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Original Research Article

A study of antibacterial action of honey against bacteria isolated from wound infection: An in-vitro study

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ABSTRACT

Introduction: As a consequence of overuse and misuse of antibiotics along with the intrinsic and acquired resistance of bacteria has given rise to multi drug resistant strains. This problem can be overcome by using naturally occurring products which have antibacterial effects. The aim of the study was to evaluate the antimicrobial activity of honey against bacteria isolated from wound infections and to compare the antimicrobial efficacy of different varieties of honey.
Materials and Methods: Prospective observational study was conducted for a period of two months. A total number of 49 bacterial isolates from clinically infected wounds were tested against the antimicrobial activity of honey on 43 bacteria isolated from wound infections. Four varieties of honey were used to test the bacteria.our kinds of honey samples.
Results: Wild variety (Apisdorsata) and Wild variety (Apisflorea) had good antibacterial activity against both gram positive and gram negative bacteria compared to Culture variety (Apiscerana) and Commercial honey (Dabur) which showed poor activity.
Conclusion: Different honeys showed diverse susceptibility patterns against the tested multi drug resistant bacteria.

1. Introduction

The development of bacterial resistance to antibiotics has called for the need to search for new antimicrobials. The World Health Organization has described alternative medicines as an inexpensive way to accomplish the goal of total healthcare coverage of the world's population and has encouraged the use of plant-based alternative medicines.[1] In India, about 70% of the rural population uses the traditional ayurvedic system of medicine.[2] The use of plants for healing purposes is well documented in ancient Vedic Indian history and forms the basis of modern medicine. Products of nature like fruits, vegetables, spices and herbs are used in the world's oldest medical systems and remains one of India's traditional health care systems.

Allen et al[3] showed that there are many types of honey with and without antibacterial action and postulated that the type of the flower which forms the source of the nectar decidesthe nature of the antibacterial action of the honey. The ability of honey to kill microorganisms has been accredited to its high content of tetracycline derivatives, peroxidases, fatty acids, phenols, ascorbic acids and amylases.[4]

Recently, honey as a natural product has evoked a lot of interest in alternative medicine.[5] In burns in particular, honey has been found effective in controlling wound infection and accelerating wound healing.[6] In the modern era of antibiotic resistance, our study focuses on finding the actions of honey on various bacteria. Hence, his study was planned to evaluate the antimicrobial activity of honey

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against bacteria isolated from wound infections and to compare the antimicrobial efficacy of different varieties of honey.

2. Materials and Methods

A prospective observational study was conducted in the Department of Microbiology of tertiary care teaching hospital for a period of 2 months after obtaining ethical clearance from the Institutional Ethics Committee. A total of 43 samples were collected aseptically from clinically infected wounds on sterile cotton swabs by rotating with sufficient pressure. Collected samples were transported to the laboratory for further processing.

2.1. Sample size

Based on the existing literature[7] and with 95% confidence and 10% allowable error, sample size was calculated as 43 based on the formula

n $=\frac{z_\alpha{}^2 p(1-p)}{e^2}$ where p= 87.3% ,prevalence of micro-organisms, z_α= 1.96 at 95% C ande= Allowable error

2.2. Microbiological workup

The pus/wound swabs were cultured on 5% sheep blood agar and MacConkey agar. Culture media were incubated at 37°C and the isolate was identified using standard microbiological procedures.[7] Antimicrobial susceptibility was done by Kirby Bauer disc diffusion method according guidelines by Clinical and Laboratory Standards Institute.[8] Antibiogram of these isolates were tested using standard antibiotics such as Ampicillin, Cefotaxime, Cefoperazone, Ciprofloxacin, Gentamicin, Imipenem.

2.3. Honey sample and preparation

4 different kinds were used; 1. Wild variety (Apisdorsata), 2. Culture variety (Apiscerana), 3. Wild variety (Apisflorea), 4. Commercial honey (Dabur) were used.

