ORIGINAL PAPER

Nig. J. Pharm. Res. 2015, 1, pp 32-39
ISSN 0189-8434

Comparative Antibacterial Activity of Some Nigerian Honey and Commonly Used Antiseptic Agents against Strains of MRSA and Other Multidrug Resistant Staphylococci Isolates From Surgical Wound Infections

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation;D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Alternative and effective means of reducing the bacterial burden of surgical wound infections are urgently required as a result of the increasing emergence of multiple-antibiotic-resistant pathogens.
Objectives: Renewed interest in honey for the treatment of infected wounds has led to the search for new and effective antibacterial honeys.
Material and Methods: Honey samples obtained from three different geographical locations in Nigeria; Benue, Osun and Oyo states, and three commonly used antiseptics agents were screened against thirty-one strains of MRSA and other multidrug resistant staphylococci isolates from surgical wound infections using the agar dilution method.
Results: The susceptibility of the organisms to the three honey samples was not affected by the degree of their resistance to antibacterial agents. Ninety percent of pathogens were sensitive to 30%v/v of one of the honey samples while the other two samples inhibited growth of 71.0% of these pathogens. At the lowest concentration of 15% tested all the honey samples were able to inhibit some of the pathogens. Only 19.4%, 25.8%, and 3.2% of the staphylococcal pathogens were sensitive to cetrimide, acriflavine and chlorocresol respectively at 32µg/ml.
Conclusions: Honey from Nigeria would be effective alternative to antiseptic agents in the management of surgical wound infections caused by MRSA and other multidrug-resistant staphylococci and may prove to be a valuable therapeutic agent.
Keywords: MRSA, Coagulase negative staphylococci, Antiseptics, Antibiotic resistance, Honey.

INTRODUCTION

Honey is the natural sweet substance produced by honeybees from the nectar of blossoms or from the secretion of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honeycomb to ripen and mature (Codex alimentarius, 2001). It has long been regarded as a valuable human food, sacred material and a medicine in various parts of the world. Therefore, it has been used to treat diverse illnesses including all kinds of infections such as respiratory, enteric, burn, eyes and wound infections (Jones, 2001; Kwakman, 2010 ; Simon et al., 2008).

Even though it is easily recognisable, an observation of the different types of honey available, either wild or cultivated, readily demonstrates that all honeys are not identical. Flora origin, bee species, geographical source and post-harvesting conditions influence its characteristics therefore its activity may not generally be assumed to be the same (Agwu and Okeke, 1997; Molan, 2002; Popa etal., 2009). Although the precise composition of honey varies according to the plant species on which the bee forages, studies have indicated that the main constituents are the same in all honeys (Kwakman et al., 2010; Omafuvbe and Akanbi, 2009).

In Nigeria, honey is relatively inexpensive and readily availabile. It is currently recognized as a good source of income since it is being used for quite a wide range of purposes. Therefore cultivators, harvesters, collectors, and hawkers of honey have significantly increased (Ayansola and Banjo, 2012; Omafuvbe and Akanbi, 2009).

Earlier studies on Nigerian honeys have indicated that they have wound healing and antimicrobial properties, *in vitro* and *in vivo* (Adeleke et al., 2007; Ayansola and Banjo, 2012; Omafuvbe and Akanbi, 2009; Yenda et al., 2010). It is however noted that these reports are still insufficient as more information on antimicrobial activities of other honeys from Nigeria is needed if they will be considered for alternative therapy. For example, reports of the antimicrobial effectiveness of Nigerian honeys on methicillin resistant *S. aureus* (MRSA) and multidrug resistant coagulase negative staphylococci (CoNS) are still

very scanty in literature. The few reports available have screened limited number of isolates from clinical specimens (Adeleke et al., 2007; Omafuvbe and Akanbi, 2009; Osho and Bello, 2010; Yenda et al., 2010). In addition, whether these honeys will present better antibacterial activities compared with various antiseptics agents used in wound disinfection are also unknown.

We report an in vitro evaluation of three Nigerian honeys against MRSA strains and other multidrug resistant staphylococci isolated from post operative wound infections and compared this activity with those obtained for some antiseptics commonly used for personal hygiene and wound disinfection. This is with a view to ascertain if these honeys will have comparative advantages for alternative therapy.

