Effects of natural honey on polymicrobial culture of various human pathogens

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Abstract

Introduction: Honey has a wide range of antimicrobial activity. All previous studies have considered honey's effect on a single microbe. The present study investigated activity of honey towards a high dose of single or polymicrobial culture.

Material and methods: 10 µl specimens of *Staphylococcus aureus* (*S. aureus*), *Streptococcus pyogenes* (*S. pyogenes*), *Escherichia coli* (*E. coli*) and *Candida albicans* (*C. albicans*) were cultured in 10 ml of 10-100% (wt/v) honey diluted in broth. Six types of polymicrobial microbial cultures were prepared by culturing the isolates with each other onto broth (control) and broth containing various concentrations of honey (10-100% wt/v). Microbial growth was assessed on solid plate media after 24 h incubation.

Results: Honey (30-70%) prevents growth of 10 µl specimens of all the isolates. Greater reduction in growth of *E. coli* was observed when cultured with *S. aureus*. Culturing of *S. aureus* with *S. pyogenes*, *C. albicans*, or *E. coli* increased its sensitivity to honey. *S. aureus* and *S. pyogenes* increased sensitivity of *C. albicans* to honey while *E. coli* and *C. albicans* decreased sensitivity of *S. pyogenes*.

Conclusions: It might be concluded that honey prevents and inhibits growth of single and polymicrobial pathogenic cultures. Polymicrobial culture affects growth of the isolates and increases their sensitivity to honey.

Key words: honey, *Candida albicans*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*.

Introduction

Honey is a drug in addition to its valuable nutrient contents. Honey was valued highly in the Middle East. It was mentioned in the Holy Quran 1400 years ago (*And thy LORD taught the bee to build its cells in hills, on trees and in men's habitations, then to eat of all the produce of the earth and find with skill the spacious paths of its LORD, there issues from within their bodies a drink of varying colors, wherein is healing for men, verily in this is a sign for those who give thought*). It is also mentioned in the Talmud. Hippocrates and Celsus used honey for wounds and ulcers. Prophet Mohammed recommended honey for treatment of diarrhea. Laboratory studies and clinical trials have shown that honey is an effective broad-spectrum antimicrobial

agent [1–9]. The antibacterial effect of honey was studied when a loopful specimen of each pathogen was inoculated in various concentrations of honey prepared in solid media or using a disc impregnated with honey. These techniques have been used to explore the effect of honey on growth of a small number of isolates usually using a 1 µl standard loop for inoculation. They did not show an influence of honey on mixed microbial cultures. In addition, most previous studies were planned to answer whether honey could prevent multiplication of a single pathogen inoculated into media prepared with various concentration of v/v honey.

Any wounds, whether surgical or traumatic, are at great risk of becoming infected. Basically, wound colonization is most frequently polymicrobial, involving numerous pathogenic microorganisms [3–12]. Managing wounds infected with a mixture of several types of microorganisms such as bacteria and fungi is a challenging clinical situation. However, no study has been conducted to investigate the antimicrobial influence of honey on mixed microbial culture.

Therefore, the purpose of the present study was to investigate: (1) antimicrobial activity of honey against single human pathogens cultured in liquid broth, (2) the influence of honey on mixed microbial cultures, and (3) the effects of mixed microbial culture on their growth and their response to honey therapy.

Material and methods

Pathogenic isolates

Cultures of various human pathogenic strains were obtained from the Microbiology Department, Dubai Specialized Medical Center and Medical Research Laboratories, Dubai. Species included *Escherichia coli* (*E. coli*), *Staphylococcus aureus* (*S. aureus*), *Streptococcus pyogenes* (*S. pyogenes*) and *Candida albicans* (*C. albicans*). These strains were isolated from human specimens. The isolates were identified by the standard bacteriological techniques. Using a 10 µl standard loop, a colony of each isolate was picked from a plate, grown in 10 ml nutrient broth, and used after 24 h incubation at 37°C. Bacterial growth was assessed visually on solid media as: 1) no growth, 2) little growth, 3) mild growth, 4) moderate growth and 5) heavy growth. The test was duplicated for each culture. Solid media included blood agar for *S. aureus* and *S. pyogenes*, MacConkey agar medial for *E. coli*, and Sabouraud media for *C. albicans*.

