. Light source

The appropriate light delivery source/device, capable of delivering the correct dosimetric parameters is essential for successful PDT. This is to both penetrate the appropriate tissues and activate the PS to a degree that sufficient ROS are generated to effect cellular damage of the target.[82, 83] Both coherent (lasers) and non-coherent (light emitting diode (LED) and lamps) light sources are currently being used and further investigated for PDT.[45]

The benefit of lasers is that they are capable of delivering a highly focussed light of an adjustable wavelength which has a high degree of monochromaticity.[45] According to Yoon *et al*[82], the most frequently used laser clinically is the Argon/ Dye laser. Other laser systems include metal vapour, KTP: YAG and diode lasers.[82] Although the diode lasers have some advantages in that they are easy to use, light-weight and less expensive than the other lasers; the major drawback with lasers in general is the cost.[84] Thus, there has been a push to develop and utilise non-coherent light sources, especially LED-based light-sources. These are user friendly, efficient, cost-effective as well being able to provide the

necessary fluence and irradiance for treatment.[84-86] The light source must also minimise ultra-violet emissions and heat to prevent mutagenesis and damage to unintended tissues.[87]

14. Treatment regimens for oral fungal infections

In a systematic review, it was found that in laboratory studies, the wavelength used for PDT are relatively consistent (660nm or 475nm).[69] However, the energy fluency and power output diverged significantly. Also, there are no reporting standards, thus parameters such as power density, number of applications, irradiation area and number of points of irradiation are not always reported on.[69]

There is no consensus on the ideal treatment oral fungal infections as the number of human studies are limited. In fact, the treatment regimens are so heterogeneous, making studies difficult to compare.[69] Three studies on the treatment of denture stomatitis made use of a similar treatment regimen.[72, 73, 88] In these studies, the denture and the palatal mucosa were treated with PDT. A haematoporphyrin derivative PS (Photogem) was applied to the palate. In two studies a 30 minute pre-irradiation time was used. Thereafter a specially designed LED device (wavelength of 440nm to 460nm) was used to irradiate the palatal mucosa for 30 minutes. The power and intensity generated in these studies was 260mW and 102mW/cm^2 respectively. Abduljabaar *et al*[72] provided only one session of PDT, whereas the two studies by Mima *et al*[70, 73] provided participants with six sessions of PDT: three times a week for 2 weeks.

Scwingel *et al*[89], Maciel *et al*[74] and Senna[90] used methylene blue as a PS.[74, 89, 90] Scwingel made use of a twin laser (λ= 660nm, P= 30mW, fluence 7.5J/cm^2) and the other two studies used a light gallium aluminium arsenide laser (GaAIAs)(λ= 660nm, P= 100mW). Thus results on the success and failure of treatment may be directly linked to the different PDT parameters used.

Therefore, the aim of this systematic review is to compare the efficacy or PDT to that of conventional antifungal medications, in the treatment of oral fungal infections in humans. We also aim to determine which PDT parameters are the most effective.

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BACKGROUND

There is an increasing prevalence of oral fungal infections and they can have a significant impact on the quality of life of those infected. Treatment of oral fungal infections conventionally make use of topical or systemic antifungal medications. However, antimicrobial resistance has limited the effectiveness of these medications. Photodynamic therapy (PDT) has been proposed as an alternative treatment modality in the treatment of oral fungal infections.

The aim of this study was to determine whether PDT, compared with standard anti-fungal treatment modalities, is effective in the treatment of oral fungal infections in humans.

METHOD

Primary experimental and observational studies comparing the effect of treatment of oral fungal infections using PDT to treatment using systemic and topical antifungal medications were included. Only human, clinical studies were included.

A comprehensive electronic database search was conducted in September 2018. The Cochrane Library, BioMed, SciELO, Scopus, EBSCOhost, PubMed/MEDLINE, ISI Web of Science, Clinicaltrials.gov, ProQuest, WorldCat and Google Scholar were used. Databases were queried using keywords, MESH and root terms (e.g. *"Candida*", "Oral fungal infections", "mycoses", "denture stomatitis", "Phototherapy", "Photodynamic therapy"). No limitations were placed on language, time-frame or publication status.

The electronic database search yielded 654 titles with an additional two titles being found by hand-searching. After duplicates were removed, 353 titles were subjected to screening by two researchers. After abstract screening, and full-text reviews, four studies were included in the review (n=112). All included studies were randomised control trials conducted on adult participants in Brazil.

The effect size was estimated and reported from continuous variables using mean difference and 95% confidence intervals. The weighting of each study was calculated using the inverse of the variance. A random-effects model was used for analysis. Where the researchers found insufficient data, they conducted a narrative report of the results. Forest plots were created for the time-points

of 7, 15 and 30 days respectively. The authors used the Cochrane test (P <0.1 cut-off for statistical significance) to determine statistical homogeneity and the I^2test was used to quantify heterogeneity. Assessment of methodological quality and risk of bias was performed using the risk of bias tools of the Cochrane Collaboration.

Pooling of the data was done to assess three outcomes: 1) clinical improvement from baseline; 2) microbiological improvement by assessing changes in *Candida* colony forming units per millilitre (CFU/ml); and 3) microbiological improvement via semi-quantification of CFU's. The studies assessed clinical change and microbiological change at different time points.

RESULTS AND DISCUSSION

This review found evidence for comparable effectiveness of PDT as compared with standard antifungal therapy at 30 days from the start of treatment as assessed by clinical improvement (risk ratio (RR) =1.59 [95% confidence interval (CI), 0.44 to 5.82]; 3 studies, n=90 participants) and mycological efficacy assessed directly using CFU's (mean difference (MD) = 0.18 [95% CI, -0.98 to 1.33]; 2 studies, n=62). This finding was supported by the semi-quantification of CFU's (RR=1.51 [95% CI, 0.45; 5.02]; 3 studies, n=72). No statistically significant difference between PDT and antifungal medication was found 15 or 30 days [15 days: RR= 1.09, 95% CI 0.63, 1.88; 30 days: [RR= 1.59, 95% CI= 0.44, 5.82]. Heterogeneity was found to be borderline high at 15 days (I^2= 47%) and very high at 30 days (I^2= 83%). Publication bias was not determined due to the sparsity of studies. We found there to be a moderate to high risk of bias for all included studies resulting in a low quality of evidence.

CONCLUSION

The findings of this review and meta-analysis, although limited due to the small number of studies and patient samples, suggest that PDT has similar effectiveness when compared with conventional therapy, in resolving oral fungal infections. Implementing PDT as an alternative management modality, however, requires refinement of treatment parameters and further research.

PROSPERO registration at CRD42017076

SHORT ABSTRACT

OBJECTIVE: Photodynamic therapy (PDT) has been proposed as an alternative treatment for oral fungal infections given the rise in antimicrobial resistance resulting in limited effectiveness of conventional antifungal medications. This study sought to determine whether PDT, compared with standard anti-fungal treatments, is effective in the treatment of oral fungal infections in humans.

METHODS: A comprehensive database search, without restrictions on language, time-frame or publication status, yielded 353 titles meeting with our inclusion criteria; after evaluation by two researchers, four studies (n=112) comparing the treatment of oral fungal infections using PDT versus standard antifungal medications, were included in the review. All were randomised control trials conducted on adult participants in Brazil. Studies varied in terms of frequency and duration of PDT sessions. Pooling of the data (random-effects meta-analysis) was done to assess clinical improvement from baseline and microbiological changes. Assessments of methodological quality, risk of bias and heterogeneity were performed using peer-reviewed criteria. PROSPERO registration at CRD42017076

RESULTS: PDT has comparative effectiveness to conventional antifungal medications from the start of therapy in clinical efficacy [30 days, risk ratio =1.59 [95% confidence interval (CI), 0.44 to 5.82]; I^2= 83%; 3 studies, n=90 participants) and mycological efficacy (30 days, mean difference = 0.18 [95% CI, -0.98 to 1.33]; I^2= 29%; 2 studies, n=62)]. Findings were consistent at all timepoints, although PDT showed near-significance favourability for mycological effectiveness at 15 days, assesed by CFU semi-quantification (RR = 1.49 [95% CI, -0.97 to 2.28]; I^2= 0%; 2 studies, n=36). There was a moderate to high risk of bias for all included studies.

CONCLUSION: The findings of this review and meta-analysis, although limited due to the small number of studies and patient samples, suggest that PDT has similar effectiveness when compared with conventional therapy, in resolving oral fungal infections.

1. BACKGROUND

Fungal infections in humans are a growing public health concern, affecting more than 300 million people annually.[1] While accurate estimates of the disease burden are not commonly available, especially from developing countries,[1-4] there is evidence of an increasing incidence and prevalence of fungal infections worldwide. [1, 3, 5] The increasing burden in part, has been due to the increasing population of immune-compromised individuals. These include those living with HIV/AIDS, established and uncontrolled diabetes mellitus, cancer and transplant patients, those who are seriously ill, malnourished and on immunosuppressive therapy and other medical conditions which affect the immune response. [5]

Furthermore, fungal infections are gaining significance as a major cause of morbidity and mortality.[6] Invasive fungal infections carry a high risk of mortality.[3] Mucocutaneous fungal infections, although rarely fatal, are very common infections in humans. [3] Some of the most common mucocutaneous fungal infections in humans affect the oral cavity and seen in the critically ill and immune-compromised.[7] Oral fungal infections are also common in neonates, babies, and denture-wearers and have a significant impact on an individual's quality of life due to oral discomfort, burning, pain, dysgeusia (altered taste) and reduced appetite.[8] HIV/AIDS predisposes the progression of oral fungal infections to the oropharynx. These individuals are particularly at risk of disseminated fungal infections if the immune system is highly suppressed. HIV/AIDS is associated with 10 million additional cases of oral candidiasis and 2 million cases of oesophageal fungal infections per annum.[9] Fungal infections are commonly overlooked and receive insufficient research attention and investment. [10]

Topical antifungals are the first line of treatment for mucocutaneous fungal infections, followed by systemic antifungal medication.[11] However, it has become well-recognized internationally that

fungi are rapidly gaining resistance to current medication [12-14] due to various reasons: (1) Multiple daily doses of triazoles, polyenes, and echinocandins are required due to limited bioavailability [7], (2). Sampling and laboratory testing is not always possible for monitoring progress [1, 13], therefore patients often do not finish their course of medication, (3). These medications often have unpleasant and dangerous side effects as well as drug interactions [8] especially important for patients who are chronically ill or HIV-positive and taking multiple medications concurrently. (4). Inherent properties of oral fungi that make them particularly difficult to treat [7] a recent study in South Africa and Cameroon found that 50% of the *Candida albicans* specimens sampled were resistant to azoles. [13] New drugs to treat fungal infections have not been developed since 2006 [12] and thus, alternative therapies are required to treat these minimally invasive fungal infections without propagating the rise in fungal antimicrobial resistance.[15] Recently, the use of photodynamic therapy has gained traction as an antifungal treatment modality.

