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ANTIBACTERIAL ACTIVITY OF HONEY ON METHICILLIN RESISTANT *Staphylococcus aureus* (MRSA) OBTAINED FROM CLINICAL SPECIMEN

Maryam Idris Muhammad[1], Hadiza Mustapha Ghazali[1], Aziza Salisu Garba[2], Bata Martins[1]

myramid1989@gmail.com

mustaphahadiza77@gmail.com

azizasalisugarba.asg@gmail.com

Department of Science Laboratory Technology, Federal Polytechnic Bauchi, Bauchi state[1]

Department of Horticultural Technology, Federal Collage of Horticulture, Dadin Kowa, Gombe State[2]

Abstract

Honey is one of the oldest traditional medicines considered as traditional remedy for microbial infections. It is also recognized as an efficacious topical antimicrobial agent in the treatment of burns and wounds. The aim of this study is to determine the antibacterial activity of honey against methicillin resistant *Staphylococcus aureus* obtained from clinical specimen. Antibacterial activity of honey was determined using Kirby Bauer disc diffusion method. The Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) were determined following standard microbiological techniques. 75% honey concentration showed maximum zone of inhibition (12mm). The MIC and MBC also indicated that honey has potential antibacterial activity against MRSA. It is recommended that future studies should focus on the use of honey in combating antimicrobial resistance.

Keywords; Antibacterial, honey, minimum bactericidal concentration, minimum inhibitory concentration

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Introduction

Honey is one of the oldest traditional medicines considered as traditional remedy for microbial infections. It is also recognized as an efficacious topical antimicrobial agent in the treatment of burns and wounds (Brudzynski, 2006). The healing effect of honey could be due to various physical and chemical properties (Snow and Monley-HAris, 2002).

The antibacterial activity of honey was first recognized in 1992, however, it has a limited use in modern medicine due to lack of scientific support. Honey is the nectar collected from flowers by bees (Sharp, 2009). Several studies demonstrated the antibacterial activity of honey against *Escherichia coli, Campylobacter jejuni, Salmonella entercolitis, Shigella dysenteria* (Adebolu, 2005 and Voidarou *et al*., 2011) and *Mycobacterium spp.* (Asadi-Pooya *et al*., 2003).

Honey has been reported to have inhibitory effects on fungi. Pure honey inhibits fungal growth and diluted honey appears capable of inhibiting toxin production (Al-Waili *et al.*, 2005). An antifungal action has also been observed for some yeast and species of Aspergillus and Penicillium, as well as all the common dermatophytes, candidiasis, caused by *Candida albicans*, may respond to honey. Cutaneous and superficial mycoses like ringworm and athletes foot are found to be responsive to honey. This responsiveness is partly due to the inhibition of bacterial infection (Bansal *et al*., 2005). In addition, some studies have reported that topical application of honey was effective in treating seborrheic dermatitis and dandruff (Al-Waili, 2005).

The antibacterial properties of honey may be particularly useful against bacteria which developed resistance to many antibiotics e.g. *Staphylococcus aureus*, which is a major cause of wound sepsis in surgical infections, burns and wound infections (Betts and Molan 2003).

Statement of Research Problem

For several decades, antimicrobial resistance (AMR) has been a growing threat to the effective treatment of an ever-increasing range of infections caused by bacteria, parasites, viruses and fungi. AMR results in reduced efficacy of antibacterial, antiparasitic, antiviral and antifungal drugs, making the treatment of patients difficult, costly, or even impossible. The impact on particularly vulnerable patients is most obvious, resulting in prolonged illness and increased mortality (WHO, 2014). Consequently, antibacterial drugs have become less effective or even ineffective, resulting in an accelerating global health security emergency that is rapidly outpacing available treatment options (WHO, 2014).

Justification

Antimicrobial resistance has been shaping the field of infectious diseases since the discovery of penicillin. Many of the advances in antimicrobial drug development have resulted from efforts to combat ever-evolving mechanisms of resistance that render existing agents obsolete, thus prompting the search for new molecules that promise to be more effective and more resilient (Kanj and Kanafani, 2011). Hence, the current study is directed to providing an insight on the antibacterial activity of honey against MRSA so as to reduce the potential risks associated with this organism.

Aim and Objectives

The aim of this study is to determine the antibacterial activity of honey on methicillin resistant *Staphylococcus aureus* obtained from clinical specimen.

