Research Article

Study of antimicrobial effects of honey in comparison to the antibiotics on the microbes isolated from infected burn wounds

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Citation

Quart ul Ain Zahra , Samina Qamer, Zahid Mahmood Nagra, Tamsil Ahsan, Raza Hassan Ch and Nisar Akber Khan
Study of antimicrobial effects of honey in comparison to the antibiotics on the microbes isolated from infected burn wounds. Pure and Applied Biology. Vol. 5, Issue 4, pp883-888. <http://dx.doi.org/10.19045/bspab.2016.50111>

Received: 14/04/2016

Revised: 03/08/2016

Accepted: 10/08/2016

Online First: 22/08/2016

Abstract

A study was conducted to evaluate the antimicrobial effect of fresh honey produced by *Apis mellifera* on the bacterial species isolated from infested burns wounds in order to compare it firstly to the antibiotics which are used for the treatment of burn infection. Secondly to assess the possible synergistic effects of bee honey when mixed to antibiotic. Blood specimens were collected with the help of cultural swap from 2-4 weeks old 30 hospitalized patients with burn infection. These blood samples were cultured on different media and four major bacterial strains including *E-coli*, *Klebsiella* spp, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were isolated and antibiotic sensitivity pattern was measured against these microorganisms by using following three types of antibiotics i.e, Amikacin (AK), Zinacef (CXM), Linzolid (LZD). The result shows that honey has a significant inhibitory growth effects on *Pseudomonas aeruginosa*'s growth (61%) followed by *E. coli* (55%), *Staphylococcus aureus* (56%), and *Klebsiella* spp (25%) as compared to the antibiotics alone.

Keywords: Honey; Microbes; Zinacef; Burn wounds

Introduction

Honey is an antique remedy for infected wounds treatment, which has in recent times been 'rediscovered' by the medical profession, particularly where predictable modern therapeutic managers are failing [1]. Recent evidence also support that honey may actively stimulate healing. In laboratory

investigations, honey's antimicrobial action has been found against broad spectrum of bacteria and fungi. *Pseudomonas aeruginosa*, a notorious organism and showed remarkable resistance to antimicrobial compounds [2]. The use of honey to treat infected wounds is 2000 years old even before the discovery of bacteria as

a cause of many infections. Honey's inhibitory effect has been reported on about 60 species of bacteria comprising aerobic, anaerobic, gram-positives and gram-negatives [3]. Its antimicrobial activity is due to the osmotic effect, acidity, production of hydrogen peroxide and phytochemical factors in it [4]. Antibacterial activity of honey fluctuates between different types of honey [2]. Honey is a complex product; it has low water activity and acidity, and high sugar content but this does not contribute significantly to the antibacterial activity of those honeys recommended for clinical use. Some of these honeys become more potent upon dilution due to production of hydrogen peroxide generated by the action of glucose oxidase deposited in honey by bees. Addition of catalase to diluted honeys of this type destroys the activity of hydrogen peroxide and reduces or eliminates antibacterial activity. Antibiotics are those molecules that stop microorganisms (both bacteria and fungi) from growing or killing them completely. There are already several non-antibiotic approaches to the treatment and prevention of infection including the use of honey. The sensitivity of organisms to different antibiotics varied depending on the isolate cultured. The present study was designed to have an idea about quick healing of burn wounds either with the use of honey or antibiotics or both.

Materials and methods

The study was carried out on 30 burn patients, according to the method published [6]. Three Antibiotics viz Linozolid (LZD-

30 μ g), Zinacef (CXM-30 μ g) and Amikacin (AK-30 μ g) were selected. The oozing blood samples were taken from the wounds of 2-4 week old burn patients in Burns Unit of Allied Hospital/Punjab Medical College, Faisalabad (Pakistan) with the help of culture sterilized swab (Figure 1a & b).

