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Original Article

Microbiology Section

Antimicrobial Activity of Honey with Special Reference to Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA)

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ABSTRACT

Introduction: Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. With the irrational and excessive use of antibiotics in underdeveloped and developing countries, there may be chances to develop and spread resistant pathogens in the community. As a result, the effectiveness of the antibiotics is diminishing. Therefore, the need for novel alternative antimicrobial strategies has renewed interest in natural products like turmeric, honey, ginger and others exhibiting antibacterial properties. This situation has led to a re-evaluation of the therapeutic use of ancient remedies like honey as no other studies are available in the state of Andhra Pradesh with the locally available honey.

Aim: To find out the efficacy of antibacterial activity of locally available honey against Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA) isolates.

Materials and Methods: A prospective study on the antibacterial activity of Bharat multi floral pasteurised honey which was locally available in the state of Andhra Pradesh, further it was conducted and evaluated against the bacterial strains of Methicillin Resistant *Staphylococcus aureus* and Methicillin Sensitive *Staphylococcus aureus*. Their antibacterial sensitivity pattern was tested using Kirby-Bauer disc diffusion susceptibility testing technique of CLSI along with other commonly used antimicrobials.

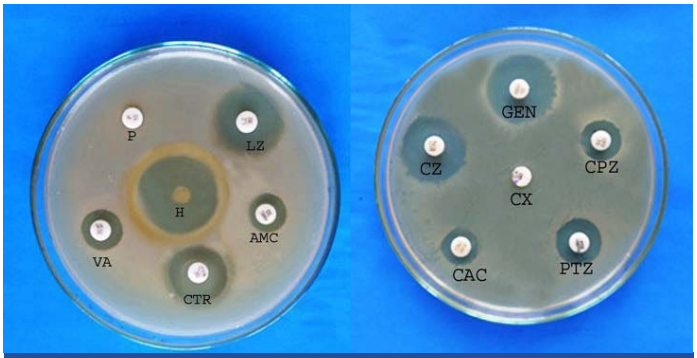
Results: Both MRSA and MSSA isolates were sensitive to honey. But MRSA were resistant to all antimicrobials tested except linezolid where as MSSA were sensitive to all except penicillin.

Conclusion: It is definitely worthy to consider honey as a promising future antimicrobial to be tested and studied. Honey, may be elaborately used in future with some more molecular studies on its method of action as an antimicrobial agent.

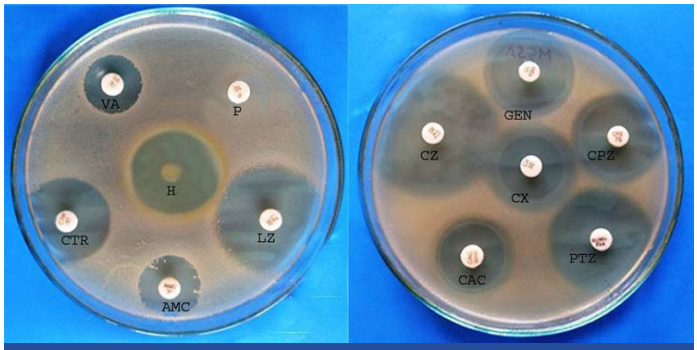
Keywords: Antibacterial activity, Kirby-bauer disc diffusion, Minimum inhibitory concentration, Zone of Inhibition

INTRODUCTION

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases [1]. The selection and spread of multi-resistant organisms in developing countries, which can often be traced to complex socio-economic and behavioural factors, contribute to the escalating problem of antibiotic resistance worldwide. In developing countries, the irrational use of antibiotics by health professionals, unskilled practitioners, laypersons, poor drug quality, unhygienic conditions and inadequate surveillance account for the spread of resistant bacteria. Nevertheless, misuse of antibiotics is one of the reasons for the increasing rates of resistance, especially in rural areas [2]. There is an emergence of drug resistance for the drugs like vancomycin and daptomycin leading to search for still newer drugs for combating the drug resistance in Staphylococci [3]. With the irrational and excessive use of antibiotics in underdeveloped and developing countries the developed resistance may spread in the community making the strains as super bugs causing difficulties in eradication [4]. As a result, the effectiveness of the antibiotics is diminished [5]. Therefore, the need for novel alternative antimicrobial strategies has renewed interest in natural products like turmeric, honey, ginger etc., exhibiting antibacterial properties. This situation has led to a re-evaluation of the therapeutic use of ancient remedies including honey [6-8]. Honey is well known as a magic drug for almost all kinds of diseases, not to mention the fact that many people do depend more on folk medicine and natural remedies which are cheap that have been known for their therapeutic effects over the past decades [6]. Honey has well established function as an effective antibacterial agent with a broad spectrum of activity against Gram-positive and Gram-negative bacteria [9-11]. The application of honey can promote the healing of infected wounds that do not respond to the conventional therapy, i.e., antibiotics and antiseptics [12] including wounds infected with methicillin-resistant *S. aureus* [13,14]. Laboratory studies have revealed that honey is effective against MRSA, β-haemolytic streptococci and Vancomycin Resistant Enterococci (VRE) [15,16]. The beneficial role of honey is attributed to its antibacterial property with regards to its high osmolarity, acidity (low pH) and content of hydrogen peroxide (H_2O_2) and non-peroxide components, i.e., the presence of phytochemical components like Methylglyoxal (MGO). The antimicrobial agents in honey are predominantly hydrogen peroxide, of which the



