COMPARATIVE STUDY OF ANTIBACTERIAL ACTIVITY OF TWO DIFFERENT TYPES OF HONEY AGAINST *Escherichia coli, Pseudomonas aeruginosa* AND *Staphylococcus epidermidis*.

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ABSTRACT

This study aimed to investigate the antibacterial activity of two different types of honey, natural and commercial, by determination the minimum inhibitory concentration (MIC) against isolates of *Staphylococcus epidermidis, E.coli and Pseudomonas aeruginosa.* In this study, fifty eight (58) human isolates of these organisms were collected from different pathological sources from both males and females with age between (9-50) years and tested for their sensitivity to honey, a natural product that is generating renewed interest for its therapeutic application, during the period from 30/10/2009 to 1/4 /2010.

In a tube dilution method, the incidence rate of bacterial inhibition was always significantly higher when natural honey was used compared to commercial honey at the three successive dilutions tested. At the highest concentration tested (1:2 dilution) all the bacterial isolates tested where inhibited (100%) by both types of honey tested. The median MIC was significantly lower for *E.coli* than *Pseudomonas aeruginosa* and *Staphylococci epidermidis* when exposed to commercial honey. The median MIC causing bacterial inhibition and mean rank was always significantly lower for natural honey compared to commercial honey in all the three types of bacterial isolates.

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Key words: MIC of natural honey, Commercial honey, *Staphylococcus epidermidis, E.coli, Pseudomonas aeruginosa.*

INTRODUCTION

The clinical use of honey has been known since ancient times, and in more recent years it has been rediscovered as a therapy for wounds (Molan, 1998). Interest in this approach stems partly from the emergence of antibiotic-resistant pathogens. Honey has been useful in the treatment of infected surgical wounds, burn wounds, and ulcers (Zumla, 1989). Honey maintains a moist wound environment that promotes healing, and its high viscosity helps to provide a protective barrier to prevent infection. In addition, the mild acidity and low-level hydrogen peroxide release assists both tissue repair (Lusby *et al*., 2005). Many publications attest to honey's antimicrobial properties (Molan, 1992), but the mechanisms by which it acts are incompletely studied. Strong solutions of honey or sugar, and sugar pastes inhibit microbial growth because of their high osmolarity Chirife *et al.*, 1982).

The potential of honey as a topical wound dressing is now recognized by the health care community, and there continues to be a search for honeys from different sources with enhanced antibacterial activity (Venkatachalam and Thangam, 2007). Manuka honey, for example, has high antibacterial activity associated with an unidentified phytochemical component (Molan, 1992) .A study by Willix *et al*., (1992), does specify the type of honey and found that antibacterial activity was primarily due to hydrogen peroxide.

In this study, we investigated the antibacterial activity in order to determine the minimum inhibitory concentration (MIC) of two different types of honey against *Staphylococcus epidermidis, E.coli, Pseudomonas aeruginosa.*

MATERIALS AND METHODS

This study include (58) isolates of multi-drugs resistant organisms isolated from urine, ear, skin, pus, 22(37.94%) of isolates were *Staphylococcus epidermidis,* 18(31.03%) of isolates were for each *E. coli* and *Pseudomonas aeruginosa*; the patients were between (9-50) years of age, the samples collected during the period of October 2009 to the April 2010**.** The organisms were subcultured on nutrient or blood agar according to species of bacteria.

The type of honey that used was standard grade. The standard grade of honey had the following properties; Specific gravity (1-37 at 27 degrees), moisture (25% max), reducing sugar (65% min), sucrose (5% max), fructose and glucose (1.0 min), acidity (0.2% max) (SeeEfem, 1988). Two types of honeys were used a natural honey and a honey of commercial source. Their antibacterial activity was determined by tube dilution method.

Different concentrations of honey were prepared in nutrient broth to give double fold dilution of 1:2, 1:4, 1:8, 1:16, 1:32 according to Pamela (1978). One tube containing 1ml of undiluted honey was also used (Pamela, 1978). Honey were measured out and diluted in sterile deionized water to prepare a 50% stock honey solution from which further dilutions in broth could be readily prepared. Appropriate volumes of stock honey and deionized water (totaling 2ml) were dispensed aseptically into 1ml samples of sterilized nutrient broth (Oxide) to produce a dilution series between 1:2 and 1:32 (v/v) of each type of honey in broth.

Twenty-four hours old cultures of S. *epidermidis*, *E.coli*, *P. aeruginosa* were inoculated in saline. The turbidity was adjusted to 0.5 McFarland's standard, and (5μl) of culture was inoculated in the dilutions and incubated at 37^{o}C for 24 hours and were observed for growth. MIC was recorded as the lowest concentration of honey that prevented growth.

