Recent advances on ocular *Demodex* infestation

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Purpose of review
To summarize recent advances on ocular *Demodex* infestation.

Recent findings
Demodex infestation is a potential cause of ocular surface inflammation. The pathogenesis of *Demodex* in eliciting ocular surface inflammation has been further clarified. Cliradex is currently the treatment of choice, it comprises the most active ingredient of tea tree oil, that is terpinen-4-ol, which helps eradicate *Demodex* mites and reduce ocular surface inflammation.

Summary
Ocular demodicosis is a common but overlooked eye disease that manifests a number of morbidities. *Demodex folliculorum* causes chronic anterior blepharitis whereas *Demodex brevis* causes posterior blepharitis, meibomian gland dysfunction, recurrent chalazia, and refractory keratoconjunctivitis. The lash sampling and microscopic counting method and in-vivo confocal microscopy are key diagnostic methods. Cliradex shows promising potential to reduce *Demodex* counts with additional antibacterial, antifungal, and anti-inflammatory actions.

Keywords
blepharitis, Cliradex, *Demodex*, meibomian gland dysfunction, ocular surface inflammation

INTRODUCTION

Demodex infestation is a common but overlooked cause of ocular surface inflammation. The prevalence of *Demodex* infestation increases with age, being observed in 84% of the population at age 60 years and in 100% of those older than 70 years [1]. The modified eyelash sampling and counting method and in-vivo confocal microscopy (IVCM) are key diagnostic methods [2,3,4▪▪]. Cliradex contains the most active ingredient of tea tree oil (TTO), that is terpinen-4-ol, which helps eradicate the mites and reduce ocular surface inflammation [5]. The present article summarizes recent advances in the diagnosis and treatment of ocular *Demodex* infestation.

PATHOGENESIS

The life cycle of the *Demodex* mite is approximately 14–18 days from the egg to the larval stage followed by the adult stage (Fig. 1) [6]. The life span of the mites is limited outside the living body and direct contact is required for trans-infestation. Two distinct species of *Demodex* mites have been identified in humans: *Demodex folliculorum* (Fig. 1a) and *Demodex brevis* (Fig. 1b). The *D. folliculorum* measures about 0.3–0.4 mm long and is primarily found in clusters around the root of the lashes and lash follicles, whereas the *D. brevis* measures about 0.2–0.3 mm long and resides solitarily in the sebaceous and meibomian glands [7]. Consequently, *D. folliculorum* is implicated in causing anterior blepharitis whereas *D. brevis* in posterior blepharitis, meibomian gland dysfunction, recurrent chalazia, and refractory keratoconjunctivitis [7,8]. The latter notion is supported by our recent study, which demonstrated a strong correlation between high prevalence of *D. brevis* infestation and chalazia [9▪▪].

During their lifespan, mites consume the lining of the hair follicles and lay eggs there, resulting in follicular distention and mal-directed lashes [10]. Debris and waste generated by mites accumulate at the root of the lashes forming cylindrical dandruff, which is pathognomonic for mites infestation [2,11–13]. Mites also mechanically block the sebaceous ducts, irritate the eyelid margin, and induce epithelial hyperplasia and hyperkeratinization

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KEY POINTS

- Suspect *Demodex* infestation in refractory blepharitis, trichiasis, chalazia, blepharoconjunctivitis, and keratitis.
- Lash sampling with microscopic counting method and in-vivo confocal microscopy are key diagnostic methods.
- Cliradex is the treatment of choice.

[2,14]. The cytoskeleton of the mites may act as a foreign body and cause granulomatous reaction as implicated in chalazia [9▪▪,15] or may elicit an inflammatory, immune response [14,16].