[Table/Fig-1]: Antimicrobial activity of honey and other common antibacterial agents on/against MRSA.



[Table/Fig-2]: Antimicrobial activity of honey and other common antibacterial agents on/against MSSA.
H=Honey, AMC = Amoxicillin + Clavulanic Acid, CTR = Ceftrioxone, VA = Vancomycin, P = Penicillin, LZ = Linezolid, GEN = Gentamicin, Cefperazone (CPZ), PTZ = Pipercillin + Tazobactam, CAC = Ceftazidime + Clavulanic Acid, CZ = Cefazolin, CX = Cefoxitine
Zone of Inhibition of Honey on/against MRSA = 36.2± 0.2
Zone of Inhibition of Honey on/against MSSA = 40.16±0.152

concentration is determined by relative levels of glucose oxidase, synthesized by the bee and catalase originating from flower pollen [17]. Hence, an attempt was made to find out the efficacy of locally available honey against MRSA which emerged as a super bug and MSSA and their antibacterial activity to commonly used antimicrobials.

MATERIALS AND METHODS

The antibacterial activity of Bharat multi floral pasteurized honey obtained from Bharat Unani Pharmacy (Bharat honey co), Hyderabad, Andhra Pradesh, India, was tested and evaluated in the month of April 2017 against the bacterial strains of MRSA and MSSA obtained from Saveetha University, Chennai, India [3] which were already confirmed phenotypically (Disc diffusion test with Cefoxitin 30 µg disc (obtained by Himedia, Mumbai) was used to differentiate MRSA and MSSA isolates and the interpretation was done by MSSA if zone size was β 22 mm. The strain was considered as MRSA if zone size was <22 mm. (ref. CLSI M100-S23) [18] and genotypically (DNA sequencing was done for MecA gene at Eurofins Genomics India Pvt., Ltd. Bangalore, Karnataka, India). Their antibacterial sensitivity pattern was tested using Kirby-Bauer disc diffusion susceptibility testing technique of CLSI [18]. MRSA ATCC strain No. 43300, MSSA ATCC 25923 were included as a positive control strains and MRSA ATCC 33591, MSSA ATCC 29213 were used as negative control strains. A six hour incubated bacterial culture suspension matching with 0.5 Mc-Farland scale standard was prepared equivalent to 1.5 x 108 CFU/ ml organisms in 5 ml peptone water and spread onto the sterile Mueller-Hinton agar (Himedia, Mumbai) plates to prepare a lawn culture. Dried in the incubator for half an hour and three such plates were prepared for each bacterial strain. Honey disks were prepared by using Watman No.1 filter paper and discs were punched with a office paper hole punching machine of 6 mm diameter and such 100 discs were taken in small glass bottle and sterilized at 160°C for two hours in a hot air oven. To these 100 sterile discs, 1 ml of pasteurized 100% V/V undiluted honey obtained for this study was added and kept for overnight for equal absorption of honey by all discs [19]. Standard antibiotic discs of Amoxicillin+ Clavulanic Acid (AMC) (20/10 µg), Ceftrioxone (CTR) (30 µg), Vancomycin (VA) (30 µg), Penicillin (P) (10 Units), Linezolid (LZ) (30 µg), Gentamycin (GEN) (10µg), Cefoperazone (CPZ) (30 µg), Pipercillin+tazobactam (PTZ) (100/10 µg), Cefpazidime+clavulonicacid (CAC) (30/10 µg), Cefazolin (CZ) (30 µg) and Cefoxitine (CX) (30 µg) obtained from Himedia laboratories Pvt Ltd., Mumbai, India, and the prepared honey discs were placed aseptically on the Mueller Hinton agar. Plates were left for one hour at 25°C to allow a period of preincubation diffusion in order to minimize the effect of variation in time between the placements of different discs. The plates were then incubated aerobically at 37°C over night to allow bacterial growth. After incubated plates were observed and the zone of inhibition was measured to evaluate the antimicrobial activity for each of the tested antibiotics and honey samples using a special scale obtained from Himedia Laboratories, Mumbai, India. The sensitivity testing plates were done in triplicates for each strain of MRSA and MSSA isolates and the zone of inhibition were measured to the nearest millimeters. To calculate the mean and standard deviation of each strain statistically using Statistical Package for the Social Sciences (SPSS) software. We took the criteria for sensitivity was as the zone of inhibition for honey is bigger than the zones any of the antimicrobials used for testing the MRSA and MSSA isolates.