Photodynamic therapy (PDT), also referred to as photodynamic antimicrobial chemotherapy (PACT), photoradiation therapy and photochemotherapy, comprises three components: a chemical photosensitizer (PS), the application of light and the presence of oxygen. Briefly, the PS is applied to the target tissue (either topically or systemically). Light of an appropriate wavelength is then used to activate the PS generating highly reactive oxygen species (ROS), including the singlet oxygen, in the target tissue. This results in cytotoxicity of the target cells and elicits an acute inflammatory response in the surrounding tissues.[16, 17] Thus, PDT is being studied as a treatment modality for a variety of clinical applications.

Evidence of the clinical applications of PDT is growing, especially in the fields of oncology[16, 18] and dermatology.[19, 20] The American Society of Dermatological Surgery (ASDS) recently published a consensus document recommending PDT as a highly effective treatment modality for precancerous lesions, superficial nonmelanoma skin cancers, inflammatory acne vulgaris and others. [21] Although

much research is being conducted on the use of PDT as an antimicrobial treatment, a majority of these studies are in-vitro or lab studies and studies on human participants are lagging.

However, more human studies are emerging, particularly from the oral health sector [22-25] suggesting PDT as a possible treatment for oral fungal infections.[22, 24, 25] In contrast, more recent studies found PDT to be inferior when compared with antifungal medication in the treatment of specific oral fungal infections.[23, 26] Given this equipoise, our systematic review aims to provide the most current evidence on the use of PDT as a treatment option for oral fungal infections in humans.

The review question:

Is photodynamic therapy compared with standard anti-fungal treatment modalities, effective for the treatment of oral fungal infections in humans?

2. OBJECTIVES OF THE STUDY

2.1 Primary:

- The primary objective of the systematic review is to determine if photodynamic therapy (PDT), when compared with standard medication, is effective in the treatment of oral fungal infections.

2.2 Secondary:

- What are the most effective photodynamic therapy regimens for the treatment of oral fungal infections (duration of treatment, pre-irradiation time, number of visits and time between visits)?
- Which photoactivators and at what concentration, are most effective in the treatment of oral fungal infections?
- Which type of light delivery device and wavelength has been shown to be most effective for the treatment of oral fungal infections?
- How do risk factors for oral fungal infections such as smoking and diabetes mellitis, affect treatment outcomes?

3. METHODS

3.1. Inclusion Criteria for studies in this review

3.1.1 Types of studies

Primary experimental and observational studies comparing the treatment of oral fungal infections using PDT to treatment using systemic and topical antifungal treatment was included. The study designs include but were not be limited to randomized control trials, quasi-randomised control trials and cohort studies. The inclusion criteria were deliberately left broad in an attempt to find as many relevant articles which relate to the specified primary and secondary objectives.

3.1.2 Types of participants

Participants had to be human, with a clinical diagnosis of an oral fungal infection. The diagnosis required microbiological confirmation. Any concomitant systemic conditions (e.g. HIV, diabetes mellitus), required that the conditions be specified and accounted for in both control and treatment groups.

3.1.3 Types of intervention

We included any human study using PDT to treat an oral fungal infection in vivo. PDT was defined as "the administration of a nontoxic drug or dye known as a photosensitizer (PS) either systemically, locally, or topically to a patient bearing a lesion, followed after some time by the illumination of the lesion with visible light, which in the presence of oxygen, leads to the generation of cytotoxic species and consequently to cell death and tissue destruction"[27].

3.1.4. Types of Controls

We included any study using conventional topical or systemic antifungal medication for the treatment of oral fungal infections. We allowed flexibility with the antifungal drugs used and dosages of the comparator as treatment regimens vary in different settings and for different

patients. The treatment regimens were noted and reported on. Clinical trials of new antifungal medications were not included.

3.1.5 Types of outcome measures

Primary

The primary objective was to determine if PDT is as effective as standard antifungal medication in the treatment of oral fungal infections. The effectiveness of therapy was determined via clinical assessment and microbiological confirmation via direct microscopy or cell cultures.

The presence or absence of *Candida* hyphae can be assessed and a change from hyphae present to absent would indicate improvement. Effectiveness was quantified by measuring the change in fungal load. The latter was quantified as *Candida* colony forming units per millilitre (CFU/mL). A decreased fungal load indicated an improvement in the condition. Some studies utilise a semi-quantification of CFU/mL which is interpreted in the same way (no change, improvement or worsening).

Secondary

The secondary objectives relate to the parameters of the PDT. The various light delivery devices, wavelengths used, photosensitisers and treatment regimens were compared to determine which is most effective in antifungal ability. The same measures of effectiveness were used as that which was used to determine the primary objective.

3.1.6 Time Frame and Language

No restrictions were placed on time frame or language in the electronic database search.

3.2. Information sources and search strategy

The protocol for this review was registered with PROSPERO, registration number CRD42017076421. A comprehensive literature search was conducted and completed in September 2018 using the following search strategy in PubMed/MEDLINE which was then modified for the other databases (Table 1): ((Phototherapy [MESH]) OR (Photodynamic Therap* [Title/Abstract])) AND ((Candid* [Title/ Abstract])

OR (fungal infect* [Title/ Abstract]) OR (Mycoses [MESH]) OR (Denture Stomatitis [other term]).

Table 1: Example of Search Strategy for photodynamic therapy for oral fungal infections

Database	PubMed/MEDLINE	
Date	4/11/2017	
Limits	None	
No	Search	Result
1	PHOTOTHERAPY[MESH]	34250
2	PHOTODYNAMIC THERAP*[Title/Abstract]	15548
3	#1 OR #2	38967
4	CANDID*[Title/Abstract]	314251
5	FUNGAL INFECT*[Title/Abstract]	18417
6	MYCOSES[MESH]	116485
7	DENTURE STOMATITIS[Other Term]	61
8	#4 OR #5 OR #6 OR#7	411274
9	(ORAL[Title/Abstract]) OR DENTAL[Title/Abstract]	682360
10	#8 AND #9	20147
11	#3 AND #10	90

The researchers used the following databases: The Cochrane Library, BioMed, SciELO, Scopus, EBSCOhost, PubMed/MEDLINE, ISI Web of Science, Clinicaltrials.gov, ProQuest, and WorldCat. The search term “photodynamic therapy oral fungal infections” was used for forward tracking in Google Scholar, ISI Citation Indices, and Web of Science ISI proceedings (conference proceedings). This was complemented by hand-searching the reference lists of the selected studies for additional relevant studies. The results of the search were documented, reported and compared between databases. The references were managed with EndNote reference manager.

3.3 Study procedure and statistical methods

3.3.1 Study selection and data extraction

The researcher (IR) conducted the search with the supervision of a senior researcher (ME). All results were collated within an online document where two researchers (IR and HH) independently performed title and abstract screening, followed by full-text evaluation and data extraction. The data extraction tool used was modified from the tool used by the Cochrane Collaboration. There was no disagreement between the authors on the studies to include.

Where it was possible to extract quantitative data from the studies, the researchers captured these results into Review Manager (RevMan version 5.3) statistical software and the data were pooled, where appropriate, to conduct a meta-analysis. Pooling of the data was done to assess three outcomes: 1) clinical improvement from baseline; 2) microbiological improvement by assessing changes in *Candida* colony forming units per millilitre (CFU/ml); and 3) microbiological improvement via semi-quantification of CFU's. The studies assessed clinical change and microbiological change at different time points. Forest plots were created for the time points of 7, 15 and 30 days respectively.

The effect size was estimated and reported from continuous variables using mean difference and 95% confidence intervals. The weighting of each study was calculated using the inverse of the variance. As expected, the studies differed in the mixes of participants and in the implementation of interventions, with different effect sizes underlying different studies; thus, a random-effects model was used for analysis [28]. Where the researchers found insufficient data, they conducted a narrative report of the results.

3.3.2 Assessment of methodological quality of trials and risk of bias

Each reviewer conducted an assessment of study quality and the risk of bias of each included study. This was performed using the risk of bias tools of the Cochrane Collaboration.[29] These assessments were summarized in a "summary of findings" table and included in the report. We followed the Grading of Recommendations Assessment, Development and Evaluation (GRADE) guidance for determining the extent of the risk of bias for the body of evidence. [30]

3.3.3 Investigation of heterogeneity

The authors used the Cochrane test (P <0.1 cut-off for statistical significance) to determine statistical homogeneity and the I^2test was used to quantify heterogeneity. The effect of patient characteristics such as age, smoking status, denture wearing and comorbidities was investigated to determine the effect on the test's performance. The effect of the photodynamic therapy on various strains of fungi were compared where possible. This was achieved by including covariates into fitted models if sufficient data are available.

3.3.4 Data Synthesis

Studies will be included in the meta-analysis if they meet the following criteria: studies require an intervention group utilising photodynamic therapy (as defined in 3.1.3) to treat an oral fungal infection (as defined by 3.1.2). Studies will also require a control group which utilises standard antifungal medications (as defined by 3.1.4) to treat oral fungal infections. Outcomes measures which will be considered for pooling will be changes in fungal load via the measure of colony forming units/millilitre (CFU/ml) or the log thereof, clinical change from baseline (as a scale of change i.e. improvement/ worsening) or as semi-quantification of fungal load which is which is a measure sometimes used in studies in the treatment of fungal infections.

3.3.5 Sensitivity Analysis

In the sensitivity analysis, we planned to assessed whether the findings are robust to the decisions made in the process of obtaining them.[29] It is not always possible to pre-specify all factors requiring sensitivity analyses as the need to do these may become more apparent as idiosyncrasies of the studies are identified.[29]

We planned to test the effect of missing data by testing various assumptions. This includes data from studies that could not be extracted and included in the review and studies which have missing participant data, including loss to follow up. We also planned to assess the effect size difference and the effect of the various study designs. However, there were insufficient data to conduct a sensitivity analysis.

3.3.6 Subgroup Analysis

We intended to use the different treatment parameters to conduct subgroup analyses. This includes a comparison of different light delivery devices and wavelengths. Different photosensitisers will be assessed as well as different treatment regimens, the various antifungal medications used, the effect of PDT on different fungal strains and the effect of comorbidities/ predisposing medical conditions such as HIV, diabetes mellitus, and dental prosthesis use. There were insufficient data to perform subgroup analyses.

