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Efficacy of treatments for Demodex blepharitis: A systematic review and meta-analysis

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ABSTRACT

Purpose: We conducted a systematic review and meta-analysis to evaluate the efficacy of different treatment for Demodex blepharitis. Parameters studied were mites count, improvement of symptoms and mites' eradication, stratified on type of treatments and mode of delivery of treatments (local or systemic).

Method: The PubMed, Cochrane Library, Embase, ClinicalTrials.gov, Google scholar and Science Direct databases were searched for studies reporting an efficacy of treatments for Demodex blepharitis.

Results: We included 19 studies (14 observational and 5 randomized clinical trials), for a total of 934 patients, 1741 eyes, and 13 different treatments. For mites count, eradication rate, and symptoms improvement, meta-analysis included fifteen, fourteen and thirteen studies, respectively. The overall effect sizes for efficiency of all treatments, globally, were 1.68 (95CI 1.25 to 2.12), 0.45 (0.26–0.64), and 0.76 (0.59–0.90), respectively. Except usual lid hygiene for mites count, Children's Hospital of Eastern Ontario ointment (CHEO) for both eradication rate and symptoms, and CHEO, 2% metronidazole ointment, and systemic metronidazole for eradication rate, all treatments were efficient. Stratified meta-analysis did not show significant differences between local and systemic treatments (1.22, 0.83 to 1.60 vs 2.24, 1.30 to 3.18 for mites count; 0.37, 0.21 to 0.54 vs 0.56, 0.06 to 0.99 for eradication rate; and 0.77, 0.58 to 0.92 vs 0.67, 0.25 to 0.98 for symptoms improvement).

Conclusion: We reported the efficiency of the different treatments of Demodex blepharitis. Because of less systemic side effects, local treatments seem promising molecules in the treatment of Demodex blepharitis.

Introduction

Blepharitis is a common eye inflammation affecting eyelash, eyelid and ocular surface with sometimes corneal resounding. Among many causes, Demodex mites are found since the 19th century with princep observation by Henle and Simon [1,2]. There are two host-specific obligate mites' species found in human being's hair follicles, sebaceous glands (Zeiss 'glands) and eyelid glands (Meibum's glands) causing anterior and posterior blepharitis: Demodex folicularum and Demodex brevis. Typically, Demodex folicularum found in clusters around the eyelash and eyelid skin whereas Demodex brevis resided alone in the deep of sebaceous and Meibomian glands [3–5]. Mites' presence may cause inflammatory process in some eyelid tissues, however the pathogenesis' role of Demodex in inflammatory process of blepharitis is discussed. Demodex would be the vector for number of bacterial and mycotic pathogens, resulting in an immunological response at the eyelid margins, with redness, itching and burning sensations [6–8]. Diagnosis of Demodex blepharitis is classically obtained by parasitologist with skin or follicles biopsies [1–6,8,9] or more recently by confocal microscopy [10,11]. Cylindrical dandruff at the base of eyelash is considered as pathognomonic of Demodex infestation [5,12,13].

This physiological lack of knowledge and saprophyte presence of Demodex in healthy eyes have an impact on therapeutics with very few studies in international scientific literature. During long years, usual lid hygiene has been used to treat this kind of resistant blepharitis, sometimes with sulphuric ointment [1], yellow mercuric ointment [1,4,9,14], pilocarpine gel [15,16] or locals' antibiotics [17] without proof of efficacy. Anthelminthics, with systemic side effects, have been

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used empirically these last years [18–21]. New local therapy based on tea tree oil (TTO) and terpinen-4-ol (T4O) have been tested recently [18,21–32], opening a new therapeutic field. To our knowledge, there are no synthesis of literature comparing Demodex treatments. To allow a future consensus or new treatment elaboration is of major interest.

Therefore, we aimed to compute a systematic review and meta-analysis to compare all efficacy of Demodex blepharitis treatments. More specifically, we aimed assess the comparative efficiency of local and systemic treatments and to evaluate influencing parameters in therapeutic efficacy.

Methods

Literature search

We have searched all articles in PubMed, The Cochrane Library, Central, Embase, ClinicalTrial.gov, Google Scholar and ScienceDirect databases from February 2018 to August 2018 with following keywords: (blepharitis OR blepharitides) AND (drug* OR pharmacotherapy OR therap* OR treat* OR administration OR patient* OR outcome* OR efficacy OR effective* OR clinical OR management OR compliance OR adherence). We limited our search to articles written in English, French, or Spanish. No minimal sample size was applied. To be included, articles needed to evaluate a therapy concerning Demodex blepharitis proved by parasitological examination or confocal microscopy or cylindrical dandruff. We imposed no limitation of regional origin or control group nature. In addition, references list of all publications was manually searched to identify any other ones not found with electronic search. The search strategy is presented in Fig. 1. One author conducted all literature searches (Valentin Navel) and collated the abstracts. Two authors (Valentin Navel and Cédric Benoist d'Azy) separately reviewed the abstracts and based on the selection criteria, decided the suitability of the articles for inclusion. A third author (Frédéric Dutheil) was asked to review the articles where consensus on suitability was debated. Finally, all authors reviewed eligible articles.

Quality of assessment

Although not created for that, the "Strengthening the Reporting of Observational studies in Epidemiology" (STROBE) criteria may be inappropriately used as an assessment tool to judge study quality, as well as the CONSORT guidelines for randomized clinical trials. STROBE and CONSORT are checklists of 22 and 30 items, respectively. We attributed one point per items, then converted into percentage to give a quality score for each included study [33–36].

We also used the SIGN criteria to also judge observational studies and randomized clinical trials, with the dedicated evaluation grids. SIGN Cohort Studies and SIGN Controlled Trials statements are a checklist of 18 and 14 items, respectively. We gave a general quality score for each include study based on the main causes of bias evaluated in section 1 of both checklists through 4 possibilities of answers (yes, no, can't say or not applicable) [37].

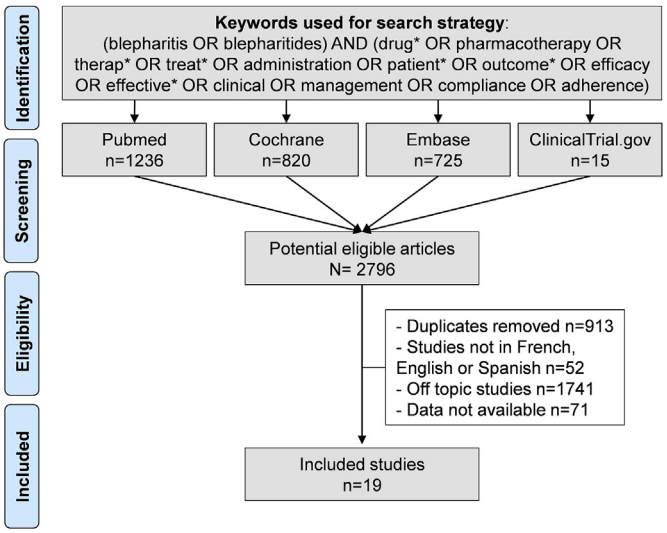


Fig. 1. Search strategy.

Statistical considerations

Statistical analyses were performed using Stata (v12, StataCorp, US) [38–44]. Parameters were reported as mean ± standard-deviation (SD) or number (%) for continuous or categorical variables. Treatment efficacy was assessed using Hedges bias corrected effect size (ES) of parasite count evolution (before-after treatment) as primary outcome. Parasite eradication rate and symptoms improvement rate were considered as secondary outcome. ES and 95% confidence interval (CI) were presented on forest plots, as a unitless measure of the effects of treatments for Demodex blepharitis on mites count, eradication rate, and symptoms improvement. An ES centered at zero means the absence of efficacy, 0.2 a small effect, 0.5 a moderate effect, and 0.8 a large effect [45]. Funnel plots assessed the publication bias. I-squared (I^2) quantified heterogeneity between studies, graded as low (< 25%), moderate (25–50%) or high (> 50%). All statistical tests were two-sided; significance was set for $p < 0.05$. When sample size was sufficient, meta-regressions (expressed as regression coefficient and 95% CI) were proposed to study relationships between parameters variation and clinically relevant parameters such as age, sex ratio and eyelash sampling method.

