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Honey: A natural potent antimicrobial remedy against wound pathogens in orthopaedic patients

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Abstract

With rise of antibiotic resistance, alternative antimicrobials are needed. Honey has been reported as one of the natural antimicrobial agents against bacteria due to its natural antibacterial property and its possession of hydrogen peroxide(H_2O_2), low pH, high osmolarity among others. Antimicrobial activity of two varieties of honey which include Oba Nuskka honey and Benue honey were compared by assessing antibacterial activities against isolates from infected wounds at National Orthopaedic Hospital Enugu Nigeria (NOHE). A total of 100 wound swabs were analysed using standard bacteriological methods. Results showed that out of the 100 samples analysed, a total of 128 isolates were detected with the highest isolate being *Pseudomonas aeruginosa* 45.3% (58) bacteria followed by, *Enterococcus spp and Proteus vulgaris* with 9.4% (12) each, *Escherichia coli* 8.6% (11), and *Actinomycetes spp, Salmonella spp, Serratia rubidae* put all being the least with 0.8% each. Total of 18 significant bacteria species were isolated of which all tested isolates were sensitive to both varieties of honey used at 100% concentration. The Oba Nsukka honey at all concentrations produced larger zones of inhibition than Benue honey. Honey represents a natural, cost-effective, and safe complementary therapy to standard wound care practices, warranting further research into its mechanisms of action and broader clinical applications. Regulatory agencies should enforce measures to ensure that pure unadulterated honey is available for patient's wound management.

Keywords: Honey; Antimicrobial Activity; Wound Infections; Natural Antimicrobial Agents; Oba Nsukka Honey; Benue Honey

1. Introduction

With the growing demand for alternative therapies against species of bacterial that are resistant to conventional therapy, the search for good and safe products still races on. Honey being a natural sweet substance is produced by bees using nectar from flowers, has been used for its medicinal properties and also as a food source for many years [1]. It's made up of sugars, water, enzymes, organic acids, vitamins, minerals, and bioactive compounds like phenols, flavonoids and methylglyoxal (MGO) mostly found in Manuka honey. Primarily, the type of sugar found include, glucose and

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fructose, in addition to water, enzymes, amino acids, vitamins, minerals, and various antioxidants [1, 2]. Its composition can vary depending on the floral sources that are visited by the bees. Honey's antibacterial activity has been widely studied and documented, and it has been demonstrated to possess potent antimicrobial properties. Honey is a natural sweetener and a source of energy due to its sugar content [3].

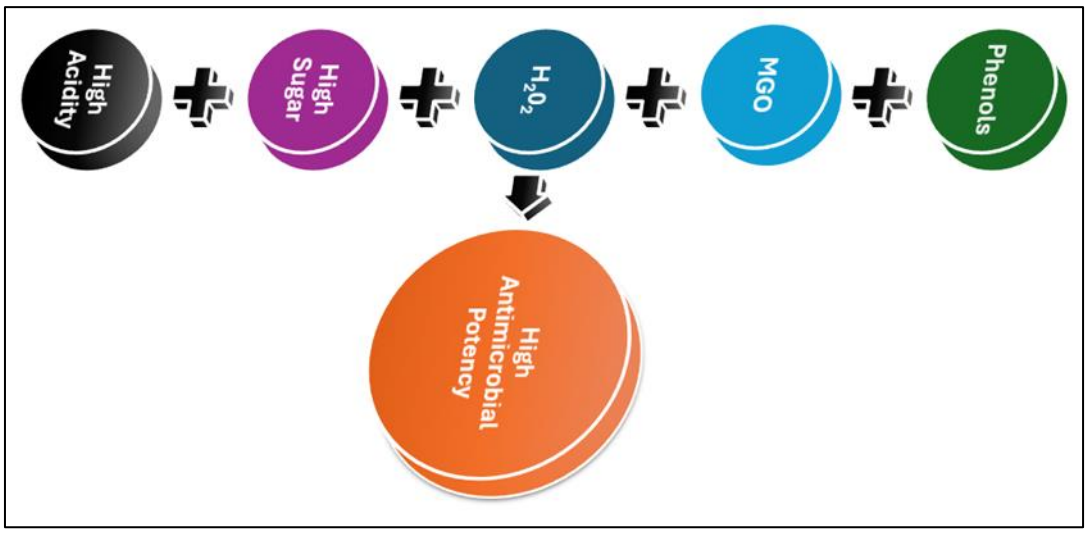

Figure 1 Antimicrobial Components of Honey

In Nigeria for example, honey has been used as a traditional alternative medicine for the treatment of various ailments, including wounds as reported by Ukwuoma and colleagues [4]. Generally, antibacterials play a crucial role in the treatment of bacterial infections, including pneumonia, meningitis, and urinary tract infections (UTIs) [5]. In addition to natural products, synthetic antibacterial agents are also being developed with the aim of diversifying treatment options. A well-known example is teixobactin, a newly discovered antibiotic that basically works by targeting the bacterial cell wall. It has been reported to show significant effectiveness and has also shown promise in preclinical studies [6]. Commonly, the use of honey in minor medical procedures is often necessitated by wounds [7].

