ASSESSING THE IN VITRO ANTIBACTERIAL ACTIVITY OF HONEY AGAINST WOUND AND RESPIRATORY TRACT INFECTING BACTERIA

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Received: 19th December, 2021 **Accepted:** 26th December, 2021 **Published:** 31st December, 2021

ABSTRACT

Background: Honey is a natural product rich in compounds with antimicrobial properties. The antimicrobial properties are being harnessed in the treatment of respiratory tract infections and wound care. Honey is locally available in Nigeria and is used in the treatment of wounds as well as respiratory tract infections. However, the antimicrobial potentials differ across honey sources and locality.

AIM: We sought to obtain laboratory based-evidence by assessing the antibacterial activities of three honey samples collected from Nsukka in Enugu State.

Methods: Neat and diluted honey was tested on six (*Escherichia coli, Pseudomonas aeruginosa, Salmonella* specie*, Staphylococcus aureus, Streptococcus faecium,* and *Streptococcus pneumoniae*) bacterial isolates from infected wound and sputum specimen using *in vitro* agar diffusion assay.

Results: The result obtained showed that the different honey samples had antibacterial activity on all the strains of bacteria tested except for *Pseudomonas aeruginosa* with minimum inhibitory concentration ranging from 10mg/ml to 27 mg/ml.

Conclusion: This suggests the usefulness of honey as an antibacterial for wound and respiratory tract infecting bacteria. However, the need for isolation and susceptibility testing of infecting bacterial organisms to honey in clinical practice is recommended.

Key words: Honey, Bacteria, Antibacterial Activity, Wound Infection, Respiratory Tract Infection

INTRODUCTION

There has been a remarkable increase in the use of therapeutically active compound extracted from natural products in the treatment of infections. This renewed interest is as a result of emergence, re-emergence and persistence of multi-drug resistant organisms, which has rendered most antibiotics therapeutically ineffective. Honey has been credited with various pharmacological properties as antimicrobial agent (Molan and Rhodes 2015; Scepankova *et al.*, 2021). Honey's antimicrobial property has been attributed to its high osmolarity, acidity, hydrogen peroxide content (inhibine), non-peroxide components or other phytochemicals (Yaghoobi *et al.*, 2013; Copper 2014). Globally, there is now a renewed interest in the use of honey as a topical agent in wound care such as treatment of wounds burns, injuries, ulcers and surgical wound infections (Al-Waili e*t al.*, 2011; Copper 2014). Interestingly wounds infected with methicillin resistant S*taphylococcus aureus* (MRSA) and those not responding to conventional treatment have been successfully treated with honey (Combarros-Fuertes *et al.*, 2020).

Citation: Onyejepu, N., Ogbonna F. N., Ikechukwu, U. E., Tukur, A. D. (2021): Assessing the *in vitro* Antibacterial Activity of Honey against Wound and Respiratory tract infecting Bacteria *BJMLS. 6(2): 62 -68*

Apart from its antibacterial and cleansing effect on wounds, honey also stimulates tissue regeneration, reduces inflammation and hastens healing with no adverse effects on the tissues (Molan and Rhodes 2015). Natural remedies such as honey has also been utilized orally in the treatment of other bacterial infections associated with respiratory tract infections ([RTIs]; El-Kased 2016). In particular, lower respiratory infections frequently caused by *Streptococcus pneumoniae* (*S. pneumoniae*) reported as a leading cause of morbidity and mortality globally (GBD 2016; Lower Respiratory Infections Collaborators 2018; Tchatchouang *et al.*, 2019) has also been treated effectively with honey (Abuelgasim *et al.*, 2021).

In the laboratory, significant antibacterial activity of honey has been demonstrated against wound infecting bacteria as well as those implicated in respiratory tract infections including some resistant species (El Kased 2016; Abuelgasim *et al.*, 2021; and Balázs *et al.*, 2021). With the increasing trend in multi-drug resistance bacteria globally, there is the need to assess the efficacy of locally available honey that may have antibacterial properties for the treatment of wound and respiratory tract infections. This is imperative as studies have shown that despite the broad-spectrum antibacterial activity of honey, there are still variations in potency between different honeys (Al-Waili *et al.*, 2011; Copper, 2014). In addition, licensed wound care products containing medical grade honey are not readily available (Copper 2014). Honey is indigenous in Nsukka, popular for commercial honey and bee hive products and a profitable venture (Onyekuru *et al.*, 2010). We therefore assessed the antibacterial activity of three different honey samples obtained at source from bee farmers in Nsukka metropolis against bacterial isolates from wound and respiratory tract infections.