3.3.7 Assessment of Reporting Bias

Reporting bias was assessed using funnel plots. The assessment was based on effective sample size and regression tests of asymmetry. There was insufficient data to conduct the assessment of reporting bias.

4. RESULTS

4.1 Study selection

Our electronic database search resulted in the identification of 654 titles (Table 2). Two additional articles were found by hand-searching the reference lists of relevant articles. The titles were collated and duplicates were excluded. The remaining 353 titles were evaluated and 273 titles were excluded. Subsequent abstract screening resulted in an additional 68 being excluded.

Table 2: Titles retrieved by electronic, manual, and reference searching

Database	Overall number of search outcomes	Number of search outcomes without duplicates
PubMed	90	90
Cochrane Library	8	8
EBSCOHOST	38	33
Medline	111	111
ISI Web of Science	134	134
ProQuest Dissertation and Theses	22	22
WorldCat	197	166
ClinicalTrials.gov	1	1
SciELO	5	5
Scopus	2	2
Science Direct	46	46
Hand Search	2	2
Total	656	620
Total after removal of duplicates between databases		353

The QUOROM flowchart was used in the study selection process (Fig.1). Nine English language articles and two Portuguese language articles were subjected to full-text screening. We were unable to find the full-text for one article. One of the Portuguese articles [26] did not fulfil the inclusion criteria and was excluded. A further seven articles were also excluded for not fulfilling the inclusion criteria (Table 3). Four full-text studies were included in the review, two of which were unpublished dissertations [22, 31] which required translation prior to data extraction.

Table 3: Clinical studies using PDT to treat Oral Fungal Infections which have been excluded

Study	Publication year	Reason for exclusion
Abduljabaar *et al* [32]	2017	Comparison between non-smokers and smokers (no antifungal control)
Alves *et al* [33]	2018	Case report
Barcessat *et al* [34]	2017	Case Report
Maciel *et al* [23]	2016	PDT followed up with light laser therapy
Mima *et al* [35]	2011	Case report
Ribeiro *et al* [36]	2012	Not related to oral fungal infections
Simonovik-Soskic *et al* [37]	2010	Outcome not relevant to this review
Cadastro & Giovani[38]	2009	Full-text not found

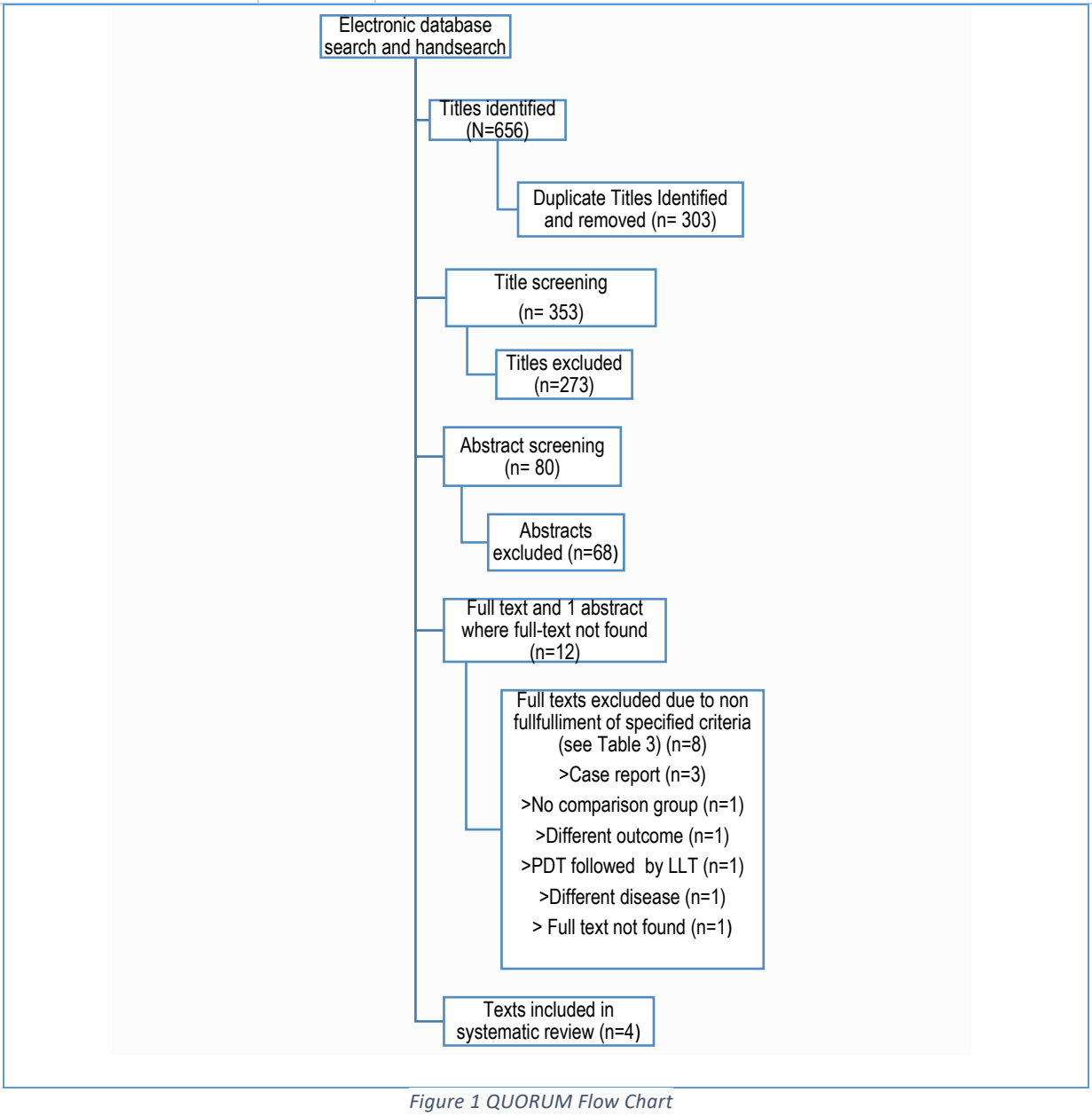

Figure 1 QUORUM Flow Chart

4.2 Study designs

We included all studies comparing PDT to conventional antifungal medications (Table 4), all of which were randomised control trials. One study had an additional arm, namely the use of light laser therapy (LLT)[25]; but data from this arm were excluded from the analyses.

4.3 Study participants (Table 4)

The four studies (n=112) included in this systematic review were all conducted in Brazil comprising between 21 and 40 enrolled participants. Three studies provided the age of the participants.[24, 25, 31] Of these, one study restricted the age range from 40-65 years [31] and the mean age in that study was 56.4 years. The second study, which did not restrict age, had a sample mean age of 62 years.[24] The single study which included only HIV-positive participants had a notably younger sample (mean age = 30 years, ± 8).[25] Two studies restricted their participants to include only immune-competent individuals.[24, 31] The remaining study excluded participants with head and neck neoplasia, or Newton type III denture stomatitis.[22]

Table 4: Design and Demographics of included study

Study	Design	Population	Sample size	Mean age, male: female	Follow up
Lopes, 2011 [22]	Randomised control trial	Patients presenting to the Dental Faculty at the University of São Paulo	Total: 22 Controls: 12 Intervention: 10	Not provided	30 days
Mima *et al*, 2012 [24]	Randomised control trial	Patients attending the Araraquara Dental School, Brazil	Total: 40 20 individuals per arm	Intervention: 62.45 (43-80) years 1:3 Control: 61.25 (41-78) 1:1.86	90 days
Scwingel *et al*, 2012 [25]	3-arm randomised control trial	Patients being seen by a customer Service Specialist at the City of Ponta Grossa (PR, Brazil)	Total: 14 7 individuals per arm	30 ± 8 years 3.2:1	30 days
Senna, 2012 [31]	Experimental analytic randomized control trial with blinding	Patients presenting to the Odontology Faculty of Instituto Tocantinense Presidente Antonio Carlos in Araguaina, Brazil.	Total 36 18 individuals per arm	Overall : 56.4 years , 1:17 Intervention: 58.1± 6 years; 1:9 Controls: 54.7 years; ±7 18 females	30 days

4.4 Treatment Parameters (Table 5)

4.4.1. Nature of the intervention

The studies had varied treatment parameters (Table 4). Two studies conducted PDT on both dentures and the oral mucosa.[24, 31] One study evaluated the treatment of oral candidiasis[25] , while the other three studies specifically evaluated the treatment of denture stomatitis [23, 25, 31] which is a form of oral candidiasis.

4.4.2. Light source and photosensitisers

Three studies (n=79) used lasers as the light source (660nm wavelength). Twin lasers were used in two studies [22, 31] and a GaAIA (Gallium aluminium arsenide) laser was used in one study [25]. The studies investigating lasers utilised methylene blue as the PS. A single study used a haematoporphyrin derivative as a PS, which was activated by an LED light of 440- 460nm wavelength[24].

The power of the LED used was 260mw, which is significantly higher than that provided by the lasers (100mw, 40mw, and 30mw respectively). Pre-irradiation time, which is the length of time between application of the PS and photoactivation, ranged from 1 minute to 20 minutes. The length application of the laser per point was between 10 seconds and 2 minutes. The length of application of the LED was 20 minutes.