Objectives include;

1. To determine the antibacterial activity of honey on Methicillin Resistant *Staphylococcus aureus* (MRSA).
2. To determine the Minimum Inhibitory Concentration (MIC) of honey on methicillin resistant *Staphylococcus aureus.*

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3. To determine Minimum Bacterial Concentration (MBC) on methicillin resistant *Staphylococcus aureus.*

Materials and Methods

Study Area

Bauchi state is located in the north eastern part of Nigeria and its over 43,837 square kilometers. Bauchi state is border by Kano and Jigawa to the north; Yobe and Gombe to the east; Kaduna to the west; and Plateau and Taraba to the south.

Sterilization of Glass Wares

All glass wares were washed, dried and sterilized in hot air oven at 160°C for 2 hours while wire loop was sterilized flaming to red hot using naked flame. The work bench was sterilized with 70% ethanol.

Sample Collection

Pure culture of MRSA was collected at the National Veterinary Research Institute (NVRI) Vom Jos Plateau state.

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Confirmatory Test on MRSA

- Mannitol Salt Agar (MSA) was prepared according to manufacturer's instructions. The media was then sterilized by autoclaving at a temperature of 121°C for 15 minutes. Once the sterilization cycle is completed, molten agar will be allowed to cool to approximately 50°C before being distributed to individual petri plates (approximately 20 to 25 mL of molten agar per plate) as described by Tille (2017). A culture of MRSA obtained was then aseptically sub cultured on MSA using the streak plating technique. The plates were then incubated aerobically at 35°C for 24 hours following the standard procedures of Cheesbrough (2006).

- Preliminary investigation based on colony morphology characteristics such as changes in physical appearance on differential media, size of colony, pigmentation, shape, surface appearance, odour, etc. as described by Tille (2017) was observed.
- Microscopic investigation based on Gram staining was carried out as described by Cheesbrough (2006) for further identification. A slide containing a smear of the overnight culture was flooded with crystal violet dye for 60 seconds; It was then washed off with water. The slide was flooded with Lugol's iodine for 30 seconds. It was washed off with water. The slide was then flooded with acetone and was rinsed immediately to avoid total washing away of the smear. The slide was then flooded with safranin for 60 seconds. It was washed off with water. The slide was observed under the microscope using X100 magnification.
- Biochemical tests such as catalase and coagulase tests were carried out according to standard procedures described by Tille (2017) for phenotypic confirmation of isolates.

  Catalase Test

  A wire loop was used to transfer a small amount of the colony to the surface of a clean, dry glass slide. A drop of 30% hydrogen peroxide (H_2O_2) was placed onto the glass slide containing the organism. It was observed for the evolution of oxygen bubbles.

  Coagulase Test

  A drop of blood plasma (preferably rabbit plasma with ethylenediaminetetraacetic acid [EDTA]) was placed onto a clean glass slide. With a wire loop, a portion of the test colony was emulsified in the rabbit plasma to create a smooth suspension. It was observed for clumping.
- Detection of MRSA

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Screening Test for Oxacillin-resistant *S. aureus* was carried out by testing 30 µg cefoxitin disc on the test organism using standard disc diffusion procedure; it was incubated at 33-35°C for 16-18 hours according to standard procedures of CLSI (2018).

3.5 Honey Collection

Honey was obtained from local bee keeper in Gwallameji, Bauchi state. The honey was collected in sterile screw cap bottle. The honey was first filtered with a sterile mesh/guage to remove debris and was streaked on blood agar plate to check the sterility and was kept at 2-8°C until used.

Preparation of Honey

Hundred percent (100%) pure honey was obtained after filtering it using sterile gauze. To get 75% honey solution (v/v), 0.75ml of honey was diluted in 0.25ml of sterile distilled water. Also, 0.50ml and 0.75ml of sterile distilled water were added to 0.50ml and 0.25ml of pure honey to obtain 50% and 25% honey solution (v/v) respectively.

Preparation of Dried Filter Paper Discs

Whatman filter paper no. 1 will be used to prepare discs approximately 6 mm in diameter, which will be placed in a Petri dish and sterilized in a hot air oven.

The loop to be used for delivering the antibiotics will be made of 20-gauge wire that has a diameter of 2 mm. This will deliver 0.005 ml of honey to each disc.