Statistical analysis

Analysis were computer aided using SPSS version 13. The statistical significance of association between type of honey and bacterial inhibition was tested by chi-square test of independence. The minimal inhibitory concentration was measured on an ordinal level (4 consecutive dilutions), therefore it was described by median and the non-parametric test of significance was used (Kruskall-Wallis and Mann-Whitney). The mean rank is a product of the test of significance used, it is useful in ordering the groups when their median seems to be almost equal.

RESULTS AND DISCUSSION

As shown in table 1, the incidence rate of bacterial inhibition was always significantly higher when natural honey was used compared to commercial honey at the 3 successive dilutions tested. At the highest concentration tested (1:2 dilution) all the bacterial isolates tested where inhibited (100%) by both types of honey tested. The incidence of inhibition obviously increases with increasing concentration (decreasing dilution) of tested honey. The same observation holds true when each type of isolated bacteria was tested separately, tables 2 to 4.

These tables showed that the effect of natural honey was more than commercial type. *E. coli* was more sensitive to both types of honey used in this study than *P. aeruginosa* and *S. epidermidis* ,these results agreed to Wilkinson and Cavanagh (2005) who found that. *E. coli* was more susceptible to inhibition by the honeys used when compared with *P. aeruginosa* , and Adebolu (2005) who explain that the inhibitory effect was highest on *E. coli* and followed by *Salmonella enterocolitis* and *Shiglla dysenteriae*. The reason for this exception is not clear because Gram negative bacteria, of which this organism is one, have been reported to be more sensitive to action of honey than Gram-positive bacteria (El-

Sukhon *et al.*, 1994). Honey works quicker than many antibiotics because it is easily absorbed in the blood stream **(**Singh and Suryanarayan, 1988).

Table 1. *The difference between natural and commercial honey in the incidence rate of bacterial inhibition at three dilutions when tested with all three types of bacterial isolates.*

Overall	**Honey dilution**					
	1:4		**1:8**		**1:16**	
	N	**%**	**N**	**%**	**N**	**%**
Natural honey (n=58)	43	74.1	19	32.8	5	8.6
Commercial honey (n=58)	23	39.7	2	3.4	0	0.0
P (Chi-square)	**<0.001**		**<0.001**		**0.022**	

Note: All bacterial isolates were inhibited by the highest concentration (1:2 dilution) of both honey types.

Table 2. *The difference between natural and commercial honey in the incidence rate of bacterial inhibition at three dilutions when tested with E.coli.*

E. coli	**Honey dilution**					
	1: 4		**1:8**		**1:16**	
	N	**%**	**N**	**%**	**N**	**%**
Natural honey (n=18)	16	88.9	8	44.4	4	22.2
Commercial honey (n=18)	12	66.7	2	11.1	0	0.0
P (Chi-square)	**0.11[NS]**		**0.026**		**0.034**	

Note: All bacterial isolates were inhibited by the highest concentration (1:2 dilution) of both honey types.

Table 3. *The difference between natural and commercial honey in the incidence rate of bacterial inhibition at three dilutions when tested with Pseudomonas aeruginosa***.**

Pseudomonas aeruginosa	**Honey dilution**					
	1:4		**1:8**		**1:16**	
	N	**%**	**N**	**%**	**N**	**%**
Natural honey (n=18)	12	66.7	4	22.2	0	0.0
Commercial honey (n=18)	4	22.2	0	0.0	0	0.0
P (Chi-square)	**0.007**		**0.034**		******	

Note: All bacterial isolates were inhibited by the highest concentration (1:2 dilution) of both honey types.

Table 4. *The difference between natural and commercial honey in the incidence rate of bacterial inhibition at three dilutions when tested with Staphylococcus epidermidis.*

Staphylococcus epidermidis	**Honey dilution**					
	1:4		**1:8**		**1:16**	
	N	**%**	**N**	**%**	**N**	**%**
Natural honey (n=22)	15	68.2	7	31.8	1	4.5
Commercial honey (n=22)	7	31.8	0	0.0	0	0.0
P (Chi-square)	**0.016**		**0.004**		**0.31[NS]**	

Note: All bacterial isolates were inhibited by the highest concentration (1:2 dilution) of both honey types.

As shown in table 5, the median MIC was significantly lower for *E.coli* than Pseudomonas and Staphylococci when exposed to commercial honey. It was also obviously lower for *E.coli* than Pseudomonas and Staphylococci when exposed to natural honey, but the difference in MIC fail short of statistical significance possibly because of small sample size. In general, whatever was the type of honey the median MIC was smallest with *E.coli* (0.25), followed by Staphylococci (0.375) and highest for Pseudomonas (0.5). In this study, both natural honey and commercially processed therapeutic honey have been shown antibacterial activity against three bacterial species.

P. aeruginosa is notoriously resistant to antimicrobial therapy it is protected from host immune effectors and can grow to sufficient levels to elaborate toxins that break down host factors. Hence, Pseudomonas is a major problem for chronic wounds and contributes to delay in healing (Scmidtchen *et al.,* 2003).