Honey

Honey was collected from United Arab Emirates. It was dark yellow in color and of multifloral origin. Different concentrations of honey were prepared. The concentrations were given as percent (w/v). The honey had a density of 1.40 g/ml, and its composition included (per 100 g of honey) fructose 38 g/dl, glucose 28 g/dl, sucrose 0.5 g/dl, moisture 20%, acidity 13%, vitamin C 2.3 g/dl, copper 0.098 g/dl, and glutathione reductase 0.52 g/dl. The amount of honey necessary to achieve the required concentrations (10–100%, w/v) was aseptically weighed into sterile test tubes. Nutrient broth was added to the tubes to make up the total volume of the concentration required. The honey broth solutions were mixed by stirring with sterile sticks.

Effects of honey on high doses of single pathogen culture

Cultures of the specimens of the isolates in broth containing different concentrations of honey were performed by using a standard loop (10 µl). A specimen of each microorganism was taken from pure culture grown in the 10 ml nutrient broth as described above. These cultures were incubated at 37°C for 24 h. Then a loopful (10 µl) of the cultures of each of the specimens of microorganisms was streaked onto agar plates to assess the viability of the isolates. The streaked plates were incubated aerobically at 37°C and inspected after 24 h.

Effects of honey on mixed microbial cultures

Six types of mixed microbial cultures were prepared: mixture 1 contained *S. aureus* and *S. pyogenes*; mixture 2 contained *S. aureus* and *E. coli*; mixture 3 contained *S. aureus* and *C. albicans*; mixture 4 contained *S. pyogenes* and *E. coli*; mixture 5 contained *S. pyogenes* and *C. albicans*; and mixture 6 contained all four isolates. A loopful specimen (10 µl) of fresh culture of each isolate was used for cultivation. Each mixture was cultured onto 10 ml broth (control) and onto tubes containing 10 ml of various honey concentrations in broth (10–100% wt/v). These cultures were incubated at 37°C for 24 h. Then a loopful (10 µl) of the cultures of each of the specimens of the mixture was streaked onto appropriate solid agar plates to assess the viability of the isolates. The streaked plates were incubated aerobically at 37°C and inspected after 24 h.

Results

The minimum concentration of honey that prevents growth of 10 ul specimens of *S. aureus* or *S. pyogenes* was 50%, growth of 10 µl specimen of *E. coli* was 30% and growth of 10 µl of *C. albicans* was 70%. The most sensitive microorganism was *E. coli*. The six kinds of mixed microbial growth were all completely prevented when cultured in various concentrations of honey (Table I). The grade of the growth of each microorganism cultured together did not differ considerably from the grade of growth of each microorganism cultured alone in the broth except for great reduction in the grade of growth of *E. coli*

Table I. Concentrations of honey prevent the growth of the microorganisms in different mixed cultures

Type of isolates	Mixed with	Control	Concentration of honey									
			10	20	30	40	50	60	70	80	90	100
S. aureus	Single	5+	4+	3+	1+	1+	0	0	0	0	0	0
	S. pyogenes	5+	3+	1+	0	0	0	0	0	0	0	0
	E. coli	2+	1+	1+	0	0	0	0	0	0	0	0
	C. albicans	5+	1+	0	0	0	0	0	0	0	0	0
	All together*	4+	2+	0	0	0	0	0	0	0	0	0
E. coli	Single	5+	2+	1+	0	0	0	0	0	0	0	0
	S. aureus	5+	3+	2+	0	0	0	0	0	0	0	0
	S. pyogenes	5+	3+	2+	0	0	0	0	0	0	0	0
	All together	4+	2+	1+	0	0	0	0	0	0	0	0
S. pyogenes	Single	5+	4+	2+	2+	2+	0	0	0	0	0	0
	E. coli	5+	4+	3+	2+	1+	1+	1+	0	0	0	0
	C. albicans	5+	4+	3+	2+	1+	1+	1+	0	0	0	0
C. albicans	Single	5+	5+	4+	3+	2+	1+	1+	0	0	0	0
	S. aureus	5+	4+	2+	1+	1+	0	0	0	0	0	0
	S. pyogenes	5+	4+	3+	1+	1+	0	0	0	0	0	0
	All together	4+	3+	2+	1+	1+	0	0	0	0	0	0

