Antimicrobial effect of bee honey on some pathogenic bacteria isolated from infected wounds in comparison to commonly used antibiotics

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Abstract

Honey produced by the honey bees is one of the ancient traditional medicines used for treatment and prevention of various illnesses .In this study the antimicrobial effect of bee honey on organisms isolated from infected wounds in comparison with commonly used antibiotics for the treatment of infection was evaluated . (50) swabs from patients with infected wounds were cultured. The most frequently isolated organism was *Psedomonas aeruginosa* representing (44%) of the isolates followed by coagulase – positive *Staphylococci* (30%) . The susceptibility of the isolated organisms to honey , antibiotics and combination of both was studied .The mean inhibition zone produced by honey when applied to isolated gram–negative bacteria (*Psedomonas aeruginosa*) and isolated gram–positive bacteria (coagulase positive S*taphylococci*) was significantly higher than that of the antibiotic used ($P<0.005$), and when combination of both honey and antibiotic was used , it showed highly significant increased sensitivity than that of honey or antibiotic alone ($P<0.001$).

Keywords :- antibiotic. Antimicrobial ,bacteria, wound ,honey.

Introduction :

The use of traditional and herbal medicine to treat infection was practiced since the origin of mankind, and in the past it was propably the only available method to be used for that [1].Various plants and their extracts have already been in use for the treatment requiring antimicrobial activity and one of the popular natural antimicrobial substances described in the ancient medicine is honey[2].

Honey is the natural sweet substance from nectar or bossom or from the secretions of the living parts or excretions of plants which the honey bees collect and store in the honey [3], it was widely used in traditional medicine but it's use in modern medicine is limited because of the lack of scientific support [4]. Among it's several uses , honey is used for the treatment of many infections , and also used effectively as wound dressing including surgical wounds , burns , and skin ulcers . It has been reported that honey speed up the growth of new tissues and so help to heal the wound, reduces pain and odour quickly[5].It has both bactreiocidal and bacteriostatic effect against various types of gram positive and gram negative bacteria such as *Staphylococcus .aureus* , *psedomonas aeruginosa* , *Escherichia .coli* , *Staphylococcus . pyogenus* and *Salmonella typhi* [6]. This antibacterial effect is dependant on the concentration of the honey used and the nature of the bacteria isolated [7] and it has been attributed to it's high osmotic effect ,high acidic mature (pH3.2 - 4.5), hydrogen penoxide concentration and it's phytochemical nature .Other constituents of honey include proteins , carbohyrates , vitamins , aminoacide , peroxide , amylase , fatyacids , phenol , and other compounds[8 and 9] .

As it is well known that infection is the most serious complication of wounds and burns accounted for 50 – 60 % of deaths due to sepsis in these patients despite improvement in antimicrobial therapy , therefore this study was planned to evaluate the invitro antimicrobial

effect of bee honey on pathogenic bacteria isolated from infected wounds compared with the effect of commonly used antibiotics in the treatment of infections .

Materials and Methods:

Honey sample was collected from the Bee Keeping unit at the college of Agriculture, University of Baghdad. The honey sample was first filtered with sterile mesh to remove debris and to check it's microbial purity it was cultured on blood agar plate and incubated overnight at 36 – 37c°, then honey discs were prepared by using dry sterile filter paper having similar thickness and size (6mm), to the antibiotic disc used and then stored at 2-8c° until use.

The clinical isolates samples used in the study was taken from (50) swabs from patients with infected wounds (surgical wound, burns and ulcers) admitted to AL-Samawa General hospital and were cultured on blood agar plates and incubated for overnight , Morphological identical colonies from these growths were picked up with an inoculating loop and suspended in 3-4 ml . of nutrient broth and incubated for 2-3 hours at 36 – 37c°, then diluted with sterile normal saline to a turbidity that matches 0.5 McFarland standard (10^6 colony for ming unit CFU/ml.), and further dilution to 1:100 in sterile nutrient broth to set an inoculum density of 1×10^4CFU / ml. which was used for this test[10] .

Six antibiotic discs were selected (oxoid), four of them were used for both gram–negative and gram–positive bacteria including (impenem (IPM)) , ciprofloxacin (CIP) , amoxicillin / clavulinic acid (AMC) , and ceftriaxone (CRO), one specific disc for gram negative bacteria (amikacin (AK)) and one specific disc for gram positive bacteria (vancomycin (VA)) .