Testing Antimicrobial Activity of Honey

Disc diffusion method: According to CLSI (2018) prepared discs were impregnated in the test's honey concentrations prepared above. MRSA was cultured in normal saline then diluted to a density visually equivalent to 0.5 MacFarland turbidity standard. The plates of Mueller Hinton Agar were dried in an incubator for 30 minutes; few drops of diluted culture

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of the test organism was floated on the agar medium and distributed all over the plate. The plates were allowed to dry for 5 minutes. Using sterile forceps, the impregnated discs were gently pressed down to ensure better contact with the agar. Unsoaked (with any honey concentration) filter paper discs of 5mm diameter were used as negative control. All plates were labelled and then incubated at 37°C for 24 hours. The zones of growth inhibition were measured as a diameter from the edge to edge of the clear area using ruler.

Determination of Minimum Inhibitory Concentration (MIC)

0.5ml of varying concentration (25, 50, 75, 100mg/ml) of honey was aseptically dispensed into four set of clean sterile test tube each containing 2ml of nutrient broth. A loopful of the bacteria 0.5McFarland turbidity standard was introduced into each of the corresponding test tube containing the nutrient broth (for the bacteria) and the reconstituted honey (0.5ml). A set of test tube containing broth only were seeded with the test organism and set as control. All the test tubes were then incubated at 37°C for 24hours. After incubation, the concentration that shows no visible growth of test organism was taken as the minimum inhibitory concentration (MIC).

Determination of Minimum Bactericidal Concentration (MBC)

To determine the MBC, incubated tubes, showing no visible growth/turbidity in MIC, were sub cultured onto sterile nutrient agar using the streak plating technique and incubated aerobically at 37^{0}c for 24 hours. The least concentration of honey that did not show growth of test organisms was considered as the MBC. Then inoculated plates were scored as bactericidal if no growth, bacteriostatic if there is light to moderate growth and no antibacterial activity if there is heavy growth.

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Result

Table 1: Morphological and Biochemical Characteristics of the Test Organism

Parameter	MRSA
Morphology	Cocci
Gram reaction	+
Catalase	+
Coagulase	+
Reaction to oxacillin disc	-

Key: - = Negative, + = Positive

Table 2: Antibacterial susceptibility of honey with different concentration against MRSA

Concentration (%)	Zone of inhibition (mm)
25	0
50	5
75	12
100	9
0 (control)	0

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Table 3: Minimum inhibitory concentration of honey against MRSA

Concentration(%)	Reaction	MICmg/ml
25	+	0.75
50	+	1.00
75	_	
100	_	

Key: - = Negative, + = Positive

Table 4: Minimum bactericidal concentration (MBC) of honey on the test organism

Concentration(%)	Reaction	MBCmg/ml
25	+	0.75
50	+	
75	-	
100	+	

Key: - = no growth , + = Growth

Discussion

The study revealed that honey has antimicrobial properties against MRSA at a concentration of 75%. This conforms with the study of Betts and Molan (2002) who revealed that diluted honey treated urinary tract infections because certain bacteria causing urinary tract infections, e.g. *E. Coli, Proteus species, Staphylococcus aureus and Streptococcus faecalis* were found to be sensitive to honey.

Previously, a small number of case studies that examined the antimicrobial activity of honey against methicillin resistant *Staphylococcus aureus* (MRSA) demonstrated that natural honey had an antimicrobial activity against the community associated MRSA organisms *in vitro*. (Wahdan, 1998)

The study revealed that the MIC of honey against MRSA is 0.75(v/v). This refutes the work of Betts and Molan (2002) who revealed that the MIC of honey was found to range from 1.8% to 10.8% (v/v), i.e. the honey had sufficient antibacterial potency to still be able to stop bacterial growth if diluted at least nine times, and up to 56 times for *Staphylococcus aureus*, the most common wound pathogen. This can be due to the fact that the efficacy of honey was dependent on the type of honey and the concentration at which it was administered.

Conclusion

Honey in this study, showed antibacterial activity against MRSA. It may however be recommended that future studies should focus on the determination of the active compound

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present in honey as the potency of honey is needed to optimize their selection and use as first-or-second line antimicrobials in various disease caused by MRSA.

Recommendation

Future studies should focus on the use of honey in combating antimicrobial resistance and treatment of wounds, ulcers and cuts, since it has antibacterial activity and enhances healing.

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