Original Article

COMPARISON (IN-VITRO) OF BEE HONEY WITH ANTIBIOTICS ON MICROORGANISMS ISOLATED FROM INFECTED BURN WOUNDS

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ABSTRACT

OBJECTIVE:
To compare the effectiveness of honey with antibiotics on microorganisms isolated from burn wound infections.

STUDY DESIGN: experimental study.

PLACE AND DURATION:

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METHODOLOGY:

The blood/pus specimen collected from fifty burn patients in Dept. of Plastic surgery and Burns,Allied Hospital/Punjab Medical College Faisalabad(from Jan. 2012 to Jul. 2012) were cultured and isolated on different media types. Isolates were then identified up-to specie level by different biochemical tests. An antibiotic sensitivity pattern against five most common isolates identified was measured by using pure honey (*Apis mellifera*) and antibiotics including Amikacin(AK), Levofloxacin(LEV), Meropenem(MER), Tazobactem(TAZ).

RESULTS:

Antibiotic sensitivity results showed that *Streptococcus spp*. gave 26%, 17%, 13%, 4% sensitivity against AK, LEV, MER and TAZ respectively and 40% against honey. *S. aureus* showed 24%, 18%, 18%, 6% sensitivity against AK, LEV, MER and TAZ respectively, while it was 34% sensitive against honey. Sensitivity showed by *E. coli* was 28% for AK, 13% LEV, 9% MER, 6% TAZ and 44% for honey. *Klebsiella spp*. growth was affected 28%, 17%, 10%, 7% against AK, LEV, MER and TAZ respectively, where as 38% against pure undiluted honey. AK (23%), LEV (15%), MER (12%), TAZ (8%) prevented *P. aeruginosa* growth as compared to (42%) honey.

KEY WORDS: Antimicrobial activity, Honey, Antibiotics, Burn Infections.

INTRODUCTION:

Burn wound can be defined as a tissue damage which may be caused by the agents such as electricity, heat, nuclear radiation etc[1]. A variety of microorganisms which infect a burn wound may be bacteria or fungi[2]. Gram positive

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microorganisms most commonly infecting a wound or burn are *Streptococcus* and *Staphylococcus*. *Pseudomonas aeruginosa*, *E. coli, Proteus* and *Klebsiella* are the gram negative pathogens[3]. Antibacterial agents are very important to reduce the ever-increasing burden of the infections but the efficacy of the antibiotics has been decreased rapidly as the resistant microbes grow and multiply. Bacterial resistance thus developed becomes a grave risk to the public-health[4,5]. Unconventional antimicrobial treatment methods are needed urgently which suggested the re-evaluation of prehistoric curative remedies including plants and their products such as honey[6,7]. Honey is a natural stuff produced after the nectar and sugary deposits are gathered from different plant sources, modified by honeybees belonging to the genera Apis and Meliponini and is then stored in honeycombs[8,9]. Antimicrobial action of honey has been investigated in a number of studies against a variety of microbes[10,11,12,13]. Using honey for the treatment of infected burns was documented first in 2000BC by Egyptians[14]. Honey inhibited a wide-range of bacteria[15,16,17,18]. Low water content/ high osmolarity, low pH/ acidity, non- peroxide component and hydrogen peroxide contributed to the antimicrobial activity of honey13,[19,20,]. It has some antibacterial activities that are predominantly valuable against several microbes which have developed resistance to numerous antibiotics e.g. *Staphylococcus aureus* (a main reason of wound sepsis in hospitals). Honey is therefore a perfect topical wound covering in burns and surgical infections[21]. The present study aimed to estimate the comparison of effectiveness of honey with antibiotics on microorganisms isolated from infected burn wounds.

MATERIALS AND METHODS:

Fresh honey *Apis mellifera* was taken from beekeeper. A sterile mesh was used to filter/remove residue and to check the microbial purity honey sample was cultured on blood and CLED agar plates and then incubated for 24 hours at 37 °C.

Burn wound samples taken from 50 burn patients in the dept. of Plastic surgery & Burns Allied Hospital/ Punjab medical College, Faisalabad using sterile swab culture, were shifted to the Pathology Laboratory Allied Hospital/ Punjab Medical College Faisalabad where they were cultured on nutrient agar and then incubated at 37 °C for 24 hours. 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, haemolysis, lactose fermenting and non- lactose fermenting, morphology and various biochemical tests including Gram's staining, catalase, coagulase, oxidase, voges-proskeur, 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[22]. Sensitivity tests were performed for different isolates by spreading them using sterile swab culture on the surface of nutrient agar then air dried for 15- 30 minutes and zones of inhibition were measured after 24 hour incubation period at 37 °C against

(i) Antibiotic discs (Amikacin-30µg, Levofloxacin- 5µg, Meropenem-µg and Tazobactem- 10µg).

(ii) Pure honey (*Apis mellifera*- 30µl immersed in sterile filter paper discs).

