ORIGINAL ARTICLE

Antimicrobial potential of Sicilian honeys against commensal *Escherichia coli* and pathogenic *Salmonella* serovar Infantis

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Key words

Honey • Escherichia coli • Salmonella

Summary

Introduction. *The purpose of this study was to investigate the antibacterial effect of 71 locally produced honeys from different botanical sources collected from apiarist's open markets in Sicily.*
Methods. *Antimicrobial activity was determined against Escherichia coli (ATCC 25922) and Salmonella serovar Infantis (ATCC 1523) by an agar-diffusion assay from the estimation of the diameter of the inhibition zone produced by the honeys. Statistically significant differences (P < .000) regarding inhibition were observed for the honeys tested.*
Results. *The chestnut and polyfloral honey samples exhibited the largest and highest inhibition (diameter of the inhibition zone > 25 mm) against both E. coli and S. Infantis. The honey of oregano origin showed intermediate or low activity against E. coli and S. Infantis, respectively. Prickly pear and erica honeys showed no antimicrobial activity against the two reference strains.*
Discussion. *The results may partially suggest the usefulness of the Sicilian honeys on treating multi-resistant enterobacteria. In light of the enormous potential for application of honey in the clinical practice, it is important that research continues not only into those honeys well recognized as antimicrobial, but also into other locally produced and yet untested honeys.*

Introduction

A re-evaluation of the therapeutic use of ancient remedies such as plants and plant-based products could be important in preventing bacterial antibiotic resistance. In fact, antibiotics are crucial in reducing the global burden of infectious diseases. Anyway, their overuse and misuse in human clinical practice as well as in veterinary medicine has been the direct cause of the emergency of multiple antibiotic resistant bacteria worldwide. One non-conventional medical treatment that has recently received much interest is honey. Honey is produced by honeybees of the genera *Apis mellifera* and *Meliponinae* [1] from many sources, and its antimicrobial activity varies greatly with processing and origin, depending on the natural vegetative flowers blooming in different seasons and in different places [1-3].
Commensal enteric bacteria in particular constitute reservoirs of genes determining antibiotic resistance [4, 5]. Especially when antimicrobial exposure occurs, commensal *Escherichia coli* can transfer their antibiotic resistance in the gastrointestinal environment to the other enterobacteria, including various *Salmonella* serovars, which are the most common bacterial pathogens causing foodborne diseases in developing as well as in developed countries [4-7]. Previous studies conducted at local scale in Sicily (South Italy) have described high prevalence of multiresistant *E. coli* together with the emergence of ampicillin resistance in *E. coli* and *Salmonella* spp. isolates from wastewater and clinical specimens [8, 9]. Thus, from a public health perspective, alternative therapies, such as honey, should draw the public's interest. In Sicily, in particular, the geomorphology and the diversity of flora give the opportunity to the local black honeybees (*Apis mellifera* ssp. sicula) of producing a wide variety of honeys from blossoming trees, shrubs and flowers which gives to the final product special sensorial properties. Anyway, despite the variety and recognized quality, as far as we know, only episodic interest has been accorded to this subject in Italy [10]. Therefore, in order to verify whether in future the clinical use of Sicilian honeys could be useful in controlling the spread of antibiotic resistances in enterobacteria, in the current study we sought to study under experimental conditions the antibacterial potency of unpasteurized honeys against *E. coli* and *Salmonella*.

Methods

Area of study and honey samples

The area of study belongs to Sicily, located in the South of Italy, where the autochthon vegetation consists of various spontaneous shrubs and cultivated plants.
Seventy-one honey samples were collected during the 2011 flowering season from apiarist's open markets in two different geographical districts, being 18 samples from centre zone and 53 samples from South-East zone. The honey samples were originated from different bo-

tanical sources, following: 12 polyfloral, 13 of chestnut (*Castanea sativa*), 11 of orange (*Citrus aurantium*), 9 of eucalyptus (*Eucaliptus*), 9 of thyme (*Thymus vulgaris*), 6 of Spanish esparcet (*Hedysarum coronarium*), 4 of citrus (*Citrus limonum*), 4 of carob (*Ceratonia siliqua*), 1 of erica (*Erica vulgaris*), 1 of oregano (*Origanum vulgare*) and finally 1 of prickly pear (*Opuntia vulgaris*) origin.

