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Microwave decontamination of eyelid warming devices for the treatment of meibomian gland dysfunction

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ABSTRACT

Purpose: The role of bacteria in meibomian gland dysfunction is unclear, yet contamination of compresses used as treatment may exacerbate this condition. This study therefore determined the effect of heating on bacteria on two forms of compress.
Methods: Cotton flannels and MGDRx EyeBags (eyebags) were inoculated by adding experimental inoculum (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas* aeruginosa; one species for each set of 3 eyebags and flannels). One of each were then randomised in to 3 groups: no heating (control); therapeutic (47.4 ± 0.7 °C); or sanitisation (68 ± 1.1 °C). After treatment, bacteria cell numbers were calculated. The experiment was repeated in triplicate.
Results: There was a statistically significant difference between each treatment with the eyebag for *S. aureus* (control = 7.15 ± 0.11 log *C*/ml, therapeutic heating = 5.24 ± 0.59 log *C*/ml, sanitisation heating = 3.48 ± 1.43 log *C*/ml; $P < 0.001$) and *S. pyogenes* (7.36 ± 0.13, 5.73 ± 0.26, 4.75 ± 0.54; $P < 0.001$). *P. aeruginosa* also showed a significant reduction ($P < 0.001$) from control (6.39 ± 0.34) to therapeutic (0.33 ± 0.26) and sanitisation (0.33 ± 0.21), but the latter were similar ($P = 1.000$). For the flannels, there was significant difference between each treatment for *S. aureus* (6.89 ± 0.46, 3.96 ± 1.76, 0.42 ± 0.90; $P < 0.001$). For *S. pyogenes,* there was a significant reduction ($P < 0.001$) from control (7.51 ± 0.10) to therapeutic (5.91 ± 0.62) and sanitisation (5.18 ± 0.8), but the latter were similar ($P = 0.07$). For *P. aeruginosa*, there was a significant difference ($P < 0.001$) from control (7.15 ± 0.36) to sanitisation (5.83 ± 0.44); but not to therapeutic (6.84 ± 0.31) temperatures ($P = 0.07$).
Conclusions: Therapeutic heating produces a significant reduction in bacteria on the eyebags, but only sanitisation heating appears effective for flannels. However, patients should be advised to heat the eyebag to sanitisation temperatures on initial use.

1. Introduction

Treatment of meibomian gland dysfunction (MGD) is typically focussed on eyelid hygiene and eyelid warming therapy to melt abnormal meibum, clear the obstructive material and therefore unblock the meibomian gland to help restore normal function. Effective eyelid warming devices reported in the scientific literature include the use of moist air goggles, combined heat and pulsatile pressure devices, and eye-masks that are heated by light-emitting-diodes or controlled chemical reactions [1–3]. In clinical practice, therapy frequently involves the recommendation of patient applied traditional warm compresses that can be performed with either a moistened flannel, or an eye-mask, such as the dry seed-filled MGDRx eyebag, that can be heated within the home using a microwave oven [4–6]. However, these patient applied procedures nearly always involve the re-use of the same device, and little information, if any at all, is provided with respect to their maintenance, storage, and decontamination.

Indeed, the role of bacteria in the pathophysiology of MGD remains unclear [7]. Previous histopathologic studies by Gutgusell et al. [8] showed that inflammatory cell infiltration was absent or minimal in MGD patients, suggesting that inflammation secondary to bacterial infection did not play an important role [8]. However, in studies on the bacteriology of healthy controls and patients with chronic blepharitis, who often present with concomitant MGD, *Staphylococcal aureus*, *Corynebacterium* spp. and *Propionibacterum acnes* was frequently isolated from the eyelid margin and expressed meibum, suggesting bacteria normally reside inside

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or within the vicinity of the meibomian gland [9,10]. Moreover, studies have shown that lipases and esterases from commensal bacteria are able to modify or degrade the meibum lipids and increase levels of free fatty acids, rather than cause direct infection and damage [7–11]. Although these lipid changes may affect the composition of meibum and increase viscosity as described above, free fatty acids have been suggested to also cause irritation, inflammation, and stimulate keratinisation of the eyelid margin epithelium in patients with chronic blepharitis [10,12]. In addition, bacterial growth may even be encouraged by the increased availability of cholesterol due to bacterial esterase [12,13]. Therefore, stasis and obstruction of the meibomian gland may lead to increased levels of pre-existing bacteria that can exacerbate MGD, rather than a primary infective cause [7].

Although heating of the compress device material at regular intervals may decontaminate it before application, temperatures obtained at therapeutic levels of heating are not sufficient to do so effectively. Temperatures that melt the abnormal meibum effectively while remaining safe (no thermal injury, cataract formation, corneal deformation, or ocular surface damage) and comfortable, typically range between 40 and 45 °C [2–6]; whereas heat sterilisation of medical instruments require a minimum of 121 °C–134 °C (ISO17665-1:2006). Thus, eyelid warming devices that make contact with the eyelids and surrounding tissue that are not sufficiently heated to decontaminate them may inadvertently prolong and or reinforce MGD. While there are no published reports showing infection caused by the use of such treatments, the presence of significant eyelid contamination is associated with an increased risk of ocular discomfort and infection in contact lens wearers [14]. However, there appears to be no reports in the scientific literature relating to the decontamination of eyelid warming devices or how this may be achieved. Therefore, this study aimed to determine the effectiveness of microwave decontamination of an eyebag and traditional compress (flannel) on bacterial isolates representative of those naturally existing on the eyelid margin.