The present study was approved by the Institutional Ethical Committee.

RESULTS

Both MRSA and MSSA were sensitive to honey with a zone of inhibition of 36.2±0.2 mm and 40.16±0.152 mm respectively. But MRSA were resistant to all antimicrobials tested except linezolid (21.03±0.152 mm) whereas, MSSA were sensitive to all except penicillin with no zone of inhibition [Table/Fig-1-3].

DISCUSSION

It has been reported that honey showed both bacteriostatic and bactericidal effect against many Gram-positive as well as Gram-negative bacteria [20-24]. The use of natural products to enhance wound healing is a common practice in many parts of the world. Honey consists of a super saturated solution of sugars and has a low pH between 3.2 and 4.5. This pH together with honey's high

Name of Organism	Diameter of Zone of Inhibition (mm)											
	Honey (H)	Amoxicillin + Clavulanic Acid (AMC) (20/10 µg)	Ceftrioxone (CTR) (30 µg)	Vancomycin (VA) (30 µg)	Penicillin (P) (10 Units)	Linezolid (LZ) (30 µg)	Gentamicin (GEN) (10µg)	Cefoperazone (CPZ) (30 µg)	Pipercillin + Tazobactam (PTZ) (100/10 µg)	Ceftazidime + Clavulanic Acid (CAC) (30/10 µg)	Cefazolin (CZ) (30 µg)	Cefoxitine (CX) (30 µg)
Methicillin resistant *Staphylococcus aureus* (MRSA)	36.2± 0.2	8.2± 0.2	16.13± 0.152	10.2± 0.2	0	21.03± 0.152	22.13± 0.152	10.1± 0.1	12± 0.1	8.166± 0.152	18.16± 0.152	0
	(S)	(R)	(R)	(R)	(R)	(S)	(R)	(R)	(R)	(R)	(R)	(R)
Methicillin sensitive *Staphylococcus aureus* (MSSA)	40.16± 0.152	23.03± 0.152	40.1± 0.1	23.03± 0.15	0	45.13± 0.152	44.1± 0.1	44.13± 0.152	46.16± 0.152	30± 0.1	48.16± 0.152	27.96± 0.152
	(S)	(S)	(S)	(S)	(R)	(S)	S)	(S)	(S)	(S)	(S)	(S)

[Table/Fig-3]: Antimicrobial sensitivity pattern of MRSA and MSSA with reference to honey and other common antimicrobials.
H=Honey, AMC = Amoxicillin + Clavulanic Acid, CTR = Ceftrioxone , VA = Vancomycin, P = Penicillin, LZ = Linezolid, GEN = Gentamicin, Cefperazone (CPZ), PTZ = Pipercillin + Tazobactam, CAC = Ceftazidime + Clavulanic Acid, CZ = Cefazolin, CX = Cefoxitine

Author/s	Ref.No.	Type of honey/Concentration	MRSA-Sensitivity/Zone of Inhibition	MSSA- Sensitivity/Zone of Inhibition
Patel A et al.,	[4]	Karapur village honey from Goa	Sensitive/NA	Sensitive/NA
Almasaudi SB et al.,	[34]	Manuka /20%,30%,40% V/V Nigella Sativa/20%,30%,40% V/V Sidr/20%,30%,40% V/V	Sensitive/NA Sensitive/NA Sensitive/NA	Sensitive/NA Sensitive/NA Sensitive/NA
Zakaria AS	[35]	Yemen Sidr Honey (YSH)/100% V/V Southern Sidr Honey (SSH) 100% V/V Multi-Flower Mountain Honey (MMH) 100% V/V	Sensitive/14±2.83 Sensitive/15±0.71 Sensitive/9±1.1	Sensitive/15±2.83 Sensitive/17±1.35 Sensitive/13±3.24
Neeraja Rani G et al.,	Present Study	Bharat Honey from Andhra Pradesh / 100% V/V	Sensitive/36.2± 0.2	Sensitive/40.16±0.152