Each honey sample was first filtered with sterile gauze to clear the debris and then streaked on a blood agar plate, which is then checked for sterility; and the honey was stored at 2–8°C until used. Hundred percent pure honeys (100% v/v) of 4 different kinds were used after filtering using sterile gauze. These honey were tested against 4 reference strains to know the resistance and sensitivity of it; E. coli ATCC 25922, K.pneumoniae ATCC 700603, P.aeruginosa ATCC 27853 and S.aureus ATCC 2592.

2.4. Determination of antibacterial activity by disc diffusion method

Antibacterial activity of various types of honey was evaluated by doing antimicrobial susceptibility testing, by disc diffusion method and zone size were measured. Susceptibility testing was performed by Kirby–Bauer disk diffusion technique according to CLSI guidelines, 2019.[8] The inoculums were prepared by picking bacteria with a sterile wire loop and suspended in sterile normal saline. The density of suspension was determined by comparing with McFarland 0.5 barium sulphate solution. A sterile swab was immersed into the suspension of the isolated bacteria, the swab was then squeezed free from excess fluid against the side of the tube, and then spread over the Muller Hinton agar plate. Then the plates were left on the bench for the excess fluid being absorbed. Using a sterile Metallic Punch with rubber teat (6mm diameter, 4 mm deep, and about 3 cm apart), four such wells were made in the agar medium. Using a micropipette which was sterile, 100 μL of honey with the 100%v/v concentration was added to the wells in the plate. The plates were incubated at 37 °C for 24 hours before the bacteria was seeded. The test organism was then uniformly seeded over the Muller–Hinton agar surface, and was kept inside incubator overnight (12-18hrs) at 37°C. The diameters of inhibition zones were then measured in mm, and the results were recorded as R: Resistant, S:Sensitive, 14mm:3+(Very active)., Pretest was conducted to check the method with ATCC strains. Sensitivity test was done against four different kinds of honey and was checked with bacteria for its reliability and validity before it was used for the actual experiment.

2.5. Statistical analysis

The collected data was analysed by frequency, percentage and chi-square test.

3. Results

A total of 43 bacterial isolates were studied in a 2 months period. The most common isolates among bacteria were Gram negative bacilli are E.coli 7(16%), Klebsiellapneumoniae 8(19%), Pseudomonas aeruginosa 8(195), Enterobacterspp 2(5%), Proteus.spp 3(7%) Citrobacterspp 2(5%), Acinetobacterspp 1(2%). Gram positive cocci were Staphylococcus aureus 6(14%), Streptococcus spp 4(5%), Enterococcus spp 2(5%).

4. Discussion

Wound infection has been a problem for a very long time in the field of medicine, and has been increasing even today due to the increasing number of antimicrobial resistant strains. This has been a problem for the public, researchers, clinicians and patients. Due to this there is constant search for newer and more effective drugs. In this study, a total of 43 samples of patients suffering from wound infection were taken. Honeys antibacterial properties may differ depending on the type of honey obtained, it's topographical location, and flower from which the final product is being derived. Hence the main objective of this test was to know the

Table 1: Antimicrobial action of honey against bacteria along zones of inhibition

