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ANTIMICROBIAL ACTIVITY OF SUDANESE HONEY AGAINEST *STAPHYLOCOCCUS AUREUS* WOUND ISOLATES COMPARED WITH MANUKA HONEY

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ABSTRACT

Background: Methicillin resistant *Staphylococcus aureus* is a major cause of nosocomial and community acquired severe human infections worldwide. The aim of this study was to determine the antimicrobial activity of some types of Sudanese honey against methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* from wound isolates in contrast with Manuka honey. **Methods:** A total of thirty wound isolates confirmed methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains were selected based on cultural characteristics, reactions in standard biochemical tests and routinely used antibiotics. The antimicrobial effect of different concentrations (v/v) of Sont (*Acacia nilotica*) and Sidr (*Ziziphus spina-christi*) Sudanese honey and Manuka honey (*Leptoapermum scoparium*) UMF® 10+ were tested using agar incorporation method in Muller-Hinton (MH) medium at 56 °C to detect the minimum inhibitory concentration (MIC). **Results:** The minimum inhibitory concentration of Manuka honey was detected at concentration 10% for both methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains. Higher minimum inhibitory concentrations, 30% and 50% were detected for Sont honey and Sidr honey respectively. **Conclusions:** Whilst Manuka honey showed better results than Sudanese honeys tested in this study nevertheless, these honeys possess reasonable antimicrobial activity against both methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* isolated from wound.

KEY WORDS: *Staphylococcus aureus*, Methicillin-resistant, Sudanese honey, Manuka honey.

INTRODUCTION

Honey is natural substance produced by bees from nectar or the secretions of plants.[1] Its has been widely used since antiquity for nutritional and medicinal proposes due to its therapeutic effects. Honey is composed mainly of carbohydrates consisting of maltose, sucrose and other sugars, many amino acids, vitamins, minerals and enzymes.[2,3] The antimicrobial properties of honey have been well recognized and natural elements of honey exhibits antimicrobial activities against a wide range of microorganisms.[4] Its activity is principally due to its ability to generate hydrogen peroxide, low pH, osmolarity and other composition of honey that have about 200 components.[5,6] In traditional medicine honey has a long history on management of infected wound, injuries, burns, digestion disorders and is valuable in the treatment of respiratory complaints and cardiovascular disease.[7]

Staphylococci, a group of facultative anaerobic, catalase positive, Gram-positive clustered cells, include the major pathogen *Staphylococcus aureus*.[8] *Staphylococcus aureus* causes both superficial and deep pyogenic infections and number of toxin mediated illnesses including skin lesions such as furuncles and carbuncles, abscesses, wound infections, pneumonia, osteomylitis, and others.[9] Methicillin resistance *Staphylococcus aureus* was reported early in 1960 shortly after methicillin (a β-lactam antibiotics) was introduced into clinical usage. This Methicillin resistance was found to be resistant to multiple antibiotics currently; there are various methicillin-resistant *Staphylococcus aureus* strains which presents a major concern to human health.[10] Thus, treatments of these cases are great challenge because numerous *Staphylococcus aureus* strains present resistance to current antibiotics.[10]

MATERIALS AND METHODS

Bacterial Isolate: A total of thirty wound isolates of *Staphylococcus aureus* were used in this study collected from patients visiting Khartoum Teaching University Hospital after reception of ethical approval from Ministry of Health Khartoum State. Additionally two reference strains of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains obtained from Microbiology Department Faculty of Medical Laboratory Sciences University of Khartoum were included. *Staphylococcus aureus* methicillin-resistant and

methicillin-susceptible were identified by using cultural characteristics, reactions in standard biochemical tests and susceptible to different antibiotics. Well identified five methicillin-resistant *Staphylococcus aureus* and other five methicillin-susceptible strains were used in this experimental study.

Honey Samples: A pure crude three types of honey were used in this study i.e. Sont (*Acacia nilotica*) and Sidr (*Ziziphus spina-christi*) Sudanese honey obtained from Bee kingdom Company (Sudan) and Manuka honey (*Leptoapermum scoparium*) UMF® 10+ obtained from Capilano Company (Australia).