The isolated pathogens were inoculated on Muller-Hinton agar . Each agar plate was divided by marker pen in to two halves , the antibiotic discs plated on one side and antibiotic discs immersed in honey was plated opposite the same antibiotic disc on the other side . At the center of the agar, sterile filter paper disc immersed in honey was placed . Plates then incubated at 37c° for 24hr and the diameter of the clear inhibition zone around each disc was measured and evaluated(11) .

Results :

(50) Swabs culture from infected wounds were cultured and showed that *psedomonas aeruginosa* was the most frequently isolated pathogen representing 22 (44%) of the isolate followed by coagulase – positive staphylococci (15 i.e 30%) then *E.coli* (8 i.e 16%) and *Klebsiella* was the least (5 i.e 10 %). Table1, fig1 demonstrate the effect of antibiotic, honey and combination of both on isolated gram–negative organisms. The isolated organisms were sensitive to IPM (82.4 %), CIP (32.5%) AMC (29.6%), CRO (37.1%) and AK (100%), while 85.2% of the isolates were sensitive to honey . The mean inhibition zone of honey (18.0 mm) was significantly higher than that of AMC and CRO (P_1=0.005) , while there was no significant increase on that of AK and CIP (P>0.05) and it was similar to that of IPM .

When honey was added to the antibiotic disc , there was significant increase in the mean inhibition zone in respect to honey for IPM & AK (P_2 < 0.001), and for CIP , AMC ,and CRO (P_2=0.005) , also there was significant increase in the mean inhibition zone of antibiotic alone , CIP , AMC ,CRO , and AK (P_3 < 0.001 for each) .While the increase was significant for IPM (P_3=0.005).

Table(2) fig(2) shows the effect of antibiotic, honey and combination of both on isolated coagulase positive *staphylococci*. All the isolates were methicillin resistant *staphylococci,* but they were sensitive to IPM (86.6 %) , CIP (53.3 %), AMC (40 %), CRO(40%) and VA (73.3 %) while all the isolated *staphylococci* (100%) were sensitive to honey . The mean inhibition zone of honey was significantly higher than that produced by antibiotics CIP, CRO and VA (p_1<0.005), while there was no significant increase in the inhibitory zone produced by honey compared to that of IPM (P>0.05) and the mean inhibition zone of honey is significantly lower than that of AMC (P_1>0.05). When honey was added to the antibiotic discs, a significant increase in the inhibition zone was noticed than that of honey alone in IPM, CIP, AMC, CRO and VA (P_2=0.005) and also shows significant increase in the inhibition zone of antibiotics mixed with honey with respect to honey in CIP , VA (P_3 <

0.001)and in IPM , AMC , CRO ($P_3 > 0.05$) ($P<0.001$).
than that produced by antibiotic alone

Table .1. Effect of antibiotic, bee honey and combination of both on isolated gram negative bacilli.

	Antibiotic			Bee honey			Bee honey + Antibiotic		
	Sensitivity %	Mean inhibition zone (mm) ± SE	P1	Sensitivity %	Mean inhibition zone (mm) ± SE	P2	Sensitivity%	Mean inhibition zone (mm) ± SE	P3
IPM	82.40	18.4 ± 0.151	> 0.05	85.2	18.0 ± 1.22	< 0.001	100	21.3 ± 1.305	0.005
CIP	32.50	18.3 ±0.126	> 0.05	85.2	18.0 ± 1.22	0.005	84.5	20.5 ± 1.334	< 0.001
AMC	29.6	16.1 ± 0.688	0.005	85.2	18.0 ± 1.22	0.005	90.2	19.9 ± 1.238	< 0.001
CRO	37.1	15.2 ± 0.633	0.005	85.2	18.0 ± 1.22	0.005	94.2	19.6 ± 1.840	<0.001
AK	100	18.1 ± 0.913	> 0.05	85.2	18.0 ± 1.22	< 0.001	100	22.4 ± 1.093	< 0.001