4.4.3 The treatment regimen

The frequency of PDT sessions was not consistent. Treatment sessions varied from one session in total [25]; to two sessions one week apart [22]; to 6 sessions over 15 days.[24] The largest number of sessions were 8 PDT sessions over four weeks.[31]

Table 5: Description of parameters investigated and technical characteristics of the photodynamic treatments used in the included studies						
Study	**Treatment arms**	**Condition treated**	**Photosensitiser and Light Source**	**PDT parameters**	**Clinical outcomes**	**Microbiological outcomes**
Lopes[22]	Control: 5ml 100000 IU topical Nystatin, 6 times a day for 2 weeks. Intervention: PDT of the lesion	Denture stomatitis	0.005% methylene chloride (Methylene blue; Chimiolux, Hipopherma) Twin laser (Twin Flex Evolution - MM Optics Ltda, São. Carlos, Brazil)	Intra-oral PDT: λ:660nm power: 40mw Energy density: 120J/cm^2 Length of application: 2 min per point Pre-irradiation time: 20 min Number of application points: varies according to extent of the lesion. On average 1 cm apart. Number of applications: 2 (1 week apart)	Procedure not specified	Quantification via counts of CFU's Species identification via germ tube, micro-culture in fermented agar and fermentation and assimilation of carbohydrates Time points: after 1st application, after 2nd application (one week later) and after 1 month.
Mima *et al* [24]	Control: Nystatin topical nystatin oral suspension 100 000 IU. Swish it for 1 min, gargle, and then expectorate it four times daily for 15 days. Intervention: PDT of the palate and maxillary denture	Denture stomatitis	Haematoporphyrin derivative (Photogem®) LED Ten LEDs uniformly distributed on a circular platform	PDT for denture and intraoral Intra-oral: λ: 440-460nm Power: 260 mW Energy density: 122J/cm^2 Intensity: 102 mW/ cm^2 Length of application: 20 minutes Number of applications: 6 sessions- 3 times per week for 15 days	Clinical assessment of infection severity using Newton's classification of denture stomatitis. Time points: 0, 15, 30, 60, 90 days	*Candida* colony counts from the palate and denture surfaces quantified as CFU/mL *Candida spp*. prevalence Time points: days 0, 15, 30, 60, 90
Scwingel *et al* [25]	Control: (fluconazole 100mg/day during 14 days). Intervention 1: light laser therapy (LLT) Intervention 2: PDT or lesion	Oral candidiasis	Methylene blue Twin Laser	λ:660nm Power: 30mw Length of application: 10 seconds Pre-irradiation time: 1 min Number of application points: 9 Number of applications: 1	Clinical efficacy- changing signs and symptoms from baseline Time points measured: every 2 days	Semiquantification of CFU of Candida spp. Time points measured: 0, 7, 15, 30 days
Senna[31]	Control: miconazole oral gel three times a day for 4 weeks. Intervention: PDT of mucosa and dentures	Denture stomatitis	Methylene blue Laser GaAlAs – Photon Lase III – DMC	λ:660nm Power: 100 mw Pre-irradiation time: 10 minutes Energy density: 28J/cm^2 Length of application: 20 seconds number of applications: 8 (twice a week for 4 weeks)	Clinical efficacy: Budtz-Jorgensen classification Time points: before treatment and 48 hours after the end of treatment.	Microbiological efficacy: response was assessed by the proposed method by Olsen (1974). Time points: before treatment and 48 hours after the end of treatment (after 4 weeks).

4.4.4 Antifungal medication

Two of the studies used nystatin suspension as the comparator. One advised rinsing with 5ml of 100000 IU suspension six times a day for two weeks [22] and the other advised rinsing with the same dosage, four times daily for two weeks.[24] The study with HIV-positive participants used 100mg of fluconazole a day for 15 days [25] as the comparator. In the fourth study, miconazole gel was applied to the affected area three times daily for four weeks.[31]

4.4.5 Method of clinical and microbiological Assessment

One study used the Newton classification [39] of denture stomatitis to assess clinical changes.[24] This was done at baseline, the end of treatment (day 15) and on follow up (days 30, 60 and 90). Another study assessed clinical efficacy by changing signs and symptoms from baseline, however, no particular assessment tool was mentioned.[25]

Quantification of colony forming units (CFUs) was used to assess the microbiological success of treatment in two studies.[24] The remaining studies made use of different means of semi-quantification of CFU/ml.[25, 31] This was either done by making a visual assessment of the medium turbidity (clear, mild or intense) of cell cultures in test tubes and then scored as low, medium or abundant growth of fungus accordingly.[25] Alternatively, the CFUs were counted and expressed in degrees of density: 0 – no growth, 1 – growth from 1 to 9 CFU; 2 – growth from 10 to 24 CFU, 3 – growth from 25 to 100 CFU, 4– growth greater than 100 CFU, 5 – confluent growth.[31]

4.5 Outcomes: Clinical and mycological

PDT showed no difference in clinical improvement as compared with standard antifungal therapy (risk ratio (RR) =1.59 [95% confidence interval (CI), 0.44 to 5.82]; 3 studies, n=90 participants) (Figure 2).

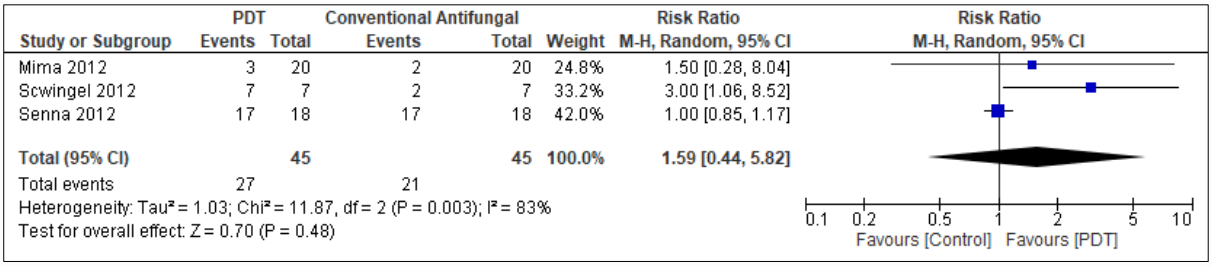

Figure 2: Forest plot of clinical efficacy at 30 days

This finding was consistent with regard to mycological efficacy as assessed using CFU's (Figure 3) (mean difference (MD) = 0.18 [95% CI, -0.98 to 1.33]; 2 studies, n=62).

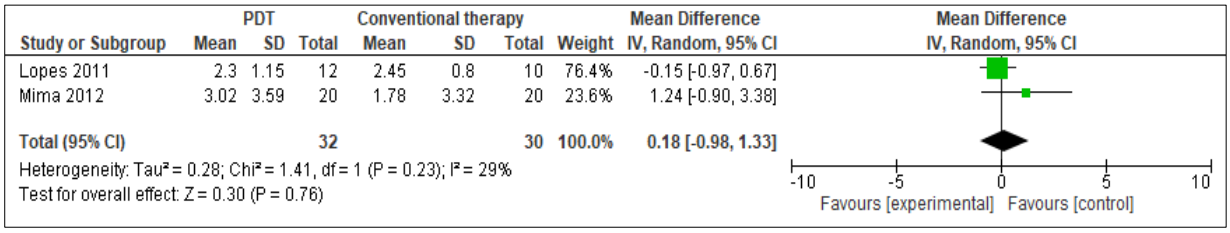

Figure 3: Forest plot of mycological efficacy using quantification of CFU's at 30 days

This finding was supported by the semi-quantification of CFU's (Table 4) (RR=1.51 [95% CI, 0.45; 5.02]; 3 studies, n=72).

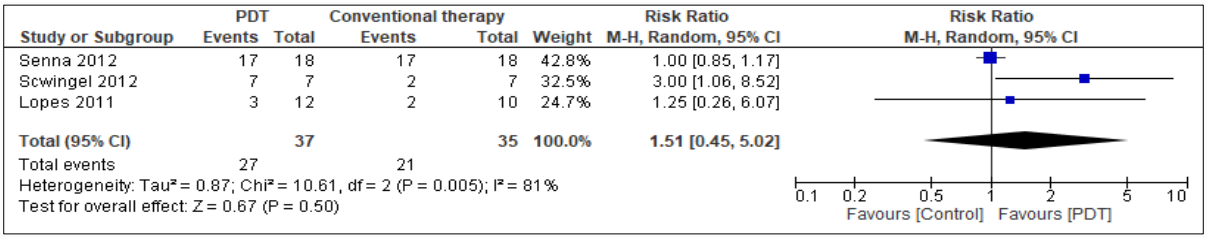

Figure 4: Forest plot of mycological efficacy of treatment using semi-quantification of CFU's at 30 days

PDT showed no difference in mycological effectiveness compared to conventional medication, assessed at 7 days from the start of treatment (Figure 5) RR= 1.07 ([95% CI 0.64; 1.77]; 2 studies; n= 36).

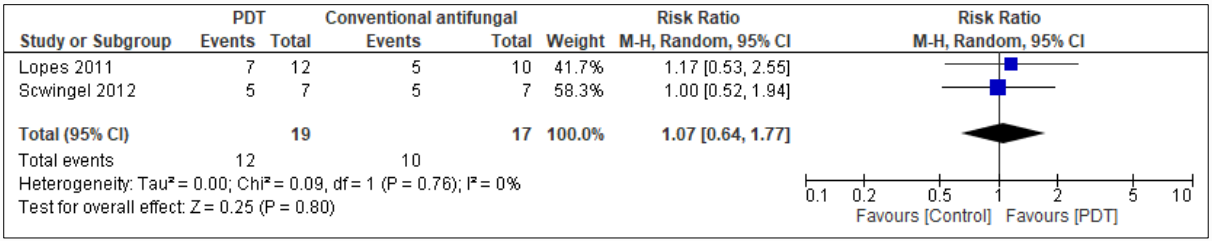

Figure 5 Mycological effectiveness of treatment at 7 days using semi-quantification of CFU's

Additionally, no clinical differences were found between therapies at 15 days (Figure 6) (RR= 1.09 [95% CI: 0.63; 1.88]; 2 studies; n=54).

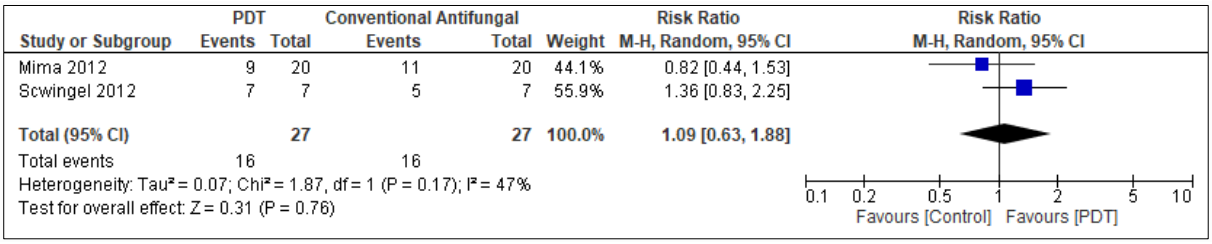

Figure 6: Clinical effectiveness of treatment at 15 days

This was in keeping with the mycological findings at 15 days (Figure 7 & 8) (MD= 0.37 [95% CI: -2.58; 3.31], 2 studies; n=62; RR=1.49 [95% CI: 0.97; 2.28]; 2 studies; n=36)

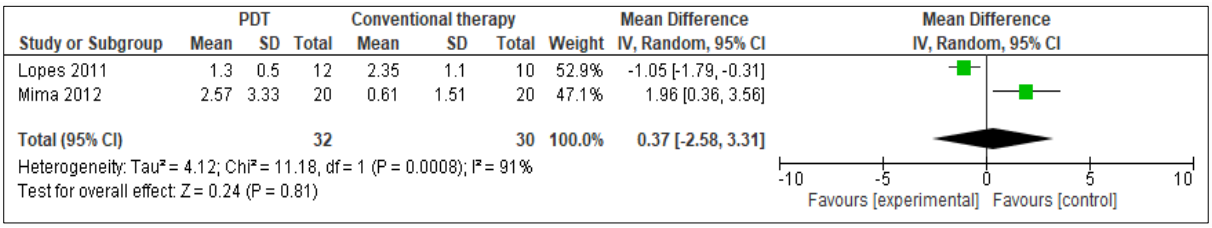

Figure 7: Mycological efficacy of treatment at 15 days using quantification of CFU's

