UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

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O efeito da desinfecção por microondas no tratamento da estomatite protética associada à *Candida spp.* e na estabilidade dimensional de resinas acrílicas para bases de próteses totais removíveis

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RESUMO

A desinfecção por microondas tem sido reportada como um método alternativo para o tratamento da estomatite protética associada a espécies do gênero *Candida*. Entretanto, além de escassa, a literatura ainda diverge sobre qual seria o melhor tratamento para a estomatite protética que, simultaneamente, não provoque alterações dimensionais nas próteses dos pacientes. Desta forma, o objetivo deste estudo foi avaliar, sistematicamente, a eficácia da desinfecção por microondas no tratamento da estomatite protética (parte 1), assim como os seus efeitos na estabilidade dimensional de resinas acrílicas para base de próteses totais removíveis (parte 2). Inicialmente, foram realizadas buscas nas bases de dados PubMed/MEDLINE, EMBASE e SCOPUS para estudos clínicos randomizados (parte 1) e estudos clínicos e *in vitro* (parte 2) que tenham comparado a desinfecção por microondas com grupos controle positivos e/ou negativos, todos publicados na língua inglesa. Os desfechos principais foram a melhora dos sinais clínicos e/ou redução do número de células de *Candida spp.* nas próteses e/ou mucosas palatinas, assim como a alteração dimensional medida em gramas. Desta forma, foram utilizados a diferença ponderada das médias, desvio padrão, risco relativo e um intervalo de confiança de 95% utilizando o modelo de desfecho randômico. A heterogeneidade foi avaliada por meio dos testes Q de Cochran e I^2, com nível de significância de $p=0.05$. Um total de 5 estudos clínicos randomizados foram incluídos na primeira parte, e 7 estudos *in vitro* na segunda. Os resultados demonstraram que a desinfecção por microondas apresentou eficácia comparável ($p>0.05$) à terapia antifúngica por meio da aplicação tópica de nistatina (100.000 IU/mL), assim como à desinfecção química com clorexidina a 2% e hipoclorito de sódio a 0.02%, e foi superior à aplicação tópica de miconazol no tratamento da estomatite protética. Adicionalmente, os resultados da meta-análise não mostraram diferença estatística entre a desinfecção por microondas e a aplicação tópica de nistatina (100.000 IU/mL) tanto para a contagem de células, assim como para as taxas de cura e recorrência ($p>0.05$). Em relação à estabilidade dimensional, a desinfecção por microondas gerou alterações significativas quando comparada à desinfecção química com hipoclorito de sódio, solução clorada, clorexidina e imersão em água ($p<0.05$). Entretanto, os protocolos de desinfecção utilizando 500 Watts por 3 minutos, e 450 Watts por 5 minutos, produziram resultados similares ($p>0.05$) ou melhores ($p<0.05$) que os grupos controle. Desta forma, a desinfecção por microondas demonstrou resultados comparáveis aos tratamentos convencionais usados para a estomatite protética, com o protocolo usando 650 Watts por 3 minutos, uma vez por semanas durante 14 dias apresentando melhor custo-benefício para a

prevenção de tratamento da estomatite protética associada a *Candida spp*. Adicionalmente , os protocolos de desinfecção utilizando 500 Watts por 3 minutos, e 450 Watts por 5 minutos, indicaram ser mais seguros em relação à estabilidade dimensional das resinas acrílicas.
Palavras-chave: Estomatite protética. Próteses totais removíveis. Microondas. Revisão sistemática. Meta-análise.

ABSTRACT

The effect of microwave disinfection in treating Candida-associated denture stomatitis and on the dimensional stability of denture base acrylic resins

Microwave disinfection has been reported as an alternative method for treating *Candida*-associated denture stomatitis (CADS). However, the information regarding the best protocol for treating *Candida*-associated denture stomatitis, and that does not promote changes on the dimensional stability of the complete dentures, is still scarce and diverging. Therefore, the objective of this study was to systematically assess the effectiveness of microwave disinfection against CADS (part 1), and its effect on the dimensional stability of denture base acrylic resins (part 2). The PubMed/MEDLINE, EMBASE, and SCOPUS databases were searched for randomized clinical trials (part 1), and clinical and *in vitro* studies (part 2) published in English, that have compared microwave disinfection to positive and/or control groups. The main outcomes were the improvement of the clinical signs and/or the decrease in the residual yeast of the dentures and palatal mucosa, and the changes on the dimensional stability measured in grams. Therefore, the mean differences, standard deviations, risk ratio, and 95% confidence interval were calculated by using the random-effects model. Heterogeneity was assessed by using Cochran's Q test and I^2 values. The level of significance was set at $p<.05$. A total of 5 randomized clinical trials were included in the first part, and 7 *in vitro* studies in the second. The results demonstrated that microwave disinfection presented comparable effectiveness ($p>0.05$) to antifungal therapy with topical nystatin (100.000 IU/mL), chemical disinfection with 2% chlorhexidine and 0.02% sodium hypochlorite, and was more effective than topical application of miconazole ($p<0.05$). In addition, the results did not show significant differences, between microwave disinfection and topical nystatin (100.000 IU/mL) for the mycological counts, and cure and recurrence rates ($p>0.05$). Regarding the dimensional stability, microwave disinfection produced significant changes when compared with sodium hypochlorite, chloride solution, chlorhexidine, and water immersion ($p<0.05$). However, disinfecting dentures at 500 Watts for 3 minutes, and 450 Watts for 5 minutes, produced similar ($p>0.05$) and better ($p<0.05$) results in comparison with control groups. Thus, microwave disinfection showed comparable results to conventional treatments protocols used against CADS, with the protocol using 650 Watts for 3 minutes,

once a week for 14 days, presenting the best cost-effectiveness for both prevention and treatment of CADS. In addition, microwave disinfection at 500 Watts for 3 minutes, and 450 Watts for 5 minutes, indicated to be safer for the dimensional stability of denture base acrylic resins.
Key words: Denture stomatitis. Complete denture. Microwaves. Systematic review. Meta-analysis.

LIST OF ABBREVIATIONS AND ACRONYMS

°C	Celsius degree
CADS	Candida-associated denture stomatitis
CASP	Critical Appraisal Skills Program
CG	Control group
CD	Complete denture
CI	Confidence interval
CFU	Colony-forming unit
CRD	Complete removable denture
DS	Denture stomatitis
g	Gram
JBI	Johanna Briggs Institute
M	Mean
MD	Mean difference
mL	Milliliter
MW	Microwave
MW1	Microwave disinfection with one irradiation per week
MW3	Microwave disinfection with three irradiations per week
MWMz	Microwave disinfection combined with miconazole
Mz	Miconazole
NYS	Nystatin
PMMA	Poly(methyl methacrylate)
ppm	Parts per million
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-analysis
PROSPERO	The International Prospective Register of Systematic Reviews
RCT	Randomized clinical trial
RR	Risk ratio
SD	Standard deviation
W	Watt

SUMMARY

1 INTRODUCTION

Complete removable denture (CRD) consists in a well stablished treatment modality that is still commonly used to rehabilitate completely edentulous patients.[1,2] Its satisfactory esthetics and reduced costs are the main reasons for its indication.[3,4] In addition, CRDs are also an important alternative for systemically compromised patients which cannot be subjected to surgical procedures for the placement of dental implants.[5]

The majority of the CRDs, approximately 90%, are constituted by poly(methyl methacrylate) (PMMA).[6] PMMA is a polymer composed of high molecular weight chains resulted from the combination of molecules of methyl methacrylate, which are successively combined in polymerization reactions.[7] Among the advantages that justifies its broad use, low cost, biocompatibility, easy manipulation and manufacturing, and good esthetics stands out.[4,8,9] However, PMMA presents some disadvantages such as low wear resistance, unsatisfactory flexural and impact strength, and high surface roughness.[3,10,11]

Treating completely edentulous patients with CRDs composed by PMMA often results in an important consequence, that is the adhesion and colonization of its surface by *Candida spp*.[12] This events are particularly influenced by inherent characteristics of PMMA, such as its hydrophobicity, surface free energy, and surface roughness.[13] In this regard, CRDs provides a favorable medium for the development of microbial biofilms, by acting as a protective environment for microorganisms against shear forces resulted from mechanical cleansing.[14,15] This is a relevant outcome, as CRDs can become potential sources systemic and local infections, such as Candida-associated denture stomatitis (CADS).[12,16,17]

Candida-associated denture stomatitis presents itself as one of the most prevalent infections among complete denture wearers, with prevalence rates ranging from 15% to 70%.[13] CADS is a chronic and multifactorial condition that is commonly associated with poor oral hygiene, nocturnal wear of the dentures, residual monomer allergy, impaired salivary flow, and systemic conditions.[13] However, several studies have been considering the primary infection of the palatal tissues by *Candida albicans* as the main etiological factor of CADS.[15,18]

Several treatment protocols against CADS have been proposed such as oral hygiene reinforcement, mechanical cleansing and chemical disinfection of the dentures, and antifungal therapy.[19] Nonetheless, some disadvantages have been correlated to these protocols. Previous studies have demonstrated that mechanical cleansing alone is not only not effective for treating CADS but may contribute to its development, as it can raise the surface

roughness of PMMA, due to mechanical trauma from brushing.[20] Moreover, antifungal therapy may induce systemic and local adverse reactions, the manifestation of resistant microorganisms, and often requires a higher commitment of the patient to the treatment, in order to obtain a proper efficacy.[15,17,18,21] On the other hand, the disinfection of the CRDs using chemical solutions has been associated with staining, allergic reactions, and with the reduction of the surface and mechanical properties of PMMA.[4]

Thus, in order to overcome this scenario, alternative treatments have been indicated, including microwave (MW) disinfection of the CRDs. Microwave disinfection consists in subjecting dentures to microwave irradiations which can be provided by a domestic microwave oven. Therefore, it is a low-cost, accessible (considering that the equipment is usually presented in homes) and has been reported as an effective method for eliminating *Candida* spp., and other microorganisms from denture's surfaces.[16]

Although its mechanism of action is still not fully understood, it is believed that the high frequency of the electromagnetic radiation may alter the cell's structure and permeability as a result from the heat generated from the interaction between cells molecules and the electromagnetic field.[20] Differently, other studies suggested that the cell damage may be a consequence of a mechanical disruption of the cells due to a high frequency oscillation induced by the electromagnetic field, therefore, exceeding the elastic limitations of the cell wall.[20,22,23] Consequently, as it consists in a physical method of disinfection, microwave disinfection has also the advantage of not allowing the possibility of the survival and selection of resistant microorganisms, as seen in antifungal therapy.[17]

Nonetheless, despite of its characteristics and advantages, previous studies have been indicating some disadvantages including deleterious effects on the flexural strength, microhardness, and specially on the dimensional stability of PMMA.[24-28] In this regard, preserving the dimensional stability of PMMA is vital, and necessary in any disinfection procedure involving CRDs, due to the direct influence of this property on the CRDs stability, retention and, consequently, on the prosthesis's performance and outcome of the prosthetic treatment.

In addition, there is a diverse range of microwave disinfection protocols described in the scientific literature.[29,30] The protocols may vary beginning with the immersion of the prostheses in distilled water (and the volume used) or not, also including several combinations among power setting, irradiation time, frequency of the irradiations and period of the treatment. Consequently, there is still no consensus regarding which microwave disinfection protocol might be effective, as a treatment, for Candida-associated denture stomatitis,

simultaneously not harming the integrity of the prostheses, especially its dimensional stability.

Therefore, the objective of this study is to systematically analyze the current literature in order to validate the effectiveness of microwave disinfection, and to propose the best evidence-based microwave disinfection protocol, guiding clinicians and patients in order to obtain an effective treatment against Candida-associated denture stomatitis and, simultaneously, a reliable protocol that does not interfere in PMMA's properties. The first null hypothesis is that there would be no differences among microwave disinfection and conventional treatments, for treating CADS. The second null hypothesis is that microwave disinfection would not affect the dimensional stability of denture base acrylic resins.

2 ARTICLES

The present research was responsible for the production of two independent manuscripts. Of those, the first manuscript was accepted and published, and the second manuscript was submitted to a journal. The detailed information about both manuscripts follows below:

1. Da Costa, RMB, Poluha RL, De la Torre Canales G, Junior JFS, Conti PCR, Neppelenbroek KH, Porto VC. The effectiveness of microwave disinfection in treating Candida-associated denture stomatitis: a systematic review and metaanalysis. Clin Oral Investig. 2020; 24(11):3821-3832. Doi: 10.1007/s00784-020-03599-5. Proof document in annex 1.
2. Does microwave disinfection affect the dimensional stability of denture base acrylic resins? A systematic review. Gerodontology. Proof document in Annex 2.

2.1 ARTICLE 1 – THE EFFECTIVENESS OF MICROWAVE DISINFECTION IN TREATING DENTURE STOMATITIS: A SYSTEMATIC REVIEW AND METAANALYSIS

The effectiveness of microwave disinfection in treating Candida-associated denture stomatitis: A systematic review and meta-analysis.

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Costa; Writing – review and editing: all authors; Supervision: Vinicius Carvalho Porto, Karin Hermana Neppelenbroek, Joel Ferreira Santiago Junior, and Paulo Cesar Rodrigues Conti.

Abstract

Objective: To evaluate the effectiveness of microwave disinfection in treating Candida-associated denture stomatitis (CADS).

Materials and Methods: The PubMed/MEDLINE, Embase, and Scopus databases were searched for reports on randomized clinical trials (RCTs) published in English until May 2020 (PROSPERO CRD42020192062) that evaluated the treatment of CADS by using microwave disinfection. The main outcomes were the improvement of clinical signs and/or the decrease in the residual yeast present on the dentures and palatal mucosa. The mean differences, standard deviations, risk ratio, and 95% confidence interval were calculated by using the random-effects model. Heterogeneity was assessed by using Cochran's Q test and I^2 values. The level of significance was set at α=0.05.