Wounds are injuries to the skin or other body tissues that can result from various causes such as cuts, scrapes, burns, bites, or surgical incisions [8]. They vary in severity, from minor injuries that can be managed at home to severe wounds that require immediate medical attention. They are a significant cause of morbidity and mortality among accident victims [9]. These infections do not just cause significant burden to patients, it can further lead to prolonged hospital stays, increased healthcare costs, and even amputations [10]. It is worth to note that, the use of antibiotics for the treatment of infections has contributed to the development of antibiotic resistance [11]. As a result, there is a growing interest in natural remedies for wound management, and honey being a natural product, has emerged as a potential alternative due to its broad-spectrum antimicrobial properties. Wound healing could be affected by several factors such as advanced age, which is well reported to slowdown the process [12], while chronic illnesses on the other hand can further impair recovery [13]. Furthermore, lifestyle habits like smoking and alcohol consumption are also reported to delay healing [14]. In addition, some drugs could also interfere with the repair process [15]. By contrast, report have also shown that proper hygiene and appropriate wound care greatly improve healing outcomes [16].

In recent times, a lot of studies have been carried out to evaluate the effectiveness of honey on wound isolates [17-19]. Due to its remarkable effect and activity, it has a lot of potent compounds used in the elimination of skin inflammation and wound complications. For example, a study conducted by Mandal and Mandal [20], showed that honey possesses significant antimicrobial capabilities, making it an indispensable alternative agent in managing several wound types, such as burns, ulcers, and post-surgical wounds, ultimately showcasing its therapeutic versatility. Furthermore, it has also been shown to be efficacious against a broad spectrum of bacterial species like *Staphylococcus aureus* and *Pseudomonas aeruginosa*, the two well know bacteria pathogens often associated with wound infections [21]. The state of honey obtain has also been reported to play a role in terms of effectiveness. Unprocessed honey (honey that were not subjected to any form of pretreatment like heating, exhibits a very wide range of antibacterial effects against various harmful bacteria, including those implicated in pathogenesis, oral microbiota, and food deterioration [22]. With honey's wide antibacterial activity against a broad range of microorganisms including, Gram-positive and Gram-negative bacteria, these makes them a very good choice for alternative therapies [20]. It is worth stating that, the antibacterial activity of honey is due to its high sugar content, low pH, and the presence of various compounds such as hydrogen

peroxide, methylglyoxal, and flavonoids [23, 24]. These compounds have been shown to disrupt bacterial cell membranes, inhibit bacterial metabolism, and induce oxidative stress, leading to bacterial death. Again, a study by Al-Jabri and colleagues [25], evaluated the antibacterial activity of honey against bacterial isolates obtained from diabetic foot ulcers. The study found that honey was effective against both Gram-positive and Gram-negative bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*.

Similarly, another study by Oladele and colleagues [26], investigated the antibacterial activity of honey against bacterial isolates that were obtained from surgical wounds. Interestingly, the study found that honey was effective against a broad range of bacterial pathogens, such as Methicillin-resistant *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, and *Acinetobacter baumannii.* This further highlights the effectiveness of honey as an alternative natural remedy.

The antibacterial activity of honey and the type of honey can vary and this depends on several factors. The two most important factors, are the plant source and the geographical location. For instance, Manuka honey from New Zealand has been extensively studied for its potent antibacterial activity against both Gram-positive and Gram-negative bacteria, including antibiotic-resistant strains [1, 27]. Other types of honey, such as Sidr honey from Yemen, Tualang honey from Malaysia, and Gelam honey from Indonesia, have also shown promising antibacterial activity [28].

Figure 2 Map of Nigeria highlighting Benue and Enugu states – sources of honey samples for research

Generally, antibacterial activity of honey on wound isolates from different hospitals in Enugu Nigeria, has been demonstrated in various studies but not much has been done in the area of comparative analysis of the two main sources of honey in two well-known regions -Enugu and Benue. A study by Eze and colleagues carried out in a tertiary hospital in Enugu state Nigeria, showed that the most common bacteria isolated from wound infections were *Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa,* and *Klebsiella species* [29]. To further support these findings, another study by Odugbemi *et al.,* carried out in a secondary healthcare facility in Enugu Nigeria, also reported similar findings, with *S. aureus, E. coli,* and *P. aeruginosa* being the predominant organisms isolated from wound infections [30]. No doubt, honey has shown promising results in the treatment of wound infections, including those caused by antibiotic-resistant pathogens. Interestingly, honey has also been found to have in addition to potent antimicrobial properties, also other beneficial effects on wound healing. Even medical-grade honey has also been shown to be effective in the treatment of infected wounds, including those infected with antibiotic-resistant bacteria [31]. Antibiotics work by targeting specific components of the bacterial cell, such as cell walls or protein synthesis machinery, and inhibiting or impeding their function [32]. One alternative to antibiotics is the use of natural products, such as essential oils and plant extracts, and they have been found to have antibacterial properties. For example, tea tree oil has been shown to have activity against a wide range of bacteria, including *Staphylococcus aureus* and *Escherichia coli* [33]. Manuka honey is

another natural product that has been found to have potent antibacterial activity, particularly against antibiotic-resistant bacteria [34].