Various researchers have shown that honey exerts an antibacterial activity against various organisms, including both gram-positive and gram-negative bacteria. The antibacterial activity of honey is mainly due to inhibiters in honey. These inhibiters are hydrogen peroxide, fiavinoids, and phenolic acids, plus many other unidentified inhibiters. A number of reasons for this have been suggested: shrinkage disruption of the bacterial cell wall due to the osmotic effect of the sugar content; induction of an unfavorable environment with low water activity (Subrahmanyam *et al.,* 2001)**.**

The median MIC causing bacterial inhibition and mean rank was always significantly lower for natural honey compared to commercial honey in all the three types of bacterial isolates, table 6.

The variations recorded in the antibacterial activity of two types of honey tested were consistent with the reports of Jeddar *et al.* (1985) and Molan *et al.* (1988) and have been attributed to delayed levels of hydrogen peroxide/ thermal stability of the glucose oxidase enzyme, non-peroxide factors, and the plant/floral source (Willix *et al.,* 1999 ; Moudoi *et al.,* 2005). The development of honey in form of a rubbery gel that can be moulded to conform to any shape will further increase the practicality of use with medical devices beyond that with the honey-impregnated dressings currently available. It remains for further clinical evaluation to be tried (Molan and Betts, 2004).

Table 5. *Difference between 3 types of bacteria in mean rank of minimal inhibitory concentration of each type of honey.*

Concentration of honey used	**Type of bacterial isolate tested**				
Type of honey used	***E. coli***	***Pseudomonas aeruginosa***	***Staphylococcus epidermidis***	**P (Kruskal-Wallis)**	**Group Total**
Commercial				**0.01**	
Range	(0.125 - 0.5)	(0.25 - 0.5)	(0.25 - 0.5)		(0.125 - 0.5)
Median	0.25	0.5	0.5		0.5
N	18	18	22		58
Mean rank	**21.06**	**34.78**	**32.09**		
Natural honey				**0.11[NS]**	
Range	(0.0625 - 0.5)	(0.125 - 0.5)	(0.0625 - 0.5)		(0.0625 - 0.5)
Median	0.25	0.25	0.25		0.25
N	18	18	22		58
Mean rank	**23.11**	**33.78**	**31.23**		
Group Total				**0.006**	
Range	(0.0625 - 0.5)	(0.125 - 0.5)	(0.0625 - 0.5)		(0.0625 - 0.5)
Median	0.25	0.5	0.375		0.25
N	36	36	44		116
Mean rank	**44.92**	**67**	**62.66**		

Table 6. *Difference between natural and commercial honey in mean rank of minimal inhibitory concentration, stratified by type of bacteria tested.*

Mean rank	***E. coli***	***Pseudomonas aeruginosa***	***Staphylococcus epidermidis***	**Overall**
Natural honey	14.5	14.06	17.39	45.39
Commercial honey	22.5	22.94	27.61	71.61
P (Mann-Whitney)	**0.014**	**0.004**	**0.004**	**<0.001**

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دراسة مقارنة للنشاطِ المضادّ للبكتيريا لنوعين مختلفينِ مِنْ العسلِ ضدّ الايشيريشيا القولونية، الزائفة الزنجارية والمكورات العنقودية الجلدية .

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الخلاصة

هذه الدراسةِ تهدف إلى اختبار نشاط نوعين مختلفين من العسل (الطبيعي والتجاري) ، كمضاد حيوي عن طريق تحديد التركيز المثبط الأدنى ضد بكتريا *Staphylococcus epidermidis, E.coli, Pseudomonas aeruginosa*. في هذه الدراسةِ، جُمِعتْ ثمانية وخمسون (58) عزلة بكتيريا مِنْ المصادرِ المرضية المختلفةِ من كلا الجنسين تتراوح أعمارهم من (9-50) سنة خلال الفترة من 2009/10/30ولغاية 2010/4/1. وتم فحص حساسية العزلات البكتيرية للعسل المنتج الطبيعي و التجاري الذي أعيد الاهتمام به في التطبيقات العلاجية.

تمت طريقة التخفيف باستخدام الأنابيب المستخدمة في هذه الدراسة، حيث خفف كلا النوعين من العسل من 2:1 إلى 32:1. أظهرت النتائج إن نسبة التثبيط البكتيري كَانتْ معنوية دائماً عند استعمال العسلَ طبيعيَ مُقَارَنة بالعسلِ التجاريِ في التخفيفاتِ المتعاقبةِ الثلاثة . عند أعلى تركيز (1:2 تخفيف) كُلّ العزلات البكتيرية قد ثبّط نموها (100 %) بكلا أنواع العسلِ المستخدمة. متوسط التركيز المثبط الأدنى الذي يسبب تثبيط نمو البكتيريا وقيمة mean rank قليلة جدا للعسل الطبيعي مقارنة بالعسل التجاري لكل من الأنواع الثلاثة للعزلات البكتيرية وذلك لمكوناته الطبيعية النقية.