**All together: mixed culture contained S. aureus, E. coli, S. pyogenes and C. albicans*

Table II. Grade of microbial growth collected from single or mixed cultures that were visualized on solid plates and their inhibition by various concentrations of honey

Type of isolates	Cultured with	Grade of growth	Honey concentration %+
S. aureus	Single	5+	50
	S. pyogenes	5+	30
	E. coli	2+	30
	C. albicans	5+	20
	All together*	4+	20
S. pyogenes	Single	5+	50
	E. coli	5+	60
	C. albicans	5+	70
E. coli	Single	5+	30
	S. aureus	5+	30
	S. pyogenes	5+	30
	C. albicans	4+	30
	All together	4+	30
C. albicans	Single	5+	70
	S. aureus	5+	50
	E. coli	5+	40
	S. pyogenes	4+	30
	All together	4+	50

**All together: mixed culture contained S. aureus, E. coli, S. pyogenes and C albicans; +minimum concentration of honey that completely inhibits microbial growth*

when cultured with *S. aureus* (Table II). However, mild reduction was observed in the growth of *C. albicans* when cultured with *S. pyogenes* and growth of *S. aureus*, *E. coli* and *C. albicans* when cultured together.

The minimum concentration of honey preventing growth of *S. aureus* decreased when *S. aureus* was cultured with *S. pyogenes*, *C. albicans*, or *E. coli* or when all four microorganisms were cultured together (Table II). Similarly, the minimum concentration of honey preventing growth of *C. albicans* decreased when *C. albicans* was cultured with *S. aureus*, *S. pyogenes*, or with all other microorganisms. In contrast, the minimum concentration of honey preventing growth of *S. pyogenes* increased when cultured with *E. coli* or *C. albicans*. No changes were observed in the minimum concentration of honey preventing growth of *E. coli* when cultured with other microorganisms.

Discussion

The main findings are: 1) honey prevents growth of a single microbe inoculated separately in broth containing honey, 2) honey prevents growth of mixed isolates inoculated together in broth containing honey, 3) polymicrobial culture affects growth of each isolate and increases their response to honey therapy except for *S. pyogenes*, and 4) the most sensitive microorganism was *E. coli* when the isolates were cultured separately in broth media and *S. aureus* when the isolates were cultured with each other. This is the first study to report the effect of honey on polymicrobial culture collected from hu-

man specimens. Honey inhibits growth of *C. albicans*, which substantiated our earlier studies showing that honey alone or honey mixed with olive oil and beeswax was useful to treat cutaneous fungal infection and seborrhoeic dermatitis [13, 14]. Clearly, previous results and the present work demonstrated that almost similar concentrations of honey could prevent growth of different doses of human pathogens, 1 µl and 10 µl [15].

Honey prevented growth of gram positive or gram negative bacteria or *C. albicans*. This property makes honey a good drug to apply when closing surgical wounds or wounds due to various traumas. It is not necessary to wait until development of signs and symptoms of established infection to apply honey. This is simply because honey is a safe natural product. Such practice is important because wounds are liable to contamination even at intensive care units. Approximately similar concentrations of honey were able to prevent growth of a smaller inoculum (1 µl) of the same isolates [15]. It seems that honey has the same potency to prevent growth of low as well as high size of inoculum (10-fold) of the isolates.