Results: Five RCTs with 245 participants were included. The descriptive investigations demonstrated that microwave disinfection was as effective ($p > 0.05$) as 0.2% chlorhexidine, 0.02% sodium hypochlorite, and topical nystatin (100.000 IU/mL), and was superior to topical miconazole in treating CADS. The meta-analysis did not show a statistical difference between microwave disinfection and nystatin (100.000 IU/mL) treatment in terms of mycological counts, cure, and recurrence rates ($p > 0.05$).

Conclusion: Microwave disinfection showed comparable results to those of conventional therapies for treating CADS. In addition**,** treatment with 650 Watts for 3 minutes once a week for 14 days had better cost-effect results, indicating both the prevention and treatment of CADS.

Clinical relevance: Our findings provide evidence regarding the treatment of CADS using microwave disinfection, and also indicating the best cost-effective option for this treatment modality.

Keywords: Denture stomatitis; Treatment; Microwaves; Randomized controlled trial; Systematic review, Meta-analysis

Introduction

Candida-associated denture stomatitis (CADS) is an opportunistic fungal infection that affects approximately 15%–70% of complete removable denture wearers [1]. Although its etiology is considered multifactorial, the tissue and denture infestation by *Candida* species, especially *Candida albicans,* is the most prominent etiological factor [1]. Biofilm accumulation on the denture is critical, as it makes the denture a reservoir for future infections, which may induce local and systemic infections, including gastrointestinal infections, aspiration pneumonia, and other respiratory infections [2-4]. The treatment of CADS includes meticulous oral and denture

hygiene, removal of the dentures at night, salivary stimulation, topical agent administration, systemic medication administration, and denture disinfection [5].

Different methods of denture disinfection have been proposed, the most common being chemical methods such as the use of glutaraldehyde, sodium hypochlorite (NaOCl), iodoform, chlorine dioxide, or alcohol solutions [4]. Moreover, chemical disinfection has some disadvantages, such as prosthesis staining and oral tissue reactions in patients [6]. As an alternative, microwave irradiation is a simple, effective, accessible (a microwave equipment is present in the majority of homes), and an inexpensive method for eradicating *Candida* spp. from the surfaces of complete dentures with less alteration [2].

Microwaves are a form of high-frequency electromagnetic radiation which either lead to cell death by altering the cell structure and its membrane permeability caused by the heat generated upon the organic matter or leads to cell destruction via an interaction between the electromagnetic field produced by the microwaves and the cell molecules [7-9]. However, there is no consensus on the efficiency of the microwave disinfection protocol on *Candida* spp. and its indication as a treatment in patients with CADS. To analyze the current literature, validating the efficacy of microwave disinfection, and suggesting which could be the best evidence-based protocol for microwave disinfection are important factors to help clinicians and patients make evidence-based decisions.

Therefore, we conducted a systematic review of the literature to validate the efficacy of microwave disinfection and to formulate the best evidence-based protocol for the treatment of CADS by microwave disinfection of dentures. The null hypothesis is that there would be no difference between microwave disinfection and the conventional treatments proposed.

Methods

Registration and protocol

The present systematic review is registered in the PROSPERO (International Prospective Register of Systematic Reviews) database with title “Does microwave disinfection effectively treat denture stomatitis: A systematic review and meta-analysis,” registration number CRD42020192062, and was elaborated based on the guidelines proposed in the PRISMA Statement (Preferred Reporting Items for Systematic Reviews and Meta-analyses) for the development of systematic reviews with meta-analyses [10], and also applying the criteria established by the Cochrane Collaboration (Cochrane Handbook for Systematic Reviews of Interventions, version 5.1.0) [11, 12]. In addition, recently published systematic review models were also adopted in this study [13-14].

Eligibility criteria

The following PICO (Population [P], Intervention [I], Comparison [C], Outcome [O] question was adopted in the data collection: “is microwave disinfection (I) an equally effective method (O) for treating patients with CADS (P), when compared to conventional protocols (C)?” where the primary outcomes were improvement in the residual yeast present on the dentures and palatal mucosa, and/or of the clinical signs of CADS.

The studies were selected according to the following inclusion criteria: (1) English language; (2) randomized clinical trials involving the treatment of CADS in complete denture wearers with microwave disinfection; and (3) studies including negative and/or positive controls.

The exclusion criteria included in vitro studies, animal studies, case reports, case series, studies with sample numbers less than 10, and studies with incomplete or absent data regarding the microwave disinfection protocol used.

Information source, search strategy, and data collection

Multiple databases including PubMed/MEDLINE, Embase, and Scopus were assessed to search for articles published until May 6, 2020. For this, the following MeSH/PubMed-based Boolean operator were adopted: "Acrylic resins", "Dentures", "Denture Bases", "Microwaves", "Disinfection" and the key words were "((Acrylic resins AND Dentures OR Denture Bases OR Complete denture) AND Microwaves) AND Disinfection".

Data collection was performed independently by two previously calibrated examiners (R.M.B.C and R.L.P). In case of any disagreement between the reviewers, consensus meetings were held, and the discrepancies were resolved by a third researcher (G.D.C). Titles and abstracts were evaluated, and inter-examiner tests (Kappa) were applied to reduce article selection bias. Finally, a definitive consensus meeting with all authors was held to evaluate the selected articles, data collection, and risk of bias.

Data items

The following data were obtained from each study: (1) author; (2) publication year; (3) number of patients; (4) mean age of the patients; (5) mean age of the dentures; (6) microwave disinfection protocols; (7) conventional treatment protocols; (8) *Candida* colony count on the palatal mucosa and dentures, (9) clinical signs of denture stomatitis, (10) treatment periods, (11) follow-up after treatment, (12) cure percentages, (13) recurrence percentages, and (14) study design.

Quality assessment

In order to increase the strength of this systematic review, all included studies underwent a quality assessment using the Critical Appraisal Skills Program (CASP) Randomized Controlled Trial Checklist [15]. This checklist consists of 11 questions to assess the validity, results, and applicability of a randomized controlled trial that can be answered with "yes," "can't tell," or "no" answers. This procedure was performed by three authors (P.C.R.C, K.H.N, and V.C.P) and consensus meetings with all authors were held to discuss any disagreements.

Summary measures

For the analyses of microwave and nystatin treatment on the *Candida* colony counts on the palatal mucosa and dentures, the mean values, mean differences (MD), and standard deviation (SD) (continuous outcome) were used, taking into consideration the time intervals (0, 14, 30, 60, and 90) and sample sizes of each group. The weight contribution of each study was also assessed. In order to compare the cure and recurrence rates of the individuals (a dichotomous outcome), the risk ratio (RR) with a 95% confidence interval (CI) was used for all analyses.

A P-value < 0.05 was considered statistically significant [12]. The software program Comprehensive Meta-Analysis (Software version 3.0 – Biostat, Englewood, NJ, USA) was used to implement the meta-analysis and to construct the forest plots [16].

Additionally, a multiple-treatment meta-analysis was performed according to the Cochrane principles. An additional subgroup analysis regarding one study [3] was also performed. In this analysis, the data regarding the

experimental groups that applied microwave (MW) disinfection protocols with one (MW1) and three (MW3) irradiations per week was gathered for only one combined group, as recommended [17, 18]. Lastly, the combination of continuous data was achieved using StatsToDo [19], an online tool.

Risk of bias in the studies

The random-effects model was adopted to reduce the potential of heterogeneity since the selected studies presented different characteristics, such as sample size of the groups [14]. Heterogeneity was evaluated using Cochran's Q test and I^2 values. I^2 values higher than 75 (range, – 0-100) were considered to indicate significant heterogeneity [13,14].

Results

One hundred and fifty-five potential articles were initially identified, of which 81 were overlapping. Thus, 74 citations were eligible. Fifty articles and seventeen articles were excluded after title and abstract editing, respectively. Based on the reported criteria, seven papers were read in full text and, after consensus, five randomized clinical trials (RCTs) [2, 3, 20-22] were included in this systematic review and two in the meta-analysis [3,22]. Information regarding the selection of the articles in the systematic review is shown in Fig. 1. The inter-rater agreement for the study selection (kappa) between reviewers was 0.84 (95% CI = 0.71.0.96).

Study findings

The total number of participants included in the systematic review (Table 1) was 245, with a range of 34 to 60 per study [2, 3, 20-22]. The mean age of the patients varied from 56.8 to 83.7 years. Three studies assessed the ages of the dentures [3, 18, 19], with a mean age range of 12.8-24.8 years. The same studies also reported a higher prevalence of CADS in females [3, 21, 22]. Concerning the risk factors, two studies reported a high frequency of patients with xerostomia, nocturnal wear of the dentures, and poor hygiene habits [3, 22]. In general, studies have evaluated patients without systemic conditions, except one, which assessed only patients with well-controlled type 2 diabetes [22]. The majority of participants were recruited in outpatient clinics at universities or hospitals. Only one study included institutionalized individuals from geriatric care hospitals [20].

Most of the interventions were carried out for 14 days [2, 3, 22]. Banting and Hill [2] treated the participants for 7 days. Neppelenbroek et al. [21] performed a longer intervention of 30 days. Most studies [2, 3, 21, 22] performed a follow-up of participants for up to 90 days.

The assessment of the experimental groups showed different microwave disinfection protocols, with power levels ranging from 350 Watts [17] to 850 Watts [2], with most studies adopting 650 Watts [3, 21, 22]. The exposure times varied from shorter periods such as 1 minute [2] and 3 minutes [3, 22] to longer periods such as 6 [21] and 10 minutes [20]. Regarding the frequency of irradiation, most studies applied weekly irradiations [3, 21, 22]. However, one study [20] performed daily irradiations and one opted for three irradiations carried along 14 days [2] (Table 1). Nystatin treatment combined with routine denture care was used in two studies [3, 22], routine denture care alone in two studies [20, 21], nystatin with chlorhexidine overnight soaking in one study [2], sodium hypochlorite overnight soaking in one study [20], and miconazole treatment in one study [21] (Table 1).

Considering the mycological outcomes, Silva et al. [3] and Sanitá et al. [22] observed that microwave disinfection was as effective as nystatin in reducing *Candida* cell counts on both palates and dentures at days 14 and 30 ($p > 0.05$). Neppelenbroek et al. [21] noticed the absence of *Candida* viable cells and mycelial forms on

the palates and dentures, at days 15 and 30, after microwave disinfection alone or combined with topical miconazole, with no statistical significance difference between groups ($p > 0.05$), but was superior to topical miconazole alone and routine denture care ($p < 0.05$). Webb et al. [20] observed that microwave disinfection and chemical disinfection using 0.02% NaOCl reduced the numbers of *Candida* in palates and dentures, and aerobic bacteria from dentures with similar results ($p > 0.05$). However, neither treatment reduced the aerobe count from palates. Banting and Hill [2] found comparable results ($p > 0.05$) from chemical disinfection with 0.2% chlorhexidine and microwave disinfection, with both treatments significantly reducing the mycelial forms of *Candida* on palates and dentures at the end treatment (Table 1).

Clinically, the studies indicated that microwave disinfection had similar results in comparison with topical nystatin ($p > 0.05$) [3, 22]. Silva et al. [3] observed an improvement of at least 65% of the clinical signs when assessing the results from all groups at the end of the treatment. Similarly, Sanitá et al. [22] found that microwave disinfection and nystatin successfully reduced the clinical signs, with cure rates of 40% for both groups on day 14. Neppelenbroek et al. [21] demonstrated that microwave alone or in combination with topical miconazole were equally effective ($p > 0.05$) in reducing the severity of inflammation and were more effective than miconazole alone and denture routine care ($p < 0.05$). Lastly, Webb et al. [20] also found similar clinical results ($p > 0.05$) in the microwave and NaOCl groups ($p > 0.05$) (Table 1).

Furthermore, the studies found clinical and mycological recurrences in a three-month follow-up. Silva et al. [3] reported recurrence of clinical signs with similar rates among groups ranging from 38.39% to 52.94% ($p > 0.05$). Similarly, Sanitá et al. [22] found comparable results comparing the recurrence rates of microwave disinfection and topical nystatin (55% and 40%, respectively) ($p > 0.05$). Neppelenbroek et al. [21] observed a recurrence of the mycelial forms only for the dentures, with recurrence rates of 33.3% for microwave disinfection alone and 40% when combined with miconazole ($p > 0.05$). The authors also observed viable cells in both groups for palates and dentures ($p > 0.05$). Additionally, 86.6% to 93% of the patients did not present clinical signs of inflammation until day 90 [21]. Lastly, over the three-month follow-up period, Banting and Hill [2] observed no significant differences ($p = 0.09$) between microwave disinfection and 0.2% chlorhexidine for mycological recurrences in denture smears. Notwithstanding, significant difference was found for palatal smears ($p < 0.001$), with only 8% of recurrence for microwave disinfection and 63% for chlorhexidine group (Table 1). Moreover, following the treatment phase, microwave irradiation also showed a significant less ($p < 0.04$) re-infestation (33%) compared to chlorhexidine soaking (58%) when considered both the palatal mucosa and tissue surfaces of maxillary dentures [2].

Quality assessment

The methodologic quality assessment of the studies, performed according to the CASP criteria for randomized controlled trials [15], is shown in Table 2. The results showed that the studies presented high scores, indicating that all studies were suitable for inclusion in the systematic review. In addition, although the five RCTs were rated as “no” on item 4 of the checklist “were the patients, health workers and study personnel ‘blind’ to treatment?”, it must be mentioned that in all studies, only the patients were not blinded.

Meta-Analysis

Two studies were selected for metaanalysis [3, 19] and the comparisons were performed taking into consideration the effect of the experimental (microwave) and control (nystatin) on the *Candida* colony count, on both the palatal mucosa and dentures, and on the percentage of cure and recurrence of the participants as well. Lastly, the metaanalysis was performed by dividing the data according to different periods (0, 14, 30, 60, and 90).

Comparison of *Candida* colony count measurements

There was no significant difference between the microwave disinfection and nystatin treatment groups in the metaanalysis regarding the data obtained at day 0 (baseline) (MD = - 0.242; 95% CI = - 0.583 to 0.099, p = 0.165) (Figure 2). This result were also observed after comparing these interventions on days 14 (MD = 0.251; 95% CI = - 0.063 to 0.564, p = 0.117), 30 (MD: 0.142; 95% CI = - 0.227 to 0.511, p = 0.450), 60 (MD: 0.210; 95% CI = - 0.181 to 0.601, p = 0.292), and 90 (MD: 0.372; 95% CI = - 0.019 to 0.764, p = 0.062) (Fig. 2).