Recent studies revealed a potential relationship between demodicosis and microbial blepharitis by different mechanisms. First, the mites may work as a vector carrying bacteria such as staphylococci and streptococci, which are common causes of anterior blepharitis. Superantigens produced by these bacteria are also implicated in the induction of rosacea [17]. Methicillin-resistant *Staphylococcus aureus* (MRSA) has been detected on eyelids of patients with *Demodex* infestation [18]. Although systemic antimicrobial therapy is effective for curing ocular inflammation in some meibomitis-related keratoconjunctivitis [19,20], it still remains unclear whether there is concomitant microbial involvement in *Demodex* infestation. The relationship between MRSA and ocular demodicosis requires further study. Secondly, mites can harbor symbiotic microbes in their intestines such as *Bacillus oleronius*, which has been found to trigger a host immune reaction by producing pro-inflammatory bacterial proteins that can stimulate proliferation of peripheral blood mononuclear cells in patients with rosacea [21]. Our prospective control study further disclosed a strong correlation among positive serum immunoreactivity to the 83 and 62-kDa bacillus proteins, ocular *Demodex* infestation, facial rosacea, and blepharitis [22,23]. In a total of 59 patients prospectively and consecutively enrolled, positive serum immunoreactivity had a significant correlation with facial rosacea ($P = 0.009$) and ocular *Demodex* infestation ($P = 0.048$).

CLINICAL MANIFESTATIONS

Demodex infestation in the face and eyelid has been implicated in causing rosacea [24–26] and blepharitis, respectively [11,27–29]. Such blepharitis frequently is associated with mite-harboring cylindrical dandruff in eyelashes [2]. Patients with *Demodex* induced rosacea have been clearly shown to have a higher *Demodex* density than controls [25,26,30,31]. However, no research has convincingly demonstrated whether a minimal number of mites must be present to produce symptoms. Moreover, there is no strong correlation between the severity of symptoms and signs and the extent of

FIGURE 1. *Demodex* mites morphology. An adult *Demodex folliculorum* has a body-to-tail ratio of 1:2 (a), *Demodex brevis* has a body-to-tail ratio of 1 : 1 (b), Larva has poorly developed legs (c), and a cone-shaped egg (d).

cylindrical dandruff or the *Demodex* count [8,11–13, 32,33▪]. Such a discrepancy sheds light on other pathogenic elements that are variable among patients with ocular demodicosis.

Symptomatic patients usually present with itching, redness, burning, foreign body sensation, eye lid crusting, and blurry vision. These symptoms are more aggressive in patients with posterior blepharitis in which the inflammation spreads over to the conjunctiva producing blepharoconjunctivitis. As mentioned above, posterior blepharitis is associated with *D. brevis* and is usually refractory to conventional medications, especially in pediatric populations [34]. In addition, *Demodex* infestation may cause unexplained keratitis, superficial corneal vascularization, marginal infiltration, phlyctenule-like lesions, and nodular corneal scarring [8,35]. These corneal manifestations are commonly associated with *D. brevis* as it resides closer to the cornea and is prone to induce ocular surface inflammation [35].

DIAGNOSIS

It is important to suspect *Demodex* infestation in all patients with chronic blepharitis or refractory ocular surface inflammation. This is particularly pertinent in dealing with clinical problems associated with recurrent trichiasis, blepharitis, chalazia, conjunctivitis, blepharoconjunctivitis, and keratitis that are refractory to conventional treatments. Slit-lamp examination is used to detect cylindrical dandruff at the root of the lashes, which is pathognomonic for *Demodex* blepharitis [2,11–13]. Lash sampling and microscopic examination provide a definitive diagnosis by identifying the mites in the lashes with cylindrical dandruff [2,3]. It allows superior evaluation of the mite species and identification of the life stage, that is ovum, larva, protonymph, nymph, or adult (Fig. 2). Recently, IVCM has been used as a noninvasive method to diagnose *Demodex* infestation [4▪▪,36] and allows a complete examination of the follicle with detecting the indistinguishable *D. brevis*, which burrows deep into sebaceous glands. However, the use of IVCM for the analysis of structures of the eyelids, meibomian glands, or the conjunctiva remains challenging because of the high reflectivity of substantia propria. Furthermore, comparative or innovative studies are needed to confirm the value of IVCM in diagnosing *Demodex* infestation especially in giving reliable quantitative assessment.