Bacterial growth was observed in all samples. Colonies differing in color, shape, size were sub cultured on different agar media such as CLED agar (cysteine lactose electrolyte deficient), blood agar, SS (*Salmonella Shiegella*) agar, MacConkey agar and incubated again for 24 hours at 37 °C. Bacterial colonies were characterized by their growth on different media, hemolysis, lactose fermenting and non- lactose fermenting, morphology and various biochemical tests including Gram's staining, catalase, coagulase, oxidase, voges-proskauer, indole test, methyl red, motility, carbohydrate utilization such as manitol, glucose, lactose. For identification purpose, characteristics of the isolates were compared using a scheme of known taxonomy [7]. *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella spp*s and *E-coli* were identified by biochemical tests. Fresh honey sample of *Apis mellifera* was taken from beekeeper. A sterile mesh was used to filter and remove residues. As a control, in order to check the microbial purity, the honey sample was cultured on blood and CLED agar plates and then were incubated overnight (37 °C). No bacterial growth was observed (Figure 2).

Figure 1a. Cultural swab

Figure 1b. Burn wound

Figure 2. Pure honey cultured on blood and CLED agar (No growth observed)

Sensitivity

Nutrient broth was prepared and stored in culture plates. Bacterial cultures were pasted on each plate. Divide the plate into 2 halves. On one side of the plate, placed antibiotics discs (Amikacin (AK 30 μ g), Zinacef (CXM 30 μ g) and Linzolid (LZD 30 μ g) with the help of sterilized forceps and on the other side (half) of the plate antibiotics along with 50 μ l honey were placed (just opposite to other half in sequence). On the middle of the plate, a filter paper dipped with honey was placed followed by incubation for 24 hours

at 37°C. Sensitivity of different bacterial strains for honey was measured in such a way that bacterial cultures were pasted on nutrient broth and then put up 50 μ l honey discs (soaked and air dried on a filter paper) with the help of forceps (Figure 3). After that, inhibition zones (cm) were noted on both sides of the plate (antibiotic disc, antibiotic + 50 μ l honey and 50 μ l honey (*Apis mellifera*) alone. The data was subjected to statistical analysis in which different physiochemical parameters were compared by ANOVA [8].

Figure 3. Sensitivity plate. (A) Antibiotics (A+H) Antibiotics + Honey with a filter paper immersed in honey in the centre of the plate

Results and discussion

Bacterial species

Biochemical tests showed that *Klebsiella* spp. *E.coli* and *Pseudomonas aeruginosa* were gram negative pathogens while *Staphylococcus aureus* is gram positive. *Pseudomonas aeruginosa* is reported as the most remarkable bacteria among burn patients which are resistant to antimicrobial complexes [2]. In this study *E. coli* (87%) and *Pseudomonas aeruginosa* (80%) were isolated in high percentage followed by *Staphylococcus aureus* (77%) and *Klebsiella* spp (57%). Same microorganisms are used to investigate the honey antimicrobial activity by others [9].

Sensitivity

Sensitivity was calculated by measuring zone of inhibitions produced by isolated microbes from burn wounds against antibiotics and honey (Table.1). *Klebsiella* spp. was isolated on blood agar (Fig: 4) and was found to be more sensitive (14% for AK, 10% for CXM and 20% for LZD) when honey mixed with antibiotics (50µl honey/30µg antibiotics) as compared to antibiotics (30µg disc) only (11% for AK, 6% for CXM and 14% for LZD) while it was 25% sensitive for 50µl pure honey (Fig.2). *E-coli* were isolated on CLED agar. It was 7%, 3% and 8% sensitive against antibiotics alone while 9%, 5% and 13% with antibiotics (AK, CXM and LZD) and honey mixture. However, pure honey inhibited 55% of *E. coli* to propagate. Likewise, *Pseudomonas aeruginosa* development was prevented 8%, 2% and 8% by antibiotics (AK, CXM and LZD) only, 10%, 3% and 8% with mixture of honey and antibiotics and 61% with pure honey. Similarly, *Staphylococcus aureus* was isolated on SS agar and antibiotics (AK, CXM and LZD) prevented only 7%, 4% and 10% of its growth, respectively, 0%, 6% and 10% by the antibiotics when mixed with 50µl honey. Whereas, same amount of pure