The use of honey as a wound-healing agent in Enugu is common; however, there is a lack of sufficient scientific comparative data on its effectiveness against bacterial pathogens commonly found in wound infections in this region. The outcomes of this research could hold significant relevance for advancing alternative therapeutic strategies against wound infections, especially those caused by bacteria that are resistant to well-known conventional antibiotics. In Nigeria, honey production is common in many states, including Enugu and Benue States. One of the areas in Enugu State where honey production is prevalent is Oba, Nsukka and mostly marketed and sold in shopping mall in Enugu state. A study by Okereke et al, reported that honey produced in Oba, Nsukka, Enugu State, Nigeria, inhibited the growth of both Gram-positive and Negative bacteria, making them good candidate for use as antibacterial agent [35]. Similarly, another study by Ogundele et al, highlighted the high nutritional and medicinal value of honey from Enugu State, attributing its effectiveness to strong antioxidant and antibacterial properties [36]. Benue State, known as the food basket of Nigeria, is also a major producer of honey. Further study evaluated honey from different regions in the state and reported high levels of phenols, flavonoids, and antioxidant activity, which contributed to its antibacterial properties [37]. The honey effectively inhibited the growth of bacteria such as *Staphylococcus aureus*, *Escherichia coli*, and *Pseudomonas aeruginosa*. Similarly, Onyeke et al., also demonstrated that honey from Benue promoted wound healing by reducing inflammation, enhancing collagen deposition, and stimulating angiogenesis [38]. Based on the evidences so far, it is safe to assert that honey indeed has a huge potential in medicine and public health in general.

In this study, we aim to determine the antibacterial effect of honey against bacterial isolates from wounds of patients at National Orthopaedic Hospital, Enugu, Nigeria. Specifically, we seek to identify bacterial isolates from infected wounds among patients seen at the hospital, assess the antimicrobial effects of honey as a potential antibacterial agent against these isolates, and evaluate the antibacterial activities of two different species of honey against the bacterial isolates. By achieving these objectives, this study will provide scientific evidence to support the use of honey in wound management, particularly in resource-limited settings where access to conventional wound care treatments may be limited. The findings of this study will contribute to the growing body of knowledge on the therapeutic properties of honey and have implications for the development of new wound care strategies for patients. Furthermore, the study may inform the development of guidelines for the use of honey in wound management, particularly in hospitals and clinics in Enugu and other regions of Nigeria.

2. Materials and methods

2.1. Study Area

This cross-sectional study was carried out from patients of National Orthopaedic hospital, Enugu State, Nigeria. The samples were collected from 4 different wards namely; Male ward, Female ward, Children ward and Outward Patient Department as shown below. The samples were collected from infected wounds in patients from each ward.

Table 1 Distribution of wound samples collected according to wards in National Orthopaedic Hospital Enugu, Nigeria

Wards	Number of samples collected
Children ward	6
Female ward	5
Male ward	19
Outward patient department	70

2.2. Ethical Consideration and Informed Consent

Ethical approval was obtained from the National Orthopaedic Hospital Enugu, Nigeria (18/07/2023). Written informed consent of willingness to participate in the study was obtained from all participants included in the study. All ethical issues involving the Identity, compensation and management of the subjects were followed according to the approved guidelines of ethical conduct.

2.3. Study Population group, Selection Criteria and Experimental Design

The study population for the research on the "Antibacterial activity of honey on bacterial isolates from wounds" consisted of individuals with bacterial wound infections, including patients from National Orthopedic Hospital, Enugu, Nigeria, with various types of wounds such as burns, surgical wounds, and chronic ulcers. Age, gender, and race were considered to ensure a diverse sample population, and a total of 20 patients suffering from wounds were selected. The study adopted a population of 100 patients, and all subjects were included due to the relatively small population size. Participation criteria required that subjects have wounds such as burns, surgical wounds, or chronic ulcers and that they freely consent to participate in the study. The test group consisted of patients meeting these criteria, including both male and female participants, while the control group comprised apparently healthy individuals with no record of malaria at the time of the research.

Convenience sampling was used, with wound swabs collected from participants at National Orthopaedic Hospital, Enugu, Nigeria, to isolate and identify bacterial strains using standard bacteriological methods. The honey samples collected from Oba, Nsukka, and Benue were then applied to the bacterial cultures to assess their antibacterial effectiveness. Data collection involved obtaining wound isolates using the Levine method, and approximately 100 sterile swab sticks were used to collect samples aseptically from infected wounds across different wards on Tuesdays and Fridays to avoid duplication. The instruments used in the study included agar plates, bacterial isolates, honey, sterile forceps, an incubator, calibrated micropipettes and sterile pipettes, Mueller Hinton agar, sterile distilled water, antibiotic disks, a spectrophotometer, a microscope, sterilized test tubes, sterilized cotton swabs, a zone of inhibition measurement scale, an autoclave, and a Bunsen burner, among others.

2.4. Laboratory investigations

2.4.1. Bacteriological Analysis and Culture Techniques

According to Collins and Lyne (2004), the media used for this analysis included Nutrient agar (Batek) MacConkey broth (Oxoid), Citrate agar, Methyl Red- Vogues Proskauer (MR-VP) broth.

All media were prepared according to the manufacturer's instructions and adequately sterilized in autoclave at 121^{o}C for 15 minutes and were dried very well before used.

This is based on the ability of bacterial organisms to grow on Nutrient agar as a universal medium for bacterial identification, also, use of MacConkey agar to grow and identify lactose fermenter bacterial organisms due its indicator-bromocresol purple and production of acid and gas.