Evaluation of antibacterial activity of honeys and bacterial strains tested

An agar diffusion method was used as described above to assess the antibacterial activity of the selected honeys against two reference strains: *Escherichia coli* ATCC 25922 and *Salmonella* serovar Infantis ATCC 1523, which are both susceptible to a wide range of antimicrobials, grow well at low temperatures, and have been shown to be stable in the laboratory following multiple passes on artificial media.

Preparation of the assay plates

E. coli and *S.* Infantis strains were inoculated into 10 mL of tryptic soy broth (TSB; Biolife, Milano, Italy) and incubated at 37°C for 18 h until growth was 0.5 optical density (450 nm). Cultures of 100 L were added to 18 mL of Mueller-Hinton Agar (Oxoid LTD; Basingstoke, Hampshire, England) previously cooled in a 50°C water bath for 30 min and immediately poured onto Petri plates, one bacterial culture per plate. A grid containing four 25 x 25 mm squares was drawn on the underside of the plates for the deposition of the honey samples as mentioned above. The plates were placed upside-down at 4°C for 24 h before being used the day after.

Honey solutions

All the honey samples were stored at room temperature. Primary honey solutions were prepared by adding 10 g of each well mixed honey to 10 mL of sterile distilled water and placed at 37°C for 30 minutes to aid mixing. To prepare secondary honey solutions, 1 mL of each primary solution was added to 1 mL of sterile distilled water. Aliquots of 100 L of each honey secondary solution were deposited at the centre of the squares drawn on the essay plates, one aliquot per square of the different essay plates. For each honey secondary solution a control plate that contained no strain culture was prepared. Plates were incubated at 37°C for 24 h.

Antimicrobial activity was determined from the estimation of the diameter of the inhibition zone produced by the honey samples, following: highest activity, diameter > 25 mm; intermediate activity, diameter ≥ 12 mm and ≤ 25 mm; lowest activity, diameter < 12 mm.

For each honey sample the experiments were repeated twice.

Statistical analysis

The differences between the antibacterial activity against *E. coli* and *S.* Infantis for each honey in results were analyzed by the Chi square test in the statistical package R (http://www.r-project.org). A critical value of $p < .000$ was considered statistically significant.

Results

The results of the assays of antibacterial activity of the 71 honeys used in this study are shown in Table I. Statistically significant differences regarding inhibition were observed for the honeys tested with the chestnut and polyfloral honey samples exhibiting the largest and highest inhibition to the two reference strains. The honey of oregano origin showed intermediate or low activity against *E. coli* and *S.* Infantis, respectively. Finally, prickly pear and erica honeys showed no antimicrobial activity against the two reference strains tested (*E. coli*: Chi-square 65.96, df 10, p = .000; *S.* Infantis: Chi-square 74.53, df 10, p = .000).

Discussion

Honey has been increasingly drawing the public's interest as alternative therapeutic remedy against a wide range of bacteria including some antibacterial-resistant species [11, 12]. In particular, in controlled susceptibility tests, most gastrointestinal bacteria are susceptible to

Tab. I. Antibacterial activity of the 71 honey samples tested against *Escherichia coli* and *Salmonella* serovar Infantis.