TREATMENT

TTO has been effectively used to eradicate ocular *Demodex* infestation [8,37,38]. Daily lid scrub with

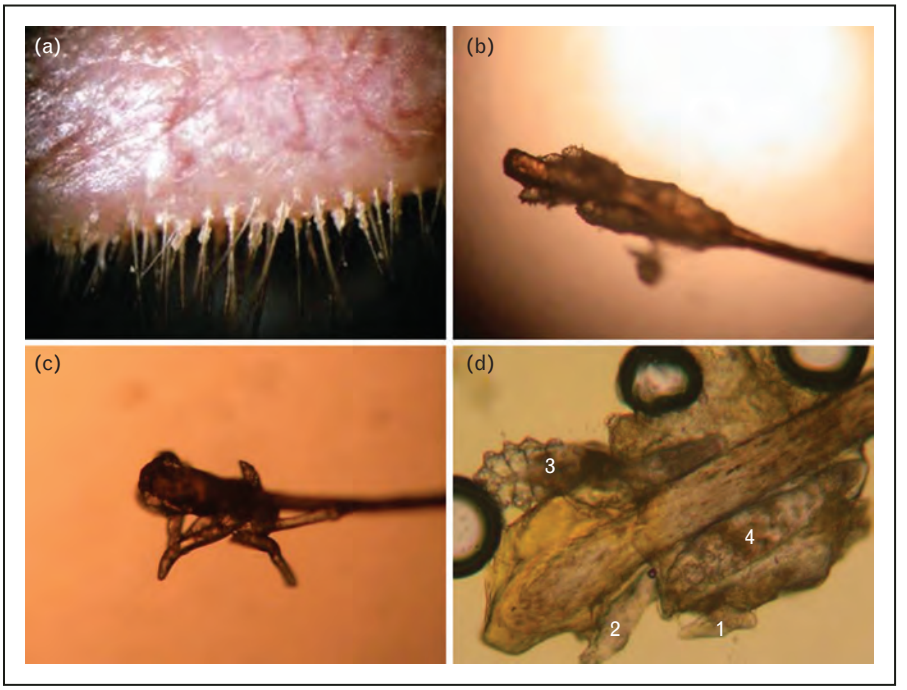

FIGURE 2. Lash sampling and microscopic examination. Lashes with cylindrical dandruff (a) are epilated and examination under light microscope shows cylindrical dandruff harboring mites (b). Fluorescein solution is usually used to dissolve the cylindrical dandruff to enhance visualization and facilitate counting (c). Different mite species at various life stages may be identified under high magnification (d): 1, egg; 2, larva; 3, adult *Brevis*; 4, adult *folliculorum*.

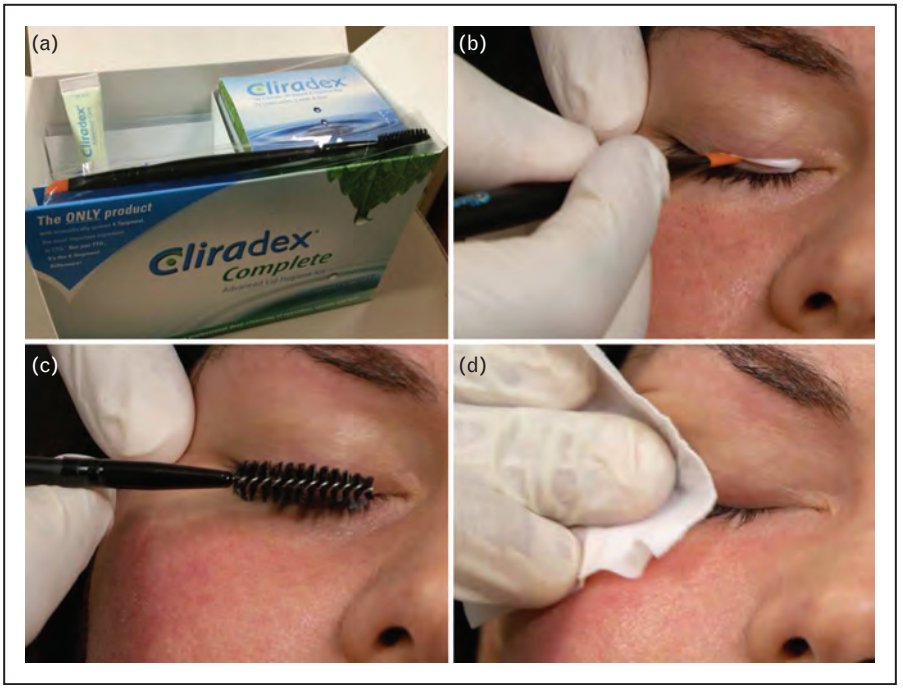

FIGURE 3. Cliradex Complete lid hygiene kit (a), allows for the initial in-office treatment by applying the T4O formulation to the lid margin (b) and removing the debris (c). The kit also contains Cliradex wipes for the patient to use at home to clean the eyelids (d).