[Table/Fig-4]: Comparative study of Antimicrobial activity of honey on/against MRSA and MSSA.
NA = Not available

osmolarity and the presence of H_2O_2 reduces the bacterial growth at the wound site. Honey in wound dressing has been reported to provide ideal environment for the rapid tissue repair and regeneration that are essential for growth of wound bed [25]. *Staphylococcus aureus* is the most frequently isolated wound pathogen and it is becoming increasingly resistant to antibiotics in common use. Honey has been reported to be effective in eradicating antibiotic resistant bacteria including MRSA [26] which is a super bug now. Any zone diameter having less than 7 mm shows that the organism is resistant to the honey sample but if the zone diameter is greater than 11 mm it suggests that the microorganism is sensitive to the honey with special reference to *Pseudomonas aeruginosa* [27]. The findings of our study together with four other previous studies [28-30] show that honey promises to be an effective wound antiseptic with broad spectrum antimicrobial activity. Some topical antimicrobials adversely affect the human skin/tissue and repair process during the treatment of wounds where as there is no need for laboratory evaluation of honey as it does not adversely affect human skin/tissue [31]. The special character of honey is the potential to limit the growth of wound pathogens, but also there is evidence that honey has the potential to promote the healing [32,33] and no other antimicrobial agent possesses these characteristics. Honey is effective even it is diluted by burn wound exudates. In burns, honey's antimicrobial and anti inflammatory properties allow a moist healing environment to be maintained that protects the wounds from deterioration and fibrosis [29]. [Table/Fig-1-3] shows the results of antibacterial activity of honey towards the two microorganisms tested. MRSA as well as MSSA were sensitive to undiluted honey samples tested with an average zone of inhibition of 36.2+0.2 and 40.16+ 0.152 mm respectively. Ogbaje EO et al., and Murthy K et al., found in their study that 100% V/V undiluted honey inhibited the growth of MRSA with a zone of inhibition of 18 mm and 11 mm respectively [22,33], whereas Patel A et al., in their study observed that diluted honey of 20% V/V, 30% V/V and 40% V/V inhibited the growth of MRSA [4] whereas, 15% V/V also inhibited the growth of MSSA in addition to other concentrations used for MRSA and Almasaudi SB et al., observed the only 50% V/V concentrated honey inhibited the growth of both MRSA and MSSA [34], whereas, Zakaria AS showed that 100% V/V undiluted honey inhibited MRSA with a zone of 14±2.83 mm and MSSA 15±2.83 mm for Yemen Sidr honey [35], MRSA with a zone of 15±0.71 mm and MSSA 17±1.35 mm for Southern Sidr honey and MRSA with a zone of 9±1.1 mm and MSSA 13±3.24 mm for multi-flower mountain honey. In our findings with our local honey, the zone of inhibitions of MRSA (36.2+0.2) and MSSA (40.16+0.152) were much bigger than the ones reported by other workers. Hence, we can say that the efficacy of our local honey was more than the honey samples of other workers. Neerajarani G et al., [36] in their study showed the antibacterial activity of undiluted honey on the isolates of *Staphylococcus aureus*, *E. coli* and *Pseudomonas aeruginosa* and all these organisms were sensitive to undiluted honey. The exact explanation for the antibacterial activity of honey is not known, but it is clear that the higher the concentration of honey the greater its usefulness as an antibacterial agent. However, it is expected that the clinical significance of the antibacterial activity in honey will be unequivocally proven only if a clinical trial is conducted to compare dressings of different sugars and selected honeys. Although more research is needed, as with many of the therapeutic interventions used in modern wound care, in the absence of data from well controlled clinical trials. Recent reviews on the successful usage of honey as a dressing on infected wounds show that many authors support the use of honey in infected wounds and some suggest the prophylactic use of honey on the wounds of patients susceptible to MRSA and other antibiotic-resistant bacteria [37]. Well documented clinical trials and researches are going on honey and nanotechnology which may provide promising results on therapeutic use of honey in the future. Comparative studies of antimicrobial activity of honey on/against MRSA and MSSA were shown in [Table/Fig-4]. Further in continuation of studies on honey we are planning to study the efficacy of honey with nano particles of gold, silver on the wound healing process in an experimental animal rat.

LIMITATION

The present study was limited to testing of 100% V/V of local honey. Testing of different dilutions is under study.

CONCLUSION

It is definitely worthy to consider honey as a promising future antimicrobial to be tested and studied. Rediscovering honey as a natural remedy for wound pathogens proved its effectiveness on antimicrobial resistant strains of bacteria including MRSA. In the present study, we tried to focus more on whether honey can be used for treating Staphylococcal infections with special reference to MRSA. Honey, the nature blessed and environmental friendly product may be elaborately used in future with some more molecular studies on its method of action as an antimicrobial agent.

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