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Original Research Article

Studies on the Effect of Some Nigerian Honey on Organisms Associated with Burn Wound Infection

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Abstract

This study was aimed at determining the susceptibility of some organisms namely *Staphylococcus* sp., *Pseudomonas* sp., *Klebsiella* sp. and *Escherichia coli* associated with burn wound infection to some Nigerian honey. Thirty (30) swabs from patients with burn wound were cultured. The most frequently isolated organism was *Staphylococcus* sp. (50.0 %) followed by *Pseudomonas* sp. (26.6%), *Klebsiella* sp. (13.4%) and *E. coli* (10.0 %). The pH values of all the honey samples were in the range of 3.63-4.09. All the honey samples tested showed strong definite antibacterial activity against the organisms associated with burn wound infection but the activity pattern was different for different honeys. The isolates were also sensitive to the antibiotics ciprofloxacin and ceftazidime at 1 mg/ml concentration (2.1 mm-5.7 mm). The MIC values of the Type I honey sample against *E. coli* and *Staphylococcus* sp. was 80% and for *Klebsiella* sp. and *Pseudomonas* sp. the value was 50% while the respective MBC values were 80% and 50%. For Type IV sample, the MIC values against *E. coli*, *Staphylococcus* sp., *Klebsiella* sp. and *Pseudomonas* sp. were 40%, 70%, 30% and 30% whereas the corresponding MBC values were also 40%, 70%, 30% and 30%.

Keywords

Antibacterial activity
Burn wound
Honey
MBC
MIC
Sensitivity

Introduction

Honey is a natural nutritious food that is produced from the nectar and pollen of plants. However, the composition of honey varies widely and depends on the botanical origin of the nectar and also on the geographical location. Honey composition includes the sugars glucose, fructose, maltose and sucrose, water and other minor components such as proteins, organic acids, amino acids, flavonoids, vitamins and minerals (Wang and Li, 2011).The antimicrobial activity of honey has been extensively studied; acidity, osmolarity and hydrogen peroxide have been identified as three components contributing to antimicrobial activity. Hydrogen peroxide is the most significant contributor followed by osmolarity and acidity. Honey is a saturated sugar solution and this high

concentration of sugars leaves very little available water for growth of microorganisms. Honey also contains many organic acids, mainly gluconic acid, produced from glucose by glucose oxidase and is characteristically acidic with a pH in the range of 3.2-4.5 (Davis, 2005). Among its several uses, honey is used for the treatment of many infections and also used effectively as wound dressing including surgical wounds, burns, and skin ulcers (Moore et al., 2001). It has been reported that honey speeds up the growth of new tissues and so help to heal the wound, reduces pain and odour quickly (Lusby et al., 2005). Honey based wound care products have been registered in Australia, New Zealand, European Union, Hong Kong and United States with the relevant regulatory authorities. In most cases, these products use manuka honey from New Zealand or the equivalent honey product from the *Leptospermum* species in Australia (Irish et al., 2011). It has both bactericidal and bacteriostatic effect against various types of Gram positive and Gram negative bacteria such as methicillin resistant *Staphylococcus aureus, Pseudomonas aeruginosa* and *Escherichia coli* (Sherlock et al., 2010). This antibacterial effect is dependent on the concentration of the honey used and the nature of the bacteria isolated (Adeleke et al., 2006).

Honey is produced from different floral sources and its antimicrobial activity varies with origin and processing. Laboratory researches/studies have revealed that honey is effective against methicillin-resistant *S. aureus* (MRSA), *beta-haemolytic streptococci* and vancomicin-resistant enterococci (VRE) (Allen et al., 1991). Yenda et al. (2010) studied on the susceptibility of MRSA isolates to some Nigerian honey. They isolated twenty MRSA from patients attending State Hospital, JimetaYola, Adamawa State. All the twenty MRSA were susceptible to different honey samples obtained from Sardauna Plateau, Hong and Abuja in Nigeria.

Microorganisms routinely isolated from burn wounds include organisms like *S. aureus, Streptococcus pyogens, E. coli, Klebsiella spp., Acinetobacter baumanii –calcoaceticus complex. Candida albicans* is the most common fungal species causing infection (Keen et al., 2010). *S. aureus*, *E. coli*, *S. pyogenes* and other bacteria have developed resistance against some commonly used antibiotics due to the indiscriminate use of the drugs and also availability of fake drugs which are substandard. Sometimes wound burn infection is hard to cure and honey can be employed to treat wound burn infection. The resurgent in the interest of honey as a treatment for infections is due to the emergence of resistant strains of bacteria to the currently available antibiotics. The present study was aimed at evaluating the *in-vitro* antimicrobial effect of bee honey collected from different sources in Kaduna, Nigeria on some microbial isolates associated with burn wound infections.