The Cochran's Q test results for heterogeneity were: at baseline (Q value = 6.522, p = 0.259, I^2 = 23.336), on day 14 (Q value = 1.526, p = 0.910, I^2 = 0.000), day 30 (Q value = 1.581, p = 0.904, I^2 = 0.000), day 60 (Q value = 1.323, p = 0.933, I^2 = 0.000), and day 90 (Q value = 3.445, p= 0.632, I^2 = 0.000). Lastly, data were also assessed by combining the subgroups MW1 and MW3 from Silva et al. [3] for the *Candida* colony count measurements (continuous outcome data). The metaanalyses were reevaluated, and they exhibited no influence or modifications on the significance level of the results comparing between the control group and the MW1 and MW3 combination, with no statistically significant difference ($p > 0.05$), Supplementary Table 1.

Comparison of cure and recurrence rates

The comparison of the rates of cure between participants, treated using microwave disinfection and nystatin, did not show significant differences for all periods assessed: day 14 (RR = 1.309; 95% CI = - 0.796 to 2.151, p = 0.288), day 30 (RR = 0.983; 95% CI = 0.587 to 1.647, p = 0.949), day 60 (RR = 1.284; 95% CI 0.668 to 2.469, p = 0.454), and day 90 (RR = 1.232; 95% CI = 0.592 to 2.561, p = 0.577). This was also seen in the recurrence rate analysis on day 90 (RR = 1.067; 95% CI = 0.716 – 1.592, p = 0.749) (Fig. 3).

The Cochran's Q test results for heterogeneity, considering the rates of cure, were: day 14 (Q value = 0.852, p = 0.653, I^2 = 0.000), day 30 (Q value = 1.469, p = 0.480, I^2 = 0.000); day 60 (Q value = 1.228, p = 0.541, I^2 = 0.000); day 90 (Q value = 0.005, p = 0.998, I^2 = 0.000) and for recurrence rates at day 90 (Q value= 1.223, p= 0.543, I^2 = 0.000). All data were also assessed by combining the subgroups MW1 and MW3 from Silva et al. [3] for the percentage of recurrence (dichotomous outcome data). All metaanalyses were reevaluated and there was no influence or modification on the significance level of the results, with all comparisons between the control group and the combination of MW1 and MW3 did not show statistically significant differences ($p > 0.05$), Supplementary Table 2.

Discussion

The null hypothesis of this systematic review was accepted, as the results obtained from the qualitative synthesis indicated that microwave disinfection was equally or more effective than the conventional treatments.

Regarding the studies included in this systematic review, it was noticed that all studies performed randomization of the patients [2, 3, 20-22] and, in general, a concern to exclude certain conditions that could knowingly influence the development of CADS such as immunosuppression, anemia, antibiotic and cancer therapy, and diabetes was observed, except in one study [22] in which the focus was to evaluate exclusively patients with well-controlled type 2 diabetes. Although most of the studies were conducted at universities [3, 21, 22], two studies assessed patients from hospitals and aged care homes [2, 20], making it difficult to control biases. In addition, although most studies verified the homogeneity of the group characteristics of patients and risk factors that may interfere with the outcomes [3, 21, 22], only two studies performed sample size calculations for group composition [3, 22]. Of these two investigations, one reported difficulties to manage the previously stablished sample size of the groups after the end of all treatments performed, i.e., during the follow-up evaluations, due to loss of participants [3].

In assessing the proposed protocols, there was a concern to equally treat the groups through the recommendation and instruction of supporting therapies, such as reinforcement of oral and denture hygiene [2, 3, 20-22]. However, one study applied the therapies separately, with the experimental group being conducted by the research personnel and the control group by the nursing staff of the hospitals and aged care homes [2]. Thus, a possible lack of compliance to the protocols by these professionals might have caused bias. A loss of patients during the studies was also reported and, although not specified by one study [2], Silva et al. [3] described a withdrawal of consent to participate and the need to be subjected to antibiotic therapy as the main reasons.

The characteristics of the 245 participants were similar with previous studies [23, 24]. Their mean age ranged from 56.8 to 83.7 years. CADS is consistently linked to the elderly, and its higher prevalence is usually correlated with limited manual dexterity and systemic conditions [25]. CADS has also been associated with the ages of the dentures. Zomorodian et al. [26] observed an occurrence of this condition in approximately 84% of patients wearing dentures for more than 5 years, in contrast to 25% of the patients with less than 1 year of denture use. This was also seen in this review with the age range of the dentures being 12.8 to 24.8 years. A higher frequency of CADS was observed among women [3, 21, 22], which has been attributed to hormonal factors, iron deficiency, and a higher search for dental treatment [22].

Among the already established risk factors for CADS, nocturnal denture wear, poor hygiene, and xerostomia have been frequently reported. Although CADS is influenced by these factors, the primary infection of the palatal tissues is by *Candida* spp., which is usually present in the oral medium as an inoffensive commensal [27, 28]. However, certain imbalances and conditions may lead to its overgrowth and the development of CADS, which is observed in 50% of denture wearers [29-31]. This association has been consistently reported indicating a strong correlation between the severity of the clinical condition with the quantitative presence of *Candida* spp. [24]. In addition, the dentures also play an important role on this process, with previous studies reporting higher microbiological colonization of the denture-supporting tissue interface, when compared with the palatal mucosa [2, 3, 21-23]. This finding has been correlated to the denture's surface characteristics as they provide a protective medium for biofilm development and growth against disinfection procedures, favoring the infection of the palatal mucosa and, consequently, the development or recurrence of CADS [4, 24, 28]. Therefore, the reduction of the levels of *Candida* cells on the palates and dentures has been considered an important outcome in the treatment of CADS. Moreover, the association of the quantitative microbiological assessment with the clinical evaluation of the patients, is imperative in order to evaluate the efficacy of any treatment proposed [32].

In this regard, we also observed higher frequencies of *Candida* spp. cells on the denture surfaces than on the palatal tissues. Two studies [2, 21] also noticed a higher number of mycelial forms, considered the invasive form of *C. albicans*, on the dentures, with one study observing the presence of these forms in all dentures and only in one-third of the palatal tissues assessed [2]. Therefore, this result reinforces the aforementioned statement that dentures act as reservoirs for *Candida* cells and potential sources of infection of the palatal tissues, indicating that adequate disinfection of dentures should be a primary point in treating CADS [3, 33-35].

Furthermore, microwave and chemical disinfections showed comparable results. A similar efficacy was observed with 0.2% chlorhexidine, which significantly reduced the mycelial forms on palates and dentures at the end of the treatment [2]. However, the authors considered the microwave disinfection as a superior method since it was 30% more effective classifying this outcome as clinically significant. Correspondingly, Webb et al. [20] did not observe differences in the reduction of viable cells of *Candida* on both palates and dentures with 0.02% NaOCl. The authors also assessed their effectiveness on aerobes, with the treatments failing to reduce the counts from the palates, suggesting that these microorganisms might compose the oral microbiota of the patients as a result of compromised health and polypharmacy, as seen in the most study participants [20]. The capacity to facilitate fungal adhesion to the dentures by co-aggregation, contributing to its overgrowth, virulence, and development of CADS has been attributed to bacteria, and its role in this scenario should be investigated in further studies [21].

Antifungal therapy is widely recommended and effective against CADS [32]. Topical antifungals, such as nystatin and miconazole, have been suggested as the first option in treating CADS [36]. Notwithstanding, it was demonstrated that microwave disinfection, combined or not with miconazole, was more effective than the antifungal only, showing no viable cells and mycelial forms of *Candida* spp. on smears from dentures and palates of all patients through and at the end of the treatment [21]. These results were in contrast with those from a previous systematic review indicating more long-term effectiveness of miconazole oral gel than other formulations for oral candidiasis [32], and may be attributed to differences in the methodologies, antifungal resistance, and difficulty in sustaining a therapeutic dose due to the diluent effect of saliva [21]. However, microwave disinfection had similar results to topical nystatin, effectively reducing the *Candida* counts of palatal tissues and dentures regardless at the end of 14 days of treatment which was also confirmed in the present metaanalysis [3,22]. In addition, no differences were found between these methods for all the evaluation periods in our metaanalysis.

The clinical assessment of the severity of the palatal infection was done in four studies [3, 20-22] and was based on the classification proposed by Newton [37], which divides the clinical condition of denture stomatitis in the absence of inflammation (0), punctiform hyperemia (type I), diffuse hyperemia (type II), and granular hyperemia (type III). Evaluations were performed through the analyses of standardized photographs of the palates, which were conducted blindly by at least two independent observers in most studies [3, 20, 22]. However, only two studies used two observed verifying inter-examiner reliability [3, 22], which is the preconized protocol in reducing bias in these evaluations.

The clinical outcomes encountered were consistent with the mycological results. Webb et al. [20] reported an equal improvement in the clinical signs of CADS, although the authors did not present sufficient data to specify this result. Neppelenbroek et al. [21] reported an 80% increase in the proportion of patients with types 0 or I inflammation severity at the end of the treatment with microwave disinfection, combined or not with miconazole. It was also noticed that microwave disinfection demonstrated clinical success for CADS, with most

of the patients presenting a reduction in the clinical signs ranging from 50-75% [3, 22]. Three studies assessed the clinical success rates at the end of the treatment, considering the absence or an improvement of the palatal inflammation from microwave groups. After 30 days of treatment, Neppelenbroek et al. [21] observed a significant improvement in inflammation severity for all patients whose denture were microwaved, with complete remission of clinical signs in 70 and 80% of palates treated, respectively, only with microwave denture disinfection or in association with miconazole. These results were in contrast with the topical antifungal therapy with miconazole, in isolation, with no significant reduction in inflammation severity for all patients up to 30 days. In addition, Silva et al. [3] and Sanitá et al. [22] noticed comparable results between the cure rates of microwave disinfection and nystatin treatments, ranging from 38.89% to 41.18% [3, 22]. Our metaanalysis indicated similar percentages of cure from these treatments in all periods evaluated [3, 22] (Figure 3). These outcomes were similar to a previous study that also assessed the efficacy of the treatment with nystatin, using a comparable protocol, with 53% of the patients obtained clinical success (cure or improvement of the palatal inflammation) at the end of the treatment (day 15) [23]. Moreover, despite the methodological differences among the included studies, it is possible to infer that the distinguished results obtained by Neppelenbroek et al. [21] are related to its extended period of treatment and time of exposure, in comparison with the others [3, 22], and a direct comparison of these protocols should be investigated in further studies.

The high recurrence rates of CADS are certainly one major challenge. Often a gradual recolonization of the dentures surfaces and palatal tissues takes place two weeks after the end of the treatment [1, 19, 38, 39]. Furthermore, failure to eliminate the colonizing strain including the development of drug resistance and reinfection by an exogenous strain has been suggested as possible reasons for this pattern of recurrence [3, 40]. The studies in this systematic review showed yeast recolonization and growth within three months of follow up [2, 3, 21, 22]. Banting and Hill [2] observed recurrence rates of 56% and 8% for dentures and palatal tissues, respectively, in the microwave group. In contrast, the patients treated with chlorhexidine presented recurrence rates of 84% for the dentures, and 63% for the palates. In addition, the authors found that the microwave group had 75% less chances of palatal reinfection. In addition, two studies noticed a significant increase in the CFU/mL values, in comparison with the end of the treatment, and showed a recurrence range of 39 to 55% of the clinical signs [3, 22]. The results from the metaanalysis also demonstrated no significant differences among the recurrence rates of the treatments with microwave disinfection and topical nystatin. However, Mima et al. observed a higher recurrence rate, with 75% of the patients treated with nystatin showing recurrence of the clinical signs [23]. Notwithstanding, the differences among these results may be explained by a lack of compliance of the patients [24, 39], thus, interfering on the risk of recurrence, as demonstrated by Cross et al. [39], which noticed a low recurrence rate in patients that sustained stringent levels of oral and denture hygiene in a three-year follow up. This reinforces the statement that patients should be constantly evaluated after the treatments with regular follow-up appointments and including professional cleaning of the dentures, if needed, in order to reduce the risk of relapse of CADS [24]. Moreover, Neppelenbroek et al. [21] obtained compelling results. Although it was noticed a subsequent growth of *Candida* viable cells on the dentures and palatal tissues, and the recurrence of few mycelial forms on, respectively, 33.3% and 40% of the dentures disinfected with microwave disinfection in isolation or associated with miconazole, mycelial forms were not observed on the palates and clinical signs of CADS at the end of three months of follow-up. However, it is important to mention that a direct comparison with the other treatments proposed by the authors [21] was not possible, as these

treatments did not achieve the parameters of success stablished by the authors and, therefore, were not included in the follow-up. Nonetheless, this is an important finding, as the *Candida* mycelial forms have been considered to have a higher pathological activity due to a better capacity to adhere and penetrate the denture fissures and palatal mucosa [24], and the effect of microwave disinfection on these pathogenic forms should be assessed in future studies.

Despite the similarities among treatments, microwave disinfection has certain advantages which are a safe, inexpensive, and practical method that can be applied at any period of day, reducing the chances of being impaired due to poor compliance of the recommendations by the patients [2, 3, 41, 42]. Moreover, topical antifungals may lead to subtherapeutic concentrations due to the diluent effect of saliva and tongue movements [3, 28]. Other factors that affect their effectiveness include local and systemic side effects, and the development of antifungal resistance by complex biofilms [3, 27, 42]. However, due to the physical mechanism of action of microwave disinfection on fungal cells, these additional shortcomings of antifungal therapies are excluded [3, 22]. Nevertheless, one potential setback of microwave disinfection is the possibility of damaging the complete dentures of the patients, such as changes in the color and dimensional stability, and on its mechanical and surface properties as well [44-47]. Although the data regarding these outcomes are still conflicting, a recent study has indicated a dry protocol of 650 Watts for 3 minutes with a maximum of three irradiations in order to avoid adverse effects on the physical mechanical properties of the dentures [42]. Nonetheless, the implications of microwave disinfection on the physical mechanical properties of denture materials should be systematically assessed in further studies.

