Honey has an antifungal effect against *Candida* species

JULIE IRISH*, DEE A. CARTER*, TAHEREH SHOKOHI† & SHONA E. BLAIR*

*School of Molecular and Microbial Biosciences, University of Sydney, New South Wales, Australia, and †Department of Medical Mycology and Parasitology, Mazandaran University of Medical Sciences, Sari, Iran

The incidence of *Candida* infections is escalating worldwide. The serious nature of these infections is compounded by increasing levels of drug resistance. We report that certain honeys have significant antifungal activity against clinical isolates of *Candida* species. Importantly, the minimum inhibitory concentration of these honeys would be achievable in a clinical setting.

**Keywords** honey, *Candida*, antifungal effect

Introduction

Honey has been used as a medicine for thousands of years, and has been found to be an effective antimicrobial agent [1]. This antimicrobial activity stems primarily from the production of hydrogen peroxide from glucose and oxygen by glucose oxidase, a bee-derived enzyme [2]. Honey from certain species of *Leptospermum* flora native to Australia and New Zealand contains additional phytochemical components that further enhance its antibacterial activity [3]. The precise nature of these components is yet to be identified.

Although several *in vitro* studies have demonstrated the antibacterial properties of honey [3–5], few have examined the action against fungi. The incidence of fungal infections is increasing in both the community and hospital environments, with *Candida* spp. among the leading organisms. *Candida albicans* causes oral infections, over 50% of candidaemia cases [6–9] and more than 90% of vaginal candidiasis [10,11]. Recently there have been increased reports of non-*C. albicans* *Candida* species in clinical studies. *Candida glabrata* has become the dominant non-*C. albicans* *Candida* species involved in bloodstream and vaginal infections [9,12–14], while *Candida dubliniensis* is often implicated in oropharyngeal candidiasis [15]. Infection with non-*C. albicans* *Candida* species has important clinical implications, as many are resistant to conventional triazole-based antifungal therapy. Other conventional antifungal therapies are either toxic or of limited efficacy, and there is a growing need for new potent antifungal agents. The aim of the current study was to determine the efficacy of various honeys against clinical isolates of *C. albicans*, *C. glabrata* and *C. dubliniensis*.

Materials and methods

Clinical isolates of *C. albicans*, *C. glabrata* and *C. dubliniensis* were tested against four different honeys: an unprocessed Jarrah honey with hydrogen peroxide activity (total antibacterial activity (as described in [16]) equivalent to 30.2% phenol [17]); Medihoney® Antibacterial Honey Barrier, a proprietary blend of *Leptospermum* and hydrogen peroxide honeys (phytochemical activity ≥18% phenol equivalent); Comvita® Wound Care 18+, a pure *Leptospermum* honey (phytochemical activity ≥18% phenol equivalent); and an artificial honey, used to simulate the high sugar levels found in honey (7.5 g sucrose, 37.5 g maltose, 167.5 g glucose, and 202.5 g fructose in 85 ml of sterile water).

The minimum inhibitory concentration (MIC) of each honey was determined based on the NCCLS microdilution method [18]. Fifty percent (w/v) stock solutions of honey in RPMI-1640 medium (with glutamine and without bicarbonate (Sigma)) were prepared immediately before each assay and filter sterilised through 0.2 μm pore filters (Millipore). Stock solutions were further diluted with RPMI-1640...
medium in 96-well microtitre plates to give final honey concentrations that increased in 1% (w/v) increments. Suspensions of Candida isolates were prepared in sterile 0.85% saline and transmittance at 530 nm was adjusted to 80–88%. Suspensions were diluted in RPMI-1640 medium, and 25 μl was added to each well of the microtitre plate immediately after preparation of the honey solutions, resulting in a final inoculum of 0.5–2.5 × 10^3 cfu/ml. Following incubation at 35°C for 24 h, the MIC was recorded as the lowest concentration of honey that prevented visible growth. Each Candida isolate was tested in duplicate and the assays were repeated on a separate day. The Mann-Whitney U test was used to evaluate statistically significant groups. Correlations were performed using the Spearman rank-order test.

**Results**

Results of the susceptibility of C. albicans, C. glabrata, and C. dubliniensis to various honeys are shown in Table 1. Jarrah honey was significantly more active against the three Candida species (P < 0.00001). The antifungal activities of the floral honeys were significantly greater than the artificial honey against C. albicans and C. glabrata (P < 0.002), but for C. dubliniensis, only Jarrah honey was significantly more active (P < 0.00001). C. dubliniensis was more susceptible to the osmotic effect of all honeys, and to the antifungal effects of Jarrah honey, exhibiting significantly lower MICs than the other species (P < 0.00001). C. glabrata, which is innately less susceptible to many conventional antifungals [19], was the least susceptible to the honeys tested (P < 0.00001).

Drug resistance profiles were available for 20 of the isolates. Twelve of these were either resistant or susceptible-dose dependent to itraconazole and/or fluconazole. All of these isolates were inhibited by honey, with no statistical relationship between antifungal susceptibility and sensitivity to honey (P > 0.05).

**Discussion**

Limited observations have found honey to have an inhibitory effect against C. albicans in vitro [20–22], however none of these studies used standardized honeys or assay methods. Only one published study has used honey with known phenol-equivalent activity to examine antifungal activity [23]. In this study the effects of pasture honey were compared with *Leptospermum* honey against dermatophyte fungi. In accordance with the results of the current study the hydrogen peroxide-type honey was found to have a greater antifungal effect.

The current study found no statistical relationship between antifungal susceptibility and sensitivity to honey. This is of particular importance considering the increasing rate of resistance toazole drugs among Candida isolates [8,24,25], and the finding that azole-based prophylaxis increases the risk of infection with non-C. albicans Candida species, which may be less responsive to usual drug dosages [6,24].

Although this study demonstrates the antifungal effect of honey in vitro there are some practical considerations for its use in vivo. Firstly, honey is limited to topical treatments, and could not be used to treat candidaemia, the most serious form of candidiasis. However, as the leading risk factor for bloodstream infection is colonization or infection of external sites, such as indwelling catheters, or the oral or vaginal mucosae [19], honey may be used prophylactically to prevent more serious infections. Whole honey placed directly around catheters was found to be at least as effective as povidone iodine [26] or mupirocin [27] in preventing exit site infection. Secondly, as honey is water soluble, it may be diluted or removed by body fluids, particularly saliva in the oral cavity. A pilot study by English et al. [28] found a significant reduction in mean plaque scores and bleeding sites in patients given a chewable ‘honey leather’; this same technique could be applied for the treatment of oral candidiasis. At other body sites, regular application of 100% honey would maintain a concentration well above the desired MIC. Honey could also be incorporated into a pessary for the treatment of vaginal candidiasis. Another practical issue is the presence of catalase in body fluids that has the potential to reduce hydrogen peroxide activity. However, case reports and clinical trials suggest sufficient activity is retained to
allow honey to be effective in the clinical setting [29,30].
The results of the current study argue for controlled clinical trials to establish honey as a topical antifungal agent.

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