Results

With the keywords described, an initial search produced 2796 articles (Fig. 1). After removal of the duplicates and applying selection criteria, we included 19 articles [14–16,18,18–32].

More details on study characteristics, quality of articles (Figs. 2 and 3), method of Demodex identification, type of treatments, protocol for each treatment, inclusion and exclusion criteria of each included study, population, aims and outcomes of included studies are described in Appendix 1.

Meta-analyses and meta-regressions

Mites count: Fifteen studies were included [14,16,19–25,27–32] with an overall ES of 1.68 (95CI 1.25 to 2.12) for all treatments. Except usual lid hygiene, all treatments decreased mites count (Fig. 4 and Fig. 5, and Appendix 2). Stratified meta-analysis did not show significant differences between local (1.22, 0.83 to 1.60) and systemic (2.24, 1.30 to 3.18) treatments (Figs. 6 and 7), or between eyelash sampling with (1.31, 0.80 to 1.81) or without (1.49, 1.02 to 1.96) cylindrical dandruff (Fig. 6, and Appendix 5). There were also no significant influences of age and gender (Fig. 6). Meta-regressions comparing treatments efficacy were not feasible due to limited number of data (most treatments were only reported in one study), despite stratified meta-analysis on each treatment demonstrated ES greater than 2.5 for oral metronidazole + oral ivermectin (3.66, 95CI 2.84 to 4.48), and 5%TTO (2.66, 2.17 to 3.15); greater than 1 for ivermectin alone (1.80, 1.10 to 2.50), 50%TTO (1.74, 0.81 to 2.67), pilocarpine gel (1.72, 0.71 to 2.73), and T4O (1.36, 0.60 to 2.11); and greater than 0.8 for Cilclar 1.9% + oxide mercuric ointment + ether application (0.81, 0.26 to 1.34), CHEO (0.53, 0.12 to 0.94) and OLSP (0.95, 0.53 to 1.37) (Figs. 4 and 5, and Appendix 2).

Eradication rate of mites: Fourteen studies were included [14,15,18,20–24,27,29–32,46] with an overall ES of 0.45 (0.26–0.64) for all treatments. Except CHEO, 2% metronidazole ointment, and

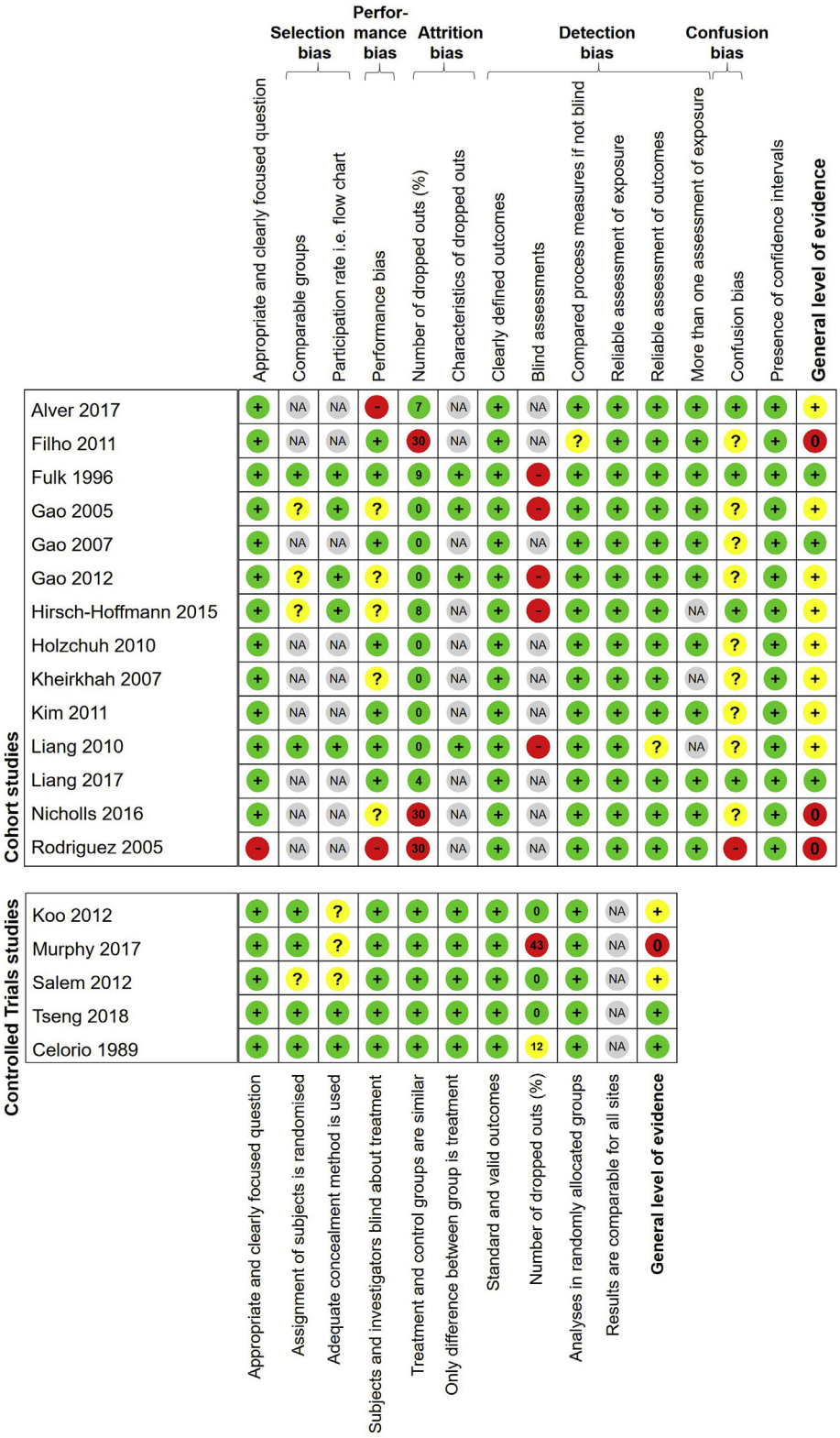


Fig. 2. Methodological quality of included cohort articles using the SIGN model.

systemic metronidazole, all treatments improved eradication rate (Figs. 4 and 5, and Appendix 3). Stratified meta-analysis did not show significant differences between local (0.37, 0.21 to 0.54) and systemic (0.56, 0.06 to 0.99) treatments (Figs. 6 and 7), or between eyelash sampling with (0.30, 0.12 to 0.51) or without (0.46, 0.25 to 0.68) cylindrical dandruff (Fig. 6, and Appendix 5). As for mites count, there were also no significant influences of age and gender (Fig. 6), and meta-regressions comparing treatments efficacy were also not feasible due to limited number of data (one study per treatment, mainly). However, stratified meta-analysis on each treatment demonstrated ES greater than 0.8 for systemic metronidazole + ivermectin (1.00, 0.80 to 1.00), and pilocarpine gel (0.92, 0.81 to 0.97); greater than 0.5 for Cilclar 1.9% + oxide mercuric ointment + ether application (0.57, 0.33 to 0.59), 50%TTO (0.54, 0.25 to 0.82), and ivermectin (0.54, 0.01 to