METHODS

Bacterial Strains

The microbial species tested were from the stock collection in our laboratory. They were staphylococci isolates earlier recovered from patients with post-operative wound infections in a Nigerian hospital which had been stored in the medium of Gibson and Khoury (1986) and in Nutrient Agar stabs in our laboratory. The staphylococci isolates comprised *S. aureus* (n=20) consisting of MRSA (n=6) and MSSA (n=14), *S. epidermidis* (n=5), *S. saprophyticus* (n=2), *S. xylosus* (n=1) and *S. haemolyticus* (n=3) making a total of thirty-one. The organisms were subcultured in fresh Nutrient Broth and Blood Agar media for use in the study. *S. aureus* reference strains: *S. aureus* NCTC 6571 and *S. aureus* NCIB 8588 were used as control.

As all the staphylococci isolates used in this study were taken directly from storage in our laboratory and studied in vitro using anonymous numbers, no patient information, codes, or names were revealed and no patient charts were identified or reviewed; therefore, no institutional review board was necessary for this study.

Honey samples

The honey samples used for the study were obtained from local producers and their characteristics are as stated in Table 1. Only honeys which had not been heated were used. The honeys were kept in amber coloured bottles tightly closed with screwed cover and kept on the laboratory shelf at room temperature prior to use.

Table 1 Characteristics of the honey samples

S/N	Source	Honey Sample	Extraction
1	Ibariba town, Oyo State, South-West Nigeria	Dark brown colour	Harvested locally cultivated
2	Jatuaka town, Benue State, South-East Nigeria	Light brown colour	Harvested wild
3	Ile-Ife town, Osun State, South-West Nigeria	Dark brown colour	Harvested locally cultivated

Tests for sterility of honey

Each honey sample was tested for sterility by streaking aseptically on nutrient agar plates and incubated at 37^{o}C for 24hours.

Sterilization was done by membrane filtration method using individual sterile 0.45µm pore size cellulose acetate membrane filters (Corning, England) with the filtration apparatus connected to an electronically operated vacuum pump. This was done after diluting the viscous honey by adding 40ml sterile distilled water to 60ml honey aseptically. The filtration process was slow.

The honey filtrate was again aseptically streaked on nutrient agar plates and incubated at 37^{o}C for 24h for sterility check. The honey filtrates form the stock solutions which were then dispensed into 5-ml sterile amber coloured bottles and used immediately for further tests.

Antibiotic susceptibility screening

Antibiotic susceptibility tests were performed according to modified Kirby-Bauer disc diffusion technique on Isosensitest agar (Oxoid, England) using the discs ofantibiotics reported in Table 2 and interpreted following CLSI guidelines (CLSI 2008). In order to detect resistance to oxacillin for identification of MRSA, Oxacillin Agar Screening technique was used as described earlier (Akinkunmi and Lamikanra, 2012).

Determination of minimum inhibitory concentrations (MICs) of the antiseptic agents and minimum active dilutions (MADs) of honey

MICs were determined by the agar dilution method. Plates of Isosensitest Agar containing 20mL of agar incorporating doubling dilutions of antimicrobial agents: cetrimide [Hopkin and Williams, England] (1-32µg/ml), acriflavine [Hopkin and Williams, England] (1-512µg/ml) and chlorocresol [Fluka Chemika, Switzerland] (1-512µg/ml) were prepared and dried at 50 ^{o}C for 15 min.

MADs of the honey samples were also determined by the agar dilution method as described by Cooper et al., (2002) with slight modifications. Briefly, further dilutions of honey were prepared by incorporating the stock honey filtrate to appropriate aliquots of molten double strength Isosensitest agar at 50 °C to produce a range of plates containing honey at concentrations of 15%v/v, 20%v/v, 25%v/v and 30% v/v which were prepared in duplicate and dried at 37 ^{o}C for 15min immediately before use. Plates containing no antimicrobials or honey were included as controls.

Table 2 Minimum Inhibitory Concentration (MIC) of antiseptic agents and Minimum active dilution (MAD) of honeys determined for the *S. aureus* and CoNS strains from surgical wound infections