STATISTICAL ANALYSIS:

The data was subjected to statistical analysis in which different physiochemical parameters were compared using ANOVA by Steel and Torrie 1981.

RESULTS:

Samples of oozing (blood or pus) were grown on different media and subjected to morphology and biochemical tests for bacterial identification up to specie level.

MICRO-ORGANISM FAUNA:

Staphylococcus aureus, Streptococcus spp, E. coli, *Klebsilla spp.* and *Pseudomonas*

aeruginosa were identified among all the samples tested.

SENSITIVITY:

Micro-organism fauna after isolation and identification was subjected to sensitivity measurements. Inhibition zones (cm) were measured on all sides of the plate around antibiotic discs and honey (Fig: 1). Inhibition zones were different for different antibiotics and honey (30µl).

S. aureus was 19%, 14%, 14%, 5% sensitive against AK, LEV, MER and TAZ respectively, while it was 48% sensitive against honey (Fig: 2A). This shows that honey has a significant effect against *S. aureus* as compared to antibiotics.

Results showed that in case of oral antibiotics *Streptococcus spp*. was 24%, 16%, 12%, 4% sensitive against AK, LEV, MER and TAZ, respectively, and 44% against honey (Fig: 2B). It shows that honey has a significant effect against *Streptococcus spp.* as compared to antibiotics.

Results indicate that *E.coli* sensitivity was 30% for amikacin, 13% for levofloaxacin, 10% for meropenem and 3% for tazobactem comparative (44%) to pure honey (Fig: 2C). It shows that honey has a significant effect against *E. coli* as compared to antibiotics.

Klebsiella spp. showed 28%, 17%, 10%, 7% sensitivity against AK, LEV, MER and TAZ, respectively, where as it was 38% sensitive against honey (Fig: 2G). It shows that honey has a significant effect against *Klebsiella spp.* as compared to antibiotics.

Honey showed (42%) maximum inhibitory effect on the growth of *P. aeruginosa* as compared to amikacin (23%), levofloaxacin (15%), meropenem (12%) and tazobactem (8%) (Fig: 2H). It shows that honey has a significant effect against *P. aeruginosa* as compared to antibiotics.

In this study it was found that out of 50 burn wound samples the occurrence of *Staphyllococcous aureus, Streptococcus spp, E. coli, Klebssila spp.* and *Pseudomonas aeruginosa* was 68%, 16%, 40%, 38%, 42% respectively.

DISCUSSION:

In spite of current advances in antimicrobial chemo-therapy and management of burn wounds, infection tends to be an important dilemma in burn wound cure. Abdel et al[23]. worked on a wide range of topical agents including sulphamylon, silver sulphadiazine and silver nitrate. They also worked on some systemic agents such as aminoglycosides, penicillins, monobactams and cephalosporins, but the problem of infection was still not completely eliminated. Some other scientists advocated that the discovery of alternative therapeutic agents was urgently required because of the ever-increasing bacterial resistance to antibiotics[21]. Since honey has the therapeutic potential, which includes the antimicrobial activity and wound healing properties. So it could be one of the best alternative medicines to be used for burn wound healing.

In this study, out of 50 burn samples, occurrence *of E. coli, Staphylococcous aureus, Streptococcus spp, E. coli, Klebssila spp.* and *Pseudomonas aeruginosa* was found 68%, 16%, 40%, 38%, and 42% respectively. the most common organisms recovered from wounds of patients were Staphylococcus aureus, pseudomonas aeurginosa and Staphylococcus epidermidis[24]. Nasir *et al*[18] also found more *Staphylococcus aureus* presence followed by *Pseudomonas spp. Klebsiella pneumoniae*, *Acinetobacter spp*. and *Streptococcus spp*. These results are quite close to the current findings.

Molan[25] and Cavanagh *et al*[26] also reported that wide range of *Pseudomonas* strains might be isolated from infected burn wounds. Their findings regarding *Pseudomonas* strains also matched with the present study that is *Pseudomonas* strain occurrence stand second after *Staphylococcus aureus*. It is supported by Jawad[27] who showed that from infected wounds and burns, the most frequently (44%) isolated gram negative microorganism was *Pseudomonas aeruginosa*. Douglas *et al*[28] and Agnihotri *et al*[29] stated that a serious cause of septic mortality and infection was *Pseudomonas aeruginosa* in burn patients and

is a commonest isolate from infected burn wounds followed by a gram positive microorganism *Staphylococcus aureus.*

Adeleke and Olaitan[30] found that *E. coli* presence was less frequent from fresh or old burn wound infections. Percentage of *E. coli* by Adeleke and Olaitan[30] is close to the percentage (38%) found in present study. Karayil *et al*[31] examined a total of 15 bacterial strains including seven *Pseudomonas* and eight *Klebsiella spp.* from various burn samples which might be different from the present study due to area and environmental changes as well as due to being chronic or fresh wound stages.