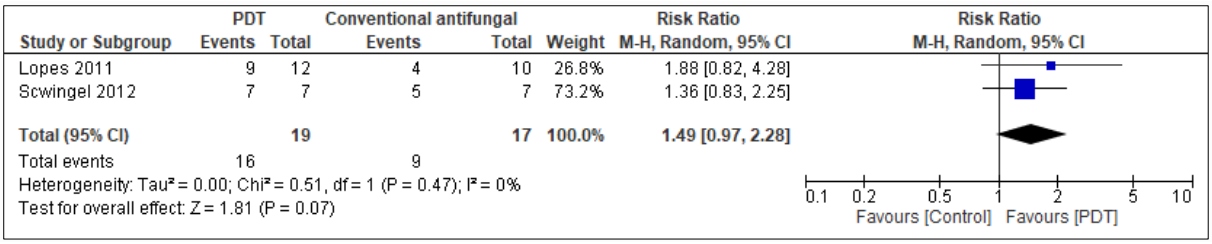

Figure 8: Mycological effectiveness at 15 days using semi-quantification of CFU's

No statistically significant difference between PDT and antfungal medication was found at either time point [15 days: RR= 1.09, 95% CI 0.63, 1.88; 30 days: [RR= 1.59, 95% CI= 0.44, 5.82]. Heterogeneity was found to be borderline high at 15 days (I^2= 47%) and very high at 30 days (I^2= 83%).

Similarly, the mycological efficacy of treatment was measured directly using log(CFU/ml) in only two studies.[22, 24] These results were pooled but no statistically significant results were found at 15 days [MD= 0.37, 95% CI=-2.58, 3.31] or 30 days [MD= 0.18, 95% CI=-0.98, 1.33]. Two of the studies used semi-quantification of the CFU's and one study provided a summary which allowed us to summarise data into a dichotomous variable. The outcome compared the mycological efficacy of the treatments by measuring the CFU's before and after treatment. This was done for timepoints 7 days, 15 days and 30 days. The meta-analysis failed to find any statistically significant differences between PDT and antifungal medication at any time point for all outcomes.

4.6. Publication bias

The researches were unable to conduct an assessment of publication bias due to the sparsity of studies.

4.7. Quality assessment

Grading of the pooled estimates using the GRADE criteria, found there to be a moderate to high risk of bias for all included studies (Figure 9). Due to these limitations, the quality of pooled estimates was found to be low.

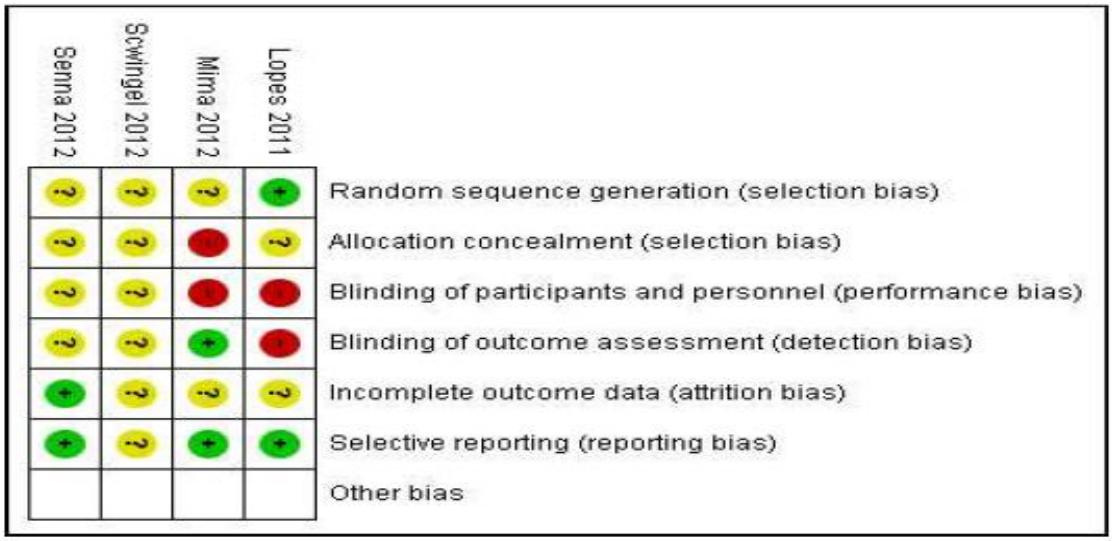

Figure 9: Risk of bias of included studies

5. DISCUSSION

This systematic review and meta-analysis found that PDT had similar effectiveness when compared with conventional therapy, in resolving oral fungal infections. Implementing PDT as an alternative management modality, however, requires refinement of treatment parameters.

This is the first systematic and meta-analysis performed on PDT and oral fungal infections analysing only human studies. Our results serve to update an earlier systematic review[40], which included experimental and animal studies, which concluded that there was a lack of sufficient evidence to support the clinical effectiveness of PDT for the treatment of oral fungal infections. Our review specifically included only human studies which utilised conventional therapy as a comparitor. We also placed no restriction on language and included unpublished studies. This enabled us to include two studies which were not included in the aforementioned review and allowed us to assess the most current evidence regarding PDT in the treatment of oral fungal infections in humans.

There has been a concerted effort to make the literature search as thorough and comprehensive as possible, limiting restrictions in the search itself. Extensive searches were done to find unpublished studies, conference papers and proceedings. In addition, the inclusion of only comparative studies enabled us to make robust comparisons with conventional therapy, thus presenting a higher level of evidence. All the included studies are randomised control trials even though it was not a prerequisite for inclusion.

The studies included in this review demonstrated heterogeneity; the biggest challenges were the lack of standardisation of methods across studies and variability in the assessment of outcomes. Studies utilised different treatment parameters which we would expect to affect treatment

outcomes. Thus, placing these treatment procedures under a single umbrella of PDT is not the most ideal means of analysis. Unfortunately, the lack of studies did not allow us to perform the desired subgroup analysis. Two studies used the quantification of colony forming units (CFUs)[22, 24] which is a direct method as opposed to the semi-quantification of CFU's used by the remaining two studies.[25, 31] This made it difficult to compare the microbiological outcomes. The methods used to combine and analyse these two outcome measures may have introduced some error into our findings. Lastly, there was a lack of detail on the measures taken to reduce bias within the included studies, especially with regard to randomization, blinding and outcome assessment. Although every effort has been made to reduce bias within our methods, these limitations should be considered when interpreting the results of this review. While we sought to undertake subgroup and sensitivity analyses, the small number of studies and variation in treatment effects precluded this step.

Finding the most effective treatment parameters was one of our secondary objectives. However, treatments varied significantly with regards to the light source, photosensitiser, wavelengths, frequency, duration of application, pre-irradiation time, number of applications and more. Thus, it is conceivable that outcomes may be influenced by these parameters. To highlight this, one recent study found contrasting findings to the four included articles.[23] This study could not be included in this review as they followed up one session of PDT with two sessions of light laser therapy. This study found a denture stomatitis cure rate of only 40% with PDT compared to 80% with miconazole treatment. It should be noted that the methylene- blue concentration and light fluence used in this study was lower than that used in other studies.[24, 31] This suggests that either one session of PDT may be insufficient to treat denture stomatitis or that the correct treatment parameters are crucial to PDT effectiveness or a combination of both. Hence, standardised PDT treatment parameters are required and one could speculate that further refinement of treatment parameters may be carried out on a case-by-case basis.

Another consideration is that the antifungal medication used as the comparators in the studies were used empirically. This implies that the medications and doses are not tailored to be most effective in treating the individual or the fungal strain. The antimicrobial sensitivity of the fungi was not accounted for and evidence exists that different fungal strains have variable sensitivity to the currently available antifungal medications.[13] This may skew data in favour of the PDT as the most appropriate antifungal medication may not have been used. Consequently, we do not know exactly which strains of fungi were being treated in each study and we do not know which were successfully eradicated by PDT or conventional therapy. Furthermore, three of the studies focused on denture stomatitis and other forms of oral fungal infections are not adequately represented. Thus, our findings should be limited to the treatment of denture stomatitis only. Future studies should therefore place more importance on determining fungal species and antimicrobial sensitivity and broadening the array of diseases being treated.

We intended to assess the effect of risk factors such as smoking, nocturnal denture wearing, pregnancy and systemic conditions predisposing to fungal infections have on treatment outcomes but were unable to do so due to our limited sample. Smoking is a risk factor for oral fungal infections and treatment outcomes tend to be inferior in smokers as compared to non-smokers.[32] The study by Senna *et al*[31] which included four smokers, found that miconazole was more effective at reducing fungal load than PDT. There was, however, only one smoker in the miconazole control group compared to 3 in the PDT group. A recent study by Abduljabaar *et al*[32] found that PDT is effective in the inactivation of oral fungal infections in smokers and non-smokers, however, at 3 months, CFUs were statistically significantly higher in smokers compared to non-smokers.[32] This may imply a greater rate of recurrence of denture stomatitis in smokers but it also suggests that smokers tend to have a higher *Candida* colony count compared to non-smokers.[32, 41] It is therefore important to account or control for patients with additional risk factors which may affect treatment outcomes.

Recurrence of fungal infection has been mentioned as a particular concern when using PDT.[22, 24] This corresponds to a recent case study done by Alves *et al*[33] who performed PDT on five patients with denture stomatitis. They used similar treatment parameters to Mima *et al*[24] and also found that all five patients had a recurrence of denture stomatitis at the end of day 45 of follow-up. More studies beyond 30 days will be required to assess if recurrence is a problem with PDT therapy in general or to determine if it is the specific treatment parameters used. If recurrence is found to be a problem with the use of PDT, it would be important to assess whether new fungal species have emerged, PDT- resistant species have developed or whether insufficient reduction of patient risk factors is a possible contributory factor to the recurrence.

The importance of finding alternatives to conventional antimicrobial medication cannot be stressed enough. PDT appears to have potential as a therapy for oral fungal infections. However, the lack of recent human studies begs to question as to why progress into this area has stalled. At present, it is still a relatively costly procedure requiring specialised equipment, not commonly available in general dental offices. However, there is an effort to create more cost-effective LED light sources.[42, 43] This would make PDT more accessible and thus have a far greater impact than would currently be possible. Moreover, one major benefit which can be noted is that no major adverse effects have been found with the use of PDT in the treatment of oral fungal infections. However, adverse effects have been noted is the treatment of skin conditions. These include erythema, pain, burns, oedema, itching, desquamation, and pustular formation.[44] Naturally, there is no risk of drug interactions which is a considerable problem with some antifungal medications. Thus, we can speculate that there is little risk to the use of PDT if one has the available resources. However, more clinical research is required on all aspects of PDT treatment parameters. There is a need for well-designed clinical trials which use standardized outcome measures, classifications and definitions to allow a more robust meta-analysis to be conducted and clinical guidelines to be developed.

