The effect of manuka honey on the structure of *Pseudomonas aeruginosa*

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Abstract. The purpose of this study was to investigate the effects of manuka honey on the structural integrity of Pseudomonas aeruginosa ATTC 27853. The minimum inhibitory concentration (MIC) and the minimum bactericidal concentration (MBC) of manuka honey for Ps. aeruginosa were determined by a microtitre plate method, and the survival of bacteria exposed to a bactericidal concentration of manuka honey was monitored. The effect of manuka honey on the structure of the bacteria was investigated using scanning and transmission electron microscopy. MIC and MBC values of manuka honey against *Ps. aeruginosa* were 9.5 % (w/v) and 12% (w/v) respectively; a time-kill curve demonstrated a bactericidal rather than a bacteriostatic effect, with a 5 log reduction estimated within 257 min. Using scanning electron microscopy, loss of structural integrity and marked changes in cell shape and surface were observed in honey-treated cultures. With transmission electron microscopy these changes were confirmed, and evidence of extensive cell disruption and lysis was found. Manuka honey does not induce the same structural changes in Ps. aeruginosa as those observed in staphylococci. Our results indicate that manuka honey has the potential to be an effective inhibitor of *Ps. aeruginosa*.

Keywords: manuka honey, Pseudomonas aeruginosa, bacterial structural integrity

Introduction.

Pseudomonas aeruginosa is an opportunist pathogen that is ubiquitously distributed throughout the environment, particularly in moist habitats. It has been implicated in a wide range of infections such as endocarditis, folliculitis, keratitis, meningitis, pneumonia, urinary tract infections and wound infections. In wounds *Ps. aeruginosa* has emerged as a multidrug resistant organism that gives rise to persistent infections in burns patients [1,2] and chronic venous leg ulcers [3]. Novel antimicrobial interventions are needed.

Honey has been used for thousands of years as a topical treatment for wounds. Although ancient remedies may have been crude preparations, modern wound care products are

honey solution to determine the effect of sugars in honey in cell structure (Cooper, Halas & Molan, 2002; Cooper, Molan & Harding, 2002). Cells were examined in scanning (SEM) (5200LV Jeol, Herts, UK) and transmission electron microscopy (TEM) (1210 Jeol, Herts, UK) by the method of Lemar, Turner & Lloyd [16], except that harvested cell pellets for TEM were embedded in Araldite resin, not Spurr.

Analysis of images

Electron micrographs of untreated and treated cells were examined to identify structural changes such as altered shape, modified surface layers, the presence of electron dense material, and cellular debris. Typically at least six photographs, each with approximately 160 cells were observed, so that more than 1000 cells were counted in total for each sample. Data was analysed for statistically significant differences by the Mann-Whitney test using Minitab (version 15).

Results.

Inhibition studies

MIC and MBC were found to be 9.5 and 12 % (w/v) manuka honey, respectively. The close proximity of these two values indicates a bactericidal mode of inhibition. This was confirmed by time-kill studies (Fig. 1) where cells exposed to manuka honey were found to lose viability with time, yet numbers of untreated cells increased. The time estimated to achieve a 5 log reduction of test organism incubated with nutrient broth containing 20% (w/v) manuka honey was 257 minutes.

Structural studies

The effect of manuka honey on cell structure was investigated in both exponential and stationary phase cultures because stationary phase cells are often less susceptible to antimicrobial agents than exponential cells. However the structural changes observed in

both of these stages of growth were similar and therefore only electron micrographs of exponential cells are presented here.

Using scanning electron microscopy the smooth surface layers of untreated cells (Fig 2a) and cells exposed to 20% (w/v) artificial honey (Fig 2b) contrasted with those of honey treated *Ps. aeruginosa* cells, which exhibited marked cell surface changes as furrows and blebs (Fig. 2c). Honey-treated cells also appeared to be shortened and to have distorted shapes (Fig. 2c). In untreated samples 2% of cells were found to have structural irregularities, whereas 80 and 60 % cells of exponential and stationary cultures, respectively exhibited irregular cell structure. These differences were statistically significant (Table 1). For exponential phase cells exposed to 20% (w/v) artificial honey, 7% of cells were found to exhibit structural irregularities. This suggests that the effect of manuka honey on *Ps. aeruginosa* is not due exclusively to the sugars contained in honey. Using TEM, untreated cells (Fig. 3a) and cells incubated in MOPS containing 20% (w/v) artificial honey (Fig. 3b) were entire cells with relatively densely stained contents. In TEM images of honey-treated *P. aeruginosa* (Fig. 3c) cellular debris was clearly evident and whole cells with evacuated areas were observed.

Discussion.

Inhibition studies

The MIC obtained in this study concurred with previous studies [6, 9, 10]. The MBC and time-kill curve (Fig.1) indicated a bactericidal rather than bacteriostatic effect of manuka honey on *Ps. aeruginosa*. The loss of viability of cells exposed to 20% (w/v) manuka honey *in vitro* provided a guide to its clinical efficacy, where in licensed wound dressings undiluted honey is usually used. Since part of the weight of the dressing is absorptive material, honey is not diluted in terms of its antibacterial activity even though the quantity of honey in terms of the total weight of dressing applied to the wound is decreased.