50% TTO was also effective in resolving ocular symptoms and inflammation in the lid margin, conjunctiva, and cornea and significantly stabilizing the lipid tear film and improving the visual acuity [8]. However, the application of TTO was not convenient for self-administration and caused irritation in some patients [8,35,37]. Recently, terpinen-4-ol (T4O) has been identified as the most active ingredient in TTO that can eradicate mites with minimal side-effects [5]. The potency of T4O was greater than TTO at an equivalent concentration and possesses anti-inflammatory [39,40], antimicrobial [41–45], and antifungal [46,47] properties. Such effects have shown promising treatment effects for hospital-acquired infections and ocular surface infections, including MRSA [44,48,49].

The above-mentioned discovery has led to the development of Cliradex and Cliradex Complete (Bio-Tissue, Inc., Miami, FL, USA; Fig. 3). Cliradex is a lid hygiene wipe that contains T4O and can be self-administered by the patient. This facilitates the treatment of a number of ocular, and cutaneous diseases, caused by demodicosis that may be associated with or without concomitant bacterial or fungal infections. Recently, Cliradex Complete was developed for in-office application that boasts a stronger concentration of the isolated T4O than the existing Cliradex. Cliradex Complete, a lid hygiene kit, allows the initial treatment in-office by thorough cleaning of the root of the lashes and removal of debris from the lid margin. The kit also includes Cliradex for the patient to take home. The recommended treatment regimen is to apply Cliradex twice a day for at least 6 weeks to cover two *Demodex* life cycles. Patients may continue using a maintenance dose of once a day for a longer period of time to ensure mites eradication and to prevent re-infestation by mites migrating from other places of the body. The integrated lid hygiene helps manage symptoms associated with *Demodex* blepharitis, meibomian gland dysfunction, rosacea, dry eye, chalazia, and other lid margin diseases.

CONCLUSION

Demodex mite plays an important role in the recurrence of a series of refractory ocular surface diseases such as blepharoconjunctivitis, chalazia, meibomian gland dysfunction, dry eye, and keratitis. It remains unclear whether *D. folliculorum* and *D. brevis* may have different pathogenic roles in the lash follicle and meibomian gland, respectively. Diagnosis of ocular demodicosis can be made by either lash sampling with microscopic counting examination or *in vivo* by confocal microscopy although the best quantitative detection method for *Demodex* is still under investigation. Cliradex, formulated wipes

soaked with T4O, is currently the treatment of choice for *Demodex* infestation.

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Conflicts of interest

Dr S.C.G.T. is an inventor, shareholder, and employee of TissueTech, Inc. He has filed two patents for the use of tea tree oil and its ingredients for treating demodecosis. No other authors have any proprietary interest in any material mentioned in this study.
Disclaimer: The content of this review is solely the responsibility of the authors and does not represent the official views of the National Eye Institute or the National Institutes of Health.

REFERENCES AND RECOMMENDED READING

Papers of particular interest, published within the annual period of review, have been highlighted as:
- ■ of special interest
- ■■ of outstanding interest

1. Post CF, Juhlin E. *Demodex folliculorum* blepharitis. Arch Dermatol 1963; 88:298–302.
2. Gao Y-Y, Di Pascuale MA, Li W, *et al.* High prevalence of ocular *Demodex* in lashes with cylindrical dandruffs. Invest Ophthalmol Vis Sci 2005; 46:3089–3094.
3. Kheirkhah A, Blanco G, Casas V, Tseng SC. Fluorescein dye improves microscopic evaluation and counting of *Demodex* in blepharitis with cylindrical dandruff. Cornea 2007; 26:697–700.
4. ■■ Randon M, Liang H, El HM, *et al.* In vivo confocal microscopy as a novel and reliable tool for the diagnosis of *Demodex* eyelid infestation. Br J Ophthalmol 2014.

This study introduced IVCM as a novel noninvasive diagnostic tool for *Demodex* infestation.