Susceptibility Test: Antibacterial effects of honey were studied in vitro. Different concentrations of honey (v/v) were diluted in a Muller-Hinton (MH) medium at 56 °C to give final concentrations of 10%, 20%, 30%, 40%, and50%.Standard bacterial/broth cultures (5 colonies/10 ml of broth) were incorporated into Muller-Hinton medium with different honey concentration whereas bacterial broth only incorporated into Muller-Hinton medium without honey act as control. The plates were incubated at 37 °C for 20 h as described by Subrahmanyam et al. [11] after overnight incubation; the plates were subsequently examined for inhibition of growth. Muller- Hinton/Honey agar medium with the lowest honey concentration entirely inhibited the growth of the all isolates considered as minimum inhibitory concentration (MIC).

RESULTS

Minimum inhibitory concentration (MIC) of Manuka honey was attained within concentration 10% since it inhibited growth of both methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains in all 10 plates (Table 1). Manuka honey was followed by Sont honey which showed complete inhibition of bacterial growth to both methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains at concentration 30% although its antibacterial effects observed within concentration 20%. Two plates out of five (40%) of methicillin-resistant *Staphylococcus aureus* strains cultured in Sont honey showed no growth at the same time growth inhibition detected in three plates (60%) cultured with susceptible *Staphylococcus aureus* strains when 20% concentration of Sont honey was used (Table 2). Sidr honey used in this study showed the highest minimum inhibitory concentration (MIC) as it completely inhibited the growth of both of methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains in concentration 50% and it effect on bacterial growth started on concentration 40%. Within concentration 40% Sidr honey inhibited growth in only one plate cultured with methicillin-resistant *Staphylococcus aureus* strains (20%) while four plates (80%) showed no growth for methicillin-susceptible *Staphylococcus aureus* strains (Table 3). Furthermore the inhibitory action of three tested honey on methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains illustrated in Fig 1and 2 respectively.

Table 1: Minimum inhibitory concentration (%) of Manuka honey against MRSA and MSSA

Honey Concentration in Muller-Hinton agar	10%	20%	30%	40%	50%
Number and percentage of MRSA strains inhibited	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)
Number and percentage of MSSA strains inhibited	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)	5/5 (100%)

Table 2: Minimum inhibitory concentration (%) of Sont honey against MRSA and MSSA:

Honey Concentration in Muller-Hinton agar	10%	20%	30%	40%	50%
Number and percentage of MRSA strains inhibited	0/5 (0%)	2/5 (40%)	5/5 (100%)	5/5 (100%)	5/5 (100%)
Number and percentage of MSSA strains inhibited	0/5 (0%)	3/5 (60%)	5/5 (100%)	5/5 (100%)	5/5 (100%)

Table 3: Minimum inhibitory concentration (%) of Sidr honey against MRSA and MSSA:

Honey Concentration in Muller-Hinton agar	10%	20%	30%	40%	50%
Number and percentage of MRSA strains inhibited	0/5 (0%)	0/5 (0%)	0/5 (0%)	1/5 (20%)	5/5 (100%)
Number and percentage of MSSA strains inhibited	0/5 (0%)	0/5 (0%)	0/5 (0%)	4/5 (80%)	5/5 (100%)