MATERIALS AND METHODS

Collection of Honey Samples and Bacterial Isolates

Honey samples were collected from three villages; Imilike, Ete and Ovoko within Nsukka Metropolis of Enugu State in labelled sterile bottles and stored at room temperature. Clinical isolates of *Escherichia coli* (*E. coli*), *Pseudomonas aeruginosa* (*P. aeruginosa*), *Salmonella* species (*Salmonella* spp), *Staphylococcus aureus* (*S. aureus*) and *Streptococcus faecium* (*S. faecium*) from wound infections were collected from stock cultures at the bacteriology and mycology laboratory of the Department of Microbiology, University of Nigeria Nsukka. The *Streptococcus pneumoniae* (*S. pneumoniae*) isolate from respiratory tract infection was obtained from Medical Microbiology and Parasitology Department, Aminu Kano Teaching Hospital, Kano. These bacterial isolates including strains of *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 used as control were sub-cultured on McConkey agar (Oxoid Ltd Basingstoke, United Kingdom) and subsequently on Mueller Hinton (Oxoid Ltd Basingstoke, United Kingdom) and incubated aerobically at 37°C for 24 hours. Nevertheless, both *S. faecium* and *S. pneumoniae* was inoculated on blood agar and incubated in a carbon dioxide atmosphere (in candle jar) at 37°C for 24 hours to obtain pure discreet colonies.

Microbiological Assay

A loopful of each honey sample was gram stained and plated out on sterile blood agar, nutrient agar (Oxoid Ltd Basingstoke, United Kingdom)and Sabouraud agar (Becton, Dickinson and Company Sparks USA) plates respectively for quality assessment. The seeded plates were incubated at 37^{o}C for eighteen hours except for Sabouraud dextrose agar that was incubated at room temperature for 3 days. Inoculation of honey samples directly on blood and nutrient agar is for the isolation of bacteria while Sabouraud dextrose agar is for fungal isolation when present.

Antibacterial Assay

Using sterile nutrient broth as diluents, different dilutions of the crude honey samples were made to provide a Neat (100mg/ml), 1/2 (50mg/ml), 1/4 (25mg/ml), 1/8 (12.5mg/ml), 1/16 (6.25mg/ml) and 1/32 (3.125mg/ml) respectively. Three isolated colonies of test bacterial isolates were picked and emulsified in 15ml of sterile peptone water and incubated overnight at 37°C. An aliquot of the bacterial suspension was then introduced into sterile peptone water and matched to turbidity standard equivalent to McFarland 0.5. A sterile swab stick was dipped each into the bacterial suspension and swabbed on the surface of Mueller Hinton agar except for *S. faecium* and *S. pneumoniae* which were plated out on blood agar plates. In addition, the various bacterial suspensions were also plated out on agar plates without honey as control plates.

Screening for Antimicrobial Activity of Honey

Agar well diffusion method was employed for the antibacterial susceptibility testing of the honey samples (Vandepitte *et al.*, 1991; Valgas, *et al*., 2007). The seeded agar plates were prepared in triplicates for each of the honey samples and controls. Wells each of 7mm diameter were bored on the already seeded agar plates using a sterile cork-borer. The wells were filled with 60µl of various dilutions of the honey samples and allowed to stand on the bench for thirty minutes for the honey samples to diffuse into the media. The plates where then incubated aerobically at 37^{o}C. Only the blood agar plates were incubated anaerobically in a candle jar at the same temperature overnight. The control strains seeded on agar plates with and without honey served as controls. The antibacterial activities of the various honey samples were determined by measuring the zones of inhibitions in millimeter (mm) after incubation. Antibacterial activity was recorded as mean of the zones of inhibition.