The minor reduction in growth of isolates when cultured together might be a result of competition for a limited nutrient resource. However, such reduction might be due to unidentified soluble suppressor factors. Suppression of *C. albicans* by human salivary bacteria and by pure cultures of human oral strains of *S. salivarius* and *S. mitior* has been reported [15, 16]. Interestingly, a great reduction was obtained in the growth of *S. aureus* when grown in the presence of *E. coli*. This reduction could not be explained by competition for nutrients because a higher grade of growth was obtained when *S. aureus* grew with other isolates. Therefore, *E. coli* might secrete a *Staphylococcus* inhibitory factor that requires further investigation. Studies have shown inhibition of *S. aureus* growth in mixed cultures with *C. albicans* [17]. Pneudomonas aeruginosa produced substances that inhibited the growth of *S. aureus* [18]. Significant suppression in the growth of *C. pylori* in the presence of *Lactobacillus acidophilus* was also observed [19].

The honey concentration that prevents growth of *S. aureus* was lower when *S. aureus* was cultured with *E. coli*, *S. pyogenes* or *C. albicans*. This means that these isolates increased susceptibility of *S. aureus* to honey. It was found that there is an increase in the in vitro susceptibility of *S. aureus* to antimicrobial agents in the presence of *C. albicans* [19]. In addition, *S. aureus* and *S. pyogenes* increased susceptibility of *C. albicans* to honey. However, higher concentration of honey was required to prevent growth of *S. pyogenes* in the presence of *E. coli* or *C. albicans*. In this case, the microbial inhibitory factor/factors in honey might be lower than the level required to prevent growth of the isolate. However, these isolates purposely protect *S. pyogenes* against the inhibitory effect of honey. This claim is supported by the decreased inhibitory effect of an antimicrobial towards a bacterial species in the presence of another species [20].

Studies have diluted honey with distilled water to obtain various volume/volume concentrations of honey [21–27]. We used broth for dilution that closely matches wounds, which was a suitable medium for microbial growth. Furthermore, the percentage of honey concentration was made as weight of honey in volume of nutrient broth [15]. This is more acceptable since various honeys have different densities. In addition, in vitro studies have mostly involved honey added to the agar plate as nutrient or brain heart infusion followed by inoculation with the microorganism or using impregnated honey discs. As honey diffuses it becomes diluted and the inhibition obtained did not match the real honey concentration used. In addition, we found that a disc impregnated with various concentrations of honey added to the agar plate became dry due to vaporization of fluid from the disc when the medium was incubated at 37°C for 24 h.

The variations in antibacterial activity of honey can be related to the amount of hydrogen peroxide and the presence of additional antibacterial components derived from the nectar source. However, we have found that honey increased nitric oxide end products in various animals and humans' biological fluids and decreased prostaglandin concentration [28–30]. We have proposed that killing of microbes by nitric oxide production might explain, in part, its antibacterial activity [15]. Interestingly, we have found that honey could enhance antibody production [31]. This might increase humoral immunity against bacterial invasion.

The main limitation of the study is that the number of isolates was not known. The weight of honey used in the experiment is known but the number of bacteria to be killed was not measured. Therefore, measuring the number of bacteria by dilution or optic density will be the next works for the authors. Most of the previous works on the effects of honey on bacteria did not include the number of viable organisms; instead they used a scoring system that included 1+, 2+, 3+ etc to assess growth of subcultures from liquid to solid media. In the next works when the number of microorganisms is known in addition to the weight of honey used, it will be easy to identify the potency of a certain dilution of honey.

In conclusion, in addition to its ability to prevent the growth of a single microbe inoculated separately in broth containing honey, honey prevents the growth of mixed isolates inoculated together in broth containing honey. The polymicrobial culture

increases the response of the isolates to the honey therapy except for *S. pyogenes*. This is the first study to report the clear effect of honey on polymicrobial culture collected from human specimens.

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