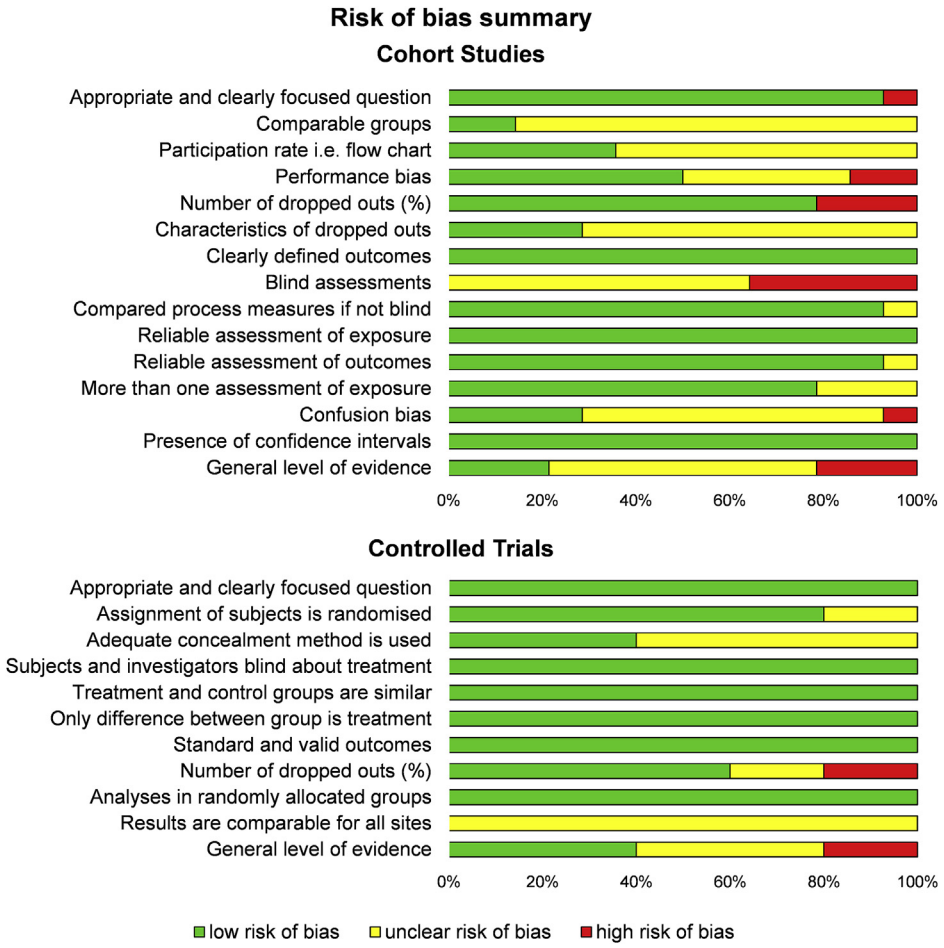


Fig. 3. Summary bias risk of included cohort studies and controlled trials articles using the SIGN model.

1.00); and greater than 0.2 for Ocusoft lid scrub (0.46, 0.28 to 0.65), 5%TTO (0.43, 0.04 to 0.87), T4O (0.41, 0.23 to 0.61), and usual lid hygiene (0.22, 0.02 to 0.53) (Figs. 4 and 5, and Appendix 3).

Symptoms improvement: Thirteen studies were included [14–16,18,19,21–24,26,28,31,32,46] with an overall ES of 0.76 (0.59–0.90) for all treatments. Except CHEO, all treatments improved symptoms (Figs. 4 and 5, and Appendix 4). Stratified meta-analysis did not show significant differences between local (0.77, 0.58 to 0.92) and systemic (0.67, 0.25 to 0.98) treatments (Figs. 6 and 7), or between eyelash sampling with (0.81, 0.37 to 1.00) or without (0.73, 0.55 to 0.89) cylindrical dandruff (Fig. 6, and Appendix 5). As for mites count and eradication rate, there were also no significant influences of age and gender (Fig. 6), and meta-regressions comparing treatments efficacy were also not feasible due to limited number of data (one study per treatment, mainly). CHEO were less efficient than usual lid hygiene with a coefficient of −1.02 (−1.33 to −0.71) (Fig. 6). However, stratified meta-analysis on each treatment demonstrated ES greater than 0.8 for T4O (1.00, 0.85 to 1.00), Ocusoft lid scrub (1.00, 0.86 to 1.00), 50% TTO (0.97, 0.86 to 1.00) and 5% TTO (0.81, 0.60 to 0.96); greater than 0.5 for Cilclar 1.9% + oxide mercuric ointment + ether application (0.79, 0.52 to 0.92), systemic ivermectin (0.78, 0.31 to 1.00), 4% pilocarpine gel (0.74, 0.60 to 0.84) and usual lid hygiene

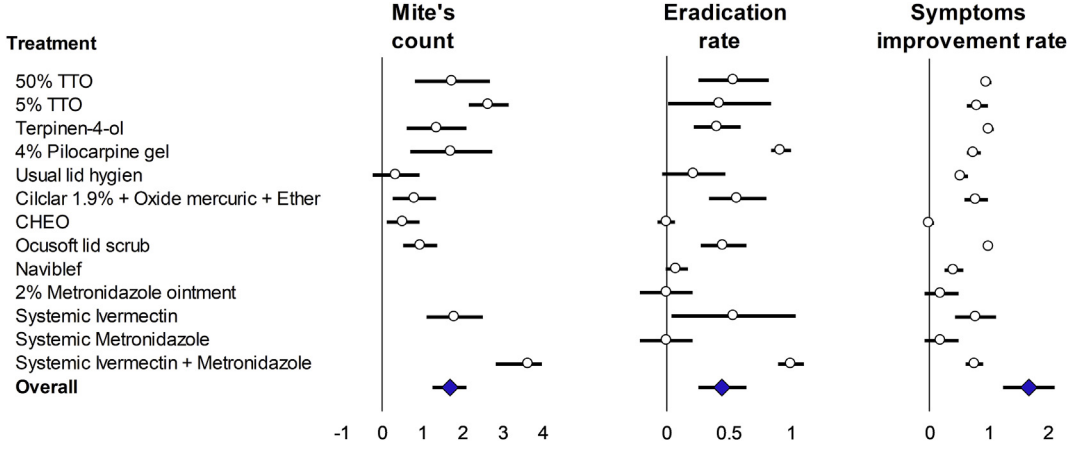


Fig. 4. Meta-analysis on mite's count, eradication rate and symptoms improvement rate in each treatment protocol (95% CI: 95% Confidence intervals).