According to the results obtained in this systematic review, it was shown that microwave disinfection can be at least equally effective as the already established treatments for CADS. However, regarding the question of which protocol may be more suitable for treating CADS, some considerations are needed. Despite the positive results from Banting and Hill [2] and Webb et al. [20] the lack of clinical assessment and follow-up, respectively, hinder the choice of these protocols. However, wet protocols seem to provide more reliable results, and are possibly related to an additional and uniform heating of the water [3, 21, 22]. Moreover, it is possible to infer that the protocol proposed by Neppelenbroek et al. [21] with 650 Watts and 6 minutes, applied three times a week for a month, showed the most compelling results in treating CADS. Nonetheless, with the possibility of its extended time and irradiations causing undesirable effects on the materials, more conservative protocols should be preferred. Based on the results of this systematic review, it can be presumed that a microwave disinfection protocol, with only one irradiation at 650 Watts for 3 minutes a week for two consecutive weeks, may be a more suitable alternative for treating and preventing CADS, simultaneously minimizing possible side effects on the dentures.

This current review has some limitations, starting from the small number of studies included, and the possibility of more non-English studies published. In addition, the differences among the methodologies and protocols restricted our metaanalysis, and standardization of the protocols is needed in further studies. In this review, the impact of microwave disinfection on dentures was not assessed and should be further investigated. Moreover, there was a lack of information regarding clinical assessment and follow up of the patients. Lastly, further evaluation of the effect of microwave disinfection on bacteria and mycelial forms of *Candida* is recommended.

Conclusions

We can conclude that for CADS treatment, microwave disinfection had comparable mycological and clinical results to overnight soaking in 0.2% chlorhexidine and 0.02% NaOCl and topical administration of nystatin, and it was more effective than topical miconazole alone. Furthermore, the microwave disinfection protocol at 650 Watts and 3 minutes once a week for two consecutive weeks was a more cost-effective, being recommended for both prevention and treatment of DS, and also minimizing possible damages to dentures.

Compliance with Ethical Standards

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Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent

For this type of study, consent is not required.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Tables

Table 1. Characteristics of the reviewed studies based on PICO-like structured reading

Study first author, year	Population (P)	Intervention (I)	Comparison (C)	Outcomes (O)		
				Mycological outcomes	Clinical outcomes	Recurrence
Silva, 2012 [3]	N=60 participants (9M, 51F, mean age range 56.8-62.5 y), patients seeking for treatment of DS (Brazil).	MW1 group (N=20, 3M, 17F, m.a. 59.5 y): routine denture care and MW disinfection (650W, 3min) 1x/week for 14 days. MW3 group (N=20, 4M, 16F, m.a. 56.8 y): routine denture care and MW disinfection (650W, 3min) 3x/week for 14 days.	NYS group (N=20, 2M, 18F, m.a. 62.5 y): routine denture care and nystatin rinsing (1 min, 1mL, 100.000I IU/mL) 4x/day for 14 days.	All groups significantly reduced the C.*albicans* cell counts on both palates and dentures at days 14 and 30, without statistical differences among them (p>0.05).	All groups effectively treated DS (p>0.05), with at least 65% of the patients presenting improvement of the clinical signs at the end of the treatment.	Recurrence of the clinical signs of DS were noticed in MW1 (52.94%), MW3 (38.89%) and NYS (50%) groups at day 90. However, the comparison among them did not show statistical differences (p>0.05).
Sanitá, 2012 [22]	N=40 participants (5M, 35F, mean age range 62.2-62.6 y), patients with well-controlled type 2 diabetes seeking for treatment of DS (Brazil).	MW group (N=20, 2M, 18F, m.a. 62.2 y): routine denture care and MW disinfection (650W, 3 min) 3x/week for 14 days.	NYS group (N=20, 3M, 17F, m.a. 62.6 y): routine denture care and nystatin rinsing (1 min, 1 mL, 100.000 IU/mL) 4x/day for 1 uk,14 days.	MW and NYS similarly reduced the C. *albicans* cell counts on both palates and dentures at days 14 (end of the treatment) and 30 (p>0.05).	MW and NYS groups were both equally successful p>0.05), with cure rates of 40% at the end of the treatment.	MW and NYS groups showed recurrence of the clinical signs after three months of follow up, with rates of, respectively, 55% and 40% (p>0.05).

Neppelenbroek, 2008 [21]	N=60 participants (12M, 48F, age range 35-90), patients seeking for treatment of DS (Brazil).	MW group (N=15, 3M, 12F, m.a. 64.5 y): MW disinfection (650W, 6 min) 3x/week for 30 days. MWMz group (N=15, 2M, 13F, m.a. 60 y): topical application of miconazole (3x/day for 30 days) and MW disinfection (same protocol as MW group)	CG (N=15, 3M, 12F, m.a. 59 y): routine denture care. Mz group (N=15, 4M, 11F, m.a. 61.4 y): topical application of miconazole (3x/day for 30 days).	MW and MWMz showed no viable cells and mycelial forms of *C.albicans* at days 15 and 30 (end of the treatment), without statistical differences between each other ($p>0.05$). However, were superior to Mz and control groups ($p<0.05$).	MW and MWMz showed similar results ($p>0.05$), significantly improving the clinical signs and with an 80% increase in patients allocated to inflammation severity types 0 and 1 at day 30. Both groups were superior to Mz and control groups ($p<0.05$).	MW and MWMz only showed reinfection of mycelial forms on, respectively, 33.3% and 40% of the dentures, in a three month follow up. Viable cells were also seen, for dentures and palates. 86.6% and 93.% patients, of MW and MWMz, did not show signs of inflammation until day 90.
Webb, 2005 [20]	N=60 participants (m.a. 83.7 y), residents of 20 aged care homes seeking for treatment of DS (Australia).	MW group (N=20): dentures were microwaved at night (350W, 10 min), daily, for 7 days.	CG (N=20): usual nursing home care. NaOCl group (N=20): overnight soaking in NaOCl (0.02%), daily, for 7 days.	MW and NaOCl similarly reduced *Candida* counts from dentures and palates ($p>0.05$) and aerobes from dentures ($p>0.05$) and more effective than CG ($p<0.05$).All groups did not reduce aerobic cell counts from palates.	Microwave disinfection and NaOCl improved the clinical signs with similar results ($p>0.05$).	Not assessed.
Banting, 2001 [2]	N=34 participants (22M, 12F, age range 62-98 y), residents of 3 aged care homes seeking for treatment of DS (Canada).	MW group (N=15): topical nystatin 3x/day for 14 days. Dentures were scrubbed with antibacterial soap and microwaved (850W, 1 min) on days 1, 5 and 10.	CG (N=19): topical nystatin 3x/day for 14 days. Overnight soaking in 0.2% chlorhexidine for 14 days, changing solution every 2 days, and scrubbed on days 1, 5 and 10.	Both protocols equally reduced the pseudohyphae counts from palates and dentures ($p>0.05$). For all groups only one denture for each group present pseudohyphae and absent for smears	Not assessed.	MW and GC recurrence rates were, respectively, 56% and 84% for dentures ($p=0.09$) and 8% and 63% for palates ($p<0.001$).

	from palatal mucosa.

CG, control group; MW, microwave; MW1, microwave disinfection one a week; MW3, microwave disinfection three times a week, Mz, miconazole; MWMz, microwave combined with miconazole; NaOCl, sodium hypochlorite; NYS, nystatin.

Table 2. CASP quality assessment of the reviewed studies

Reference	Item 1	Item 2	Item 3	Item 4	Item 5	Item 6	Item 7	Item 8	Item 9	Item 10	Item 11
Silva et al., 2012 [3]	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Sanitá et al., 2012 [22]	Yes	Yes	Yes	No	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Neppelenbroek et al., 2008 [21]	Yes	Yes	Yes	No	Can't tell	Yes	Yes	Yes	Yes	Yes	Yes
Webb et al., 2005 [20]	Yes	Yes	Can't tell	No	Can't tell	Yes	Yes	Yes	Yes	Yes	Yes
Banting and Hill, 2001 [2]	Yes	Yes	No	No	Yes	Yes	Yes	Yes	Yes	No	Yes

Figures

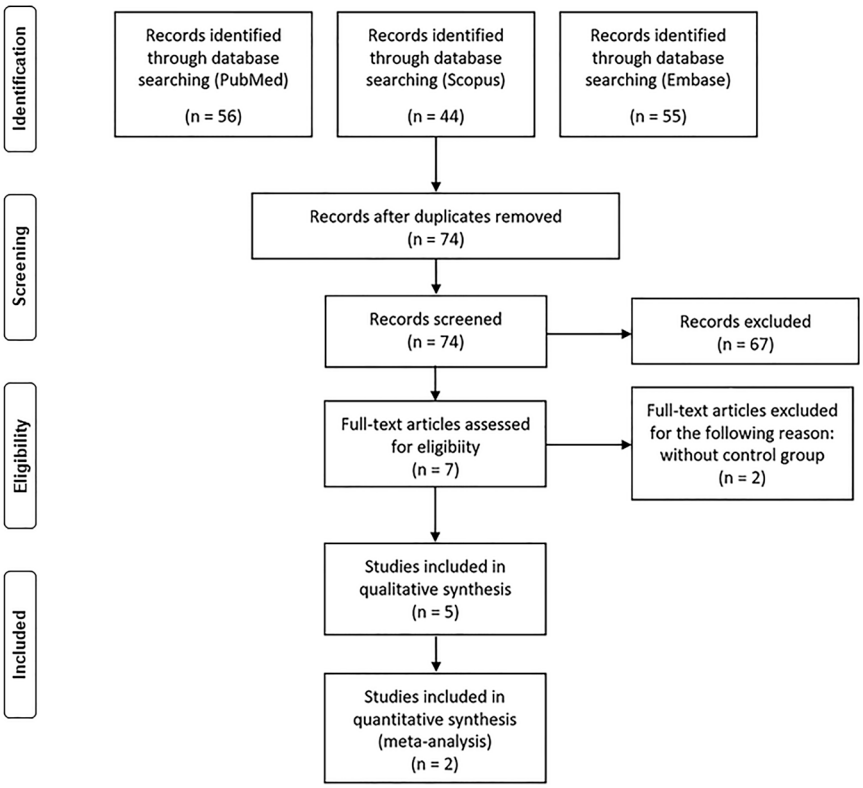

Fig. 1 Flow diagram of the search strategy.

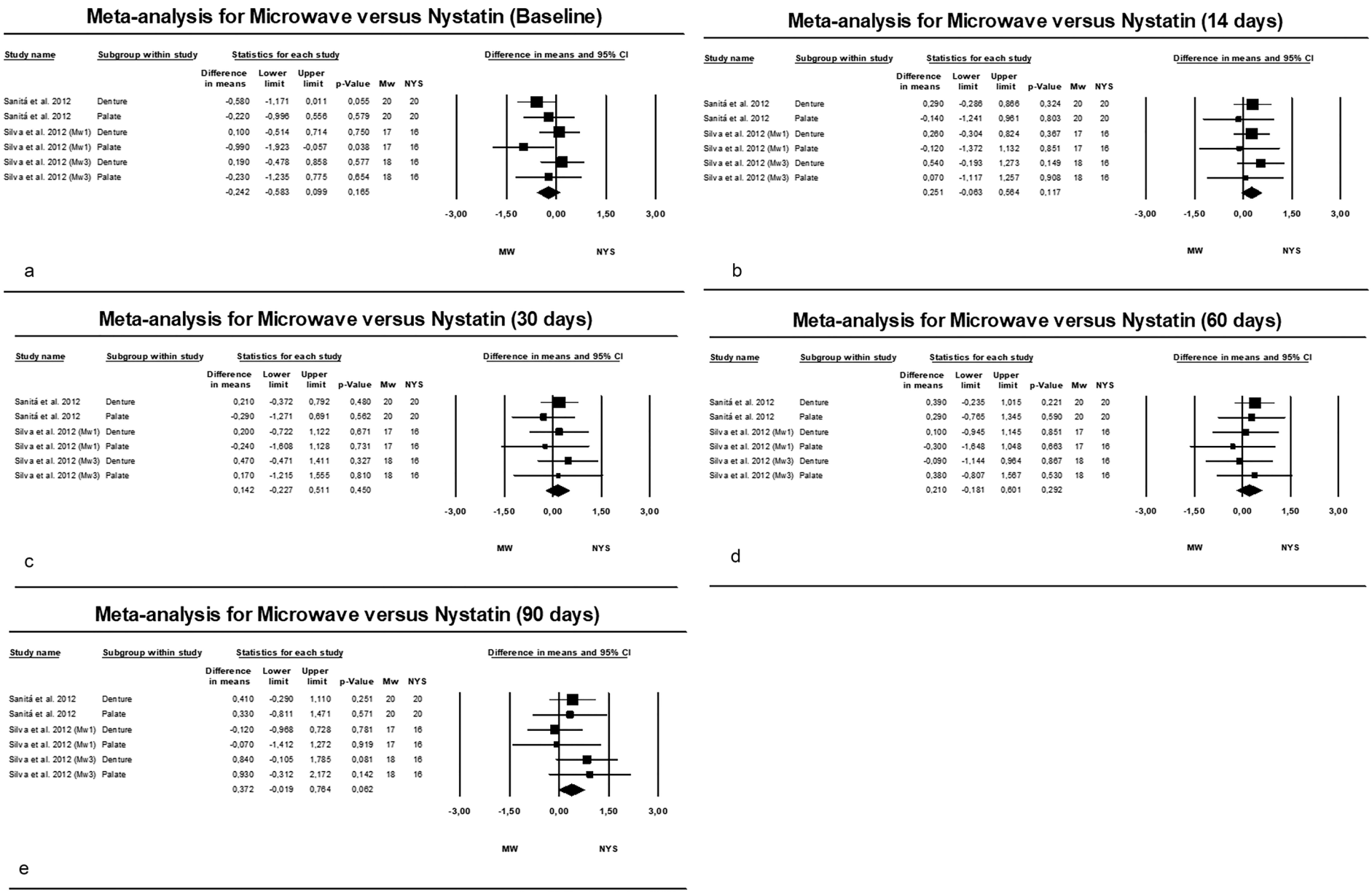

Fig. 2 Forest plots of the comparison between microwave disinfection and nystatin treatments for *Candida* colony counts at a, baseline; b, day 14; c, day 30; d, day 60 and e, at day 90.