honey prohibited 56% of *Staphylococcus aureus* to grow. CXM proved to be least effective antibiotic compared to AK and LZD for all four isolated bacterial species under clinical conditions. LZD had maximum synergistic effect of added honey to the antibiotic discs followed by AK and CXM on isolated microbes. A significant increase in growth sensitivity of gram-negative isolates has found when mixed with honey as compared to either antibiotic discs alone or with honey only [6]. According to a study [10] *Staphylococcus epidermidis* was most sensitive to amikacin. Further a specified noteworthy increased in inhibition zones of antibiotics compounds mixed with honey in comparison to inhibition areas of antibiotics only and same is examined when undiluted pure honey is added to the antibiotics during present study [9]. In another research synergistic effect of honey with antibiotics in has been noticed particularly on *Pseudomonas aeruginosa*, multidrug-resistant microbes and among the commonest bacterium that is the basic cause of infection in burn wounds [12]. The antimicrobial activity of honey on *E.coli*, *Pseudomonas aeruginosa* and *Staphylococcus aureus* is also investigated by [13].

Antimicrobial action of antibiotics and honey on infected burns

Present study indicates that pure honey could be a significant treatment for infected burns as it inhibits *Pseudomonas aeruginosa*'s growth (61%) followed by *E.coli* (55%), *Staphylococcus aureus* (56%), and *Klebsiella* spp (25%). It was observed [14] that honey is capable of eliminating malodors from wounds, eradicating antibiotic-resistant strains of bacteria from wounds and acting as an effective prophylactic agent honey has the potential to be an effective treatment option for burns infected or at risk of infection with *P. aeruginosa* [15]. Commercial

antibacterial honeys, can inhibit *E. coli* and *P. aeruginosa* [16]. Honey is suggested as an effective natural item for consumption in

overcoming the widespread antibiotic resistance of *P. aeruginosa* which is close to the present findings [16].

Table 1. Measurement of inhibition zones (cm) with antibiotics, mixture of honey+ antibiotics and pure honey

S. No	Antibiotics used	Means of inhibition zones (cm) of four bacterial Species			
		<i>Klebsiella spp</i>	<i>E.coli</i>	<i>Pseudomonas aeruginosa</i>	<i>Staphylococcus aureus</i>
1	AK	0.49±0.14	0.51±0.55	0.59±0.52	0.52±0.46
2	AK + Honey	0.62±0.3	0.62±0.3	0.71±0.48	0.82±0.52
3	CXM	0.28±0.31	0.2±0.2	0.13±0.14	0.34±0.43
4	CXM+ Honey	0.43±0.34	0.38±0.25	0.21±0.15	0.48±0.4
5	LZD	0.63±0.4	0.57±0.37	0.54±0.38	0.59±0.39
6	LZD+ Honey	0.92±0.5	0.87±0.46	0.58±0.40	0.81±0.43
7	Honey	*1.13±0.3	*1.53±0.44	*1.4±0.53	*1.46±0.32

Abbreviations: *AK=Amikacin, **CXM=Zinacef, ***LZD=Linozolid, each disc contains 30µg of antibiotic. P*≤0.05, P**≤0.05, P***≤0.05

Figure 4. Sensitivity of *Klebsiella spp.* against honey (H)

Conclusion and recommendations

The results of this work support that microbial resistance in infected burn wounds is becoming a grave threat and the honey possibly, could represent a reasonable complementary or alternative cure which is cheaply available in all places and is non-toxic. It inhibited the growth of both, gram-negative and positive pathogens. It has an effect which is more bacteriostatic and bactericidal in comparison to certain

antibiotics against many wound infecting microorganisms.

Authors' contributions

Conceived and designed the experiments: S Qamer Performed the experiments: QUA Zahra Analyzed the data: QUA Zahra & S Qamer, Contributed reagents/ materials/ analysis tools: ZM Nagra, T Ahsan, RH Ch & NA khan, Wrote the paper: S Qamer.

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