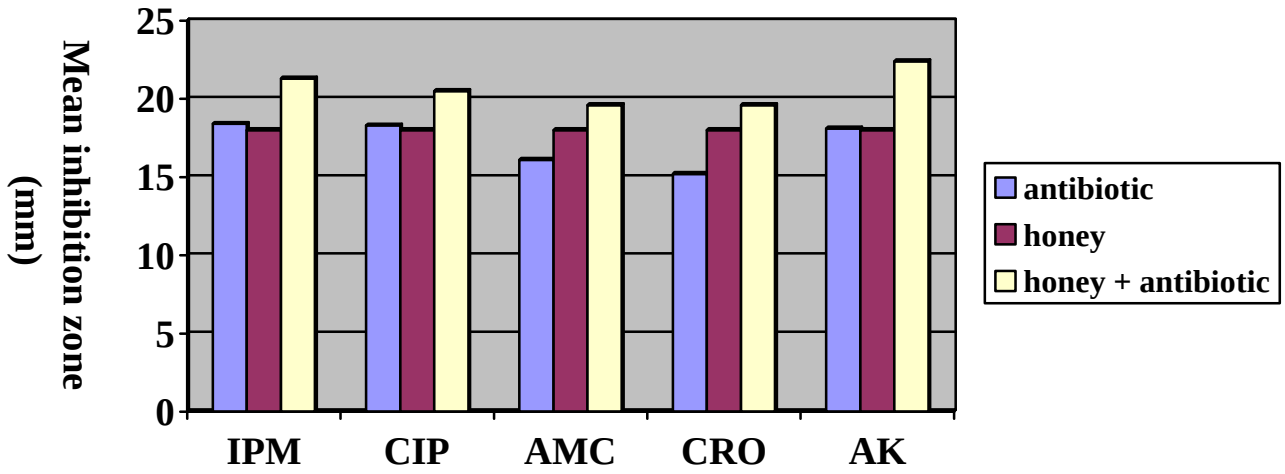

Figure1. Mean inhibition zone of antibiotic, honey and combination of both on isolated gram - negative bacilli.

Table . 2. Effect of antibiotic, bee honey and combination of both on isolated gram positive *Staphylococcus*

	Antibiotic			Bee honey		Bee honey + Antibiotic			
	Sensitivity %	Mean inhibition zone (mm) ± SE	P1	Sensitivity %	Mean inhibition zone (mm) ± SE	P2	Sensitivity%	Mean inhibition zone (mm) ± SE	P3
IPM	86.6	17.4 ± 0.947	> 0.05	100	18.1 ± 0.792	0.005	100	20.3 ± 1.114	> 0.05
CIP	53.3	16.7 ± 1.080	< 0.005	100	18.1 ± 0.792	0.005	100	21.1 ± 2.222	< 0.001
AMC	40.0	19.8 ± 1.846	> 0.05	100	18.1 ± 0.792	0.005	100	22.3 ± 0.975	> 0.05
CRO	40.0	16.2 ± 1.423	< 0.005	100	18.1 ± 0.792	0.005	100	22.7 ± 1.254	> 0.05
VA	73.3	15.4 ± 1.005	< 0.005	100	18.1 ± 0.792	0.005	100	21.3 ± 1.326	< 0.001

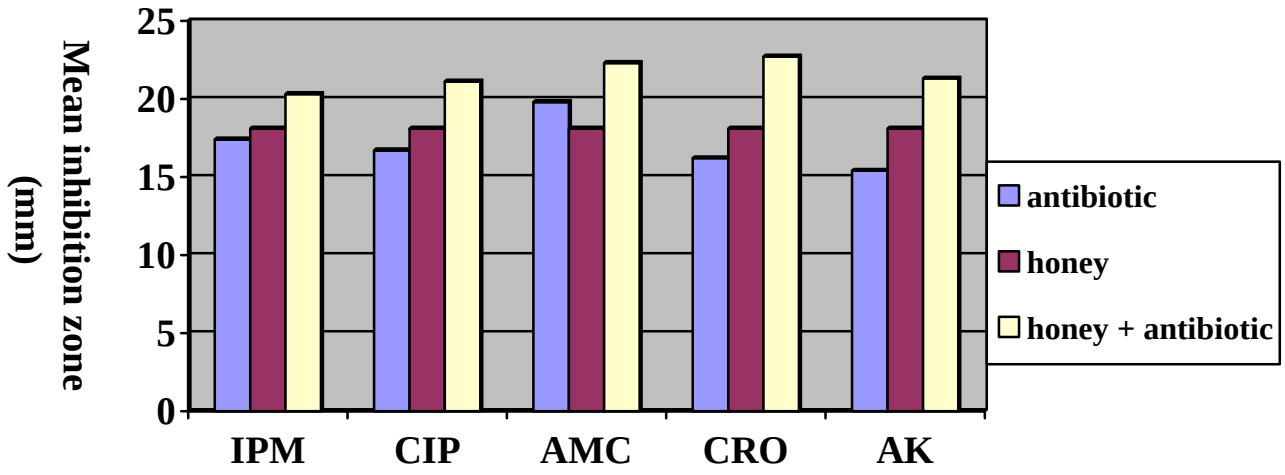

Figure 2. Mean inhibition zone of antibiotic, honey and combination of both on isolated coagulse- positive *staphylococci* .