S.No.	Sample	Honey 1	Honey 2	Honey 3	Honey 4
1	E.coli n=7	2+= 7(100%)	2+ =3(42.86%) 1+ =2(28.58%) S =1(14.29%) R =1(14.29%)	3 =1(14.29%) 2 =5(71.53%) R =1(14.29%)	3+ =1(14.29%) 2+ =1(14.29%) 1+ =3(42.86%) R =2(28.58%)
2	K.pneumoniae n=8	2+=4(50%) 1+=2(25%) R =2(25%)	2+ =1(12.50%) R =7(87.50%)	2+=4(50%) 1+ =3(37.50%) R =1(12.50%)	2+ =1(12.5%) 1+ =1(12.5%) R =6(75%)
3	P.aeruginosa n=8	3+ =1(12.50%) 2+ =4(50%) 1+ =1(12.50%) R =2(25%)	3+ =1(12.50%) 2+ =2(25%) 1+ =1(12.50%) R =4(50%)	3+ =1(12.50%) 2+ =5(62.50%) 1+ =1(12.50%) R =1(12.50%)	3+ =1(12.50%) 2+ =1(12.50%) 1+ =1(12.50%) R =5(62.50%)
4	Staphylococcus.spp n=6 a)S.aureus(n=1) b)CoNS(n=2) c) MRCoNS n=1) MRSA(n=2)	2+=4(66.67%) 1+=2(33.34%) a) 1+=1(100%) b) 2+=2(100%) c) 2+=1(100%) d) 2+=1(50%) 1+=1(50%)	2+=1(16.67%) R=5(83.34%) a) R=1(100%) b) 2+=1(50%) R=1(50) c) R=1(100%) d) R=2(100%)	2+=3(50%) 1+=1(16.67%) R=2(33.34%) a) 1+=1(100%) b) R=2(100%) c) 2+=1(100%) d) 2+=2(100%)	2+=4(66.67%) 1+=2(33.34%) a) R=1(100%) b) R=2(100%) c) R=1(100%) d) R=2(100%)
5	Streptococcus.spp n=4	2+ =2(50%) 1+ =1(25%) R =1(25%)	2+ =2(50%) R =2(50%)	2+ =1(25%) R =3(75%)	2+ =1(25%) 1+ =1(25%) R =2(50%)
6	Enterobacter.spp n=2	2+ =2(100%)	1+ =1(50%) R =1(50%)	2+ =2(100%)	2+ =1(50%) R =1(50%)
7	Proteus.spp n=3	2+ =3(100%)	2+ =1(33.34%) 1+ =1(33.34%) R =1(33.34%)	2+ =3(100%)	3+ =2(66.67%) R =1(33.34%)
8	Enterococcus.spp n=2	2+ =2(100%)	S =1(50%) R =1(50%)	1+ =2(100%)	1+ =2(100%)
9	Citrobacter.spp n=2	2+ =2(100%)	2+ =1(50%) R =1(50%)	3+ =2(100%)	3+ =1(50%) R =1(50%)
10	Acinetobacter.spp n=1	S=1(100%)	R=1(100%)	S=1(100%)	R=1(100%)

Honey 1: Wild variety (Apisdorsata; Honey 2: Culture variety (Apiscerana); Honey 3: Wild variety (Apisflorea) Honey 4: Commercial honey (Dabur)
R: Resistant; S: Sensitive<6mm; Interpretation: 6-9mm:-1+ 10-13mm:-2+ >14mm:-3+

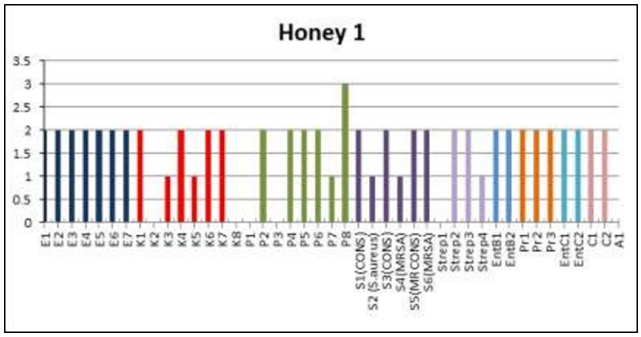

Fig. 1: Bar graph of antimicrobial action of Honey 1 against the 43 samples of bacteria isolated from wound infection in X-axis (0.5cm) and zones of inhibition in Y-axis (1cm).

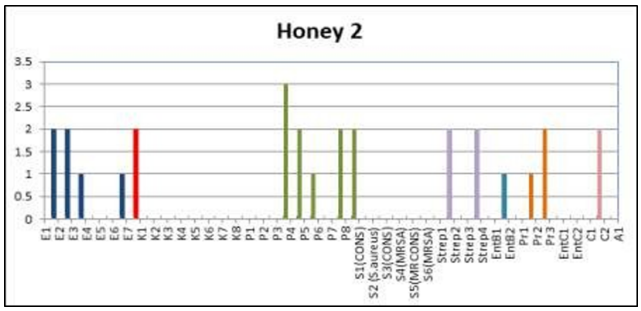

Fig. 2: Bar graph of antimicrobial action of Honey 2 against the 43 samples of bacteria isolated from wound infection in X-axis (0.5cm) and zones of inhibition in Y-axis (1cm).