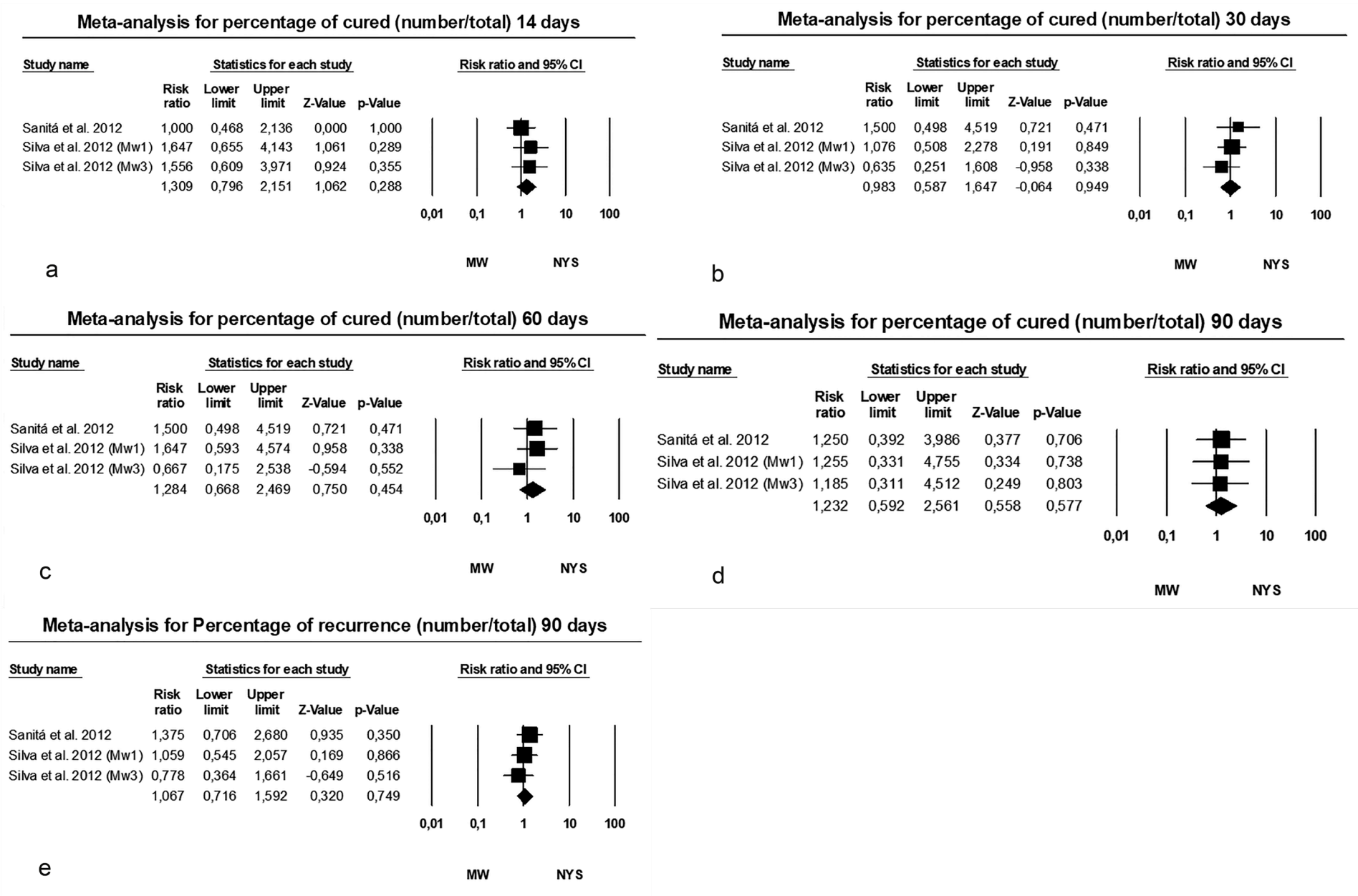

Fig. 3 Forest plots of the comparison between microwave disinfection and nystatin treatments, considering the cure rates at a, day 14; b, day 30; c, day 60, d, day 90 and

recurrence rate at e, day 90.

2.2 ARTICLE 2 – DOES MICROWAVE DISINFECTION AFFECT THE DIMENSIONAL STABILITY OF DENTURE BASE ACRYLIC RESINS? A SYSTEMATIC REVIEW.

Does microwave disinfection affect the dimensional stability of denture base acrylic resins? A systematic review

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DECLARATIONS

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest to disclosure.

AUTHORS CONTRIBUTIONS

All authors made significant contributions regarding the conceptualization and design of the research. Material preparation, data collection and analysis were performed by Rodrigo Moreira Bringel da Costa, Helena Sandrini Venante, Ana Paula Chappuis-Chocano, Karin Hermana Neppelenbroek, and Vinicius Carvalho Porto. The first draft was written by Rodrigo Moreira Bringel da Costa and Mariana Domingues Pordeus. In addition, all authors commented and reviewed the manuscript. Lastly, the authors also declare that any subject, who fits the authorship criteria, was excluded from the list of authors of this study.

Abstract

Objective: To systematically evaluate the effect of microwave disinfection on the dimensional stability of denture base acrylic resins.

Background: Microwave disinfection has been considered as an alternative method for disinfecting complete dentures to help prevent and treat denture stomatitis. However, data on the impact of microwave disinfection on the dimensional stability of acrylic resins are still scarce.

Methods: The PubMed/Medline, SCOPUS, and EMBASE databases were searched in order to assess articles published in English up to January 2021 (CRD42021212267). We included studies that have assessed the effect of microwave disinfection, on the dimensional stability of acrylic resins, comparing them with negative or positive controls.

Results: A total of seven *in vitro* studies were included. The qualitative synthesis demonstrated that, in general, microwave disinfection produced more distortion on the materials than do immersion in sodium hypochlorite, chloride solution, chlorhexidine, and water immersion. However, considering the dimensional stability of the specimens, microwave disinfection at 500 W for 3 minutes, and at 450 W for 5 minutes, produced similar or better outcomes than did control groups.

Conclusion: In general, microwave disinfection promotes changes in the dimensional stability of denture base acrylic resins, and should thus be used with caution. However, microwave disinfection protocols at lower power settings (500 W and 450 W) and exposure times (3 and 5 minutes) produces similar or less distortion than chemical disinfection. More studies are still required in order to evaluate the clinical and long-term implications of microwave disinfection.

Keywords: complete denture, acrylic resins, microwaves, systematic review.

1 INTRODUCTION

Edentulism is the complete loss of teeth[1,2]. The prevalence of edentulism is widely variable, ranging up to 78% in people aged 65 years and over. Although its occurrence has been reducing, the older population in developed countries has been increasing due to increasing life expectancy, and so, the number of people with edentulism is also increasing[1,2]. The complete loss of teeth comes with an extensive number of consequences, including the collapse of orofacial tissues and lower chewing efficiency, and oral-health related quality of life. Complete dentures remain the most frequently used treatment modality because they are cheaper than implant-supported prostheses. Despite this advantage, oral infections such as denture stomatitis remains common in complete denture wearers[3-5].

Denture stomatitis is one of the most common infections observed in patients with complete dentures, and it often presents as a chronic and multifactorial condition[3-5]. Denture stomatitis has been associated with several predisposing factors, such as poor oral and denture hygiene, nocturnal denture wear, denture age, xerostomia, immunosuppression, and various systemic conditions[3,5]. However, infection of the palatal tissues by *Candida* spp. has been considered the main etiological factor[5]. In this regard, the upper denture plays an important role because its surface and physicochemical characteristics favor microbial colonization and the development of biofilms on its denture base, leading to infection and reinfection processes in the palatal tissues, contributing to the development of and relapses in denture stomatitis[6,7]. Thus, dentures can act as reservoirs for microorganisms and be potential sources of localized and perhaps systemic infections, including aspiration pneumonia and gastrointestinal infections[6,7].

Several disinfection protocols have been developed and tested to reduce or eliminate microorganisms on dentures. In this regard, chemical disinfection, through the immersion of dentures in solutions such as sodium hypochlorite, chlorhexidine, alkaline peroxides, and glutaraldehyde, has been the first choice among clinicians for disinfecting dentures[6]. However, these protocols have some disadvantages: they do not eliminate microbial colonization in depth in acrylic resin, and they may also affect the physicomechanical properties of the polymers. In addition, previous studies have also reported oral tissue reactions resulting from these disinfection protocols[8,9,10]. Lastly, it is a time-consuming process, and its effectiveness strictly depends on the commitment of the patients to the disinfection protocol guidelines[8,10,11]. To overcome these issues, studies have proposed alternative protocols for disinfecting dentures, such as using microwave irradiation[9].

Microwave disinfection is a simple and accessible procedure in which the dentures, immersed in water or not, are subjected to a predetermined quantity of microwave irradiation under specific power and time settings[8]. Moreover, microwave effectiveness has been consistently reported by previous studies[10,12,13], including as an effective protocol for both prevention and treatment of *Candida*-associated denture stomatitis[14], since it is physical method of disinfection that eliminates the risk for the survival of microorganisms and selection of resistant yeasts. However, data on the effects of microwave disinfection on the dimensional stability of polymers are still conflicting[9]. There have been reports of distortion of the dentures after microwave irradiation, attributed to the additional polymerization of monomers, leading to shrinkage, or the high temperatures achieved during these disinfection processes[15-17]. In addition, there is a lack of consensus on the gold standard microwave disinfection protocol. Therefore, the objective of this systematic review was to assess

the effect of microwave disinfection on the dimensional stability of denture base acrylic resins and provide evidence on a safe microwave protocol for disinfecting dentures. The hypothesis is that the dimensional stability of the denture base acrylic resins can be affected according to different microwave disinfection protocols.

2 MATERIALS AND METHODS

2.1 Registration, protocol, and eligibility criteria

This systematic review was registered in the International Prospective Register of Systematic Reviews (PROSPERO), under the registration number CRD42021212267 and the title that follows: “Does microwave disinfection affect the dimensional stability of denture base acrylic resins? A systematic review and meta-analysis”. In addition, the present study was developed according to the guidelines proposed by the PRISMA (Preferred Reporting Items for Systematic Reviews)[18] and Cochrane Collaboration (Cochrane Handbook for Systematic Reviews of Interventions, version 5.1.0)[19,20] organizations for the elaboration of systematic reviews and according to similar studies previously published[14,21,22].

The eligibility criteria applied in this study included clinical and *in vitro* studies that have assessed the effect of microwave disinfection protocols on the dimensional stability of denture-base acrylic resins. Moreover, we also involved studies that used positive or negative controls to compare to the microwave disinfection method. Thus, studies without control groups were excluded. We also included only studies with complete description of the microwave disinfection protocol applied.

This study was also conducted based on the PICO strategy by adopting the following question: “Does microwave disinfection (I) promote more changes on the

dimensional stability (O) of denture base acrylic resins (P) than other disinfection methods, or immersion of these materials in water (C)?

2.2 Information source, search strategy, and data collection

The search was conducted using three different databases (PubMed/Medline, SCOPUS, and EMBASE) to assess articles published in English until January 22, 2021. The following MeSH/PubMed terms were used for the search: “Acrylic resins,” “Denture,” “Denture bases,” “Complete denture,” “Removable” and “Microwaves.” After this, the aforementioned terms were combined with Boolean operators, and the following search phrase was used: “Acrylic resins OR denture OR denture bases OR complete denture OR removable AND Microwaves”. Two examiners (RMBC and HSV) independently collected the data. In addition, a third examiner (KHN) clarified and resolved any disagreements between the two examiners. The titles and abstracts of the studies selected based on the previously mentioned inclusion and exclusion criteria were evaluated, and an inter-examiner test (Kappa) was applied to minimize bias during this stage of article selection. Finally, a definitive consensus meeting with all the authors was held to assess the data collection, articles selected, and the risk of bias.

2.3 Data items

The following data were collected: (1) authors and year of publication; (2) study design; (3) property evaluated; (4) sample size; (5) material and polymerization method applied; (6) negative or positive controls used for comparison; (7) and the microwave disinfection protocols used.

2.4 Quality assessment

The methodological quality of the selected studies was assessed using the Johanna Briggs Institute (JBI) Critical Appraisal Checklist for Quasi-Experimental Studies

(non-randomized experimental studies)[23,24], which has been adapted for *in vitro* studies. The referred checklist contains nine questions that can be answered as “Yes,” “No,” “Unclear,” and “Not applicable” to evaluate the possibility of biases in the design, conduct, and analysis of the studies. The quality assessment of the selected studies was undertaken by two of the authors (RMBC and APCC), and a third author (VCP) was engaged to resolve any disagreement among the results.

3 RESULTS

3.1 Study findings

The initial online database search yielded 1086 studies, 554 of which overlapped. Five hundred and twelve articles were excluded after screening the titles and abstracts. Twenty studies were provided in full-text, and, after the application of the eligibility criteria, seven studies were selected for qualitative synthesis[15-17,25-28] (Fig. 1). Of the seven studies, due to significant differences among the methodologies, only two studies were elected for the quantitative synthesis[27,28]. However, after the revision of these studies, we agreed that the inclusion of two studies would restrict the power of the meta-analysis, therefore reducing its contribution to this systematic review. In addition, both studies were conducted by the same research group, using very similar methodologies, and results. Therefore, in light of these findings, we decided to not perform a quantitative synthesis in this review.

All included articles were for *in vitro* studies that presented positive and negative control groups. Among them, five evaluated the linear dimensional stability[15-17,25,26], and two assessed the tridimensional stability[27,28]. The selected studies included disinfection control methods such as immersion in sodium hypochlorite solution, diluted sodium hypochlorite solution, gluconate chlorhexidine, effervescent tablets, immersion in tap water, distilled water, active chlorine solution, and glutaraldehyde.