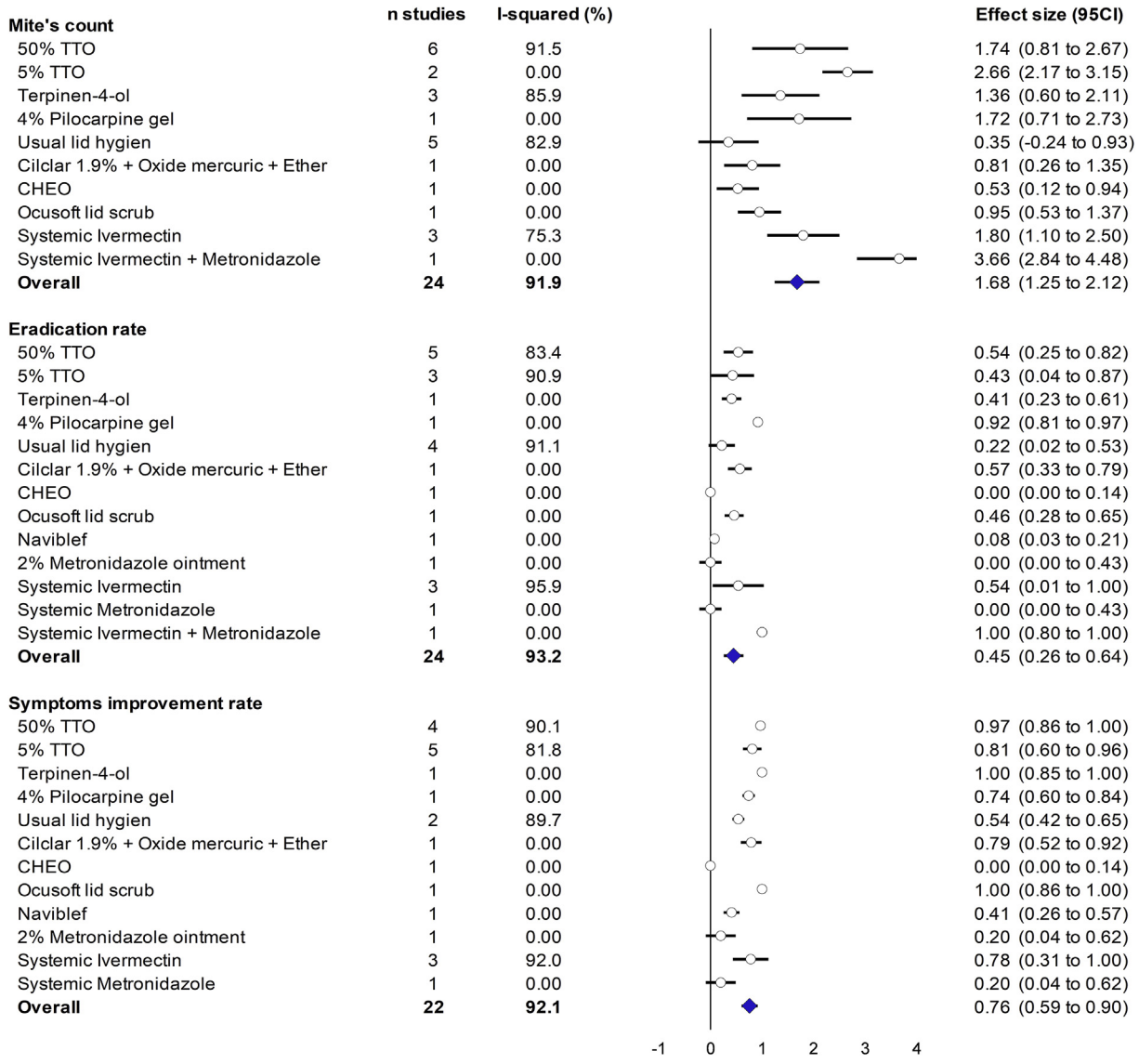


Fig. 5. Meta-analysis on mite's count, eradication rate and symptoms improvement rate in each treatment protocol (95% CI: 95% Confidence intervals).

(0.54, 0.42 to 0.65); and greater than 0.2 for Naviblef (0.41, 0.26 to 0.57), 2% metronidazole ointment (0.20, 0.04 to 0.62) and systemic metronidazole (0.20, 0.04 to 0.62) (Figs. 4 and 5, and Appendix 4).

Discussion

Our study is the first systematic evaluation of treatments for Demodex blepharitis. Physiopathology of this commensal parasite were a hindrance to the development of various therapies. We reported the efficiency of the different treatments of Demodex blepharitis. More interestingly, stratified meta-analysis did not show significant differences between local and systemic treatments. Because of less side effects, local treatments seem promising to manage Demodex blepharitis. We did not demonstrate influence of sociodemographic in the efficacy of treatments.

Rational of study

Despite Demodex was first identified 150 years ago, it only attracted wider interest recently, over the last 10 years [1]. In fact, the relative current ignorance of physiopathology is a drawback in therapeutics' evaluations. Initially, Demodex was considered as a saprophyte parasite normally colonising the eyelashes. Current consensus proposed to consider as physiological a number of mites < 5 mites/cm2 for skin lesions or < 3 mites at the root of each eyelash [4,20,47]. However, mites outbreaks may play a role in the pathophysiology of the infection, causing a local inflammatory reaction and a repercussion on the ocular surface [1,5,8,9,13,48–50]. Therefore, several therapeutics were used such as antiparasitic, antiseptics, or anti-inflammatory drugs. Our meta-analysis was needed because most treatments were used without sound proof of efficiency and without randomized controlled trials comparing efficiency of treatments. We chose Demodex count as primary judgement criteria because the presence of some mites may be considered as normal and outbreaks pathological. Eradication rate was chosen as a secondary judgement criteria to evaluate the in vivo killing effect in parallel of mites count decrease.

Interest molecules

Initially, usual lid hygiene has been used to treat resistant blepharitis with sulphuric ointment [1], yellow mercuric ointment [1,4,9,14] or pilocarpine gel [15,16]. Sulphuric ointment or yellow mercury treatments were poorly supported and are now obsolete (last publications are more than twenty years old) [2,5,13,51,52] whereas

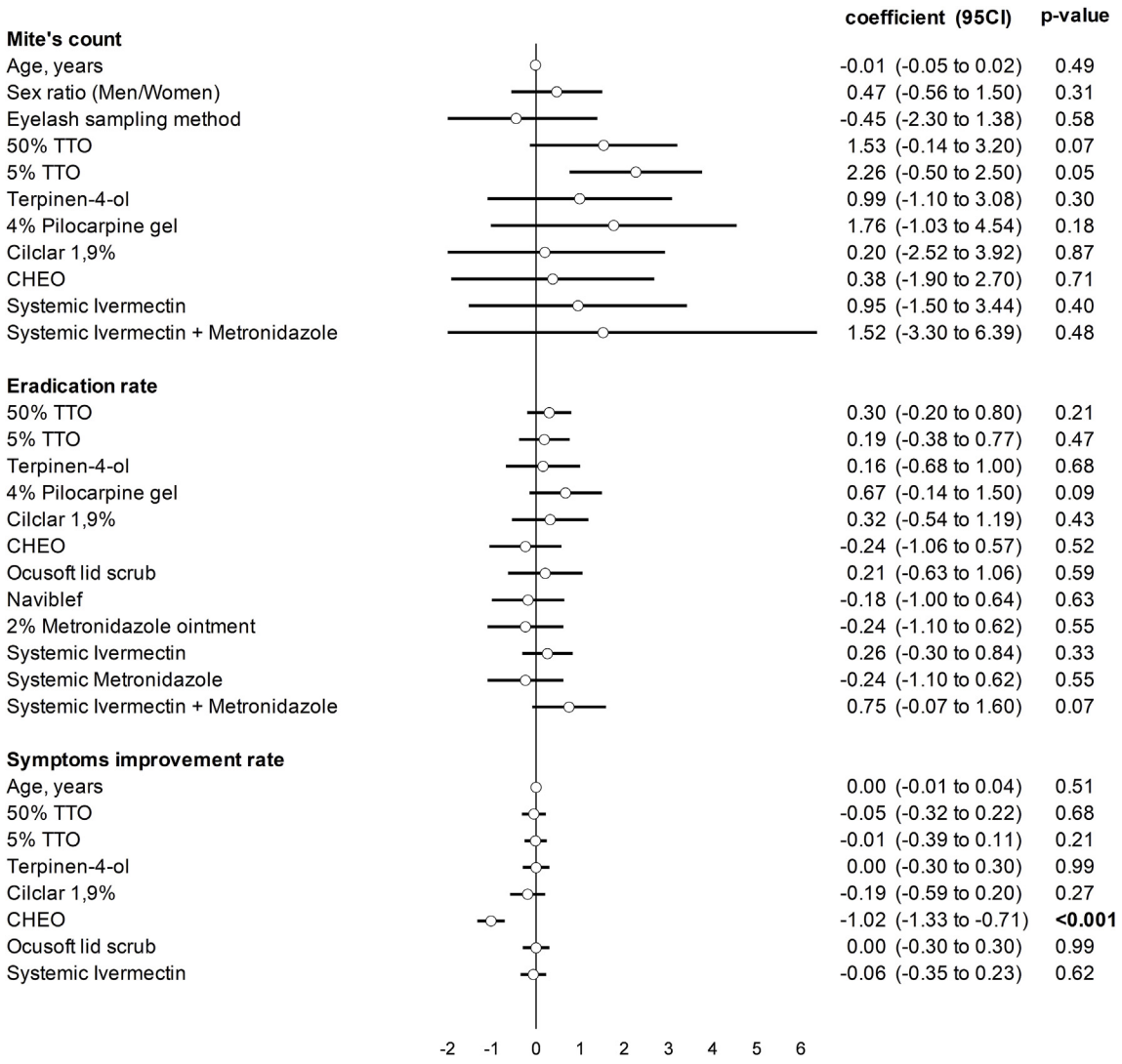


Fig. 6. Meta-regressions about parameters of analyses (95% CI: 95% Confidence intervals).

pilocarpine, a well-known molecule in glaucoma, showed interesting results with gel form [15,16]. Its antiparasitic effect may be based on parasympathomimetic action resulting in paralysis of mites' respiration and mobility [15,16]. Over the last three decades, anthelminthics, such as ivermectin or metronidazole, were used empirically to treat Demodex blepharitis, as an off-label drug prescription outside marketing authorisation [18–21]. Ivermectin is an effective orally administered antiparasitic drug, known since several years. Whereas the acaricidal effect of metronidazole on the Demodex mite is unknown [20,53,54], the parasitic killing effect of ivermectin is well known, through a selective activity against glutamate-gated chloride ion channels from the peripheral nervous system of invertebrates. These last years, news locals' therapies based on TTO and T4O have been tested [18,21–32], opening a new therapeutic field. TTO is a natural substance extracted from the leaves of the Melaleuca alternifolia, a plant of the Myrtaceae family. This product was known for a long time by Australian indigenous concerning antiseptic properties [55,56]. Some studies concerning TTO proved its antifungal, antibacterial, antiviral and antiparasitological effects [57–61]. T4O is the most active ingredient of TTO with concentration from 30 to 48% [56]. The results of TTO, T4O and pilocarpine uses corroborated the results of in vitro killing effect [27]. To our knowledge, in most countries, no treatment based on TTO or T4O are available to clinicians with marketing authorisation to treat Demodex blepharitis. It would be interesting to evaluate these news locals' treatments in clinical trials to prove their efficacy, and to consider these molecules in therapeutic association.