2.4.2. Culture and Macroscopy

Freshly prepared nutrient agar (Batek) and marking agar (Oxoid) were dried and incubated with canister jar. Methods for laboratory study were in accordance with Cheesbrough (2006). On completion of 24 hours incubation period, the nutrient agar plate and MacConkey agar plate were examined and growth observed were read macroscopically. Identification of isolates were carried out using the methods of Cheesbrough (2006).

2.4.3. Gram Staining Method and Biochemical tests

The Gram staining technique was used to differentiate between Gram-positive and Gram-negative bacteria isolated from all samples collected. Gram-positive bacteria appear purple, while Gram-negative bacteria appear red. Morphological and biochemical characteristics of the bacterial isolates were used for the identification of isolates according to Cheesbrough (2019).

- Indole Test: Used to detect the production of indole from tryptophan. Test organisms were inoculated in peptone water and incubated at 37°C for 48 hours. Kovac's reagent was added, and a red color at the surface indicated a positive result.
- Citrate Utilization Test: Assessed the ability of bacteria to utilize citrate as a carbon source. Organisms were streaked on Simmons citrate agar and incubated at 37°C for 48 hours. A blue color indicated a positive test.
- Methyl Red Test: Differentiated bacteria based on acid production from glucose fermentation. Organisms were incubated in MR broth at 37°C for 48 hours, followed by addition of methyl red indicator. Red color indicated a positive result.
- Voges-Proskauer Test: Determined the production of acetoin by bacteria. Organisms were incubated in VP broth at 37°C for 48 hours. Addition of Barritt's reagents produced a pink-red color for positive results.

- Catalase Test: Identified bacteria producing catalase. Colonies were mixed with hydrogen peroxide on a slide. Bubble formation indicated a positive result.
- Coagulase Test: Differentiated *Staphylococcus aureus* from other Staphylococcus species. Bacterial suspension was mixed with plasma; immediate clumping indicated a positive result.

2.4.4. Preparation of Different Concentration of Honey

Honey samples were obtained from Oba, Nsukka, Enugu, and Benue. The samples were characterized based on their geographical origin and physical properties, including color, consistency, and pH. The honey was sieved through sterile gauze to remove debris. Serial dilutions were prepared using distilled water as the diluent to obtain five concentrations: 100%, 50%, 25%, 12.5%, and 6.25%. To prepare the dilutions: 1 mL of 100% honey was mixed with 1 mL of distilled water to obtain 50% honey. Similarly, 1 mL of 50% honey was mixed with 1 mL of distilled water to produce 25% honey; 1 mL of 25% honey was mixed with 1 mL of distilled water to produce 12.5% honey; and 1 mL of 12.5% honey was mixed with 1 mL of distilled water to obtain 6.25% honey.

2.4.5. Agar Well Diffusion Assay and Measurement of Zone of Inhibition

The antibacterial activity of the honey extracts was evaluated using the agar well diffusion method. Sterile plates containing freshly prepared Mueller-Hinton agar were inoculated with bacterial isolates from wounds using sterile swab sticks. Five wells, each 6 mm in diameter, were bored into the agar using a sterile cork borer. The wells were then filled with the previously prepared honey concentrations and labeled as follows: Well 1 – 100% honey, well 2 – 50% honey, well 3 – 25% honey, well 4 – 12.5% honey, and Well 5 – 6.25% honey. The plates were allowed to stand for 7 hours to permit pre-incubation diffusion of the honey before being incubated at 37°C for 24 hours. After incubation, the plates were examined for zones of inhibition around the wells, indicating antibacterial activity. The diameters of the zones of inhibition were measured in two dimensions (horizontal and vertical), and the average value was recorded in millimeters.

2.5. Statistical Analysis

Data obtained from this study were analyzed using the Statistical Package for Social Sciences (SPSS) version 22. Data were presented as means and standard deviations. Student's t-test was used to calculate differences between the means. Analysis of Variance (ANOVA) was used to estimate differences between group means, while estimation of relationship between parameters was carried out using Pearson's correlation analysis. All hypotheses' tests were performed using two-tailed test and p-value <0.05 considered statistically significant.

3. Results

The table below presents the general distribution of bacteria isolated from wounds in National Orthopaedic hospital, Enugu. A total of 128 bacteria were isolated, with *Pseudomonas aeruginosa* having the highest occurrence, with a frequency of 58 (45.3%), followed by *Proteus vulgaris* and *Enterobacter spp* and *Escherichia coli* with frequencies of 12 and 11 respectively. When compared across the different wound sources, there was a statistically significant difference in the bacteria isolates according to wound sources ($p < 0.05$). $p = 0.113$. (As shown in fig 3).