Materials and methods

Collection of samples

Four different types of honey from different sources were collected for this study. The three samples of the honey harvested as natural honeys were labeled as A_1 (Kachia), A_2 (Kagoro) and A_3 (Kajuru) which were collected from three Local Government Area of Kaduna State and dispensed into sterile cork-screwed containers. Fresh, dark and unadulterated were the criteria for purchase. In the laboratory, all the three honeys were extracted by hand pressing and then filtered with a sterile mesh to remove debris. Their purity and confirmation of non-adulteration through physical examination of color, viscosity and non-infected with insects was performed. The fourth type of honey used for this study was purchased as processed honey (A_4) and was bought at supermarket.

Finally, each of the four honey samples collected was streaked on nutrient agar and Sabouraud Agar (SDA) plates, and then incubated at 37^0C for 24 hrs. Each plate was examined in turn for absence of microbial population. In order to determine the catalase activity of the different honeys, slide catalase test was performed for each of four tested honey samples (Benson, 2005).

Isolation and identification of clinical isolates used for this study

Selection of Hospitals for Collection of Clinical Specimens: Two (2) hospitals were used for specimen's collection, St. Gerald's Hospital, Kakuri, Usman Dantosho Hospital, Tudun Wada Kaduna. From each of these, fifteen burn patients

were swabbed. Only patients with less than one month of the burn wound were swabbed.

Cultivation of Isolates from Clinical Specimens: Samples were cultivated by streaking swabs on Eosin Methylene Blue agar (EMB), Nutrient Agar (NA), MacConkey agar (MAC) and Mannitol Salt Agar (MSA) and then inculcated for 24 hours at 37^0C. Pure cultures were obtained by sub-culturing each distinctive colony growth on EMB, NA, MSA and MAC agar slants.

Biochemical tests for identification of isolates: Different biochemical tests namely indole, coagulase, oxidase, motility, urease, citrate utilization**,** **t**riple sugar Iron, MR-VP were performed in order to identify the isolates (Benson, 2005)

Determination of antibacterial activity using cup plate method

Each of the honey samples at original concentration (100%) were used for this purpose. All the tested honey samples were adjusted to 40^oC in a water bath in order to aid pipetting. Each of the isolated organisms was picked up with an inoculating loop and suspended in 3-4ml of nutrient broth and incubated for 2-3hours at 36-37^oC, then diluted with sterile distilled water to a turbidity that matches 0.5 McFarland standard as a suspension. Using a swap stick, each of the test pathogens that was standardized were surface spread on Muller Hinton agar (24ml) in petri dish. A single cup was bored using a 6mm sterile cork borer into each dried plate. About 0.2ml of each honey type was aseptically introduced into the well of each plate using a sterile pasture pipette or 2ml syringe.

Ceftazidime and ciprofloxacin antibiotics were used as the control in order to compare the activity between the honey samples and control. The plates were allowed to dry on the laboratory bench as well as allowing the honey to diffuse for about 2-3hours and then incubated at 28-30 ℃ for 24-48 hrs.

The antibacterial activity was expressed as the diameter of the zone of inhibition calculated as the difference in diameter of the observed zone and the diameter of the well or disc.

Determination of minimum inhibitory concentration of the four different honey (Type I, II, III, and IV)

All the four honey types were used for this purpose. The organisms used here were *E.coli*, *Staphylococcus sp.*, *Pseudomonas sp.* and *Klebsiella sp.* Different dilutions of the honey were made using glycerol and all the honey types were adjusted to 40^oC in a water bath in order to aid pipetting during preparation of the various diluted honey solutions. The dilution of 90%, 80%, 70%, 60%, 50%, 40% and 30% (v/v) were made for this study. In each test tube containing 1ml of Muller Hinton broth, 1ml of each of the prepared diluted honey was added. Then a loopful of the test organism that was equivalent to 0.5 McFarland standards was introduced into the appropriate broth media.

A set of test tubes containing only 1ml of 70% diluted honey and 1ml of Muller-Hinton broth as control were also incubated along with each batch of the different honey samples. The highest dilution of honey that showed no visible growth of test organism was taken as the minimum inhibitory concentration (Brock and Madigan, 2002).

Determination of minimum bactericidal concentration (MBC) of the honey

For each set of the test tubes in MIC determination, a loopful of broth was collected from those which did not show any growth and inoculated on sterile nutrient agar plates. The inoculation was carried out using streak method. The plates were then incubated at 37^oC for 24hours. All the plates examined for the presence of growth. The concentration at which no visible growth was observed after incubation period was noted as minimum bactericidal concentration (Brock and Madigan, 2002).