Type of honey	Antibacterial activity			
	N	*E. coli*	*N*	*S.* Infantis
Chestnut	10	H[1]	10	H
(N.13)	3	L[3]	1	I[2]
			2	L
Polyfloral	9	H	11	H
(N.12)	3	R[4]	1	I
Orange	7	H	11	H
(N.11)	1	I		
	3	L		
Eucalyptus	6	H	7	H
(N.9)	1	L	1	L
	2	R	1	R
Thyme	6	H	6	H
(N.9)	3	R	1	L
Spanish esparcet	4	H	3	H
(N.6)	2	R	1	L
			2	R
Citrus	3	H	2	H
(N.4)	1	R	2	R
Carob	1	H	2	H
(N.4)	3	R	2	R
Erica	1	R	1	R
(N.1)				
Oregano	1	I	1	L
(N.1)				
Prickly pear	1	R	1	R
(N.1)				

[1] = highest activity, diameter > 25 mm

[2] = intermediate activity, diameter ≥ 12 mm and ≤ 25 mm

[3] = lowest activity, diameter < 12 mm

[4] = no activity

the antimicrobial activity of manuka honey but not to artificial honey [13].
Nonetheless, it has also been suggested that other honeys, both commercially and locally produced, have equivalent activity for some, but not all, bacteria [14-16]. In the present study we have described that Sicilian locally obtained unprocessed honeys may be active against *E. coli* and *S.* Infantis. In fact, our data show that all but two (erica and prickly pear) of the 71 honey samples tested have some antibacterial action, the activity ranging from 'high' to 'low'. These results are in accordance with a previous study on the concentrations of the major 1,2-dicarbonyl compounds in Sicilian commercial honey samples from 12 different floral origins [17].
Obviously, careful should be applied in generalizing these results to all the Sicilian honeys for some main reasons. First, the relatively low number of honey samples tested. Anyway, the overall good activity showed by the majority of the honeys (chestnut, polyfloral, orange, eucalyptus, thyme and Spanish esparcet), which together account to 84.5% of the honey samples tested, could indicate a good activity of Sicilian honeys against enterobacteria.
Second, although significant inhibition of bacterial growth was noted for the majority of the honeys tested, it is doubtful whether the activity observed under experimental conditions would be clinically significant. In fact, in this study we have used an agar-diffusion assay rather than an agar dilution method that could better mimic the situation where the honey dress directly in contact with the infected mucosa [18]. For this reason, we think that further research is required to assess the correlation between the described antibacterial activity *in vitro* and the actions *in vivo* of the Sicilian honeys.
Third, our results underline that *E. coli* and *S.* Infantis showed also a certain degree of resistance to some of the honeys tested. Some bacterial species can be inhibited by low levels of osmolarity, so inhibition by honey may be due to the sugar content rather than to hydrogen peroxide or non-peroxide factors [19]. From this perspective, further experimental essays could be useful in order to standardize the reported antibacterial activities of the Sicilian honeys. In particular, the use of a reference antiseptic or the comparison with an artificial honey as a reference could be useful in distinguishing the efficacy of antibacterial factors other than the osmolarity. Anyway, there is evidence that the activity of honeys can vary greatly among different floral types [20]. Moreover, even the antibacterial activity of honeys sharing the same floral origin could greatly differ in activity depending, for example, from the storing conditions. Thus, because the antibacterial activity of honey is sensitive to light and to heat [21], differences in the observed antibacterial activity could be due to the fact that our honey samples were stored at room temperature and not in a dark refrigerator.
Finally, although the limited number of strains considered in our study their sensitivity to Sicilian honeys may partially suggest the usefulness of these honeys on treating multi-resistant *E. coli* and *Salmonella* spp. previously isolated from wastewater and clinical specimens in Sicily [8, 9]. For this reason, we think that it would be of value to further investigate the potency of these honeys with more antibiotic resistant bacterial species in future in the view of a possible clinical use.

Conclusions

In conclusion, in light of the enormous potential for application of honey in the clinical practice, it is important that research continues not only into those honeys well recognized as antimicrobial, but also into other locally produced and yet untested honeys. Although the number of antibacterial resistant strains that have been tested with honey in our study is limited and although it was not been evaluated whether or not bacteria would eventually develop resistance to honey, the sensitivity of *E. coli* and *S.* Infantis to the honeys tested may partially suggest the usefulness of the Sicilian honeys on treating multi-resistant enterobacteria. It would be of value to further investigate the potency of the Sicilian honeys with more antibiotic resistant bacterial species in future.

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