No	Staphylococcal isolate	Strain of isolate	Antibiotic resistance pattern	MIC of Antiseptics (µg/mL)			MAD of Honey (%v/v)		
				Cetrimide	Acriflavin	Chlorocresol	Honey 1	Honey 2	Honey 3
1	1A	*S. aureus*	PV, DX, AMX, EM, CL, GEN, COT, TET, NAL, OX	>32	512	128	20	15	20
2	8B	,,	PV	8	8	32	30	30	30
3	11E	,,	PV, DX, AMX, AUG, EM, GEN, TET, NAL, OX	>32	>512	128	>30	20	>30
4	12A	,,	PV, DX, AMX, EM, GEN, COT, NAL, OX	>32	>512	64	20	15	20
5	14D	,,	PV, DX, EM, NAL	>32	512	64	25	15	20
6	17A	,,	PV, DX, AMX, EM, CL, GEN, COT, TET, NAL, OX	>32	>512	64	>30	25	30
7	19D	,,	PV, DX, AMX, NAL	>32	16	64	30	30	>30
8	23C	,,	PV, DX, AMX, AUG, EM	>32	128	64	30	>30	>30
9	24C	,,	PV, DX	8	>512	64	25	20	25
10	26B	,,	PV, DX, TET, NAL,	16	1	64	15	25	>30
11	46A	,,	PV, DX, AMX, EM, CL, GEN, TET, NAL	>32	128	64	25	20	25
12	28B	,,	PV, DX, AMX, AUG, CL, COT, NAL	8	256	128	>30	30	30
13	31C	,,	PV, DX, AMX, AUG, EM,GEN, COT, NAL, OX	>32	512	128	20	15	20
14	34B	,,	PV, DX, CL, TET, NAL	>32	2	64	20	20	20
15	36D	,,	PV, DX, AMX, EM, GEN, COT, NAL	>32	512	64	25	20	25
16	41B	,,	PV, DX, EM, CL, GEN, COT, TET, NAL, OX	>32	>512	64	>30	>30	>30
17	48B	,,	PV, DX, EM, CL, TET, NAL	>32	512	64	30	15	30

18	55A	,,	PV, DX, AMX, AUG, EM, NAL	>32	32	64	25	15	20
19	56B	,,	PV, DX, AMX, AUG, EM	>32	128	64	25	15	20
20	59A	,,	PV, DX, AMX, EM, GEN, TET, NAL,	>32	32	64	>30	30	>30
21	13B	*S. epidermidis*	PV, DX, AMX, EM, CL, GEN, COT, TET, NAL, OFL, CI, OX	>32	128	128	20	15	25
22	20A	,,	PV, DX, AMX, EM, CL, GEN, TET, NAL, OX	>32	128	64	30	30	>30
23	22D	,,	PV, DX, NAL	>32	512	64	>30	20	25
24	27E	,,	PV, DX, EM, CL, GEN, COT, TET, NAL, OX	>32	>512	64	30	>30	>30
25	36A	,,	PV, DX, AMX, EM, CL, GEN, COT	>32	>512	64	>30	15	25
26	18B	*S. saprophyticus*	PV, DX, EM, GEN, COT, NAL	4	1	64	15	15	15
27	21D	,,	PV, DX, AMX, AUG, EM, CL, GEN, COT, TET, OX	>32	>512	128	>30	25	30
28	2A	*S. haemolyticus*	PV, DX, AMX, EM, CL, GEN, COT, TET, NAL, OX	>32	128	64	>.30	20	>30
29	3B	,,	PV, DX, AMX, AUG, EM, GEN, COT, TET, NAL, OX	32	1	64	15	15	15
30	29C	,,	PV, DX, AMX, EM, GEN, TET, NAL	>32	512	64	30	20	25
31	15A	*S. xylosus*	PV, DX, EM, NAL	>32	512	64	30	20	30

PV: Penicillin V; DX: Cephadroxil; AMX: Amoxicillin; EM: Erythromycin; CL: Chloramphenicol; GEN: Gentamicin: COT: Cotrimoxazole; TET: Tetracycline; NAL: Nalidixic acid; OX: Oxacillin; AUG: Augmentin; OFL: Ofloxacin; CI: Ciprofloxacin

Four colonies of each test and reference strain grown overnight on Blood Agar plates were inoculated into 2ml of Nutrient broth for 18 hours. A 1 in 1000 dilution of each organism was prepared in sterile distilled water and the resultant suspensions containing approximately 2 x 10^5 cfu/ml (by comparison with McFarland standard 0.5) were applied to the surface of the antiseptic, honey and control plates in duplicate with a multipoint inoculator applying an approximately 1µl of the suspension. The plates were allowed to dry at room temperature for 15 minutes before incubating for 24 hours at 35^{o}C. The lowest concentration of agents and honey inhibiting growth in both replicates was taken as the MIC for the antiseptic agents and MAD for the honey.

Statistics

Results were compiled and analysed by calculating percentages.

RESULTS

All the honey samples were found to be non-sterile when streaked on agar plates. The honey filtrate passed the sterility tests.