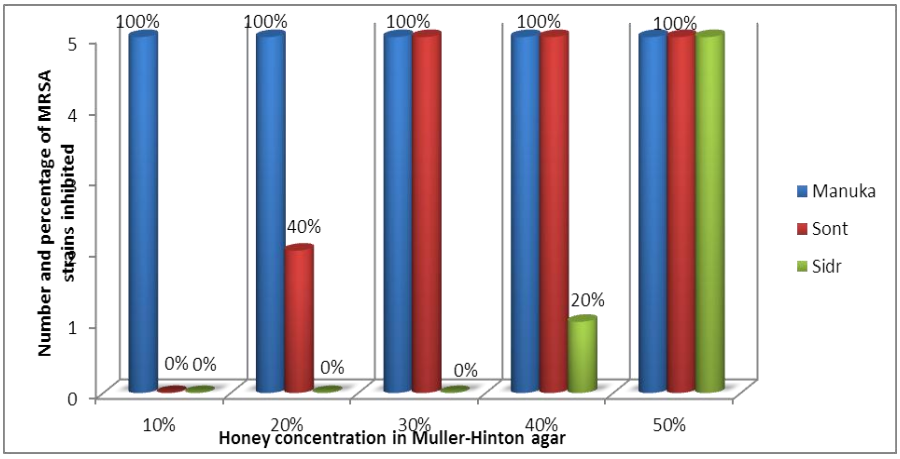

Fig 1: Minimum inhibitory concentration (%) of Manuka, Sont and Sidr honey against MRSA strains

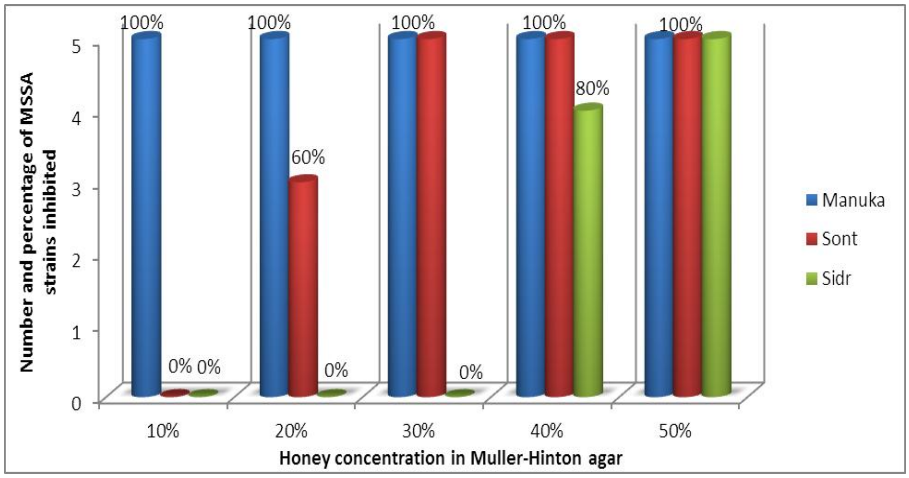

Fig 2: Minimum inhibitory concentration (%) of Manuka, Sont and Sidr honey against MSSA strains

DISCUSSION

This study was proved that three types of honey used at 10 - 50 % concentration showed inhibition of bacterial growth. Best result in this study achieved by Manuka honey which showed maximum antibacterial activity against methicillin-resistant and methicillin-susceptible *Staphylococcus aureus* strains. Comparable others findings in agreement of our study results concerning Manuka honey antimicrobial activity were reported by others authors in lots of studies conducted worldwide in past few years. [12-17] However by searched in Websites of Science for studies assessing the antimicrobial activity of Sudanese honey against microorganisms, there were limited published data. In agreement with our study similar results were found by Al- Naama et al. in study conducted to evaluate inhibitory effect of honey on bacterial isolates in Sudan, whereas best result obtained by Hamza et al. showed that the minimum inhibitory concentration of Sudanese honey was detected at 25% when tested against *Staphylococcus aureus*.[18-19] Many factors contributed in efficacies of different types of honey against the same type of bacteria. These factors involved geographical, seasonal, weather conditions, the bees' source of nectar as well as harvesting, and the location of the flowers, the storage time and conditions. [20-21]

CONCLUSION

Low effectiveness of Sudanese honey used in current study compared to Manuka honey may be due to the methods of beekeeping, harvesting, packaging process and storage conditions. Further studies needed to carry out in Sudan to assess in vitro and in vivo antimicrobial activity of Sudanese honey to encourage apply of honey for treatment of wound infections as a part of introducing traditional application of honey in modern medicine.

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