Proposal of recommendations for the treatment of Demodex blepharitis

In our meta-analysis, all Demodex blepharitis included from individual studies were resistant to the first-line treatment such as usual lid hygiene and local antibiotics [14–16,18–32,46]. Thus, negative results of usual lid hygiene were expected. However, its mechanical effects have been proved and should at an early stage [17,62–64]. More interestingly, we demonstrated that local and systemic treatments had comparable efficiency (1.22, 0.83 to 1.60 vs 2.24, 1.30 to 3.18 for mites count; 0.37, 0.21 to 0.54 vs 0.56, 0.06 to 0.99 for eradication rate; and 0.77, 0.58 to 0.92 vs 0.67, 0.25 to 0.98 for symptoms improvement). As mentioned upper, Demodex mites are present in healthy eyelids so it could be unnecessary to employ toxic or very effective systemic treatment. In included studies, clinical side effects or hepatic toxicity were not observed with systemic ivermectin or metronidazole [18,19,21]. However, hypersensitivity reaction is more common with systemic treatments compared with local treatments. Serious reactions were observed using ivermectin or metronidazole in other parasitic infections such as Mazzotti reaction (tachycardia, hypotension, arthralgias, oedema, and abdominal pain), Steven-Johnson and Lyell disease, fatal encephalopathy, increased INR (International Normalized Ratio) with

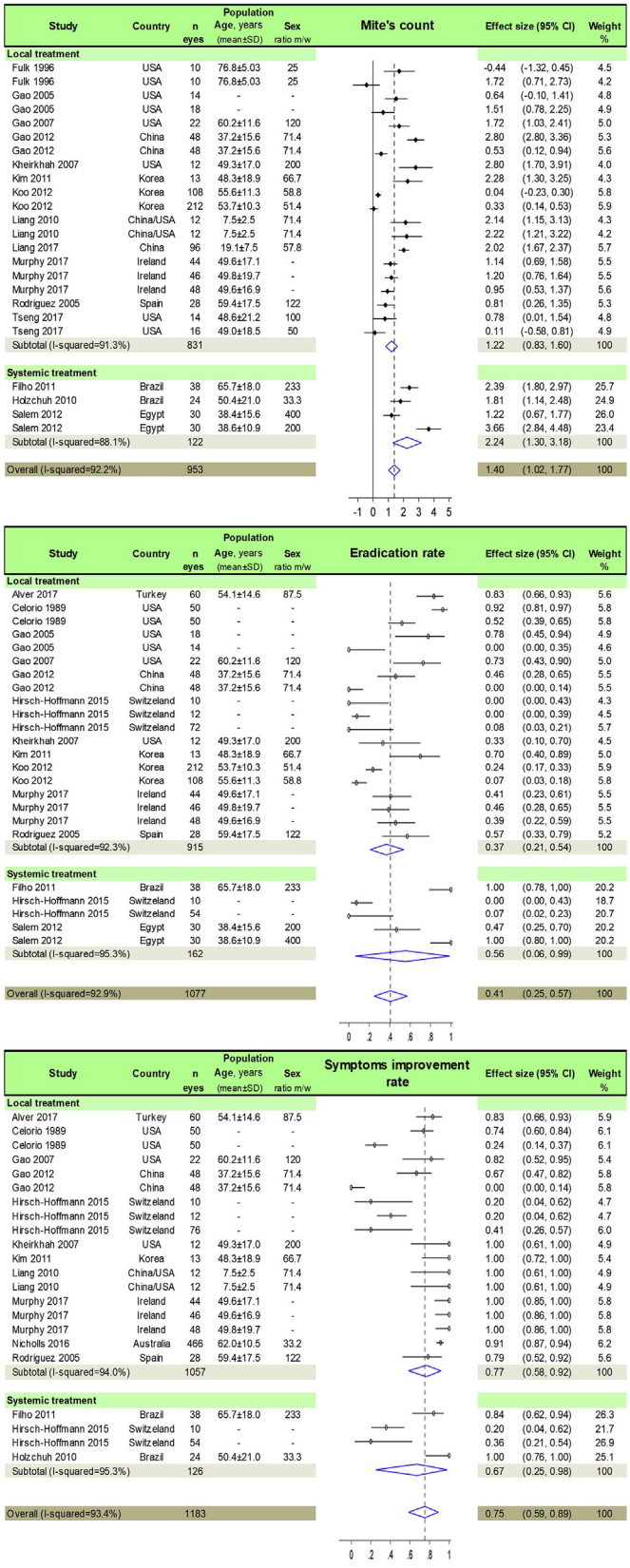


Fig. 7. Meta-analysis on mite's count, eradication rate and symptoms improvement rate in each treatment type (95% CI: 95% confidence intervals).

hemorrhage, decrease in leukocyte count and anemia, hepatitis, elevation of liver enzymes, and elevation of bilirubin. Ivermectin should not be used during pregnancy since safety in pregnancy has not been established [20,21,53,54]. In blepharitis Demodex, the sides effects with local uses of TTO, T4O or pilocarpine were rare and benign, such as eyes irritations, redness eyelid, cutaneous eczema, itching or burning sensations, but never systemic reactions [14,18,22–32,46]. Therefore, considering that cylindrical dandruffs at the base of the eyelashes are pathognomonic of Demodex blepharitis [5,13,27], and considering our results, we propose to treat blepharitis with cylindrical dandruffs with antiparasitic local first-line treatment i.e. the association of TTO, T4O or pilocarpine gel with usual lid hygiene once or twice daily during 1–3 months. In second-line or in severe cases, systemic treatment such as ivermectin or metronidazole could be added, which may also decrease recurrence – although not proved –, without severe side effects reported with systemic low dose in the treatment of Demodex blepharitis. Severe cases refer to severe ocular repercussions such as keratitis, corneal ulcer, severe itching with skins lesions, trichiasis, ectropion or entropion with corneal lesions. Combination of both systemic and local treatment may also be interesting in some putative facial extensive Demodex outbreaks, such as rosacea [65–67].

Parameters influencing therapeutics

In epidemiological studies, the influence of socio-demographic parameters on mites count was controversial. It was described a higher prevalence of infestation in people with oily or mixed skin than with dry or neutral skin [68–71], in humid-tropical climate [72], in immunocompromised patients [73–77], or in childhood malnutrition [78]. Majority of studies concluded on an increase of mites count with age [1,68,79,80], which may be explained by the decreasing activity of the glands of Zeiss and the Meibomian glands with age [1,50,81]. However, in paediatric and teenage population, Demodex mites could played a pathological role in recurrent chalazia, itching and redness eyelid [28,30]. Differences between socio-demographic results could be explained by variability of inclusion criteria. According to meta-regression results, we did not find significant influences of age and gender on mites count. Most of included studies were epidemiological and recruited patients during conventional examination for refractive or pre-surgical consultations. Many patients in these consultations may have not complained of any symptoms whereas all patients in our study were recruited because of chronic blepharitis (thus with a high probability of complaints).