Denture-base materials, including heat-polymerized and microwave-polymerized acrylic resins, were assessed in all studies. Several microwave disinfection protocols were reported in this systematic review, with power settings ranging from 450 W to 900 W and exposure time from 3 to 15 min (Table 1).

Al-Saadi et al[25], when evaluating the effectiveness of six disinfection methods against Candida spp. and the influence of these methods on the adaptation of maxillary dentures, reported that the mean values of the posterior palatal gaps, from the microwave oven at medium power (450 W) for 5 min (M = 240) and sodium hypochlorite (5.25 %) (M = 183.33) were close to those of tap water (control group). However, microwaving at full power (900 W) (M = 1.013) for 5 min promoted changes in the dimensional stability of the heat-polymerized acrylic specimens.

Moreover, Nirale et al[15] and Seo et al[16] assessed a microwave disinfection protocol involving a 650 W power setting and an exposure time of 6 min. The authors reported that with one and seven cycles, microwave disinfection, compared with other control groups, promoted significant changes in the dimensional stability of the heat-polymerized denture bases. Polychronakis et al.[17] applied the same disinfection protocol (650 W for 6 min) and determined a similar pattern of dimensional changes. The authors noticed a reduction in all the distances measured; the highest dimensional changes were observed on the denture bases after exposure to seven microwave disinfection cycles (wet protocol). By contrast, the authors observed that the microwave disinfection dry protocol promoted smaller and clinically insignificant dimensional changes.

Sartori et al[27,28] assessed the effect of microwave disinfection (690 W and 6 min) on the internal adaptation of the denture bases and observed a gradual and significant increase in the distortion of the denture bases with time relative to the chloride

solution and control groups. Lastly, Polyzois et al[26] observed linear changes in denture bases after microwave disinfection at 500 W for 3 and 15 min. Microwaving for 3 min produced significantly fewer changes than the other protocols. Overall, all groups demonstrated statistically significant dimensional changes, although they were not enough to be clinically relevant.

3.2 Quality assessment

The results from the assessment of the risk of bias of the studies (Table 2) indicated that the selected studies had adequate methodological quality, since the majority of the items were answered as “Yes.” Item 5 of the checklist was checked as “Unclear” for three studies[15,17,25], as the authors did not provide sufficient information on whether there were multiple measurements for the specimens, or whether the measures were performed before and after the interventions. In addition, item 6, which covered the assessment of the adequacy of follow-up, was checked as “Not applicable,” as the seven studies selected were *in vitro*.

4 DISCUSSION

The hypothesis of this systematic review was accepted, as the majority of the microwave disinfection protocols evaluated produced significant changes in the dimensional stability of the denture base acrylic resins.

Concerning the characteristics of the included studies, the majority of the studies used denture bases as specimens[15-17,25,27,28]; only one study used rectangular specimens[26]. Complete dentures have complex structures, with a wide variation in thickness accompanied by different areas[15]. Therefore, denture-base specimens are more reliable for assessing the effect of disinfection protocols, as regular-shaped specimens may introduce bias related to the response of the material to the interventions[15,16].

In addition, most studies evaluated linear stability, which was assessed through successive measurements among pre-established points on the specimens[15-17,25]. In contrast, two studies[27,28] assessed the tridimensional stability of the specimens, which consisted of lightweight polysiloxane impression material films between the dentures and the master casts. In this regard, the evaluation of tridimensional stability is preferred, as it allows the detection of several distortions along the specimen. In contrast, a linear dimensional evaluation may not detect distortion along the pre-established segments, but the specimen may still demonstrate dimensional changes in other areas that may not be accounted for. Thus, the tridimensional stability of denture-base acrylic resins should be investigated in further studies.

In general, the studies reported that most of the microwave disinfection protocols promoted significant changes in the dimensional stability of the specimens when compared with the control groups[15-17,25,27,28]. This finding was also highlighted in two studies, which showed that one cycle of microwave disinfection at 690 W for 6 min produced a significant distortion given the baseline state and the outcome of chemical disinfection with 0.01% chloride solution[27,28]. All these studies used wet microwave disinfection protocols. The immersion of complete dentures during microwave disinfection has been preconized by several studies as a more effective method than dry protocols because of the additional heat generated by the water, which uniformly heats the complete dentures[10,12-14]. On the other hand, the distortion may become apparent when water reaches high temperatures near the glass transition temperature of acrylic resins, thereby promoting the release of stress within the polymeric matrix of the material from its initial polymerization, which results in its shrinkage[15-17]. It has been theorized that heat may also induce the diffusion of the

residual monomer to the active sites of the polymeric chains, leading to further polymerization and polymerization shrinkage[16,29].

Thermopolymerized acrylic resins may suffer distortion at temperatures ranging from 71 to 90°C[30]. Most of the studies reported that the water the specimens were immersed in reached its boiling point after 90 seconds[15-17]. This event also occurred after 80 seconds[25] and at the end of the irradiation (6 min)[28]. Therefore, subjecting the specimens to high and sustained temperatures may be strongly related to the distortions encountered.

Conversely, the wet protocol with 450 W for 5 min did not promote significant changes in the linear stability of the specimens when compared with the other groups[25], which is in agreement with the reports of previous studies[31-33]. Consani et al[31] did not notice deleterious effects on the dimensional stability of denture-base specimens immersed in 150mL after one microwave disinfection cycle at 650 W for 3 min. This pattern was also reported by a similar study using the same protocol after five disinfection cycles repeated daily[32]. Senna et al[33] assessed the effect of cumulative microwave disinfection and observed no significant differences after six cycles using wet disinfection protocols with power settings of 450 W and 630 W for 3 min. Although the authors encountered changes after 36 cycles, they concluded that the respective protocols were safe for polymethyl methacrylate polymers. Although these studies were not included in this systematic review, as they did not meet the inclusion criteria, these findings indicate that protocols with reduced power settings and exposures may be safe. However, this should be addressed in future studies, including the relevance of the water volume and the impact of long-term microwave disinfection on acrylic resins.

Similarly, Polyzois et al[26] observed unimportant linear changes using two dry microwave disinfection protocols with 500 W and exposure times of 3 and 15 min. Microwave disinfection of the specimens at 500 W for 3 min produced less distortion among all groups tested. These results are similar to those of a previous study[34] that also observed insignificant changes using a dry protocol at 650 W for 15 min. However, the extrapolation of these results to a complete denture is impractical, as the specimens used in these studies were rectangular and cylindrical. Nonetheless, Polychronakis et al[17] observed significantly less distortion after the dry protocol than after the wet protocol for the posterior region of the denture-base specimens, and this should be addressed in future studies.

The extent of distortion observed in this study ranged up to -2.36%. It has been reported that dimensional changes of up to 1% are not clinically relevant. However, the available data are still insufficient, as this statement is based on a clinical report[35]. In a more recent clinical study[36], it was reported that wet protocols at 650 W for 3 min, with one and three irradiations per week (for one month), produced dimensional changes of up to -0.8% without clinical significance or damage to the dentures. Nevertheless, it is still difficult to draw a direct correlation between the results obtained in this review and its clinical implications. The shrinkage of the dentures leads to inadequate compression forces on the supporting tissues and discomfort to the patients. Therefore, possible adjustments to the dentures after microwave disinfection procedures may be required.

In light of the findings of this systematic review, it can be concluded that microwave disinfection protocols of 450 W for 5 minutes[25] or 500 W for 3 minutes[26] may be safe for disinfecting complete dentures. These protocols not only produced less distortion but also showed outcomes comparable to previously established chemical

disinfection protocols[25,26]. Despite these conclusions, the microbiological effectiveness of these protocols is of the utmost importance in order to help prevent and treat *Candida*-associated denture stomatitis. Attwa et al[37] observed that microwave irradiation at 500 W for 3 minutes was effective at decreasing the number of microorganism in both mucosal and denture swabs. Similarly, Al-Saadi et al[25] found that disinfecting acrylic resin denture bases at 450 W for 5 minutes reduced *Candida* colony counts by more than 4 $\log_{10}$, which is considered as standard for adequate disinfection. In addition, this protocol showed comparable results to microwave disinfection at 900 W for 5 minutes and immersion in sodium hypochlorite 5.25% for 5 minutes[25]. However, these findings should be interpreted with caution, because more data on the microbiological outcomes of these protocols are still required, particularly from clinical studies comparing different protocols and their long-term effects on complete dentures.

The present systematic review has some limitations, including the limited number of included studies and the likelihood that most studies are published in languages other than English. Moreover, due to the eligibility criteria, only *in vitro* studies were selected, and the clinical implications of microwave disinfection, as well as its long-term effects, should be investigated in further studies. In addition, differences regarding the disinfection protocols and methodologies among the studies restricted the possibility of performing a meta-analysis. In fact, only two studies[27,28], performed by the same research group with very similar methodologies could be considered for meta-analysis, which limited its power and contribution for the study. Therefore, we opted to not include a meta-analysis in this review. Lastly, further studies should also consider the possibility of applying digital methods of measurement, such as 3D

digital superimposition of dentures and master casts, to provide more complete, precise, and standardized data.

5 CONCLUSIONS

Within the limitations of this review, we conclude that microwave disinfection produced significant changes in the tridimensional and linear stability of denture-base acrylic resins in general. Only two microwave disinfection protocols were considered safe for disinfecting complete dentures, and the findings were comparable to those of chemical disinfection protocols, including immersion in 5.25% NaOCl, 125 ppm NaOCl, 0.12% gluconate chlorhexidine, effervescent tablets, and 2% glutaraldehyde. Nevertheless, further studies are required to provide more insights into the effects of microwave disinfection on the dimensional stability of complete dentures.

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TABLES

TABLE 3 Characteristics of the studies reviewed based on PICO-like structure reading

Authors, year	Study design	Property evaluated	Sample size (n)	Material/ Polymerization	Control group	Microwave disinfection protocol	Conclusions
Al-Saadi et al., 2014	In vitro	Linear dimensional stability	5	Triplex Hot/Conventionally polymerized.	CG1: immersion in 5.25% NaOCl for 5 min. CG2: immersion in diluted NaOCl (125ppm) for 5 hours. CG3: immersion in gluconate chlorhexidine 0.12% for 5 hours. CG4: immersion in effervescent tablets for 15 min. CG5: immersion in tap water for 15 min.	MW group 1: microwave disinfection at 900W for 5 min. MW group 2: microwave disinfection at 450W for 5 min.	Microwaving at full power (900W) for 5 minutes promotes changes on the dimensional stability of the complete dentures ($P < .001$). However, microwave disinfection at 450W for 5 minutes did not promote significant changes ($P > .05$), being considered a safe disinfection method.
Polychronakis et al., 2014	In vitro	Linear dimensional stability	6	Paladon 65/Conventionally polymerized.	CG: immersion in distilled water (at 37ºC) for 1 week.	MW group 1: microwave disinfection at 650W for 6 min daily for a week (immersed in water). MW group 2: microwave disinfection at 650W for 6 min (without immersion in water).	Microwave disinfection promoted a similar pattern of shrinking on the denture bases. Microwave disinfection immersing the denture bases promoted significant changes in comparison with the other groups for one segment of measure (AB).
Nirale et al., 2012	In vitro	Linear dimensional stability	10	DPI/ Conventionally polymerized.	CG: immersion in NaOCl 0.525% for 10 min.	MW group: microwave disinfection at 650W for 6 min.	Microwave disinfection produced significant changes in the dimensional stability of denture bases

							(p<0.001). NaOCl disinfection did not promote significant changes (*P* = .058).
Sartori et al., 2008	In vitro	Tridimensional stability	12	Veracryl/Microwave polymerized.	CG1: without treatment. CG2: immersion in 0.01% active chloride solution for 24 hours.	MW group: microwave disinfection at 690W for 6 min two times per week for two weeks.	Microwave disinfection showed significant distortion in comparison with the other groups after two irradiations (*P* < .05).
Seo et al., 2007	In vitro	Linear dimensional stability	10	Lucitone 550/Conventionally polymerized.	CG1: without treatment. CG2: immersion in distilled water (37ºC ± 1) for 7 days.	MW group 1: immersion in distilled water and microwaved at 650W for 6 min, once. MW group 2: microwave disinfection at 650W for 6 min daily, for 7 days.	Microwave disinfection, with one and seven cycles, promote significant changes (p=0.003 and p=0.007, respectively) in comparison with other groups (*P* < .001).
Sartori et al., 2006	In vitro	Tridimensional stability	6	Veracryl/Microwave polymerized.	CG1: without treatment. CG2: immersion in 0.01% active chloride solution for 24 hours.	MW group: microwave disinfection at 690W for 6 min two times per week for two weeks.	Microwave disinfection promoted significant changes on the denture bases, after two cycles, in comparison with the other groups (*P* < .05)
Polyzois et al., 1995	In vitro	Linear dimensional stability	10	Paladon 65/Microwave polymerized.	CG1: immersion in water for 1 hour. G2: immersion in water for 12 hours. CG3: immersion in 2% glutaraldehyde for 1 hour. CG4: immersion in 2% glutaraldehyde for 12 hours.	MW group 1: microwave disinfection at 500W for 3 min. MW group 2: microwave disinfection at 500W for 15 min.	Microwave disinfection at 500W for 3 min produced significant less changes in comparison with other groups (*P* < .05). All specimens presented changes,

	although without clinical importance.