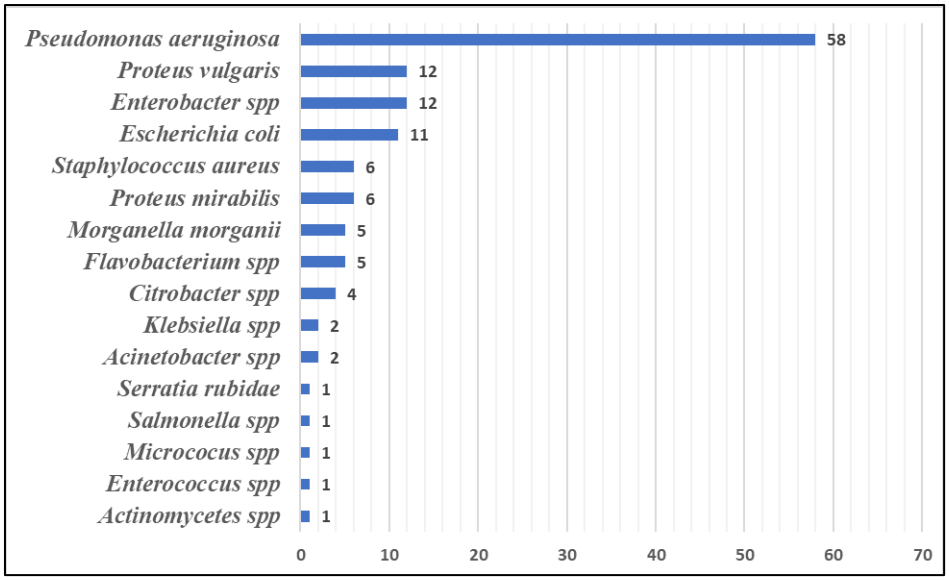

Figure 3 General distribution of bacteria isolated from wounds

Table 2 Percentage distribution of number of bacteria isolated from different wound types in national orthopaedic hospital, Enugu

Bacteria	Total	Percentage
Pseudomonas aeruginosa	58	45.3%
Enterobacter spp	12	9.4%
Proteus vulgaris	12	9.4%
Escherichia coli	11	8.6%
Proteus mirabilis	6	4.7%
Staphylococcus aureus	6	4.7%
Flavobacterium spp	5	3.9%
Morganella morganii	5	3.9%
Citrobacter spp	4	3.1%
Acinetobacter spp	2	1.6%
Klebsiella spp	2	1.6%
Actinomycetes spp	1	0.8%
Enterococcus spp	1	0.8%
Micrococus spp	1	0.8%
Salmonella spp	1	0.8%
Serratia rubidae	1	0.8%
	128	100.0%

The table below, presents the total number of bacteria isolated according to wound types in National Orthopaedic hospital, Enugu. 128 bacteria were isolated from 10 different wound sources, with accident wounds having the highest occurrence, with a frequency of 35 (27.3%), followed by burns and venous ulcers with frequencies of 26 (20.3%) and 20 (15.6%) respectively. When compared across the different bacteria isolates, there was statistically significant difference in the of the total number of isolates according to wound sources ($p > 0.05$). $p = 0.80$. (As shown in fig 4)

Table 3 Percentage distribution of number of bacteria isolated from different wound types in national orthopaedic hospital, Enugu

Bacteria	**Accident**	**Burns**	**Venous Ulcer**	**Trauma**	**Graft Ulcer**	**Surgical**	**Amputation**	**Rectal Ulcer**	**Osteomyelitis**	**Stab**	**Total**
Pseudomonas aeruginosa	15	12	8	10	4	5	1	2	1	0	58
Enterobacter spp	5	3	3	0	1	0	0	0	0	0	12
Proteus vulgaris	5	1	1	0	2	2	1	0	0	0	12
Escherichia coli	4	3	0	3	0	0	0	0	1	0	11
Proteus mirabilis	3	1	1	1	0	0	0	0	0	0	6
Staphylococcus aureus	0	1	3	0	1	1	0	0	0	0	6
Flavobacterium spp	1	0	1	1	2	0	0	0	0	0	5
Morganella morganii	1	1	1	0	0	1	1	0	0	0	5
Citrobacter spp	1	1	0	1	0	0	0	0	0	1	4
Acinetobacter spp	0	1	0	0	1	0	0	0	0	0	2
Klebsiella spp	0	1	1	0	0	0	0	0	0	0	2
Actinomycetes spp	0	0	0	1	0	0	0	0	0	0	1
Enterococcus spp	0	0	0	0	0	0	0	0	0	1	1
Micrococus spp	0	0	1	0	0	0	0	0	0	0	1
Salmonella spp	0	0	0	1	0	0	0	0	0	0	1
Serratia rubidae	0	1	0	0	0	0	0	0	0	0	1
	35 (27.3%)	26 (20.3%)	20 (15.6%)	18 (14.1%)	11 (8.6%)	9 (7.0%)	3 (2.3%)	2 (1.6%)	2 (1.6%)	2 (1.6%)	128