Limitations

Our study had some limitations. Data collections and inclusion/exclusion criteria were not identical within each studies, which may have affected our results, as well as heterogeneity due to different study designs – retrospective [18,22,24,30] or prospective studies, randomized [15,20,25,29,31] or not [14,16,19,21,23,26–28,32,46]. Nevertheless, we combined a large number of patients and procedures to permit a large overview, with sensitivity analyses (data not shown) demonstrating similar results whatever study designs. Studies included small samples and were exclusively monocenter, precluding generalizability. Though, all continents and all ethnicities were included. Moreover, we cover nearly 30 years of treatments of Demodex blepharitis, with a wide range of therapeutics. However, the apparition of new treatments precluded efficacy analyses of same treatments over time. All studies used conventional parasitological examination to prove Demodex infestation. Despite different number of eyelashes sampled between included studies, and thus difference between studies concerning mite's count before treatment, it did not influence our results because meta-analysis were on mites count changes. Other parameters evaluating efficacy of treatments (e.g. tears quality [19,23], specific questionnaires [19,29,31,32,46], infrared thermography [23]) were limited to few studies and differing, precluding further analyses.

Conclusion

Except usual lid hygiene for mites count, CHEO for both eradication rate and symptoms, and CHEO, 2% metronidazole ointment, and systemic metronidazole for eradication rate, all treatments were efficient. TTO, T4O and pilocarpine gel are interesting molecules to elaborate new eyewashes as first-line local treatment of Demodex blepharitis. As second-line treatment or in severe cases, systemic treatment as ivermectin or metronidazole could be used in association with local treatments.

Contributors

CBA and FC were responsible for the design and conception of the study. VN and CBA searched and collected studies and data. VN, CBA, FC and FD analysed and interpreted data. AM and FD were in charge of statistical analyses. VN, CBA and FD wrote the manuscript. All authors made critical revisions to the article. All authors gave their final approval of the article.

Funding

None.

Conflicts of interest

The authors declare that they have no competing interests.

Data sharing statement

No additional data are available.

Financial disclosures

None reported.

Abbreviations and Acronyms

CHEO Children's Hospital of Eastern Ontario (cholestyramine 5% ointment)
CONSORT consolidated standards of reporting trials
ES effect size;
INR International Normalized Ratio
OLSP Ocusoft lid scrub plus
SIGN Scottish Intercollegiate Guidelines Network
STROBE strengthening the reporting of observational studies in epidemiology
TTFW Dr Organic Tea Tree Face Wash
TTO tea tree oil
T4O terpinen-4-ol
US United State of America
95CI 95% confidence interval

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtos.2019.06.004.

Appendix 1

Details on study characteristics, quality of articles (Figs. 2 and 3), method of Demodex identification, type of treatments, protocol for each treatment, inclusion and exclusion criteria of each included study, population, aims and outcomes of included studies.

All articles were written in English except one in Spanish [14]. Included studies came from all continents: 6 from Asia [22,28–30,32,46], 3 from Europe [14,18,31], 2 from South America [19,21], 6 from North America [15,16,23–25,27], 1 from Oceania [26] and 1 from Africa [20].

Quality of articles

Quality assessment of the 19 included studies was performed by STROBE and SIGN Cohort Studies criteria concerning observational studies, CONSORT and SIGN Controlled Trials criteria concerning the randomized clinical trials. There were 14 observational studies [14,16,18,19,21–24,26–28,30,32,46] and 5 randomized clinical trials [15,20,25,29,31]. Results of STROBE criteria varying from 63.6 [14] to 84.8% [24], with a mean score of 75.1 ± 6.08. Results of CONSORT criteria varying from 72.9 [31] to 78.3% [20], with a mean score of 75.6 ± 2.70. Overall, the studies performed the best in methods and introduction sections and worst in the discussion section. Results of SIGN Cohort Studies criteria varying from 46.1 [24] to 92.3% [16] for Yes responses, with a mean score of 63.2 ± 10.9. Results of SIGN Controlled Trials criteria varying from 55.5 [31] to 77.7% [20,25,29] for Yes responses, with a mean score of 71.1 ± 9.9 (Figs. 2 and 3).

Method of Demodex identification

All studies used conventional parasitological examination to prove Demodex infestation in the base of eyelash [14–16,18,18–32]. Eyelashes were sampled on all eyelids of both eyes for all included studies [14–16,18,18–32], with [22–25,27–29,32] or without [14–16,18–21,30,31,46] cylindrical dandruff. All studies using eyelashes with cylindrical dandruff sampled two eyelashes per eyelid [22–25,27–29,32]. For studies sampling eyelashes without cylindrical dandruff, the number of eyelashes sampled per eyelid was three [21,46], five [14], or six [19]. When the eyelashes were sampled, different conservations' solutions were used like glycerine or oil [20,46], saline solution [14,23,25,27,28,32], 2% methylcelluloses [19] or a mix of 20 μL saline solution + 20 μL 100% alcohol [22,24,29]. The examination unfolded by × 50 and × 100 magnification under light microscopy [14,16,18,20,23,25,27,32,46] or × 100 and × 400 magnification [14,29] or slip lamp microscopy [22,24,26,28,30,31]. All studies have measured and evaluated infestation in naked eye except one [46].

Type of treatments

A total of 13 different treatments were used: 6 studies used 50% TTO [22–24,27–29], 4 used 5% TTO in their treatment protocol [18,32,46], 3 used T4O eyewash [25,30,31], 2 used 4% pilocarpine gel [15,16], 6 used usual lid hygiene as principal treatment or control [15,16,25,27,29,31], 1 used Cilclar (1.5% boric acid) + 2% yellow mercury oxide ointment + ether application [14], 1 used 5% cholestyramine ointment called CHEO (because it was developed initially by the Children's Hospital of Eastern Ontario) [32], 1 used OcuSoft Lid Scrub Plus (OLSP)(1,2-octanediol) [31], 1 used Naviblef (0.02% TTO) [18], 1 used 2% metronidazole ointment [18], 4 used systemic ivermectin [18–21], 1 used systemic metronidazole lonely [20], and 1 used systemic metronidazole + ivermectin association [18]. In total, 4 studies used systemic treatments [18–21] and 16 studies used local treatments [14–16,22–32,46], with one study using both systemic and local treatments [18].

Protocol for each treatment

50% TTO was used once a week during one month, in the hospital office, to scrub the lash roots for 3 sessions (10 min interval) with a drop of 0.5% proparacaine because of eyes irritation and burning sensation of 50% TTO. In addition, at home, TTO shampoo and eyelid hygiene massage were used twice daily during 1 month and then one daily thereafter [22–24,28,29].

5% TTO was used in two different packaging: in eyelid gel [46] or in eyelid oil [18,26,28,32]. Patients applied TTO at home on the eyelash, twice daily [18,32,46] or once after washing the face before sleeping [26], during 1 [18,32,46] or 3 months [26].

T4O, a major component of TTO, was used in two devices. Cliradex lid scrub device applied twice daily and Dr Organic Tea Tree Face Wash (TTFW) containing 38% of T4O and applied twice daily, both during 3 months [25,30,31].

4% pilocarpine gel was spread once in the evening on the base of eyelashes, and removed in the morning, for 2 weeks [15,16].

Usual lid hygiene consisted of scrubbing eyelashes with saline solution, warm massage and soap solution once or twice daily [15,16,25,27,29]. No other treatments were applied except in one study where BlephEx™ microblepharoexfoliation device was used at home to provide debridement and exfoliation at the lash margin [31].

Cilclar 1.9% (Novartis) and 2% oxide mercuric ointment were used twice and once daily at home, respectively, during 6 weeks, and ***ether*** was applied once a week in hospital office [14].

CHEO ointment, containing 0.5% cholestyramine in petroleum jelly, was spread by lid massage for 4 weeks [32].

OcuSoft Lid Scrub Plus (OLSP) contained 1,2-octanediol. This substance which has been shown to have pediculicide potential was scrubbed on the base of eyelashes in circular movements, once daily at home during 4 weeks [31].

Naviblef lid foam, containing 0.02% diluted TTO, was administered once in the morning to clean the lids, eye brow and face skin during 2 months [18].

2% metronidazole ointment was administered to the margins of the lower and upper lids once daily at bed time for 2 months [18].

Ivermectin was administered per os, 6 mg twice at a 14-day interval [18], 6 mg twice at a 14-day interval [21], or 200 μg/kg at a 7-day interval [19,20].