RESULTS

The preliminary gram staining and microscopy of isolates obtained from culture of the crude honey samples on both nutrient agar and Sabouraud dextrose agar showed the presence of gram-positive rods and gram-positive hyphae respectively. The different diameter (mm) for the zones of inhibition obtained for the neat (100%) and the serial dilutions of the various honey samples are highlighted in Table I. All the honey samples presented evidence of antibacterial activity to the ATCC standard and typed isolates of *E. coli, Salmonella spp, S. aureus* and *S. faecium* and *S. pneumoniae* except for *P. aeruginosa that* showed no activity to the honey samples. All the neat (100%) honey and the honey at dilution 12.5mg/ml was inhibitory to the test isolates with zones of inhibition ranging from 20-27mm and 10-23mm respectively except for *S. faecium*. The control strains, *S. aureus* ATCC 25923 had diameter of 20mm, 19mm and 23mm while that of *E. coil* ATCC 25922 were 15mm, 13mm, and 20mm to honey samples A to C respectively. *Streptococcus faecium* had demonstrable activity at dilution 25mg/ml with diameter of the zones of inhibition ranging from 15-18mm. The undiluted honey exerted more inhibitory effect on all the isolates (especially for *S. aureus*) and did not differ markedly when compared to the standard strains *S. aureus* ATCC25923 and *E. coli* ATCC 25922.

Table 1: Antibacterial susceptibility profile of honey samples against wound and respiratory tract bacterial pathogens using agar well diffusion assay.

Honey	DIAMETER OF ZONES OF INHIBITION (MM)								
	***Dilutions* (mg/ml)**	**SA**	**EC**	**SF**	**PA**	**SAL**	**SP**	**ATTC *Staphylococcus aureus***	**ATTC *Escherichia coli***
Honey Sample A	100 (Neat)	25	24	25	0	21	24	27	21
	50	23	23	20	0	19	20	24	18
	25	18	20	18	0	14	17	21	17
	12.5	14	18	0	0	10	13	20	15
	6.25	0	0	0	0	0	0	0	0
	3.125	0	0	0	0	0	0	0	0
Honey sample B	100 (Neat)	26	23	24	0	21	22	25	21
	50	22	21	20	0	17	19	24	17
	25	20	18	15	0	14	16	21	15
	12.5	17	17	0	0	10	13	19	13
	6.25	0	0	0	0	0	0	0	0
	3.125	0	0	0	0	0	0	0	0
Honey Sample C	100 (Neat)	26	25	26	0	20	24	26	25
	50	23	21	24	0	16	24	24	23
	25	17	18	18	0	15	20	24	21
	12.5	13	17	0	0	10	16	23	20
	6.25	0	0	0	0	0	0	0	0
	3.125	0	0	0	0	0	0	0	0

Key: SA = *Staphylococcus aureus,* EC = *Escherichia coli, SF = Streptococcus faecium, PA = Pseudomonas aeruginosa, SAL = Salmonella spp, SP = Streptococcus pneumoniae*

DISCUSSION

The study accessed the antibacterial activity of honey obtained from Nsukka metropolis to wound and respiratory tract infecting bacteria using the agar well diffusion technique. All the test isolates from wound and respiratory tract infections did not vary markedly in their response to the antibacterial activity of the honey samples. This suggests an antibacterial effect of honey obtained from this environment. However, this is a cautious assumption since the study did not compare the zones of inhibition to the critical diameter of a standardized antibiotic (Vandepitte *et al*, 1991).Wide range of bacterial species implicated in wound and respiratory tract infections are susceptible to the antibacterial effect of honey thereby supporting its clinical use (Mandal and Mandal, 2011; Al-Waili *et al.*, 2011; Balázs *et al.*, 2021). However, the antibacterial activity and level of potency of honey vary even within countries (Irish *et al.*, 2011; Bazaid *et al.*, 2021). Various studies in Nigeria and other countries on wound and respiratory bacterial pathogens seem to support this trend (Irish *et al.*, 2011; Mshelia *et al.*, 2017; Oluboyo, *et al.*, 2017; Ede *et al.*, 2017). The physical property, geographical distribution and different floral sources may play important role in the antimicrobial activity of honey in particular the undiluted neat honey which exhibited the widest zones of inhibition supporting earlier reports (Mandal and Mandal, 2011; Bazaid *et al.*, 2021).