6. CONCLUSION

6.1. Implications for Practice:

The findings of this review and meta-analysis, although limited due to the small number of studies and patient samples, suggest that Photodynamic therapy (PDT) is as effective at treating oral fungal infections compared with conventional antifungal medications and may be used as an alternative treatment with caution due to the ideal treatment guidelines not yet being established. Despite the criticism of the currently available evidence, there is a growing number of clinical applications for the use of PDT. However, the major drawback is the cost of light-sources. Consequently, although the use of PDT as an antifungal treatment modality appears encouraging, far too little is known about the treatment parameters to fully endorse its clinical use.

6.2. Implications for Research

Oral fungal infections is a common disease which can seriously impact the quality of life of those affected. Resistance to antifungal medication is occurring at an alarming rate and suitable alternatives need to be found with urgency. Thus, if a treatment modality shows as much promise as PDT and shows potential in reducing the reliance on antifungal medications, more effort is required to research it and add to the greater body of evidence on the use of PDT as an antimicrobial therapy. Although many laboratory studies are being conducted showing positive results, this study shows that there has been a failure to translate this into clinical studies. This has resulted in many factors regarding PDT being indeterminable at present, especially regarding the treatment parameters. It is imperative that more high-quality research is conducted with a standardised methodology, low risk of bias, adequate sample sizes and with longer follow-up periods.

7. CONFLICT OF INTEREST

None to declare.

8. FUNDING

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PART D: APPENDIX CONTENTS

APPENDIX A: LIST OF TERMS i
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APPENDIX A: LIST OF TERMS

AIDS- Acquired Immune Deficiency Syndrome

HIV- Human Immunodeficiency Virus

PACT- photodynamic antimicrobial chemotherapy

PDT- Photodynamic therapy

PS- Photosensitiser

CFU/mL-Colony Forming Units per millilitre

GRADE- Grading of Recommendations Assessment, Development and Evaluation

APPENDIX B: COMPREHENSIVE DATABASE SEARCH STRATEGY

Number	Search term	Yield
	PubMed **Date: October 2017** **Limits: none**	
1	PHOTOTHERAPY[MESH]	34250
2	PHOTODYNAMIC THERAP*[Title/Abstract]	15548
3	#1 OR #2	38967
4	CANDID*[Title/Abstract]	314251
5	FUNGAL INFECT*[Title/Abstract]	18417
6	MYCOSES[MESH]	116485
7	DENTURE STOMATITIS[Other Term]	61
8	#4 OR #5 OR #6 OR#7	411274
9	ORAL[Title/Abstract]) OR DENTAL[Title/Abstract	682360
10	#8 AND #9	20147
11	#3 AND #10	**90**
	Cochrane **Date: November 2017** **Limits: None**	
1	"oral fungal infections" (Word variations have been searched)	15
2	"oral candidiasis" (Word variations have been searched)	1301
3	"denture stomatitis" (Word variations have been searched)	100
4	"photodynamic therapy" (Word variations have been searched)	1383
5	"phototherapy" (Word variations have been searched)	2215
6	#1 or #2 or #3	398
7	#4 or #5	3545
8	#6 AND #7	**8**
	EBSCOHOST **Date: November 2017** **Limits: None**	
1	"oral fungal infection" OR “oral candid*” OR “denture stomatitis”	5926
2	photodynamic OR phototherapy	39289
3	#1 & #2	**39**
	Medline **Date: November 2017** **Limits: None**	
1	((kw: oral and kw: fungal and kw: infection)) or ((kw: oral and kw: candid*)) or ((kw: denture and kw: stomatitis))	22581
2	kw: photodynamic or kw: phototherapy	29861

3	kw: oral and kw: fungal and kw: infection) or ((kw: oral and kw: candid*)) or ((kw: denture and kw: stomatitis)) and (kw: photodynamic or kw: phototherapy	**111**
ISI Web of Science Date: November 2017 Limits: None		
1	TS= (Oral fungal infection OR Oral Candid* OR Denture stomatitis)	16989
2	TS= (Photodynamic or phototherapy)	40478
3	#2 AND #1	**134**
ProQuest Date: November 2017 Limits: None		
1	(oral fungal infection) OR (oral candid*) OR (denture stomatitis)	281,059
2	Photodynamic OR phototherapy	7,510
3	((“oral fungal infection”) OR (“oral candid*”) OR (“denture stomatitis”)) AND (Photodynamic OR phototherapy)	**22**
WoldCat Date: November 2017 Limits: None		
	kw:("Oral fungal infection" OR "Oral candid*" OR "denture stomatitis") AND (photodynamic OR phototherapy)	**197**
	*31 duplcates removed	**166**

Total 601 titles

Delete 251 exact duplicates

Total 351

PRISMA-P (Preferred Reporting Items for Systematic review and Meta-Analysis Protocols) 2015 checklist: recommended items to address in a systematic review protocol*

Section and topic	Item No	Checklist item
ADMINISTRATIVE INFORMATION		
Title:		
Identification	1a	Identify the report as a protocol of a systematic review
Update	1b	If the protocol is for an update of a previous systematic review, identify as such
Registration	2	If registered, provide the name of the registry (such as PROSPERO) and registration number
Authors:		
Contact	3a	Provide name, institutional affiliation, e-mail address of all protocol authors; provide physical mailing address of corresponding author
Contributions	3b	Describe contributions of protocol authors and identify the guarantor of the review
Amendments	4	If the protocol represents an amendment of a previously completed or published protocol, identify as such and list changes; otherwise, state plan for documenting important protocol amendments
Support:		
Sources	5a	Indicate sources of financial or other support for the review
Sponsor	5b	Provide name for the review funder and/or sponsor
Role of sponsor or funder	5c	Describe roles of funder(s), sponsor(s), and/or institution(s), if any, in developing the protocol
INTRODUCTION		
Rationale	6	Describe the rationale for the review in the context of what is already known
Objectives	7	Provide an explicit statement of the question(s) the review will address with reference to participants, interventions, comparators, and outcomes (PICO)
METHODS		
Eligibility criteria	8	Specify the study characteristics (such as PICO, study design, setting, time frame) and report characteristics (such as years considered, language, publication status) to be used as criteria for eligibility for the review
Information sources	9	Describe all intended information sources (such as electronic databases, contact with study authors, trial registers or other grey literature sources) with planned dates of coverage
Search strategy	10	Present draft of search strategy to be used for at least one electronic database, including planned limits, such that it could be repeated
Study records:		
Data management	11a	Describe the mechanism(s) that will be used to manage records and data throughout the review

Selection process	11b	State the process that will be used for selecting studies (such as two independent reviewers) through each phase of the review (that is, screening, eligibility and inclusion in meta-analysis)
Data collection process	11c	Describe planned method of extracting data from reports (such as piloting forms, done independently, in duplicate), any processes for obtaining and confirming data from investigators
Data items	12	List and define all variables for which data will be sought (such as PICO items, funding sources), any pre-planned data assumptions and simplifications
Outcomes and prioritization	13	List and define all outcomes for which data will be sought, including prioritization of main and additional outcomes, with rationale
Risk of bias in individual studies	14	Describe anticipated methods for assessing risk of bias of individual studies, including whether this will be done at the outcome or study level, or both; state how this information will be used in data synthesis
Data synthesis	15a	Describe criteria under which study data will be quantitatively synthesised
	15b	If data are appropriate for quantitative synthesis, describe planned summary measures, methods of handling data and methods of combining data from studies, including any planned exploration of consistency (such as I^2, Kendall's τ)
	15c	Describe any proposed additional analyses (such as sensitivity or subgroup analyses, meta-regression)
	15d	If quantitative synthesis is not appropriate, describe the type of summary planned
Meta-bias(es)	16	Specify any planned assessment of meta-bias(es) (such as publication bias across studies, selective reporting within studies)
Confidence in cumulative evidence	17	Describe how the strength of the body of evidence will be assessed (such as GRADE)

*** It is strongly recommended that this checklist be read in conjunction with the PRISMA-P Explanation and Elaboration (cite when available) for important clarification on the items. Amendments to a review protocol should be tracked and dated. The copyright for PRISMA-P (including checklist) is held by the PRISMA-P Group and is distributed under a Creative Commons Attribution Licence 4.0.**

From: Shamseer L, Moher D, Clarke M, Ghersi D, Liberati A, Petticrew M, Shekelle P, Stewart L, PRISMA-P Group. Preferred reporting items for systematic review and meta-analysis protocols (PRISMA-P) 2015: elaboration and explanation. BMJ. 2015 Jan 2;349(jan02 1):g7647.

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APPENDIX D: PRISMA CHECKLIST[2]

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	

Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	
DISCUSSION			

Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

2. Moher D, Liberati A, Tetzlaff J and Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA Statement. *BMJ*. 2009; 339.