Results and discussion

Characteristics of different honey samples

The source, appearance, pH and catalase activity of different honey samples are listed in Table 1. All the unprocessed honey collected from local sources in Kaduna state of Nigeria had strong

aroma. Type 2 and 3 were thin, dark in colour and not so clear. Type 1 was thick, clear and light in color, clear thin and strong aroma while type 4 (processed) was light in color, clear, thin and strong aroma too. The pH values of the different honey samples were in the range of 3.63-4.09. The results also have shown that all four different honey samples possess catalase.

Identification of isolates from clinical specimens

The isolated organisms were identified as *Staphylococcus* sp., *Pseudomonas* sp., *Klebsiella* sp. and *E. coli* (Table 2). The percentage distribution of organisms was *Staphylococcus* sp. (50.0%) followed by *Pseudomonas* sp. (26.6%), *Klebsiella* sp. (13.4%) and *E. coli* (10.0%).

Antibacterial activity of the honey samples and the selected antibiotics against the test organisms

Table 3 shows the observed antibacterial activities of all the honey types and two antibiotics namely Ceftazidime and ciprofloxacin against the four organisms. Table 4 shows the observed antibacterial activities of only four honey samples against the isolates obtained from burn wound infection.

Table.1 Information on honey samples collected from different sources

Honey type	Source	Colour and appearance	pH	Catalase
Type 1	Kachia local Government Kaduna	Comb honey (unprocessed), light colour, clear, thick and strong aroma	3.63	+
Type 2	Kagoro local Government Kaduna	Comb honey (unprocessed), light colour, not clear , thin and strong aroma	3.76	+
Type 3	Kajuru local Government Kaduna	Comb honey (unprocessed), light colour, clear , thin and slight aroma	3.93	+
Type 4	Laser, England	processed honey light colour, clear , thin and strong aroma	4.09	+

Table.2 Gram staining, morphology and biochemical characteristics of isolates obtained from burn wound infections

GR	Morphology	C	Co	Ur	In	Mo	L	G	MR	VP	Ox	Organism
-	Rods	+	n/c	-	+	+	+	+	+	-	-	*E. coli*
+	Cocci	+	+	+	-	-	+	+	-	-	-	*Staphylococcus* sp.
-	Rods	+	n/c	+	-	-	+	+	-	+	-	*Klebsiella* sp.
-	Rods	+	n/c	-	-	+	+	-	-	-	+	*Pseudomonas* sp.

GR- Gram reaction; C-catalase; Co-coagulase; In-indole; L-lactose; G-glucose; MR-Methyl red; VP-Voges-Proskauer; Ox-oxidase; n/c-not carried out.

Table.3 Sensitivity profile of isolates against different honey samples and antibiotics

Honey Type/ Antibiotic used	*E. coli*	*Staphylococcus sp.*	*Pseudomonas sp.*	*Klebsiella sp.*
Type 1	4.8	3.1	2.3	4.5
Type 2	5.0	2.8	2.7	5.3
Type 3	4.1	4.3	4.3	4.1
Type 4	4.4	4.9	4.9	4.3
Ciprofloxacin	2.1	4.8	5.7	4.5
Ceftazidime	3.0	4.3	4.1	4.5

$F_{observed} = 0.393$; $F_{cal} = F_{5,24,0.95} = 2.6207$. Since F_{obs} value is lower than F_{cal} value, we can say that there is no significant difference between the diameters of zones of inhibition of the isolates against four honey samples and the two antibiotics.

Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of honey Type 2 (unprocessed) and Type 4 (processed) against the four test organisms.

Table 5 shows the MIC and MBC of the honey Type 1 and 4 against S*taphylococcus sp.*, *E. coli*, *Pseudomonas sp.* and *Klebsiella sp.* *E. coli* and *Staphylococcus sp.* showed 80% of MIC and MBC in Type 1 honey sample whereas MIC and MBC values of 40 and 70% respectively in Type 4 honey. For *Klebsiella sp.* and *Pseudomonas sp.* the MIC and MBC values were 50 and 30% in Type 1 and Type 4 honey respectively. For both honey samples and antibiotics, biostatistical studies reveal that F_{obs} (0.393) value is lower than F_{cal} (2.6207) value and there is no significant difference between the diameters of zones of inhibition of the isolates against four honey samples and the two antibiotics. Also, for the four honey samples, F_{obs} (0.476) value is lower than F_{cal} value (3.4903), indicating that there is no significant difference between the diameters of inhibition zones of the isolates.