2. Materials and methods

The study was designed as a randomised, controlled, examiner masked in-vitro experiment.

2.1. Experimental protocol

Separate *S. aureus* (NCTC10788; National Collection of Type Cultures, Public Health England), *S. pyogenes* (NCTC8198), and *P. aeruginosa* (NCTC10332) experimental inoculum were prepared by placing a few colonies of the bacteria in 10 ml of nutrient broth and incubating at 37 °C for 24 h. A haemocytometer (Neubaur, Germany) was used to count the number of cells (*C*/ml) in each overnight culture, before diluting in nutrient broth to obtain a bacterial count of 2–6 × 107 per ml for each species.

A set of 9 of each new and unused eyebags and flannels (unfolded 30 cm × 30 cm 100% cotton flannels; soaked in sterile water for 10 s and then excess water wrung until no further water was liberated) were microwaved (centre of the carousel tray) for 60 s at 800 W and left to cool to 25 °C), before being inoculated by adding ten evenly spaced 0.1 ml drops of the experimental inoculum (one species for each set of 3 eyebags and flannel compresses) and left for five minutes to allow the inoculum to be absorbed. One of each inoculated eyebag and flannel in each set was randomised into one of the following:

Control: did not receive any heat treatment. This group not only served as a control, but also allowed the sensitivity of the bacterial recovery method to be determined.

Therapeutic: heated for 30 s in an 800 W microwave oven to an immediate surface temperature of 47.4 ± 0.7 °C (ThermoTracer 7102MX, NEC, Japan).

Sanitisation: heated for 60 s in the same 800 W microwave oven to produce an immediate surface temperature of 68.0 ± 1.1 °C

After treatment, all eyebags and flannels were placed into separate sterile stomacher bags and left to cool for 70 min. Nutrient broth (200 ml) was then added to each bag and kneaded for 2 min using a stomacher machine (Stomacher 400 Circulator, Steward, West Sussex, UK). A stomacher is a laboratory homogeniser, where a series of paddles manipulate the contents of the bag creating a washing effect to drive more microorganisms from the sample into the surrounding diluent, thereby providing better recovery for analysis. A 0.1 ml sample of this solution was then taken and 1:10 dilution series was created in 0.85% sodium chloride for each bag. A 0.1 ml sample of each dilution was spread evenly on separate nutrient agar (*S. aureus*; *P. aeruginosa*) or blood agar (*S. pyogenes*) plates (Oxoid, Basingstoke, UK) and incubated at 37 °C for 24 h before counting and calculating the number of cells. This experimental protocol was repeated in triplicate using new and unused eyebags and flannels each time.

Due to the destructive nature of the testing, an additional 9 eyebags and flannels that were neither inoculated nor heat-treated underwent microbiological analysis to determine the pre-existing bacterial load. Here, the eyebags and flannels were placed into separate stomacher bags and 200 ml of nutrient broth was added before kneading for 2 min using the stomacher machine to release any organisms. The solution from each bag was then inoculated on to a range of selective culture media (nutrient agar CM0003, violet red bile glucose agar CM1082, manitol salt agar PO0151, and *Pseudomonas* agar base CM0559 with selective supplement SR103). Any cultures of characteristic morphology were observed using microscopy and staining to arrive at a presumptive identification. Further investigation with biochemical profiling tests (API20E and API20NE, BioMerieux UK Ltd., Hampshire, UK) was performed to confirm the bacterial species. Another 9 un-inoculated eyebags and flannel compresses had the sanitisation protocol applied and then also underwent the same analysis to determine if sanitisation temperatures affected the levels of bacteria previously identified.

In all cases sterile disposable gloves were worn at all times while handling the eyebags and compresses.

2.2. Statistical analysis

As the data were normally distributed (Kolmogorov–Smirnov test $P > 0.05$), one-way ANOVA was performed to determine if there were any significant differences between the levels of each bacterium in the controls and after heat and sanitisation treatment. Tukey's post-hoc test was performed to determine which group differed from the others for each bacterium if appropriate. Unpaired Student's *t*-test was performed to determine if there were any significant differences in the change in bacteria levels between the eyebags and flannel compresses, and before and after treatments. Statistical significance was taken as $P < 0.05$.

3. Results

3.1. Effect of heating on pre-existing bacteria

Microbiological analysis of the 9 un-inoculated and un-heated eyebags revealed pre-existing bacteria including the species *Bacillus* spp. (mean 1.63 ± 0.98 log *C*/ml) *Enterobacter sakazakki* (4.13 ± 1.56 log *C*/ml) and *S. aureus* (4.40 ± 0.42 log *C*/ml). Heating of the other 9 un-inoculated eyebags for 60 s sat 800 W (sanitisation protocol) demonstrated a statistically significant