Data Extraction and Assessment Template for Photodynamic Therapy for Oral Fungal Infections

Study ID:	**Report ID :**	Date form completed:
First author:	Year of study:	Data extractor:
Citation:		

1. General Information

Publication type	Journal Article ☐ Abstract ☐ Other (specify e.g. book chapter)_____
Country of study:	
Funding source of study:	Potential conflict of interest from funding? Y / N / unclear

2. Study Eligibility

Study Characteristics			**Page/ Para/ Figure #**
Type of study (Review authors to add/remove designs based on criteria specified in protocol)	☐ Randomised Controlled Trial (RCT) ☐ Cluster Randomised Controlled Trial (cluster RCT)	☐ Controlled Before and After (CBA) study • Contemporaneous data collection • Comparable control site • At least 2 x intervention and 2 x control clusters	
	☐ Interrupted Time Series (ITS) • At least 3 time points before and 3 after the intervention • Clearly defined intervention point	☐ Other design (specify):	
	☐ A process evaluation of an included study design	*Does the study design meet the criteria for inclusion?* Yes ☐ No ☐ →**Exclude** Unclear ☐	
	Description in text:		

Participants (Review authors insert inclusion criteria as defined in Protocol)	Describe the participants included:		
	Are participants defined as a group having specific social or cultural characteristics?	Yes ☐ No ☐ Unclear ☐ Details:	
	How is the geographic boundary defined?	Details: Specific location (e.g. state / country):	
	Do the participants meet the criteria for inclusion?	Yes ☐ No ☐ →**Exclude** Unclear ☐	

Types of intervention (Review authors insert inclusion criteria as defined in Protocol)	Strategies included in the intervention		
	Focus of the intervention		
	Parameters of PDT	**Wavelength (nm)**	**Power (mW)**
	Beam Area cm^2	**Light type:**	
	Photosensitisers*:*	**Length of application**	
	Number of applications:	**Pre-irradiation time:**	
	Fluence (J/cm^2)	**Number of application points:**	
	Does the intervention meet the criteria for inclusion?	Yes ☐ No ☐ →**Exclude** Unclear ☐	
Duration of intervention	Start date:	Stop date:	Intervention duration:
	Is the duration of intervention adequate for inclusion?	Yes ☐ No ☐ →**Exclude** Unclear ☐	
Types of outcome measures (Review authors insert inclusion criteria as defined in Protocol)	List outcomes:		
	Outcome measured at a population level or individual level?	Details:	
	Do the outcome measures meet the criteria for inclusion?	Yes ☐ No ☐ →**Exclude** Unclear ☐	

Summary of Assessment for Inclusion

Include in review ☐	**Exclude from review** ☐
Independently assessed, and then compared? Yes ☐ No ☐	Differences resolved Yes ☐ No ☐
Request further details? Yes ☐ No ☐	Contact details of authors:
Notes:	

DO NOT PROCEED IF PAPER EXCLUDED FROM REVIEW

3. Study details

Study intention	**Descriptions as stated in the report/paper**	**Page/ Para/ Figure #**
Aim of intervention	*What was the problem that this intervention was designed to address?*	
Aim of study	*What was the study designed to assess? Are these clearly stated?*	
Equity pointer: Social context of the study	*e.g. was study conducted in a particular setting that might target/exclude specific population s? See also Inclusion/exclusion criteria under Methods, below.*	
Start and end date of the study	*Identify which elements of planning of the intervention should be included*	
Total study duration		

Methods	**Descriptions as stated in the report/paper**	**Page/ Para/ Figure #**
Method/s of recruitment of participants *(How were potential participants approached and invited to participate? Where were participants recruited from? Does this differ from the intervention setting?)*		

Inclusion/exclusion criteria for participation in study		
Representativeness of sample: Are participants in the study likely to be representative of the target population?		
Total number of intervention groups		
Assumed risk estimate (*e. .baseline or population risk noted in Background)*	*References:*	
Sample size calculation: What assumptions were made? Were these assumptions appropriate?	 *(Yes/No/Unclear)*	
What was the unit of randomisation? Allocation by individuals or cluster/groups		
What was the unit of analysis? **Is this the same as the unit of randomisation?**	 *(Yes/No/Unclear)*	
Statistical methods used and appropriateness of these methods	*(Check with your statistician if unsure about appropriateness)*	

Results

Participants *Include if relevant*	**Include information for each group (i.e. intervention and controls) under study**	**Page/ Para/ Figure #**
• What percentage of selected individuals agreed to participate?		
• Total number randomised (or total pop. at start of study for NRCTs)		
• Number allocated to each intervention group (no. of individuals)		

• For cluster trials, number of clusters, number of people per cluster		
• Where there any significant baseline imbalances?	Yes ☐ No ☐ Unclear ☐ Details:	
• Number and reason for (and sociodemographic differences of) withdrawals and exclusions for each intervention group		
• Were patients who entered the study adequately accounted for?		
• What percentage of patients completed the study?		
• What percentage of participants received the allocated intervention or exposure of interest?		
• Is the analysis performed by intervention allocation status (intention to treat) rather than the actual intervention received? Have any attempts been made to impute missing data?		
• Age (median, mean and range if possible)		
• Sex		
• Race/Ethnicity		
• Principal health problem (incl. stage of illness)		
• Diagnostic criteria		
• Co-morbidity		
• Other sociodemographics (eg. Educational level, literacy level, soci-economic status, first language. Also consider possible proxies for these e.g. low baseline nutritional status)		
• PROGRESS categories reported at baseline (indicate letters of those reported: Place of residence, race, occupation, gender, religion, education, SES, social capital)		

Subgroups	*Enter a description of any participant subgroups from this paper to be analysed in the review.*	

Intervention Group 1

(copy and paste table for each Intervention group)

<table>
<tr><td>Group name:</td><td>(State brief name for this intervention group.)</td><td>Page / Para / Figure #</td></tr>
<tr><td colspan="3">Details of intervention or control condition (Include if relevant in sufficient detail for replication)</td></tr>
<tr><td>• Setting eg multicentre, university teaching hospitals, rural, metropolitan, school, workplace, community, GP clinic, etc.</td><td></td><td></td></tr>
<tr><td>• Theoretical basis (include key references)</td><td></td><td></td></tr>
<tr><td>• Content (list the strategies intended and delivered)</td><td></td><td></td></tr>
<tr><td>• Did the intervention include strategies to address diversity/disadvantage?</td><td>Enter a description of any relevant strategies</td><td></td></tr>
<tr><td>• Delivery (eg. Stages (sequential or simultaneous), timing, frequency, duration, intensity, fidelity – process indicators)</td><td>
<table>
<tr><td>Parameters of PDT</td><td>Wavelength (nm)</td><td>Power (mW)</td></tr>
<tr><td>Beam Area cm^2</td><td colspan="2">Light type:</td></tr>
<tr><td>Photosensitisers:</td><td colspan="2">Length of application</td></tr>
</table>
</td><td></td></tr>
</table>

	Number of applications: / **Pre-irradiation time:** **Fluence (J/cm^2)** / **Number of application points:**	
• Providers (who, number, education/training in intervention delivery, ethnicity etc. if potentially relevant to acceptance and uptake by participants		
• Co-interventions		
Duration of intervention		
Duration of follow-up		
Was sustainability discussed by the authors? Was is a consideration in study development?		
Economic variables ie costs of the intervention, and changes in other (eg health care) costs as result of intervention♠	Yes ☐ →**List in Outcome section if appropriate** No ☐ Unclear ☐ Details:	
Other economic information (from a societal, non-healthcare view – e.g. lost wages, time)	Yes ☐ No ☐ Details:	
Resource requirements to replicate intervention (e.g. staff numbers, hours of implementation, equipment?)		
Subgroups	*Enter a description of any intervention subgroups from this report to be analysed in the review.*	
What are the moderators/mediators of changes stated in the study?		

♠ Costs associated with the intervention can be linked with provider or participant outcomes in an economic evaluation (depends on the type of economic evaluation)

Do the authors describe any political or organisational context?	*List relevant dot points*	
Were any partnerships referred to?	*List these as dot points*	
Was a process evaluation conducted?	*What components were included in the process evaluation? (eg. dose, frequency, consistency, implemented as intended etc)*	
Control/comparison (what information is provided about what the control or comparison group received?)	*Enter a description of what was provided for the control group, if applicable*	

Outcomes

(This table is set up for 2 outcome measure to save spaces, copy and paste table as often as required)

Question	Outcome 1	Page/ Para/ Figure #	Outcome 2	Page/ Para/ Figure #
Is there an analytic framework applied (e.g. logic model, conceptual framework)?				
Outcome definition (with diagnostic criteria if relevant)				
Type of outcome: Is this a modifiable variable (Community level, neighbourhood level, individual level) or desired health outcome				
Time points measured				
Time points reported				
Is there adequate latency for the outcome to be observed?				
Is the measure repeated on the same individuals				

or redrawn from the population / community for each time point?				
Unit of measurement (if relevant)				
For scales – upper and lower limits and indicate whether high or low score is good				
How is the measure applied? Telephone survey, mail survey, in person by trained assessor, routinely collected data, other				
How is the outcome reported? Self or study assessor				
Is this outcome/tool validated?				
…And has it been used as validated?				
Is it a reliable outcome measure?				
Is there adequate power for this outcome?				
Were PROGRESS categories analysed by outcome? Indicate the letters of those that outcomes were analysed by (place of residence, race, occupation, gender, religion, education, SES, social capital)				

Results

Copy and paste the appropriate table for each outcome and subgroup at each timepoint, including baseline

For RCT/CCT

Dichotomous outcome

page/para/fig

Comparison					
Outcome					
Subgroup					
Timepoint					
Results	**Intervention**		**Comparison**		
	Events	No. participants	Events	No. participants	
No. of missing participants and reasons					
Any other results reported					
Reanalysis required? (specify - (e.g. correlation adjustment)					
Reanalysis possible?	*yes/no/unclear*				
Reanalysed results					

For RCT/CCT

Continuous outcome page/para/fig

Comparison		
Outcome		
Subgroup		

Timepoint							
Post-intervention or change from baseline?							
Results	**Intervention**			**Comparison**			
	Mean	SD (or other variance)	No. participants	Mean	SD (or other variance)	No. participants	
No. missing participants and reasons							
Any other results reported							
Reanalysis required? (specify)							
Reanalysis possible?	*yes/no/unclear*						
Reanalysed results							

For RCT/CCT

Generic inverse variance method

Page/para/figure

Comparison				
Outcome				
Subgroup				
Timepoint				
Results	Effect estimate	SE (or other variance)	Intervention no.	Control no.
No. missing participants and reasons				

Any other results reported		
Reanalysis required? (specify)		
Reanalysis possible?	*yes/no/unclear*	
Reanalysed results		

For CBA

Page/para/fig

Comparison			
Assignment	How were control and treatment groups selected?? Is there likely to be an effect if these were the opposite way?		
	Contemporaneous data collection?		
Outcome			
Subgroup			
Timepoint			
Post-intervention or change from baseline?			
	Intervention	**Comparison**	
No. participants measured			
No. missing participants and reasons			
Baseline result (with variance measure)			
Post-intervention			

results (with variance measure)			
Change (Post – baseline) (with variance measure)			
Difference in change (intervention – control) (with variance measure)			
Any other results reported			
Reanalysis required? (specify)			
Reanalysis possible?	*yes/no/unclear*		
Reanalysed results			

For ITS

Generic inverse variance method

Page/para/fig

Comparison		
Outcome		
Subgroup		
Length of timepoints measured		
Snapshot or interval measured		
No. participants measured		

No. missing participants and reasons					
	Pre-intervention			Post-intervention	
No. of timepoints measured					
Mean value (with variance measure)					
Difference in means (post – pre)					
Percent relative change					
Result reported by authors (with variance measure)					
Reanalysis required? (specify)					
Reanalysis possible?	*yes/no/unclear*				
Individual time point results					
Read from figure?	*yes/no*				
Reanalysed results	Change in level	SE	Change in slope	SE	

Other relevant information

Were outcomes relating to harms/unintended effects of the intervention described? Include any data for these in the outcomes tables above		
Potential for author conflict *ie. evidence that author or data collectors would benefit if results favoured the intervention under study or the control*		
Key conclusions of the study authors		
Could the inclusion of this study potentially bias the generalisability of the review? Equity pointer: Remember to consider whether disadvantaged populations may have been excluded from the study.		
Is there potential for differences in relative effects between advantaged and disadvantaged populations? (e.g. are children from lower income families less likely to wear bicycle helmets)		
Are interventions likely to be aimed at the disadvantaged? (e.g. school meals aimed at poor children).		
Issues affecting directness (*Note any aspects of population, intervention, etc. that affect this study's direct applicability to the review question)*		
References to other relevant studies		
Additional notes by review authors		
Correspondence required for further study information (from whom, what and when)		

Risk of bias assessment

Please refer to Chapter 8 - *Table 8.5.c: Criteria for judging risk of bias in the 'Risk of bias' assessment tool and to the Cochrane EPOC Group's guidance for assessing* **Risk of bias for studies with a separate control group (RCTs, CCTs, CBAs) and Risk of bias for interrupted time series studies** (Appendix 3) for additional guidance for scoring Yes/No/Unclear. Note that the table below includes items from both EPOC tools. The ITS tool has been incorporated into the bottom of the table and all items for ITS studies are denoted by ITS preceding the risk of bias question.