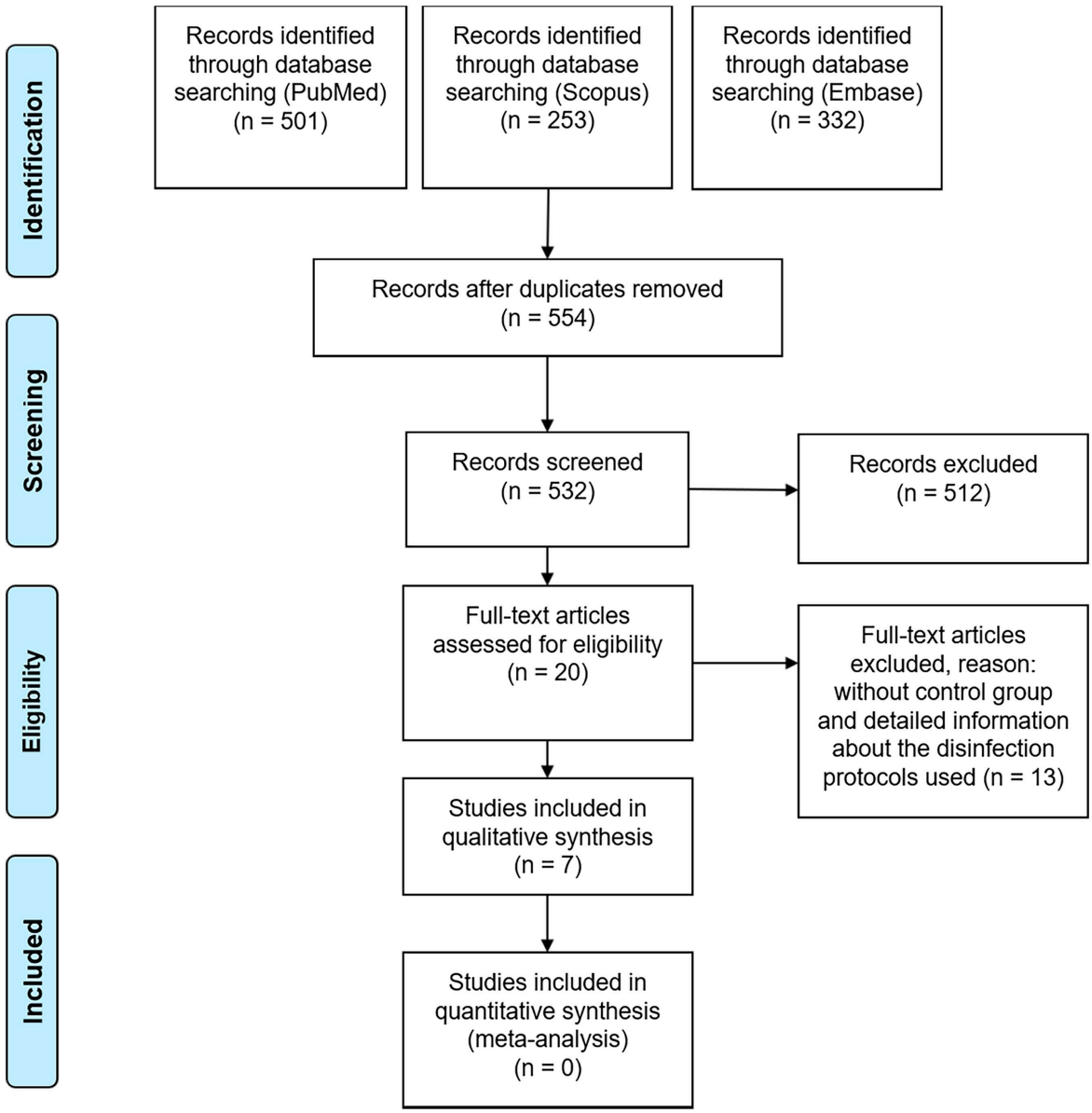
Abbreviations: CG1, control group 1; CG2, control group 2; CG3, control group 3; CG4, control group 4; CG5, control group 5; NaOCl, sodium hypochlorite; min, minutes; MW, microwave; W, Watts.

TABLE 2 JBI quality assessment of the reviewed studies

JBI Checklist questions	Al-Saadi et al[25]	Polychronakis et al[17]	Nirale et al[15]	Sartori et al[28]	Seo et al[16]	Sartori et al[27]	Polyzois et al[26]
1. Is it clear in the study what is the "cause" and what is the "effect" (i.e. there is no confusion about which variable comes first)?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
2. Where the participants included in any comparisons similar?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
3. Were the participants included in any comparations receiving similar treatment/care, other than the exposure or intervention of interest?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
4. Was there a control group?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
5. Were there multiple measurements of the outcome both pre and post the intervention/exposure?	Unclear	Unclear	Unclear	Yes	Yes	Yes	Yes
6. Was follow up complete and if not, were differences between groups in terms of their follow up adequately described and analyzed?	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable	Not applicable
7. Were the outcomes of participants included in any comparisons measured in the same way?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
8. Were the outcome measured in a reliable way?	Yes	Yes	Yes	Yes	Yes	Yes	Yes
9. Was appropriate statistical analysis used?	Yes	Yes	Yes	Yes	Yes	Yes	Yes

FIGURES

FIGURE 1 Flow diagram of the search strategy



3 DISCUSSION

The first null hypothesis of the present research was accepted, as microwave disinfection was equally or more effective, for treating CADS, in comparison with conventional treatments. On the other hand, the second null hypothesis was rejected as the results demonstrated that microwave disinfection affected the dimensional stability of denture base acrylic resins.

Microwave disinfection of complete denture was first introduced in 1985, by Rohrer and Bulard[31] (1985). The authors observed consistent sterilization for *Candida albicans* and aerobic bacteria after 8, 10 and 15 minutes of exposure, using a power setting of 720 Watts. Since then, several protocols have been suggested for disinfecting acrylic resins against a variety of microorganisms. Webb et al.[32] (1998) achieved complete sterilization for *C. albicans* with a power setting of 650 Watts and with exposure times ranging from 2 to 10 minutes. A similar result was also reported by Sanitá et al.[33] (2009), which achieved sterilization of complete dentures contaminated with *C. albicans* after microwave irradiation at 650 Watts for 3 minutes. In addition, complete sterilization was also noticed for *Porphyromonas aeruginosa*, *Staphylococcus aureus*, *Candida albicans* and *Bacillus subtilis*, using a power setting of 650 Watts and an exposure time range of 3 to 5 minutes[34]. However, it is worth mentioning all of these finding was obtained from in vitro studies.

Contrasting with a variety of in vitro studies, few studies assessed the clinical implications of microwave disinfection against CADS. In the present research, this issue was addressed by 7 studies, with two being excluded for not meeting all the inclusion criteria, specifically, the comparison of microwave disinfection protocols with positive and/or negative control groups. In this regard, 5 studies from three different countries (Brazil, Australia, and Canada), involving 245 participants, were included.[16,17,20,30,35] Most of the included studies were performed at universities,[17,20,30] while two were conducted in hospitals and aged care homes,[16,35] therefore a less controlled environment in order to avoid biases. All studies performed the randomization of the participants and, in general, a concern for excluding risk factors, that may influence the development of CADS, and for verifying the homogeneity, among the experimental and control groups, was also seen. However, only two studies performed the sample size calculation.[17,30] Moreover, two studies reported the loss of participants,[16,17] due to withdrawal of consent to participates and the necessity of being subjected to antibiotic therapy.[17]

The characteristics of the participants were similar to previous reports, as the majority of them were elderly (age range of 56.8 to 83.7 years), especially in females.[17,20,30] In addition, it was observed that most of the patients used their dentures for long periods (age range of 12.8 to 24.8 years), higher than the 5 years usually preconized, event that has been correlated to the development of CADS.[36] Other commonly observed risk factors were nocturnal denture wear, poor denture and oral hygiene, and xerostomia. Lastly, a higher frequency of Candida spp. cells on the dentures,[17,32] including its mycelial forms,[16,20] was also seen. This event has been associated with the surface characteristics of PMMA, as its roughness, hydrophobicity, and surface free energy, combined, acts as a favorable and protective medium for the adhesion of microorganisms, and the development of its biofilms.[14,15,19] This is an important finding, as the dentures may be a source for infection or reinfection processes of the palatal tissues, therefore, indicating that its disinfection is crucial when treating patients with CADS.[17,37-39]

The main outcomes of the studies consisted in the decrease of the residual yeast present on the dentures and palatal tissues (microbiological), and/or the improvement of the clinical signs of CADS (clinical). Therefore, regarding the microbiological results, microwave disinfection showed comparable results with the chemical disinfection protocols proposed (immersion in 0.2% chlorhexidine and 0.02% NaOCl).[16,35] Although Banting and Hill[16] (2001) reported no statistically significant differences between the two interventions, the authors notice that microwave disinfection was 30% more efficient than 0.2% chlorhexidine. Similarly, Webb et al.[35] (2005) observed equal effectiveness of microwave and 0.02% NaOCl protocols against *Candida spp*. from dentures and palates, and aerobic bacteria from dentures. However, both interventions failed to reduce the number of cells of aerobes from the palates.[35] Additionally, the results obtained in the qualitative synthesis of the present study demonstrated that microwave disinfection was as effective as topical nystatin, for reducing both the *Candida* spp. cell counts on the palates and dentures, at the end of the treatment (14 days).[17,30] In fact, this outcome was also confirmed in the meta-analysis, which did not show any statistical differences among the treatment protocols, for all periods assessed. Notwithstanding, Neppelenbroek et al.[20] (2008) noticed significantly superior results from microwave disinfection, associated or not, to topical miconazole, in comparison with miconazole in isolation. Moreover, the authors did not observe any viable cells or mycelial forms of *Candida spp*. after the end of the treatment (30 days).[20] Despite these results, antifungal therapy with miconazole has been usually recommended against CADS, and its effectiveness has already been demonstrated in a previous study.[40] Therefore, differences in the

methodology, subtherapeutic doses due to the diluent effect of the saliva, and antifungal resistance may be related to these outcomes.

Clinically, the outcomes were similar to those encountered on the microbiological assessment. In general, the studies used the classification proposed by Newton[41] (1962) for denture stomatitis (DS), in order to standardize the analyses. This classification divides the progression of DS in stages: absence of inflammation (0), punctiform hyperemia (type I), diffuse hyperemia (type II), and granular hyperemia (type III). Webb et al.[35] (2005) provided a breath description of the clinical outcomes obtained, reporting that both microwave disinfection and chemical disinfection with 0.02% NaOCl similarly improved the clinical signs of CADS. On the other hand, Silva et al.[17] (2012) and Sanitá et al.[30] (2012) reported significant and comparable improvement, of the clinical conditions of the participants, for microwave disinfection and topical nystatin, reaching cure (absence of palatal inflammation) rates ranging from 38.89% to 41.18% at the end of the treatment. This outcome was, again, confirmed in the meta-analysis which showed comparable results from both treatments, for all periods assessed. These results are similar to a previous study,[42] which reported a success rate (cure or clinical improvement) of 53%, with a similar antifungal therapy protocol with topical nystatin for 15 days. Nevertheless, the most noticeable outcomes were reported by Neppelenbroek et al.[20] (2008) which demonstrated that, whether associated or not to topical miconazole, microwave disinfection achieved complete remission of the clinical signs in, respectively, 80% and 70% of the participants after 30 days (end of the treatment). Contrastingly, all patients treated with topical miconazole, in isolation, did not show significant reduction of the clinical signs. These distinctive outcomes are possibly related to methodological differences such as longer time of exposure of the irradiation (6 minutes), and extended period of treatment, in comparison with the other studies.

However, the recolonization of the palates and dentures, and the clinical relapse of CADS, were reported within a follow-up period of three months.[16,17,20,30] Silva et al.[17] (2012) and Sanitá et al.[30] (2012) observed recurrence rates of the clinical signs varying from 39% to 55%. Banting and Hill[16] (2001) noticed lower recurrence rates on the microwave group, with 56% for dentures and 8% for the palates, with the authors concluding that this group had 75% less chances of reinfection of the palatal tissues, in comparison with the chlorhexidine group. Additionally, Neppelenbroek et al.[20] (2008) observed recolonization of a few mycelial cells in 33.3% and 40% of the dentures subjected to microwave disinfection alone, or associated to miconazole, respectively. The recurrence of the clinical signs, associated with the reinfection of the palates and dentures, is frequent drawback in the treatment of CADS, often occurring

two weeks after the end of the treatment, which may be attributed to antifungal resistance, colonization and infection by a new strain, or failure to eliminate the current colonizing strength.[13,43-45] In addition, the recurrence of CADS is also related to the maintenance of an adequate oral and denture hygiene, as demonstrated by Cross et al.[45] (2004), which noticed lower recurrence rates in patients that managed rigorous levels of hygiene for 3 years.

Despite the similar outcomes among the abovementioned treatment protocols, microwave disinfection has some advantages over the other therapies, as it consists in a simple and inexpensive method of disinfection.[16,17] In addition, its practicality reduces the chances of impairment of the treatment, due to lack of commitment of the patients.[16,21,46] Moreover, its physical mechanism of action prevents the appearance of resistant fungal cells, in contrast with the possibility within treatments involving antifungal therapy.[17,30] Nonetheless, previous studies have been indicating disadvantages of this method, including deleterious effects on the mechanical and surface properties of PMMA, and changes on the dimensional stability of complete dentures.[24-28] However, the current data, concerning this scenario, from the scientific literature remains controversial.

In this regard the systematic assessment of the effect of microwave disinfection, on the dimensional stability of denture base acrylic resins, showed more enlightening results. Of the seven in vitro studies included, five studies assessed the linear stability of denture base acrylic resins,[26,47-50] and two studies evaluated the tridimensional stability.[51,52] The assessment of the later is preferred and a more reliable method, as distortion may occur in different parts of the specimens which are out of the pre-established measurement segments which may introduce misleading results.[26] In addition, this is more prone to occur in complex structures such as presented by complete dentures, which may suffer uneven distortion after the disinfection procedures due to variations in thickness along its parts.[26,47-49,51,52] In this sense, the majority of the studies have used denture bases as specimens, with only using rectangular shaped specimens.[50]

The results of the present study demonstrated that most of the microwave disinfection protocols tested promoted significant distortion on the specimens, which involved the irradiations with the specimens immersed in water.[51,52] Wet protocols have been reported as more effective than dry protocols due to an additional and uniform heating of the water.[17,20,30] In fact, this effectiveness was demonstrated in the previous studies discussed in the present study. However, the high temperatures of the water may induce distortion on the materials, as they can reach the glass transition temperature of the acrylic resins, therefore, leading to the release of residual polymerization stresses within the polymeric matrix, and shrinkage of the

material.[26,48,49,53] According to Craig[54] (1997), thermopolymerized resins may encounter distortion under a temperature range of 71ºC to 90ºC. In fact, the water boiling was reported in some studies and occurred mostly after 90 seconds,[26,48,49] but was also reported after 80 seconds,[47] and after 6 minutes.[52] Thus, it is possible to infer that subjecting the specimens under high temperatures and prolonged periods might played an important role on the dimensional changes encountered.