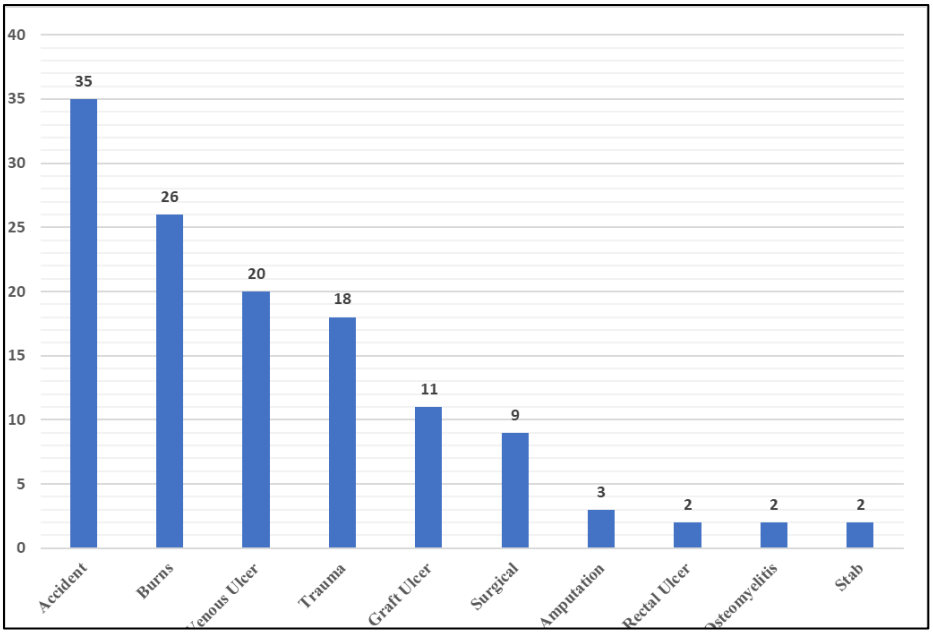

Figure 4 General distribution of bacteria according to wound type

The table below highlights the zone of inhibition of each bacterium against the different concentrations of honey gotten from Oba. When compared across different concentrations, there was a statistically significant difference across the different concentrations of oba honey for each bacteria isolate ($p < 0.05$). $p = 0.104$. (As shown in fig 5)

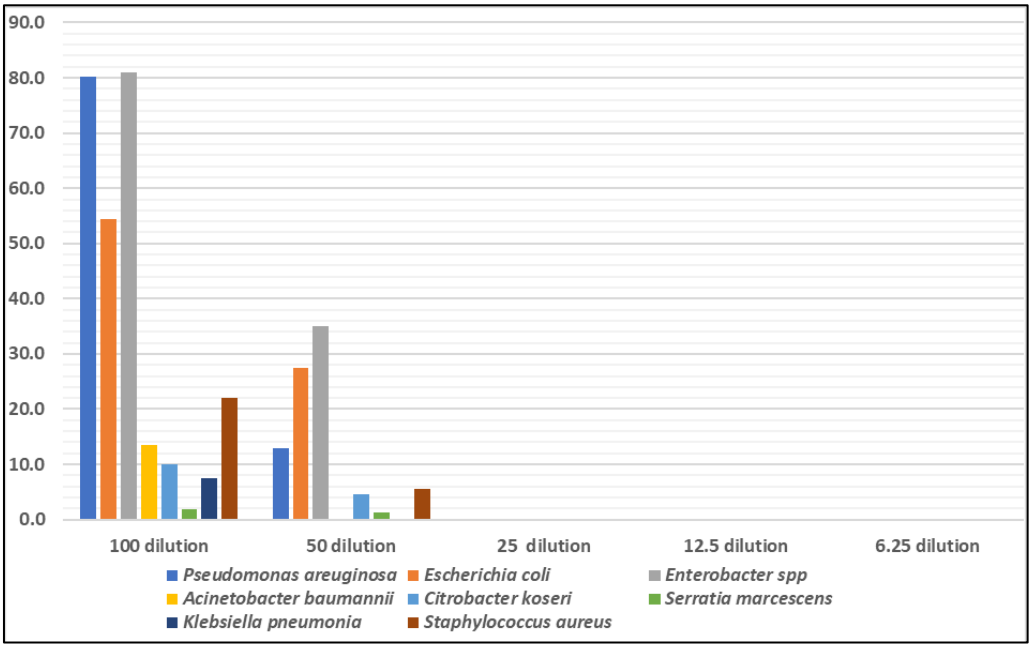

Figure 5 Zone of inhibition of each bacteria isolate treated with honey from OBA

Table 4 Comparison of the different dilutions of honey gotten from Oba for each isolate

	Wound source	**100 DILUTION Oba**	**50 DILUTION oba**	**25 DILUTION oba**	**12.5 DILUTION oba**	**6.25 DILUTION oba**
Pseudomonas aeruginosa	Trauma wound	5.3	0	0	0	0
Pseudomonas aeruginosa	Surgical wound	6.5	0	0	0	0
Pseudomonas aeruginosa	Venous ulcer	6	0	0	0	0
Pseudomonas aeruginosa	Surgical wound	13	0	0	0	0
Pseudomonas aeruginosa	Trauma wound	13.5	0	0	0	0
Pseudomonas aeruginosa	Trauma wound	12	0	0	0	0
Pseudomonas aeruginosa	Rectal ulcer	16	8.5	0	0	0
Pseudomonas aeruginosa	Burn wound	8	4.5	0	0	0
Escherichia coli	Trauma wound	7.5	7	0	0	0
Escherichia coli	Accident wound	8.5	0	0	0	0
Escherichia coli	Osteomyelitis Pus wound	38.5	20.5	0	0	0
Enterobacter spp	Accident wound	16	0	0	0	0
Enterobacter spp	Accident wound	10.5	7.5	0	0	0
Acinetobacter baumannii	Burn wound	13.5	0	0	0	0
Citrobacter koseri	Burn wound	10	4.5	0	0	0
Serratia marcescens	Burn wound	1.95	1.3	0	0	0
Klebsiella pneumonia	venous ulcer	7.5	0	0	0	0
Staphylococcus aureus	venous ulcer	22	5.5	0	0	0