The antibiotics resistance profiles of organisms are as stated in Table 2. The MADs of the three honeys samples and the MICs of the antiseptic agents for each organism are also shown in Table 2.

The different species of staphylococci demonstrate variable degree of susceptibility to the antibiotics. No two isolates exhibited similar antibiogram. More than 90% of the isolates showed resistance to more than three of the antibiotics tested. One of the isolates demonstrated resistance to up to twelve antibiotics including the fluoroquinolones, ciprofloxacin and ofloxacin (Table 2).

There are very considerable differences in the activities of the antiseptics on the MRSA and MSSA isolates as none of the antiseptic agents could inhibit the growth of the MRSA at 32µg/ml. On the other hand, 7.1%, 28.6% and 48.9% of the MSSA were susceptible to chlorocresol, cetrimide and acriflavine respectively at the same concentration (Table 3). In contrast to what obtained for the antiseptic agents, the honey samples demonstrated considerable activities against the MRSA strains with as much as 83.3% of the MRSA strains susceptible to the Honey Sample 2 with the highest activities, 66.7% to Honey Sample 3and 50.0% to the Honey Sample 1.

Only 19.4%, 25.8%, and 3.2% of the staphylococcal pathogens were sensitive to 32 µg/ml of cetrimide, acriflavine and chlorocresol respectively. On the other hand, up to 90% of pathogens were sensitive to one of the honey samples. The two remaining honey samples showed similar activities on the organisms as 71.0% of these pathogens were sensitive to each of them.

DISCUSSION

The results of this study clearly show that the Nigerian honey samples have the potential to be used as antibacterial agent to prevent and control surgical wound infections caused by MRSA and other staphylococci in a degree higher than some commonly used antiseptic agents. Staphylococci isolates showed relatively consistent susceptibilities to the three samples of natural honeys screened and the effectiveness of the honeys is shown to be independent of antibiotic susceptibility of these pathogens. The lack of considerable differences in susceptibility to honey by any of the isolates tested may indicate that other well-known wound pathogens such as *Pseudomonas aeruginosa* and *Enterococcus species* are likely to be equally as susceptible (Cooper et al., 2002a; Cooper et al., 2002).

It has been suggested by Molan (2002) that to explore the potential of honey in treating and preventing wound infection, clinical isolates should be tested. For this reason clinical isolates of staphylococci from post operative wound infections were employed in this study. Staphylococci and especially MRSA have been implicated as the main etiologic agents in surgical wound infections (Akinkunmi *et al.*, 2014).

When honey is applied to wounds, it will be diluted by body fluids. Hence, if honey is to be an effective wound antiseptic, it must retain inhibitory activity on dilution. In this study the three honeys tested each retained considerable inhibitory activity in vitro even after dilution. This might indicate that these honeys will be effective in vivo when used to treat such wounds and may support the reported effectiveness of some Nigerian honey in the treatment of wound infections in some hospitals in Nigeria (Adesunkanmi and Oyelami, 1994; Okeniyi et al., 2005). The results also confirm an earlier report by French et al., (2005) on the effects of honey on CoNS where it was indicated that honey could be diluted by exudates up to 20-fold and still inhibit the growth of CoNS.

Results show that none of the staphylococci, MRSA, MSSA and CoNS, was 100% sensitive to all the antibiotics. This result is comparable to other studies (Ayansola and Banjo, 2012; Hafeez et al., 2004). MRSA infections are noted to be often life-threatening and to be recalcitrant to other antimicrobial agents (Hafeez et al., 2004; Harbath et al., 2008). Honey is promising to be effective in these cases as results indicate that it shows activities which appears not to be affected by methicillin resistance.

The demonstratively higher inhibitory activity of samples of honey in comparison with the antiseptic agents, cetrimide, acriflavin and chlorocresol tested in this study against multidrug resistant pathogens is in agreement with earlier reports by other researchers on honey in Nigeria. For example Karayil et al., (1998) found honey to be superior to cephaloridine, ampicilin, gentamicin, nitrofurantoin, nalidixic acid and co-trimoxazole in inhibiting growth of nine strains of pathogenic organisms. Similarly, Adeleke et al., (2007) has reported a higher antibacterial activity for honey over cloxacillin and ampicillin against clinical strains of *S. aureus* and *S. albus*. Yenda et al., (2010) has also shown that ciprofloxacin and ofloxacin used in concentrations of 5µg/ml were less active on their MRSA isolates compared with three honey samples inNigeria from Sardauna Plateau, Hong and Abuja. Furthermore, Molan (2006) had reported a more effective treatment of bacterial infected burn wounds with honey than with silver sulphadiazine, a recognized

antibacterial ointment. The present study has thus shown that Nigerian honeys have comparative advantages not only over some antibiotics but also over these antiseptic agents used for prevention of wound infections. The non susceptibility of some of the test organisms to the honey samples could be due to the emergence of resistant strains, a subject which deserve further investigations.