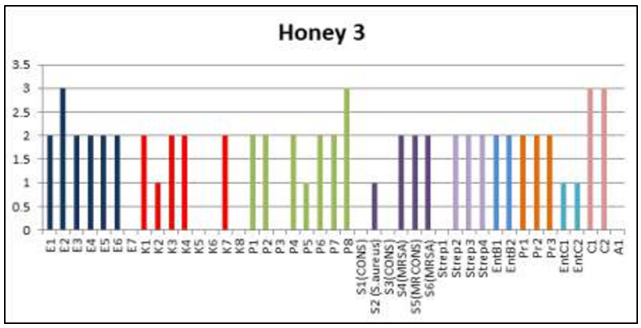

Fig. 3: Bar graph of antimicrobial action of Honey 3 against the 43 samples of bacteria isolated from wound infection in X-axis (0.5cm) and zones of inhibition in Y-axis (1cm).

antimicrobial activity of honey against bacterial isolates from the wound infection. In this study, all the tested samples of honey showed various results ranging from 6 mm to greater than 14mm of clear zone of inhibition against the tested organisms at 100%v/v of honey.This difference in the antibacterial action might be due to the different species of honey bees, variations in the antimicrobial activities of the honey, and different geographical locations in which the honey was found. Our study results showed Wild variety (Apisdorsata) and Wild variety (Apisflorea) had good

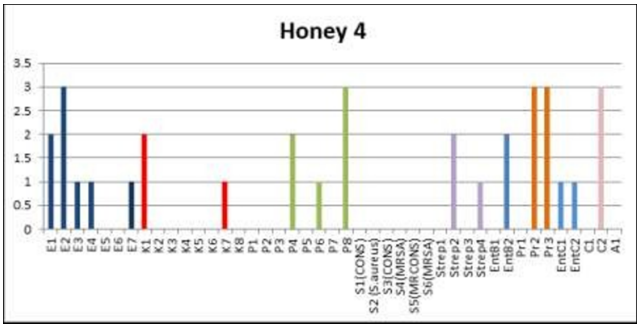

Fig. 4: Bar graph of antimicrobial action of Honey 4 against the 43 samples of bacteria isolated from wound infection in X-axis (0.5cm) and zones of inhibition in Y-axis (1cm).

antibacterial activity against both gram positive and gram negative bacteria compared to Culture variety (Apiscerana) and Commercial honey (Dabur) which showed poor activity. The results show commercial variety may be obtained by the cultured bees. Wild honey(Apisdorsata and Apisflorea) exhibited a broad-spectrum of antibacterial activity against both Gram-positive bacteria and Gram-negative bacteria, including antibiotic-resistant MRSA (Methicillin resistant Staphylococcus aureus) ones. Cultured variety showed action against E,coli, Pseudomonas aeruginosa, Proteus.spp and to an extent on Streptococcus pyogenes. It was resistant to Klebsiella and Staphylococcus. Similar findings were noted by Wasihun, et al.[9] Despite the fact that tested honey showed antibacterial activity, many studies have demonstrated that not all honey samples may not have the same degree of antibacterial action. The study on honey done by Mama M, et al[10] showed the antimicrobial properties against Methicillin resistant Staphylococcus aureus(MRSA) .Visavadiaet al,[11] in their study observed pathogenic bacteria causing wound infections such as Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli and Streptococcus pyogenes were found to be sensitive to honey . From this study it conveys that the honey has potential in the decontamination of the wound colonized strains of bacteria. This holds up to the fact of using honey as a local practice to treat wound infection.

5. Conclusion

The results of this study emphasize that honey has the antibacterial property against the commonly isolated bacteria from wounds tested. The antibacterial potency of wild honey on the bacterial isolates was good compared to culture variety at 100%v/v. To obtain stronger evidence in association with this, further analysis with larger sample sizes are required. Different honeys showed diverse susceptibility patterns against the tested multidrug resistant bacteria. However, pharmacological standardization and

clinical evaluation on the effect of honey are essential before using honey as a therapeutic agent for common bacterial species.

6. Conflict of Interest

The authors declare no relevant conflicts of interest.

7. Source of Funding

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