Honey samples A and B were obtained from felled logs and is usually referred to locally as golden honey because of its colour while that of honey sample C is referred to as brown honey which was obtained from castor oil tree. The source of honey did not seem to have effect on the susceptibility of the test organisms to the honey samples. However, the physical properties such as acidity, osmotic effect, moisture and phytochemicals found in honey from Nsukka environ are important antibacterial properties that may suggest the antibacterial effect of these honey (Nweze, *et al.*, 2017).

The antibacterial effect on both the Gram-positive and Gram-negative bacteria assessed in this study suggest the antibacterial broad-spectrum potentials of honey despite the lack of activity against *P. aeruginosa.* The honey samples showed the highest activity against *S. aureus* notorious for its multiple drug resistance (MRSA) to existing antibiotics. Many studies have reported high susceptibility by *S. aureus* including MRSA to honey (Huttunen *et al.,*2013; Ede *et al.,* 2017;Almasaudi*et al.*, 2017). *Staphylococcus aureus* are usually osmotolerant to honey and are therefore completely inhibited even when honey was diluted to a much lower concentration (Bang *et al.*, 2003). Similarly, other Gram-positive organisms including *Streptococcus* spp*,* (*S. pneumoniae*), coagulase negative staphylococci also showed activity to honey including that from Nsukka, various localities in Nigeria and elsewhere (El Kased 2016; Okeke *et al.*, 2018; Almasaudi *et al.*, 2017). Several studies have documented activity of honey on wound infections caused by *P. aeruginosa* in contrast to our study that observed resistance to the different dilutions of the honey samples (Scepankova *et al*., 2021). Ede *et al.* (2017) reported sensitivity up to 50mg/ml while Shenoy *et al*. (2012) reported bactericidal activity even when honey was diluted five-folds *in-vitro* for wound infecting *P. aeruginosa*. Now *P. aeruginosa* resistant to treatment with honey has also emerged. Camplin and Maddocks (2014) reported that biofilm-forming *P. aeruginosa* showed increased resistance when treated with honey and other antimicrobials. It is however interesting to note that some studies have also reported reduced antibacterial activity or effectiveness of honey in the treatment of respiratory tract infections caused by *P. aeruginosa* (El Kased 2016; Oluboyo et al., 2017; Balaz *et al*., 2021).

The antibacterial effect of honey is dependent on concentration of honey used and the nature of the bacteria as observed in this study (Al-Waili *et al.*, 2011). This indicates that antibacterial effect varies across the spectrum of honey collected from Nsukka metropolis. This portends the need to isolate, characterize the infecting bacteria and assess for antibacterial activity before use of honey products in the management of wound and RTIs. Taken together, studies have shown comparable antibacterial activity of honey to both Gram-positive and Gram-negative bacteria demonstrating broad spectrum antibacterial potential. This is credited to anti-microbial and other anti-infective properties abundant in honey (Mohd Kamal 2021). Although we did not evaluate the antibacterial properties of the honey samples in order to determine variance in their antibacterial activity but scientific literatures reviews have documented the effectiveness of honey as an antimicrobial agent (Al-Waili *et al.*, 2011; Scepankova *et al.*, 2021).

CONCLUSION AND RECOMMENDATION

Assessment of antibacterial activity of honey sourced from Nsukka metropolis have demonstrated the broad-spectrum antibiotic potentials of these honey and thus its value in wound care and management of respiratory tract infections. This supports the use of honey as an add-on to management of wound and respiratory tract infections. However, isolation of the infecting bacteria and its susceptibility to the honey should be assessed. Agar well-diffusion assay for susceptibility testing offers a simple, low cost, easy to interpret results in this setting.

ACKNOWLEDGEMENTS

The authors wish to express their heartfelt thanks to the staff of the Microbiology and mycology laboratory of the University of Nigeria, Nsukka for making available the isolates used for the study and for their invaluable contributions to study methodology.

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