The floral sources of honey have been reported to be responsible for some of the antibacterial activities of honey (Akharaiyi and Omoya, 2005). Studies have shown that most Nigerian higher plants have high antimicrobial activities (Akharaiyi and Omoya, 2005; Omafuvbe and Akanbi, 2009). These activities have been reported for the different parts including roots, stems, barks, leaves, fruits and flowers. This could be responsible for the high antibacterial potency that has been reportedly observed in most honey from Nigeria since bees majorly obtained their materials from these plant parts (Akanmu et al., 2011; Ayansola and Banjo, 2012). This is confirmed in this study as the flora source of most Nigerian honey samples were plants that have been reported to have high antimicrobial activities (Akanmu et al., 2011; Agwu and Okeke, 1997).

It has been shown that the antimicrobial activity of honey may range from concentrations lower than 3% to concentrations of 50% and higher (French, 2005; Osho et al., 2010; Wilkinson and Cavanagh, 2005). This study confirmed these reports. These levels of concentrations have been reported to be standardised in some honeys such as the Manuka (Leptospermum) honey from New Zealand (Comvita Ltd, Bay of Plenty, New Zealand), the Canadian Clover and Buckwheat honeys (Farmboy Inc., Ottawa, Canada) and the Sidr honey (in Yemen). These honeys are now available in commercial grades in licensed products on sale for wound care in Australia, Europe and New Zealand.

The honey samples investigated in this study could therefore be developed to such commercial scale. Additional support of this proposition is the fact that the physico-chemical properties of natural honey from different geographical locations of Nigeria have been reported to compare favourably with honeys from other countries (Omafuvbe and Akanbi, 2009).

There are other advantages honey has over other antiseptics when applied to the traumatized tissue around medical devices. It has been reported to possess anti-inflammatory activity which can be expected to prevent serous exudates that can serve as medium for bacteria to colonize (Molan, 2002).

Furthermore, honey has been reported to have a stimulatory action on growth of wound repair tissues and to provide moist conditions ideal for healing (Kwakman et al., 2010). In addition to this, unlike other antiseptics, it has no harmful effects on tissues and its slow enzymic release of hydrogen peroxide has been reported to give about one thousandth of that in a 3% hydrogen peroxide solution (Brudzynski, 2006; Kwakman et al., 2010). It is therefore an additional advantage to discover that these honey samples have better or comparable activities to antiseptics against known wound pathogens.

CONCLUSION

From the results presented in this report we conclude that these honey samples possess high antimicrobial activity and can be used as a means of reducing the bacterial burden of surgical wound infections and for treatment of such infections. Investigations of the mechanisms of the antibacterial activities of these honeys should constitute the subject of further studies.

ACKNOWLEDGEMENT

The abstract of this work has been partly published in the Book of Events and Abstracts of the 13th Annual National Conference and Scientific Meeting of the Nigeria Association of Pharmacists in Academia (NAPA), 10-14 August, 2015 page 52.

Table 3: Percentage of organisms susceptible to antiseptic agents and honey at stated concentrations and dilutions

Staphylococcus spp (Number)	Agents (Concentrations)					
	Cetrimide (=32µg/mL)	Acriflavin (=32µg/mL)	Chloocresol (=32µg/mL)	Honey 1 (=30%w/v)	Honey 2 (=30%w/v)	Honey 3 (=30%v/v)
S. aureus (20)	20.0	30	5	75.0	90.0	70.0
MRSA (6)	0.0	0	0	50.0	83.3	66.7
MSSA (14)	28.6	42.9	7.1	85.8	92.9	71.4
S. epidermidis (5)	0.0	0	0	60.0	80.0	60.0
S. saprophyticus (2)	50.0	50	0	50.0	100.0	100.0
S. xylosus (1)	0.0	0	0	100.0	100.0	100.0
S. haemolyticus (3)	33.3	33.3	0	66.7	100.0	66.7
Total (31)	19.4	25.8	3.2	71.0	90.3	71.0

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Conflict of Interest: None declared
Received: 31 August, 2015
Accepted: 9 November, 2015