Metronidazole was administered per os, 1 g per day during 10 days [18], or 750 mg per days during two weeks in association with 200 μg/kg of ivermectin at a 7-day interval [20].

Inclusion and exclusion criteria

All studies included patients diagnosed with chronic and treatment-resistant blepharitis, and with a proven parasitological ocular demodicosis [14–16,18,18–32]. All studies included adults (> 18 years old) [14–16,18–27,29–32,46], except one study without age criteria [28]. Most studies excluded the use of topical or systemic anti-inflammatory and antibacterial medications [16,19,20,23,25–27,29,31,32] and any kind of surgery prior to inclusion [19,20,25,29,31].

Population

Sample size: We included a total of 934 patients, ranging from 5 [18] to 233 [26], for a total of 1741 eyes treated for Demodex blepharitis, ranging from 10 [16,18] to 266 [26] in each included studies.

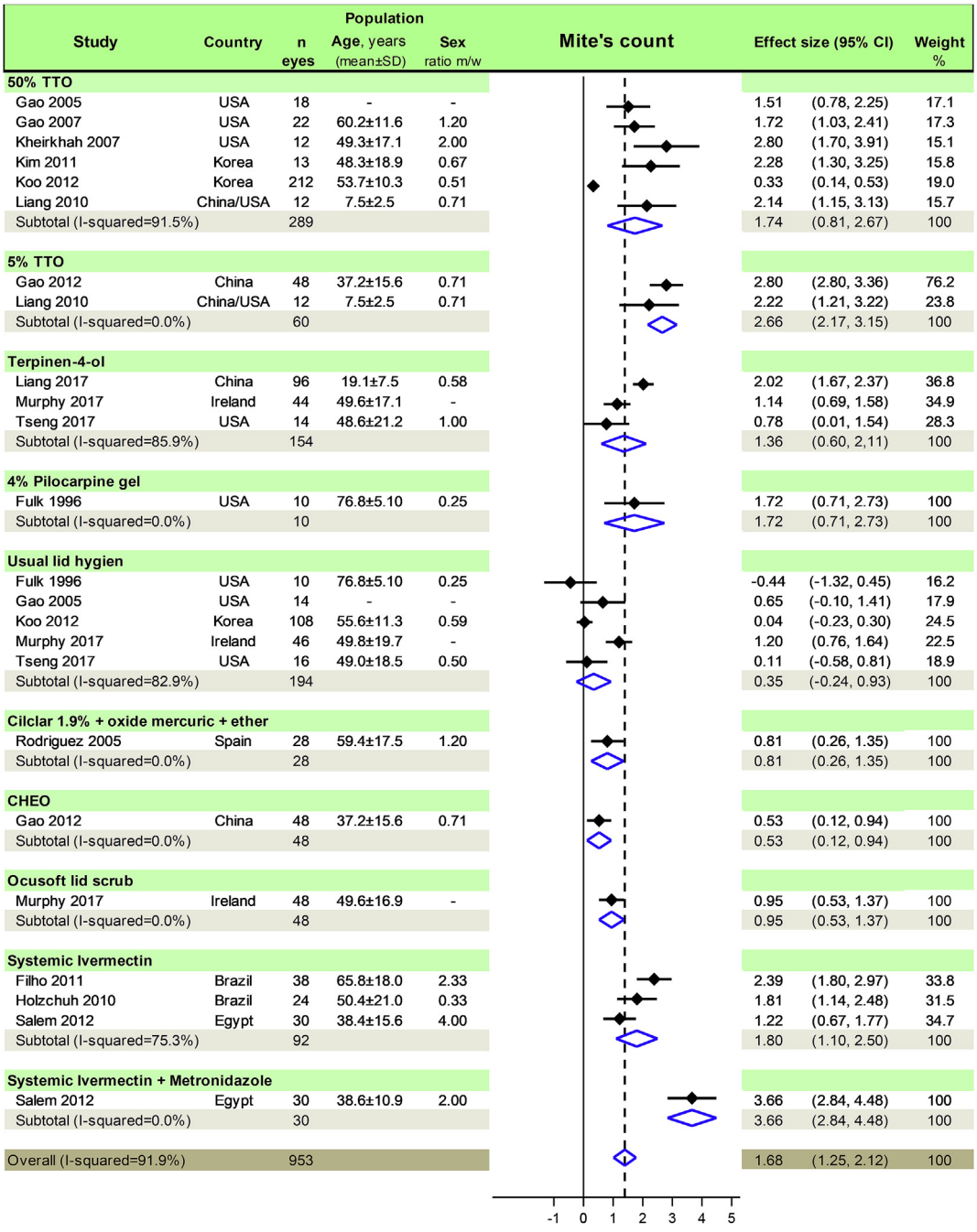
Gender: A total of 280 men and 521 women were included with a proportion of female ranging from 20 [20] to 80% [16]. Four studies did not specify gender [15,18,27,31].

Age: All studies included adults [14–16,18–27,29–32,46], except one which included children [28]. Within each study, mean age ranged from 7.5 ± 2.5 [28] to 76.8 ± 5.0 years [16]. Age of patients for each study is reported in supplemental files (Appendix 2 to 5).