According to a study by Abdel et al[23] topical application of honey to isolated gram negative bacteria produced inhibition zones (mm) that were significantly higher than some antibiotics which support the results of this research where it was observed that honey has more antimicrobial effect against *Staphylococcus aureus, Streptococcus spp. Pseudomonas aeurginosa, E. coli* and *Klebsilla spp.* although varied with the type of antibiotics.

Moore *et al*[32] noticed that recovery or wound healing time is changed by applying honey on them as compared to antibiotic treatments. Similar results have been obtained through current research where mean inhibitory zone created by honey was 1.10cm for *S. aureus*, 1.18cm for *Streptococcus spp*. and *Pseudomonas aeruginosa,* 1.13cm for *E.coli*, 1.22cm for *Klebsiella spp*.

The present research findings that honey significantly effect bacterial growth comparative to antibiotics are also strengthened by Al-naama[33] who worked on antimicrobial activity of honey (*in vitro*) against some pathogens. He stated that honey showed a considerable antimicrobial activity against certain microbial isolates. Willix *et al*[34] found that honey inhibited the growth of both gram positive and gram negative microbes such as *Staphylococcus aureus, Eschereria coli, Pseudomonas aeruginosa* and is in agreement with Bilal *et al*[35] who describe that practically a good antimicrobial activity was exhibited by honey sample against a number of microorganisms including *Pseudomonas aeruginosa* and *Staphylococcus aureus.* These results are also in accordance with Molan[25] who bring into being that *Staphylococcus aureus*, was most susceptible to the antibacterial activity exhibited by honey.

CONCLUSION:

The results of this work advocated that microbial resistance in infected burn wounds is posing an increasing threat to wound healing. Honey which is an ages old medication, has been rediscovered in recent times. It is made up of a vast number of different compounds carrying numerous nutritional and health benefits. Its therapeutic potential has been attributed to its antimicrobial, anti-inflammatory and anti-oxidant properties as well as boosting up of the immune system. Moreover, the effectiveness of honey against antibiotic sensitive and resistant microorganisms, the ease of administration as well as lack of its side effects culminate to the fact that honey possibly shall represent a reasonable complementary or alternative cure to burn wounds. It inhibited the growth of both gram-negative and positive pathogens in comparison to certain antibiotics.

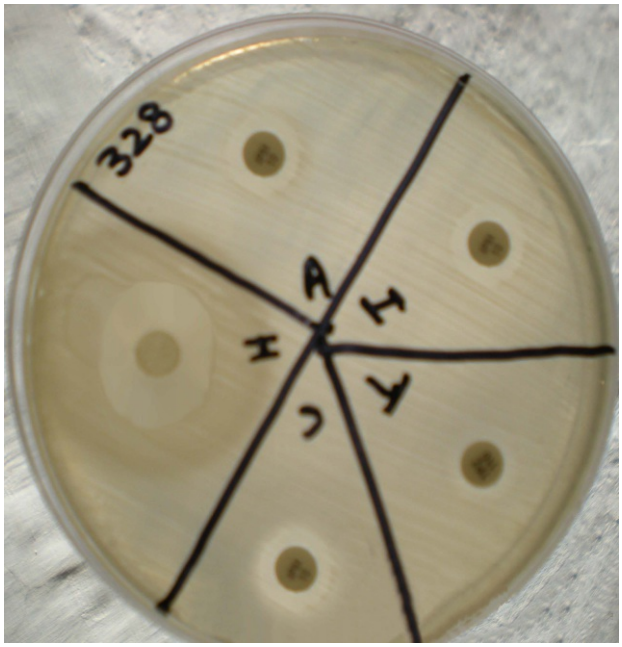

Fig: 1. Senstivity plates. Amikacin (A), Meropenem (I), Tazobactem (T) Levofloaxacin

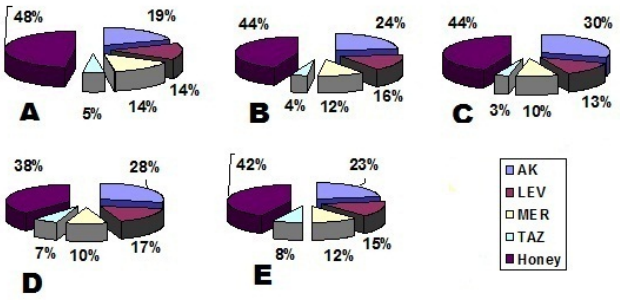

Fig: 2. Percentage effect of antibiotics and honey (A, B, C, D, E) on isolated *Staphylococcus aureus, Streptococcus spp, E. coli, Klebsiella spp., and Pseudomonas aeruginosa* respectively.

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Blessed is the man who always kept the life after death in his view, who remembered the Day of Judgment through all his deeds, who led a contented life and who was happy with the lot that Allah had destined for him.

Hazrat Ali (Karmulha Wajhay)