5. Tighe S, Gao YY, Tseng SC. Terpinen-4-ol is the most active ingredient of tea tree oil to kill mites. Transl Vis Sci Technol 2013; 2:2.
6. Rufli T, Mumcuoglu Y. The hair follicle mites *Demodex folliculorum* and *Demodex brevis*: biology and medical importance: a review. Dermatologica 1981; 162:1–11.
7. English FP, Nutting WB. Demodicosis of ophthalmic concern. Am J Ophthalmol 1981; 91:362–372.
8. Gao YY, Di Pascuale MA, Elizondo A, Tseng SC. Clinical treatment of ocular demodecosis by lid scrub with tea tree oil. Cornea 2007; 26:136–143.
9. ■■ Liang L, Ding X, Tseng SC. High prevalence of *Demodex brevis* infestation in chalazia. Am J Ophthalmol 2014; 157:342–348.

This study demonstrated a strong correlation between *Demodex brevis* and multiple chalazia.

10. Liu J, Sheha H, Tseng SC. Pathogenic role of *Demodex* mites in blepharitis. Curr Opin Allergy Clin Immunol 2010; 10:505–510.
11. Coston TO. *Demodex folliculorum* blepharitis. Trans Am Ophthalmol Soc 1967; 65:361–392.
12. English FP. *Demodex folliculorum* and oedema of the eyelash. Br J Ophthalmol 1971; 55:742–746.
13. Norn MS. *Demodex folliculorum*. Incidence and possible pathogenic role in the human eyelid. Acta Ophthalmol Suppl 1970; 108:7–85.
14. Bevins CL, Liu FT. Rosacea: skin innate immunity gone awry? Nat Med 2007; 13:904–906.
15. English FP, Cohn D, Groeneveld ER. Demodectic mites and chalazion. Am J Ophthalmol 1985; 100:482–483.
16. Lacey N, Kavanagh K, Tseng SCG. Under the lash – *Demodex* mites in human diseases. Biochemist 2009; 31:2–6.
17. Wolf R, Ophir J, Avigad J, *et al.* The hair follicle mites (*Demodex* spp.). Could they be vectors of pathogenic microorganisms? Acta Derm Venereol 1988; 68:535–537.
18. Lee SH, Chun YS, Kim JH, *et al.* The relationship between *Demodex* and ocular discomfort. Invest Ophthalmol Vis Sci 2010; 51:2906–2911.
19. Suzuki T, Mitsuishi Y, Sano Y, *et al.* Phlyctenular keratitis associated with meibomitis in young patients. Am J Ophthalmol 2005; 140:77–82.
20. Suzuki T. Meibomitis-related keratoconjunctivitis: implications and clinical significance of meibomian gland inflammation. Cornea 2012; 31 (Suppl 1):S41–S44.
21. Lacey N, Delaney S, Kavanagh K, Powell FC. Mite-related bacterial antigens stimulate inflammatory cells in rosacea 1. Br J Dermatol 2007; 157:474–481.
22. Li J, O'Reilly N, Sheha H, *et al.* Correlation between ocular *Demodex* infestation and serum immunoreactivity to *Bacillus* proteins in patients with facial rosacea. Ophthalmology 2010; 117:870–877.
23. O'Reilly N, Menezes N, Kavanagh K. Positive correlation between serum immuno-reactivity to *Demodex*-associated *Bacillus* proteins and erythema-totelangiectic rosacea. Br J Dermatol 2012; 167:1032–1036.
24. Basta-Juzbasic A, Subic JS, Ljubojevic S. *Demodex folliculorum* in development of dermatitis rosaceiformis steroidica and rosacea-related diseases. Clin Dermatol 2002; 20:135–140.
25. Erbagci Z, Ozgoztasi O. The significance of *Demodex folliculorum* density in rosacea. Int J Dermatol 1998; 37:421–425.
26. Forton F, Seys B. Density of *Demodex folliculorum* in rosacea: a case-control study using standardized skin-surface biopsy. Br J Dermatol 1993; 128:650–659.
27. English FP. *Demodex*: a cause of blepharitis in Australia. Med J Aust 1969; 1:1359–1360.
28. Fulk GW, Clifford C. A case report of demodicosis. J Am Optom Assoc 1990; 61:637–639.
29. Heacock CE. Clinical manifestations of demodicosis. J Am Optom Assoc 1986; 57:914–919.
30. Bonnar E, Eustace P, Powell FC. The *Demodex* mite population in rosacea. J Am Acad Dermatol 1993; 28:443–448.
31. Forton F, Germaux MA, Brasseur T, *et al.* Demodicosis and rosacea: epidemiology and significance in daily dermatologic practice. J Am Acad Dermatol 2005; 52:74–87.
32. Uyttebroeck W, Nijs I, Maudgal PC, Missotten L. Incidence of *Demodex folliculorum* on the eyelash follicle in normal people and in blepharitis patients. Bull Soc Belge Ophtalmol 1982; 201:83–87.
33. ■ Wesolowska M, Knysz B, Reich A, *et al.* Prevalence of *Demodex* spp. in eyelash follicles in different populations. Arch Med Sci 2014; 10:319–324.