The table below highlights the zone of inhibition of each bacterium against the different concentrations of honey gotten from Benue. When compared across different concentrations, there was a statistically significant difference across the different concentrations of Benue honey for each bacteria isolate ($p < 0.05$). $p = 0.054$. (As shown in fig 6)

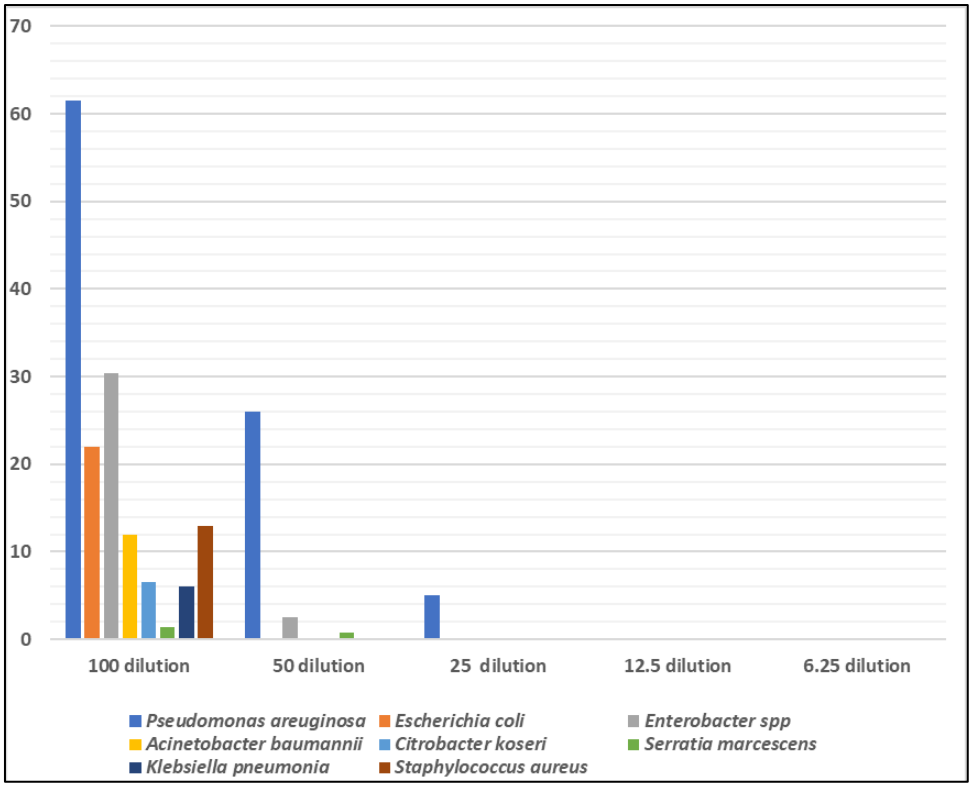

Figure 6 Zone of inhibition of each bacteria isolate treated with honey from Benue

Table 5 Comparison of the different dilutions of honey gotten from Benue for each isolate

	Wound Source	**100 DILUTION Oba**	**50 DILUTION Oba**	**25 DILUTION Oba**	**12.5 DILUTION Oba**	**6.25 DILUTION Oba**
Pseudomonas aeruginosa	Trauma wound	7	0	0	0	0
Pseudomonas aeruginosa	Surgical wound	5	0	0	0	0
Pseudomonas aeruginosa	Venous ulcer	4	0	0	0	0
Pseudomonas aeruginosa	Surgical wound	12.5	9.5	0	0	0
Pseudomonas aeruginosa	Trauma wound	12	9	5	0	0
Pseudomonas aeruginosa	Trauma wound	5.5	0	0	0	0
Pseudomonas aeruginosa	Rectal ulcer	10	7.5	0	0	0
Pseudomonas aeruginosa	Burn wound	5.5	0	0	0	0
Escherichia coli	Trauma wound	6.5	0	0	0	0
Escherichia coli	Accident wound	5	0	0	0	0

Escherichia coli	Osteomyelitis Pus wound	10.5	0	0	0	0
Enterobacter spp	Accident wound	1.4	0	0	0	0
Enterobacter spp	Accident wound	7	2.5	0	0	0
Acinetobacter baumannii	Burn wound	12				
Citrobacter koseri	Burn wound	6.6	0	0	0	0
Serratia marcescens	Burn wound	1.4	0.8	0	0	0
Klebsiella pneumonia	venous ulcer	6	0	0	0	0
Staphylococcus aureus	venous ulcer	13	0	0	0	0

3.1. Statistical Analysis of Differences Amongst the Bacteria Organisms of Oba Nsukka and Benue Honey

Decision Rule: P-value less than or equal to 0.05 (5%) indicates no statistically significant difference while p-value above 0.05 (5%) indicates a statistically significant difference.

Fig 7: Compares the ZID for the honey gotten from Oba and that gotten from Benue. When compared across the different isolates, there was statistically significant difference between the two different honeys ($p > 0.05$). $p = 0.281$.