Table.4 Sensitivity profile of isolates against different honey samples

Honey Type	*E. coli*	*Staphylococcus sp.*	*Pseudomonas sp.*	*Klebsiella sp.*
Type 1	4.8	3.1	2.3	4.5
Type 2	5.0	2.8	2.7	5.3
Type 3	4.1	4.3	4.3	4.1
Type 4	4.4	4.9	4.9	4.3

$F_{observed} = 0.476$; $F_{3,12,\ 0.95} = 3.4903$; Since F_{obs} value is lower than F_{cal} value, we can say that there is no significant difference between the diameters of zones of inhibition of the isolates against four honey samples.

Table.5 Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of honey Type 1 (unprocessed) and Type 4 (processed) against the isolates (%v/v)

Organism	M1	M2	M3	M4
E. coli	80%	80%	40%	40%
Staphylococcus sp.	80%	80%	70%	70%
Klebsiella sp.	50%	50%	30%	30%
Pseudomonas sp.	50%	50%	30%	30%

M1 and M2- MIC and MBC of Type 1 samples; M3 and M4- MIC and MBC of Type 1V sample.

The pH values of all the four honey samples collected from different sources were in the range of 3.63-4.09. This agrees with the work of Davis (2005) and Molan (1992) that honey is characteristically acidic (pH 3.2-4.5) and is low enough to be inhibitory to many animal pathogens. Results in Table 1 show that all the tested honey samples possess catalase and it confirms the fact that non-peroxides factors may also contribute to antibacterial properties of these honeys. It agrees with the work of Weston et al. (2000) that non-peroxide components such as flavonoids, lysozyme, phenolic acid, etc. present in the honey and also contribute to the antibacterial activity of honey. In this study, four types of organisms namely *Staphylococcus* sp., *Pseudomonas* sp., *Klebsiella* sp. and *E. coli* were isolated from the sites of wound burn infection. The results of this study show that *Staphylococcus* sp. was the most commonly isolated organism (50%) from burn wound infection which is in agreement with the findings of Nasir et al. (2010) who reported that the most common microorganism identified from burn wound infection was *S. aureus* followed by CONS, *K. pneumonia*, *Streptococcus* sp., *E. cloacae*, *Pseudomonas* sp. and *Acinetobacter* sp. Church et al. (2006) reported that *P. aeruginosa* emerged as a predominate number of burn wound flora and in the absence of topical therapy is cultured from the burn injuries of 70% patients by the third week.

All the honey samples tested showed strong definite antibacterial activity against the organisms associated with burn wound infection but the activity pattern was different for different honeys.

Honey samples differ in quality on account of various factors such as season, the origin of honey, the activity of the bee, the food of the bee, the period and technique of extraction of honey, conditions of storage and the freshness of honey. Kwakman et al. (2011) have reported that two major medicinal honeys (RS honey and manuka honey) have different mechanisms of bactericidal activity due to the fact that RS and manuka honey have highly distinct compositions of antibacterial factors, resulting in large differences in bactericidal activity. Bhat (1998) studied the effect of honey in the treatment of wound infection and found that honey had almost uniform bactericidal effect on *S. aureus, P. aeruginosa, E. coli, K. pneumoniae and P. mirabilis.* Bogdanov (1997) reported that the antibacterial activity of honey correlated significantly with the acidity of honey.

Ahmad and Othman (2013) observed that Tualang honey (TH), a multifloral jungle honey found in Malaysia, inhibits the growth of several bacterial strains such as *S. pyogenes*, *S. typhi*, *S. aureus*, coagulase negative streptococcus sp. and *E. coli* at concentrations of 6.25-25%. TH is more effective than Manuka honey against some gram negative bacterial strains in burn wounds management. The antibacterial activity of honey samples provided by apiarists and honey packers was tested against microorganisms usually isolated from skin wounds. Most of the undiluted honey samples inhibited the growth of *S. aureus* and *S. epidermidis*. Undiluted honey samples also inhibited the growth of *S. uberis*, *P. aeruginosa*, *E. coli* and *K. pneumoniae* although to a lesser extent (Basualdo et al., 2007). Table 3 shows that types III and types IV honey had the highest activity against virtually all the tested isolates. Types II honey showed strongest activity against *E. coli* and *Klebsiella species*. Type IV honey possess strongest activity against *Pseudomonas spp.* and *Staphylococcus* spp. Type 1 honey also showed greatest activity on specie of *Klebsiella* and *E. coil*. This research work shows that the antibacterial used as controls (C_1 and C_2) had high antibacterial activity against *Staphylococcus, Pseudomonas, Klebsiella* and had low activity against *E. coli* compared to the activity of the four honey types. Table 3 shows the MIC and MBC activity of honey samples against the bacterial isolates.

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