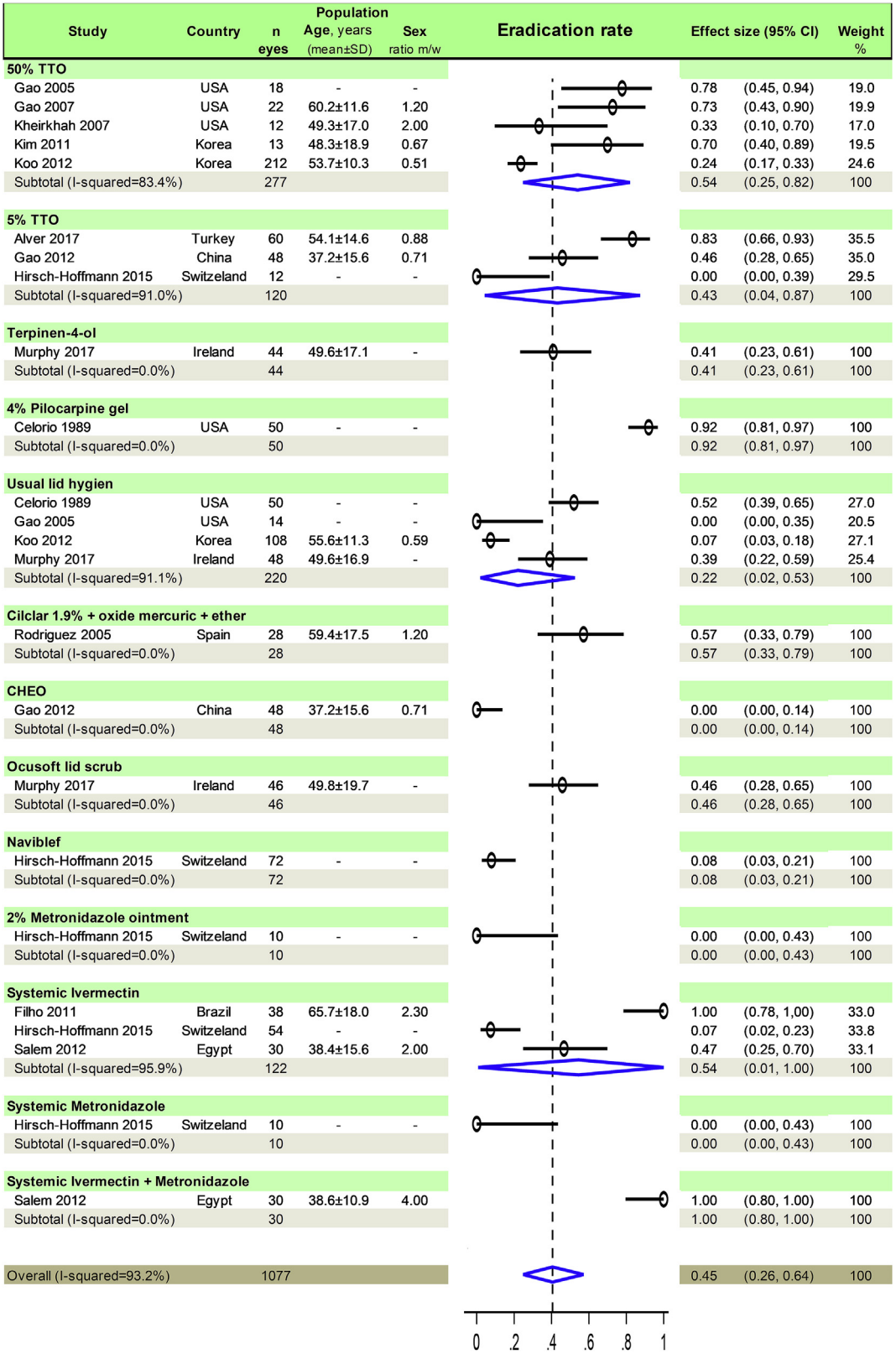
Aims and outcomes of included studies

All included studies aimed to evaluate efficacy and safety of treatments for Demodex blepharitis, based on clinical outcomes [14–16,18,18–32]. All studies reported mites count before and after the different treatments, eradication rate (no mites after treatment), and improvement of symptoms, except four, six and six studies which did not report mites count [15,18,19,25,26,28]. eradication rate [16,19,25,26,28], and improvement of symptoms [16,20,25,27,29,30], respectively.

Appendix 2. . Meta-analysis on mite's count in each treatment protocol (95% CI: 95% confidence intervals)

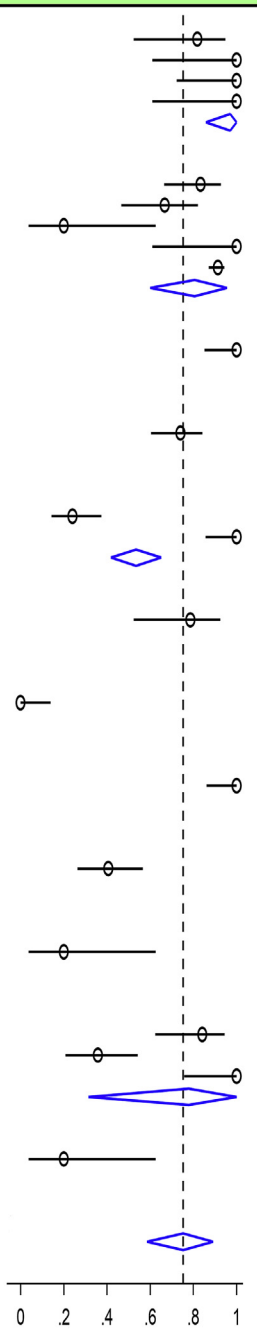


Appendix 3. . Meta-analysis on eradication rate in each treatment protocol (95% CI: 95% confidence intervals)

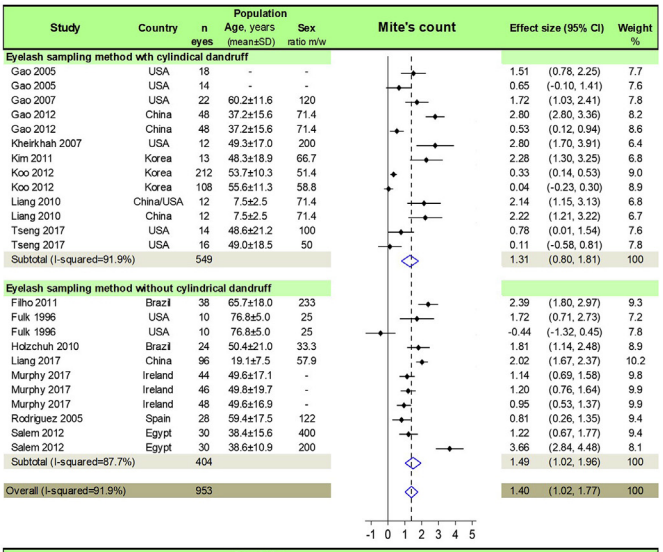


Appendix 4. . Meta-analysis on symptoms improvement rate in each treatment protocol (95% CI: 95% confidence intervals)

Study	Country	n eyes	Population Age, years (mean±SD)	Sex ratio m/w	Effect size (95% CI)		Weight %
50% TTO							
Gao 2007	USA	22	60.2±11.6	1.20	0.82	(0.52, 0.95)	32.9
Kheirkhah 2007	USA	12	49.3±17.0	2.00	1.00	(0.61, 1.00)	18.6
Kim 2011	Korea	13	48.3±18.9	0.67	1.00	(0.72, 1.00)	30.0
Liang 2010	China/USA	12	7.5±2.5	0.71	1.00	(0.61, 1.00)	18.6
Subtotal (I-squared=90.1%)		59			0.97	(0.86, 1.00)	100
5% TTO							
Alver 2017	Turkey	60	54.1±14.6	0.88	0.83	(0.66, 0.93)	23.2
Gao 2012	China	48	37.2±15.6	0.71	0.67	(0.47, 0.82)	22.3
Hirsch-Hoffmann 2015	Switzeland	12	-	-	0.20	(0.04, 0.62)	13.0
Liang 2010	China/USA	12	7.5±2.5	0.71	1.00	(0.61, 1.00)	14.2
Nicholls 2016	Australia	466	62.0±10.5	0.33	0.91	(0.87, 0.94)	27.3
Subtotal (I-squared=81.8%)		598			0.81	(0.60, 0.96)	100
Terpinen-4-ol							
Murphy 2017	Ireland	44	49.6±17.1	-	1.00	(0.85, 1.00)	100
Subtotal (I-squared=0.0%)		44			1.00	(0.85, 1.00)	100
4% Pilocarpine gel							
Celorio 1989	USA	50	-	-	0.74	(0.60, 0.84)	100
Subtotal (I-squared=0.0%)		50			0.74	(0.60, 0.84)	100
Usual lid hygien							
Celorio 1989	USA	50	-	-	0.24	(0.14, 0.37)	68.2
Murphy 2017	Ireland	46	49.6±16.9	-	1.00	(0.86, 1.00)	31.8
Subtotal (I-squared=89.7%)		96			0.54	(0.42, 0.65)	100
Cilclar 1.9% + oxide mercuric + ether							
Rodriguez 2005	Spain	28	59.4±17.5	1.22	0.79	(0.52, 0.92)	100
Subtotal (I-squared=0.0%)		28			0.79	(0.52, 0.92)	100
CHEO							
Gao 2012	China	48	37.2±15.6	0.71	0.00	(0.00, 0.14)	100
Subtotal (I-squared=0.0%)		48			0.00	(0.00, 0.14)	100
Ocusoft lid scrub							
Murphy 2017	Ireland	48	49.8±19.7	-	1.00	(0.86, 1.00)	100
Subtotal (I-squared=0.0%)		48			1.00	(0.86, 1.00)	100
Naviblef							
Hirsch-Hoffmann 2015	Switzeland	76	-	-	0.41	(0.26, 0.57)	100
Subtotal (I-squared=0.0%)		76			0.41	(0.26, 0.57)	100
2% Metronidazole ointment							
Hirsch-Hoffmann 2015	Switzeland	10	-	-	0.20	(0.04, 0.62)	100
Subtotal (I-squared=0.0%)		10			0.20	(0.04, 0.62)	100
Systemic Ivermectin							
Filho 2011	Brazil	38	65.7±18.0	2.33	0.84	(0.62, 0.94)	33.5
Hirsch-Hoffmann 2015	Switzeland	54	-	-	0.36	(0.21, 0.54)	34.4
Holzchuh 2010	Brazil	24	50.4±21.0	0.33	1.00	(0.76, 1.00)	32.1
Subtotal (I-squared=92.0%)		116			0.78	(0.31, 1.00)	100
Systemic Metronidazole							
Hirsch-Hoffmann 2015	Switzeland	10	-	-	0.20	(0.04, 0.62)	100
Subtotal (I-squared=0.0%)		10			0.20	(0.04, 0.62)	100
Overall (I-squared=92.1%)		1183			0.75	(0.59, 0.90)	100.00



Appendix 5. . Meta-analysis on mite's count, eradication rate and symptoms improvement rate in each eyelash sampling method (95% CI: 95% confidence intervals)



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