Discussion:

This study shows that *pseudomonas aeruginosa* was the most frequently isolated organism from infected wounds and burns (44%) , a result that is consistent with other studies like that of Douglas et al [11 and 12] in which he stated that *P. aeruginosa* continued to be serious cause of infection and septic mortalily in burn patients , also Agnihotri ,et al [13], who stated that *P. aeruginosa* was the commonest isolate from infected wounds followed by *Staphylococcus aureus* .

The inhibitory effect of honey on the growth of isolated gram–negative bacteria (*Pseudomonas aeruginosa*) was evident , as the mean inhibition zone of honey was significantly higher than that of AMC and CRO (P_1=0.0005) ,

but there was no significant increase when compared with AK($P>0.05$). This effect was attributed to the presence of hydrogen peroxide (H_2O_2) in bee honey , however the powerful antioxidant and low pH that inhibit the bacterial growth[14] when honey was added to the antibiotic discs , there was high synergistic effect as the mean inhibition zone was significantly higher than that of AK ($P<0.001$) , this result is similar to that stated by Karayil et al [15].who showed in vitro synergistic of honey when added to antibiotic especially aminoglycoside on resistant organisms particularly *Pseudomonas aeruginosa* , and this observation is important in prevention of the development of resistant strains of these organisms against the commonly used antibiotics .

Regarding the effect of honey on isolated coagulase positive staphylococci which is considered to be the commonest gram–positive coccus affecting wounds , this study shows significant increase in the mean inhibition zone of honey compared to that of antibiotics CIP ,CRO and VA ($P<0.005$) and when used in combination with antibiotics it shows clear synergistic effect on methicillin resistant Staphylococci ($P<0.001$) and this is consistent with the fact that combination of antibiotics may be used effectively against resistant bacterial strains [16].

Conclusion :

Honey is famous rediscovered remedy which is cheap and nontoxic .It showed high inhibitory effect on the growth of isolated gram–negative and gram–positive bacteria and when used in combination with antibiotics it shows good synergistic effect on resistant bacteria.

However, Pharmacological standardization and clinical evaluation of the effect of honey is necessary before using it as preventive or curative measure.

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تاثير العسل كمضاد ضد البكتيريا المعزولة من الجروح الخمجة ومقارنته مع فعل المضادات الحياتية

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الخلاصة

لقد استعمل العسل المنتج من قبل نحل العسل في الطب التقليدي القديم لعلاج الامراض المختلفة والوقاية منها . صمم هذا البحث لدراسة التاثير التثبيطي لعسل النحل على انواع مختلفة من البكتريا المرضية التي تم عزلها من (50) عينة ماخذوة من جروح متقيحة ومقارنة ذلك مع التاثير التثبيطي لمضادات حيوية شائعة الاستعمال .

اظهرت الدراسة بان بكتريا *Psedomonas aeruginosa* هي اكثر البكتريا المعزولة من العينات المفحوصة حيث مثلت نسبة (44%) من عدد العينات ، تليها جرثومة *Staphylococci* S coagulase positive وبنسبة (30%) ، ثم اجري فحص الحساسية لكل من مادة العسل ، المضادات الحيوية وخليط العسل مع المضادات الحيوية على الاطباق الزرعية ، وعند قياس قطر منطقة التثبيط لكل حالة وجد بان قطر منطقة التثبيط المحدثة باستعمال مادة العسل فقط على الاطكباق الزرعية لجرثومتي *Psedomonas aeruginosa*،*Staphylococci* S coagulase positive هي اكبر من قطر منطقة التثبيط عند استعمال المضادات الحيوية فقط وبفارق معنوي ($P<0.005$) ، وعند استعمال خليط العسل مع المضادات الحيوية ظهر بان التاثير التثبيطي لهذا الخليط اكبر بكثير من التاثير لمادة العسل او المضادات الحيوية لوحدهما وبفارق معنوي كبير ($P<0.001$) .