Domain	Review authors' judgement*	Description	Page/ Para/ Figure #
Was the allocation sequence adequately generated?	**Yes / No / Unclear**	***Describe the method used to generate the allocation sequence in sufficient detail to allow an assessment of whether it should produce comparable groups.***	
Was allocation adequately concealed?	**Yes / No / Unclear**	***Describe the method used to conceal the allocation sequence in sufficient detail to determine whether intervention allocations could have been foreseen in advance of, or during, enrolment.***	
Were baseline outcome measurements similar?	**Yes/No/Unclear**	***Note whether baseline outcome measurements were reported and whether there were any important differences between groups. If there were important differences between groups, note whether appropriate adjusted analysis was performed to account for this.***	
Were baseline characteristics similar?	**Yes/No/Unclear**	***Note whether baseline characteristics were reported and whether there were any important differences between groups.***	
Were incomplete outcome data adequately addressed? ***Assessments should be made for each main outcome (or class of outcomes).***	**Yes / No / Unclear**	***Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. State whether attrition and exclusions were reported, the numbers in each intervention group (compared with total randomized participants), reasons for attrition/exclusions where reported, and any re-inclusions in analyses performed by the review authors.***	
Was knowledge of the allocated intervention adequately prevented during the study?	**Yes / No / Unclear**	***Describe all measures used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective, or whether blinding was appropriate.*** • Participants – yes, no, unclear *[record supporting statement from study].*	

Separate assessments should be made for relevant groups of people involved in the study i.e participants, outcome assessors, investigators, data assessors etc		• Investigators – yes, no, unclear *[record supporting statement from study].* • Outcomes assessors – yes, no, unclear *[record supporting statement from study].* Data assessors – yes, no, unclear *[record supporting statement from study].*	
Was the study adequately protected against contamination?	**Yes/No/Unclear**	***State whether and how the possibility of contamination was minimised by the study design/implementation.***	
Are reports of the study free of suggestion of selective outcome reporting? ***Assessments should be made for each main outcome (or class of outcomes).***	**Yes / No / Unclear**	***State how the possibility of selective outcome reporting was examined by the review authors, and what was found.***	
Other sources of bias •	**Yes / No / Unclear**	***State any important concerns about bias not addressed in the other domains in the tool.***	
ITS: Was the intervention independent of other changes?	**Yes/No/Unclear**	***Describe whether or not the intervention occurred independently of other changes over time and whether or not the outcomes may have been influenced by other confounding variables/historic events during the study period.***	

ITS: Was the shape of the intervention effect pre-specified?	**Yes/No/Unclear**	***State whether or not the point of analysis was the point of intervention. If not, describe whether a rationale for the shape of the intervention effect was given by the study authors.***	
ITS: Was the intervention unlikely to affect data collection?	**Yes/No/Unclear**	***Describe whether or not the intervention was likely to affect data collection and what the potential impact might have been.***	
ITS: Was knowledge of the allocated interventions adequately prevented during the study? ***Separate assessments should be made for relevant groups of people involved in the study i.e participants, outcome assessors, investigators, data assessors etc***	**Yes/No/Unclear**	***Describe all measures used, if any, to blind study participants and personnel from knowledge of which intervention a participant received. Provide any information relating to whether the intended blinding was effective, or whether blinding was appropriate.*** • Participants – yes, no, unclear *[record supporting statement from study].* • Investigators – yes, no, unclear *[record supporting statement from study].* • Outcomes assessors – yes, no, unclear *[record supporting statement from study].* Data assessors – yes, no, unclear *[record supporting statement from study].*	
ITS: Was incomplete outcome data adequately addressed? ***Assessments should be made for each main outcome (or class of outcomes).***	**Yes/No/Unclear**	***Describe the completeness of outcome data for each main outcome, including attrition and exclusions from the analysis. State whether attrition and exclusions were reported, the numbers in each intervention group (compared with total randomized participants), reasons for attrition/exclusions where reported, and any re-inclusions in analyses performed by the review authors.***	
ITS: Was the study free from selective reporting?	**Yes/No/Unclear**	***State how the possibility of selective outcome reporting was examined by the review authors, and what was found.***	

ITS: Was the study free from other risks of bias?	**Yes/No/Unclear**	***State any important concerns about bias not addressed in the other domains in the tool.***	

* Note: For each section above 'Yes' indicates a 'low risk of bias'; 'No' indicates a 'high risk of bias'; 'Unclear' indicates an 'uncertain risk of bias'. When entering the data into RevMan, the options to choose from will be 'Low', 'High' and 'Unclear'

Results

Comparison: _____

Outcome: _____

Subcategory: _____

Treatment group:		**Control group:**	
Observed (n)	total (N)	observed (n)	total (N)

	Treatment group:	**Control group:**
Total randomised		
excluded*		
Observed		
lost to follow up*		

*Reasons for loss/exclusion:

_____

_____

Subcategory: _____

Treatment group:		**Control group:**	
Observed (n)	total (N)	observed (n)	total (N)

	Treatment group:	**Control group:**
Total randomised		
excluded*		
Observed		
lost to follow up*		

PHOTODIAGNOSIS AND PHOTODYNAMIC THERAPY

ELSEVIER

AUTHOR INFORMATION PACK

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ISSN: 1572-1000

DESCRIPTION

Official Journal of the European Platform for Photodynamic Medicine
Affiliated with the International Photodynamic Association
Also affiliated with the British Medical Laser Association and the Polish Society for Photodynamic Medicine

INDEXED in MEDLINE/PubMed, SciSearch/Science Citation Index Expanded, Current Contents/Clinical Medicine.

Aims and Scope:
Photodiagnosis and Photodynamic Therapy is an international journal for the dissemination of scientific knowledge and clinical developments of **Photodiagnosis** and **Photodynamic Therapy** in all medical specialties. The journal publishes original articles, review articles, case presentations, "how-to-do-it" articles, Letters to the Editor, short communications and relevant images with short descriptions. All submitted material is subject to a strict peer-review process.

AUDIENCE

Professionals in all medical disciplines with an interest in medical and biological applications of lasers and light sources, and photodiagnosis/photodynamic therapy in the treatment of human disease.

IMPACT FACTOR

2017: 2.895 © Clarivate Analytics Journal Citation Reports 2018

ABSTRACTING AND INDEXING

Current Contents / Clinical Medicine
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GUIDE FOR AUTHORS

INTRODUCTION

Scope

Photodiagnosis and Photodynamic Therapy is an international journal for the dissemination of scientific knowledge and clinical developments of Photodiagnosis and Photodynamic Therapy in all medical specialities. The journal publishes original articles, review articles, case presentations, "how-to-do-it" articles, Letters to the Editor, short communications and relevant images with short descriptions. All submitted material is subject to a strict peer review process.

Types of manuscript

Research Papers should report original clinical studies or research not previously published or being considered for publication elsewhere. Work in Progress may also be submitted. See below for the standard layout. Submission of a manuscript to this journal gives the publisher the right to publish that paper if it is accepted. Manuscripts may be edited to improve clarity and expression.

Review articles, including institutional reviews of recent developments are welcome, and will undergo peer review. Reviews should have an abstract of up to 250 words.

Editorials
Although most Editorials in the journal are commissioned, authors may contact the Editor-in-Chief to request submission of their own Editorial.

Correspondence. Readers are encouraged to write about any topic that relates to photodiagnosis or photodynamic therapy, clinical, scientific, educational, social or economic. Letters should be no longer than 500 words and may include discussions on material previously printed in the Journal.

Case report will be considered if formatted as a research letter with 2 figures maximum. Maximum length is up to 1000 words with up to 6 references and 2 tables or figures. There should be no Abstract and no headings.

Short Communications should not exceed 1000 words and should consist of a background section (not to exceed 100 words), aims (not to exceed 50 words), methods (not to exceed 250 words), results (not to exceed 250 words) and conclusion (not to exceed 250 words). An abstract of 150-200 words should also be provided. The editorial team reserves the right to decide which tables/figures submitted are necessary. No abstract is necessary.

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:
- E-mail address
- Full postal address

All necessary files have been uploaded:
Manuscript:
- Include keywords
- All figures (include relevant captions)
- All tables (including titles, description, footnotes)
- Ensure all figure and table citations in the text match the files provided
- Indicate clearly if color should be used for any figures in print

Graphical Abstracts / Highlights files (where applicable)
Supplemental files (where applicable)

Further considerations
- Manuscript has been 'spell checked' and 'grammar checked'
- All references mentioned in the Reference List are cited in the text, and vice versa

• Permission has been obtained for use of copyrighted material from other sources (including the Internet)
• A competing interests statement is provided, even if the authors have no competing interests to declare
• Journal policies detailed in this guide have been reviewed
• Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

BEFORE YOU BEGIN

Ethics in publishing

Please see our information pages on Ethics in publishing and Ethical guidelines for journal publication.

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors must disclose any interests in two places: 1. A summary declaration of interest statement in the title page file (if double-blind) or the manuscript file (if single-blind). If there are no interests to declare then please state this: 'Declarations of interest: none'. This summary statement will be ultimately published if the article is accepted. 2. Detailed disclosures as part of a separate Declaration of Interest form, which forms part of the journal's official records. It is important for potential interests to be declared in both places and that the information matches. More information.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify originality, your article may be checked by the originality detection service Crossref Similarity Check.

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