BIOLOGICAL IMPACT OF POLYFLORAL HONEY ON ANTIBIO-RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM CANINE DERMATITIS

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Summary

The antibacterial activity of honey was evaluated in vitro against *Staphyococcus aureus* strains isolated from canine patients with dermatitis, compared to an artificial honey solution.

Performing a broth microdilution protocol, supported by agar plate cultivation, we determined the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Antimicrobial activity was observed in the polyfloral honey tested (MIC - 1.563% and MBC - 12.5%)(v/v), when compared to the artificial honey, weaker in its antibacterial efficacy.

In conclusion, the results showed that floral honey could be considered, even diluted, included in topical medication, as an alternative treatment in canine recurrent dermatitis.

The medicinal use of honey in wound treatment is derived from diverse ancient civilizations (Jones, 2001). The antibacterial properties of honey were recognized more than a century ago and have subsequently been extensively studied (Molan, 1992a, 1992b). Now, it is possible to determine quantitatively the antibacterial activity of a honey (Allen et al., 1991) and also to discriminate between honeys, whose mode of action involves in limiting bacterial growth factors beyond their osmolarity (Allen et al., 1991).

The aim of this *in vitro* study was to evaluate and compare the sensitivity (MICs and MBCs) of multiresistant strains of *Staphyococcus aureus* towards floral honey compared to artificial honey.

Materials and methods

Honey samples: 1) *Artificial honey* was prepared by dissolving 1.5g sucrose, 7.5g maltose, 40.5g fructose and 33.5g glucose in 17 ml sterile saline solution (100 g). This solution presents the proportions of the four predominant sugars in natural honey samples (Cooper et al. 2000). 2) *Polyfloral honey* was purchased from a private certificated producer, and was obtained in a hilly region of Romania, with a various pasture flora.

Bacterial strains: Four strains of *Staphylococcus aureus* strains were collected from clinical cases of canine pyodermitis, identified by bacteriological laboratory means, and used in comparison to a reference strain of *Staphylococcus aureus* (ATCC 25923). The field isolates were resistant to antibiotics such as penicillin, tetracyclin, cephalotine, oxacilline, enrophloxacine, gentamicin, amoxycillin with clavulanic acid and ciprophloxacine. All bacterial strains included in this research were cultivated for 24h at 37◦C on agar plates (Merck). 24h bacterial culture was diluted in sterile saline solution and the optical density was adjusted according to the tube 0.5 of McFarland' scale in order to prepare a standardized inoculum (1.5×10^8cfu/ml).

Antimicrobial assay: Three different methods were used to evaluate the antimicrobial activity of honey: broth microdilution method, well and disc diffusions, respectively. The broth microdilution method, established previously by Carson *et al.* (1995) was finally used to determine the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). Using Mueller Hinton broth (Difco^{TM}), series of twofold dilutions of each honey were performed in sterile 96 wells microtitre plates. 100 µl of each resulted dilutions ranging from 1.563 (1/64) to 50% (1/2) (v/v) were mixed with equal volume of bacterial suspension. Positive (broth and inoculum) and negative (simple broth) growth controls were prepared. The polyfloral honey effects were screened in comparison to artificial honey. The plates were incubated for 24h, at 37◦C. The MIC was defined as the lowest concentration (highest dilution) of honey that inhibited the visible growth (no turbidity) compared to the control (simple broth). The results were read spetrophotometrically. After this, 10µl of each well was transferred to Mueller Hinton agar plates and incubated at 37◦C for 24h.The MBC was considered as the lowest concentration of honey associated with no visible growth of bacteria on the agar plates. All samples were tested in duplicate. Mean values of growth inhibition were calculated.

Results and discussions

In recent studies, the susceptibility of wound pathogens (Willix et al., 1992) and bacteria isolated from infected wounds (Cooper and Molan 1999; Cooper et al., 1999) to honeys of known floral source and defined antibacterial activity has been reported. However, the inhibition of antibiotic-resistant bacteria by honey has not been fully explored. In most honeys, the antibacterial activity depends on the enzyme catalyzed generation of hydrogen peroxide to varying degrees (Molan 1992a) but, in some honeys, there are additional phytochemical antibacterial factors (Molan, 1992a).

In the research, five *Staphylococcus aureus* strains were tested in order to evaluate the antimicrobial potential of honey, in comparison with artificial honey. The MICs values for polyfloral honey, determined using a broth microdilution

method, were presented in comparison to artificial honey as the average in tables I and Fig. 1.

Table I

Bacterial growth inhibition under polyfloral and artificial honey *in vitro* treatments

	Polyfloral honey		Mean	Artificial honey		Mean
Dilution1	0.073	0.058	0.066	0.046	0.045	0.046
Dilution2	0.088	0.096	0.092	0.057	0.026	0.042
Dilution3	0.087	0.096	0.092	0.079	0.05	0.065
Dilution4	0.061	0.101	0.081	0.035	0.032	0.034
Dilution5	0.045	0.05	0.048	0.05	0.024	0.037
Dilution6	-0.002	0.002	0.000	0.319	0.589	0.454

As shown in the table, for natural honey, the MIC values were somewhat higher than in the artificial one, but close to those. Interestingly, the dilution degree negatively influenced the antibacterial activity up to dilution 3 (1/8) and afterwards the effect turned to positive, the most diluted polyfloral honey sample being the most effective. At the highest dilution the artificial honey proved to be totally ineffective. This influence of the polyfloral honey resembles a homeopathic one.

A wide range of microbial species has been shown to be inhibited by honey, but reported susceptibilities are not consistent. It is remarkable that ancient physicians were selective in the honeys that they utilized in their remedies (Jones, 2001), although the underlying principles would have been obscure.

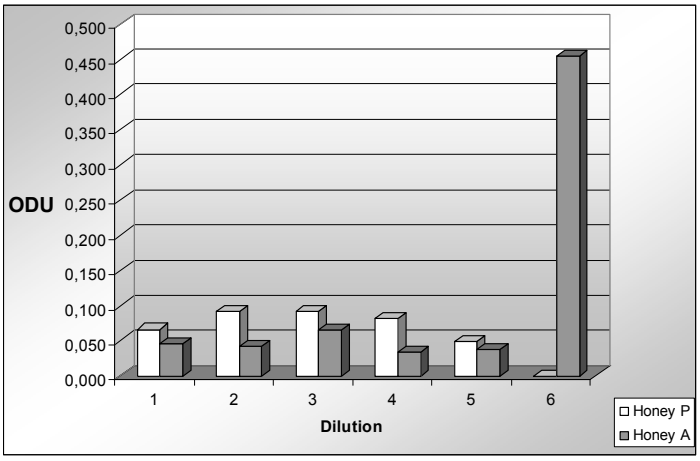

Fig.1. Mean minimal inhibitory concentrations' values for the two types of honey against *S. aureus* strains

Figure I underlines the very wide difference between the efficacy of the two types of honey at the highest (1/64) dilution.

The minimal bactericidal concentrations were close for the two types of honey, but somewhat higher in the natural one (12.5% dilution), when compared to the artificial one (25%).

These data, obtained for the antibacterial usefulness of Romanian honey versus *S. aureus*, were related to those described in literature for the antibacterial efficacy of various honeys, from Europe (Germany) and mainly Australia and New-Zeeland.

Conclusions

The results obtained in this experiment supported the idea of a bactericidal and meanwhile homeopathic effect of the natural honey that proved active at very low concentrations versus *S. aureus* from canine dermatitis, when compared to artificial honey. Floral honey could be considered, even diluted, included in topical medication, as an alternative treatment in canine recurrent dermatitis.

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