Contrastingly, Al-Saadi et al.[47] (2014) did not noticed distortion using a wet protocol with 450 Watts for 5 minutes, finding that is in consonance with previous studies. Senna et al.[55] (2011) reported no significant differences after 6 cycles, using wet disinfection protocols with power settings of 450W and 630W for 3 minutes. The authors encountered changes only after 36 cycles, concluding that these protocols were safe for poly(methyl methacrylate) polymers. In addition, the irradiation of specimens at 650 Watts for 3 minutes, immersed in 150 mL of water, did not promote significant changes on the dimensional stability of acrylic resins.[56] Despite these studies have not been included in this research, it is possible that wet protocols with moderate power and lower time exposure may be a safer option for disinfecting dentures, specially taking into consideration the microbiological and clinical outcomes observed using a 650 Watts for 3 minutes disinfection protocol. Therefore, this issue should be addressed in future studies, including the relevance of the water volume, and the impact of long-term microwave disinfection on the acrylic resins.

Similar results were obtained in the study conducted by Polyzois et al.[50] (1995), as the authors did not notice any important change on the dimensional stability of the specimens after dry irradiations at 500 Watts for 3 and 15 minutes, with the first protocol producing the best result among all groups. This was also observed by Burns et al.[57] (1990), with a dry protocol with 650 Watts for 15 minutes. Notwithstanding, extrapolation of these data is difficult taking into consideration that both studies used regular shaped (rectangular and cylindrical, respectively) specimens. Nevertheless, Polychronakis et al.[49] (2014) indicated significantly less dimensional changes in favor of a dry protocol, specifically in the posterior area of the denture base specimens. Therefore, further studies should also address the comparison of wet and dry protocols.

However, a direct correlation between the results observed, and its clinical implications, is difficult to be drawn. A previous study stated that dimensional changes up to 1% does lead to clinically relevant outcomes.[58] However, information regarding this issue is still missing, as this conclusion was based only in a clinical report. In a more recent clinical study, Basso et al.[59] (2010) noticed clinically insignificant changes up to -0.8%, using a wet protocol with a

power setting of 650 Watts for 3 minutes, with irradiations once and three times a week, for one month. Nevertheless, the amount of distortion observed ranged from -2.36 to 0.009%. Therefore, it is possible to infer that clinicians should be aware that adjustments on the dentures may be required along the treatments involving microwave disinfection, in order to avoid pressure points on the supporting tissues and, consequently, discomfort for the patients.

In light of the results of this study, it was demonstrated that microwave disinfection is an alternative treatment for CADS with similar or better efficiency, when compared with conventional treatments. However, considering the lack of information regarding follow-up and clinical assessments, the protocols proposed by Banting and Hill[16] (2001), and Webb et al.[35] (2005), still needs further investigations. In addition, although with distinctive results, irradiating complete dentures three times a week, for one month, using a power setting of 650 Watts for 6 minutes[20] may provoke deleterious effects on the prostheses. Therefore, it can be presumed that a microwave disinfection protocol, with only one irradiation at 650 Watts for 3 minutes a week for two consecutive weeks, may be a more suitable alternative for treating and preventing CADS, simultaneously minimizing possible side effects on the dentures.[17] Unfortunately, all studies that have assessed the effect of this protocol on the dimensional stability of acrylic resins did not meet the criteria for inclusion on the second part of the present study, which would have provided a more complete understanding of the outcomes of this protocol, and this should be subject of further studies. Nonetheless, disinfecting dentures with irradiations, at 450 Watts for 5 minutes[47] or 500 Watts for 3 minutes,[50] may be a safe alternative, as these protocols were similar to already stablished chemical disinfection protocols. Notwithstanding, more studies, assessing the long-term and clinical effects of different protocols, on the complete dentures, are still necessary to completely validate these results.

The present study has some limitations, to begin with the small number of studies included, and the possibility of other relevant studies being published in languages other than English. Also, according to the inclusion and exclusion criteria, only in vitro studies were included for the assessment of the dimensional stability, and further clinical studies are required in order to provide more information regarding this issue. Additionally, the differences in the methodologies and disinfection protocols reduced the scope of the meta-analyses performed, thus, standardization is an issue that should be addressed in further studies. Lastly, due to the eligibility criteria, it was not possible to evaluate a microwave disinfection protocol, in order to perform a direct correlation between the microbiological and clinical results, and the effect on the dimensional stability of the materials.

4 CONCLUSION

Within the limitations of the present study, it can be concluded that microwave disinfection was as effective as antifungal therapy with topical administration of nystatin, overnight soaking in 0.02% NaOCl, 0.2% chlorhexidine, and was more effective than topical miconazole in isolation. In addition, the microwave disinfection protocol at 650 Watts and 3 minutes, once a week for two consecutive weeks, proved to be a better option for the prevention and treatment of CADS, and with less chances of damaging the prostheses. Overall, microwave disinfection produced statistically significant changes on the linear, and tridimensional stability of acrylic resins. Of the different microwave disinfection protocols assessed, only two protocols (450 Watts for 5 minutes and 500 Watts for 3 minutes) did not promote changes on the dimensional stability of the materials, showing similar results with other chemical disinfection protocols, such as 5.25% NaOCl, 125 ppm NaOCl, 0.12% gluconate chlorhexidine, effervescent tablets, and 2% glutaraldehyde.

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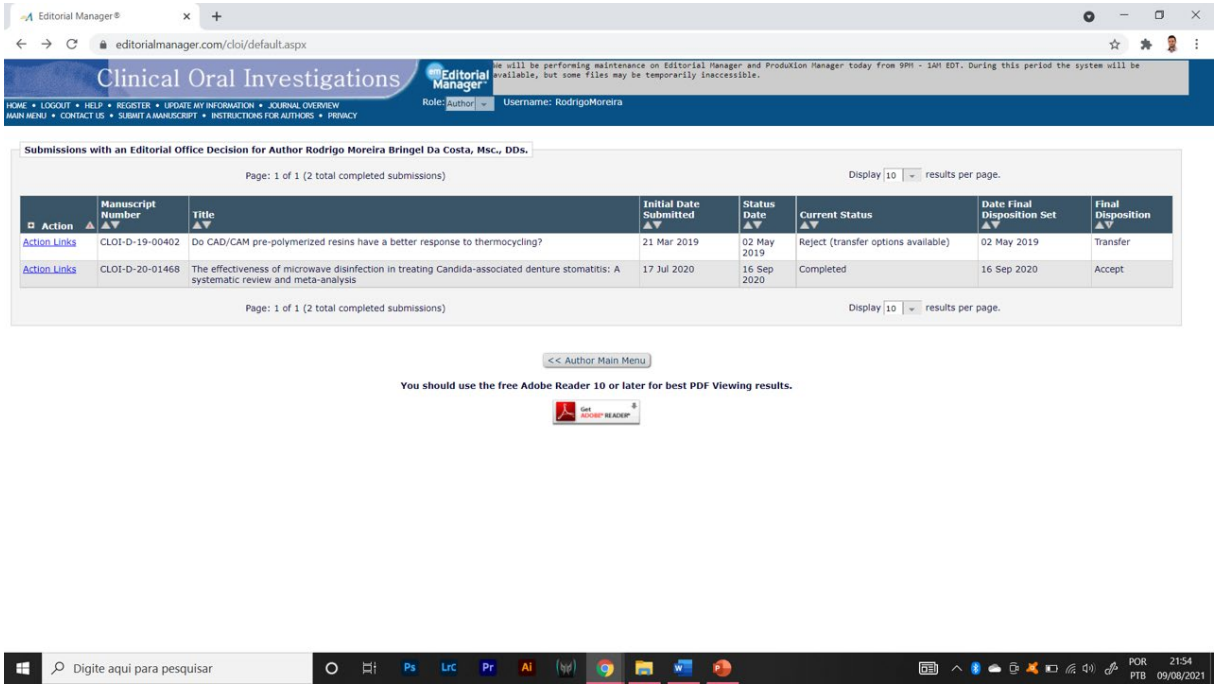
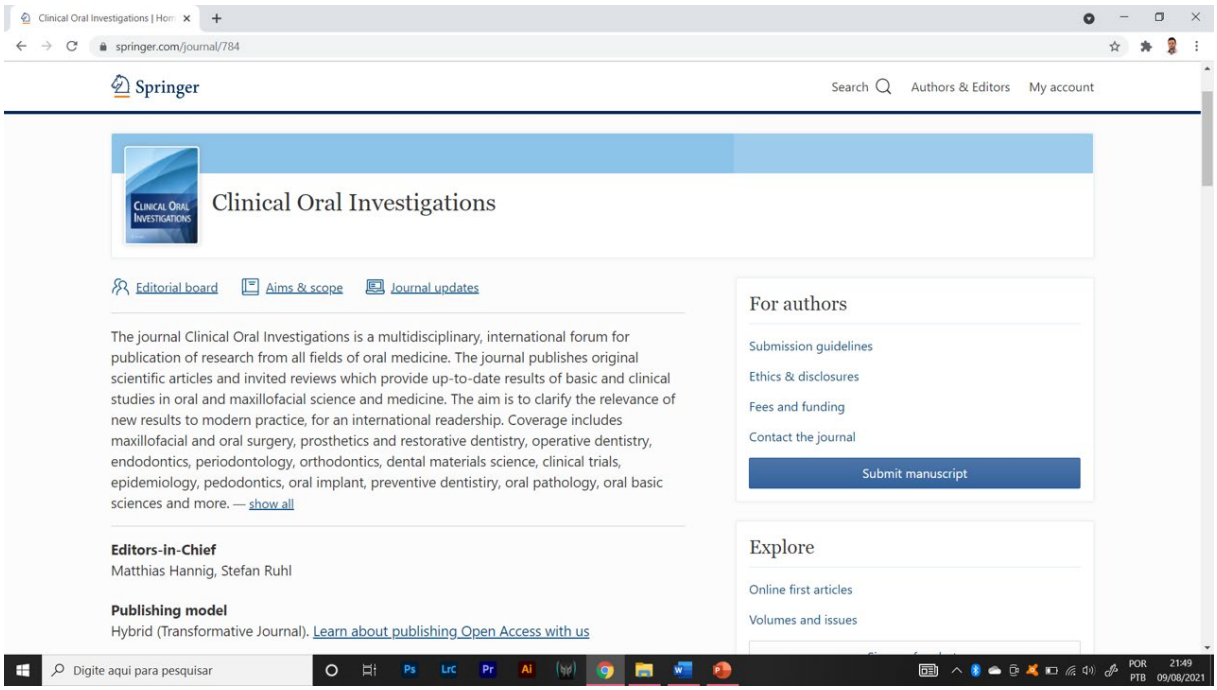
ANEXXES

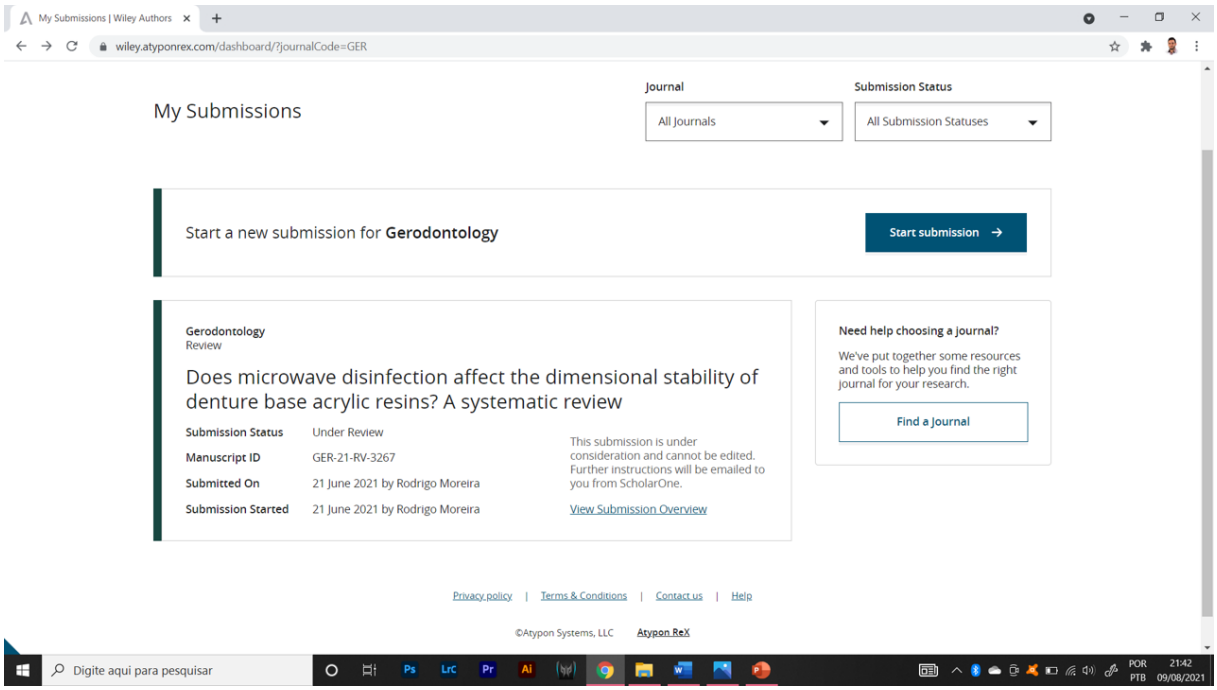
ANNEX A – Clinical Oral Investigations Proof of Submission



ANNEX B - Clinical Oral Investigations Index



ANNEX C – Gerodontology Proof of Submission



ANNEX D - Gerodontology Index