This study highlights the discrepancy between *Demodex* count and the severity of symptoms.

34. Liang L, Safran S, Gao Y, *et al.* Ocular demodicosis as a potential cause of pediatric blepharoconjunctivitis. Cornea 2010; 29:1386–1391.
35. Kheirkhah A, Casas V, Li W, *et al.* Corneal manifestations of ocular *Demodex* infestation. Am J Ophthalmol 2007; 143:743–749.
36. Sattler EC, Maier T, Hoffmann VS, *et al.* Noninvasive in vivo detection and quantification of *Demodex* mites by confocal laser scanning microscopy. Br J Dermatol 2012; 167:1042–1047.
37. Gao Y-Y, Di Pascuale MA, Li W, *et al.* In vitro and in vivo killing of ocular *Demodex* by tea tree oil. Br J Ophthalmol 2005; 89:1468–1473.
38. Gao YY, Xu DL, Huang LJ, *et al.* Treatment of ocular itching associated with ocular demodicosis by 5% tea tree oil ointment. Cornea 2011; 31:14–17.
39. Brand C, Ferrante A, Prager RH, *et al.* The water-soluble components of the essential oil of *Melaleuca alternifolia* (tea tree oil) suppress the production of superoxide by human monocytes, but not neutrophils, activated in vitro. Inflamm Res 2001; 50:213–219.
40. Hart PH, Brand C, Carson CF, *et al.* Terpinen-4-ol, the main component of the essential oil of *Melaleuca alternifolia* (tea tree oil), suppresses inflammatory mediator production by activated human monocytes. Inflamm Res 2000; 49:619–626.
41. Cox SD, Mann CM, Markham JL. Interactions between components of the essential oil of *Melaleuca alternifolia*. J Appl Microbiol 2001; 91:492–497.
42. Hammer KA, Carson CF, Riley TV. Effects of *Melaleuca alternifolia* (Tea Tree) essential oil and the major monoterpene component terpinen-4-ol on the development of single- and multistep antibiotic resistance and antimicrobial susceptibility. Antimicrob Agents Chemother 2012; 56:909–915.
43. Hidron AI, Edwards JR, Patel J, *et al.* NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. Infect Control Hosp Epidemiol 2008; 29:996–1011.
44. Thomsen NA, Hammer KA, Rileya TV, *et al.* Effect of habituation to tea tree (*Melaleuca alternifolia*) oil on the subsequent susceptibility of *Staphylococcus* spp. to antimicrobials, triclosan, tea tree oil, terpinen-4-ol and carvacrol. Intl J Antimicrob Agents 2013; 41:343–351.
45. Wong VW, Lai TY, Chi SC, Lam DS. Pediatric ocular surface infections: a 5-year review of demographics, clinical features, risk factors, microbiological results, and treatment. Cornea 2011; 30:995–1002.

46. Hammer KA, Carson CF, Riley TV. Antifungal activity of the components of *Melaleuca alternifolia* (tea tree) oil. J Appl Microbiol 2003; 95:853–860.

47. Hammer KA, Carson CF, Riley TV. Antifungal effects of *Melaleuca alternifolia* (tea tree) oil and its components on *Candida albicans, Candida glabrata* and *Saccharomyces cerevisiae*. J Antimicrob Chemother 2004; 53:1081–1085.

48. Caelli M, Porteous J, Carson CF, *et al.* Tea tree oil as an alternative topical decolonization agent for methicillin-resistant *Staphylococcus aureus*. J Hosp Infect 2000; 46:236–237.

49. Carson CF, Cookson BD, Farrelly HD, Riley TV. Susceptibility of methicillin-resistant *Staphylococcus aureus* to the essential oil of *Melaleuca alternifolia*. J Antimicrob Chemother 1995; 35:421–424.