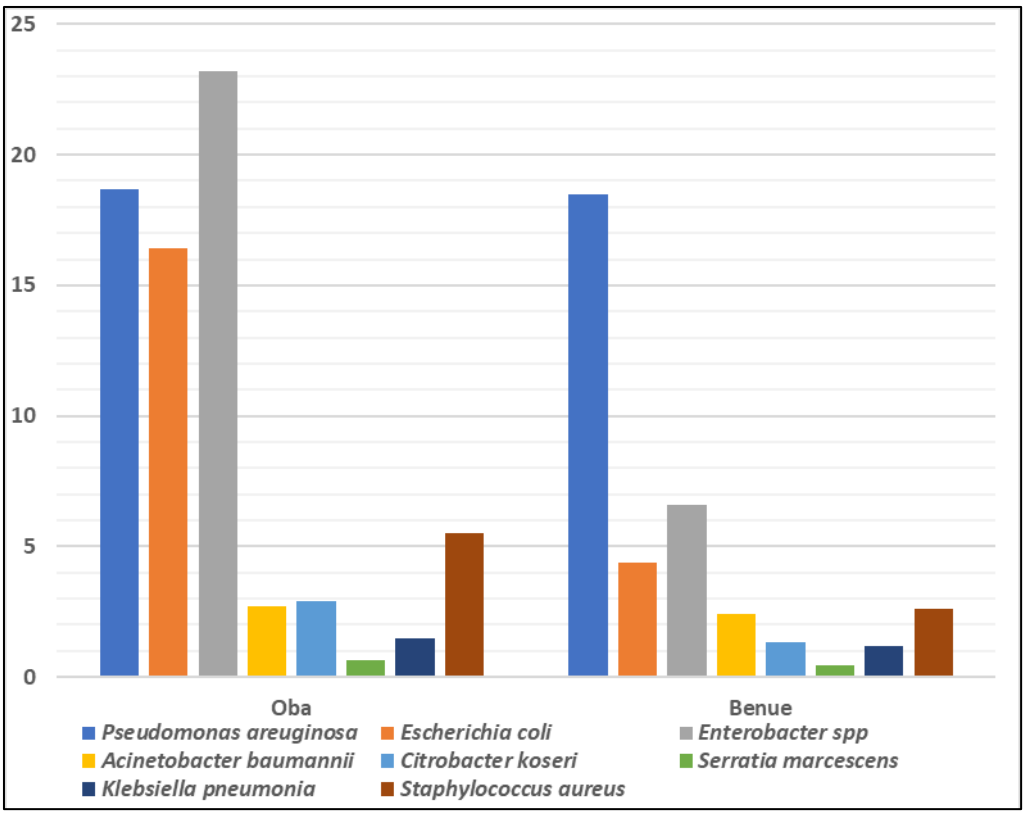

Figure 7 Comparison between the ZID of Oba and Benue honey for all bacteria isolates

Fig 7: Compares the ZID for the honey gotten from Oba and that gotten from Benue. When compared across the different isolates, there was statistically significant difference between the two different honeys ($p > 0.05$). $p = 0.281$.

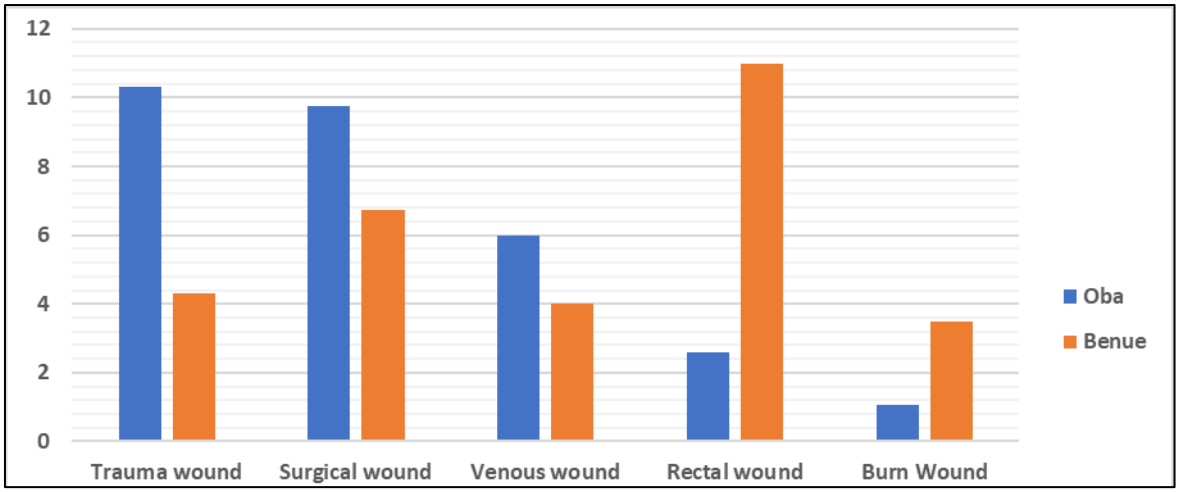

Figure 8 Comparison of the ZID of *Pseudomonas aeruginosa* of the two honeys for the different wound type

Fig 8, compares the ZID of *Pseudomonas aeruginosa* for the honey gotten from Oba and that gotten from Benue. When compared across the two honeys, there was statistically significant difference between the two different honeys ($p > 0.05$). $p = 0.071$.

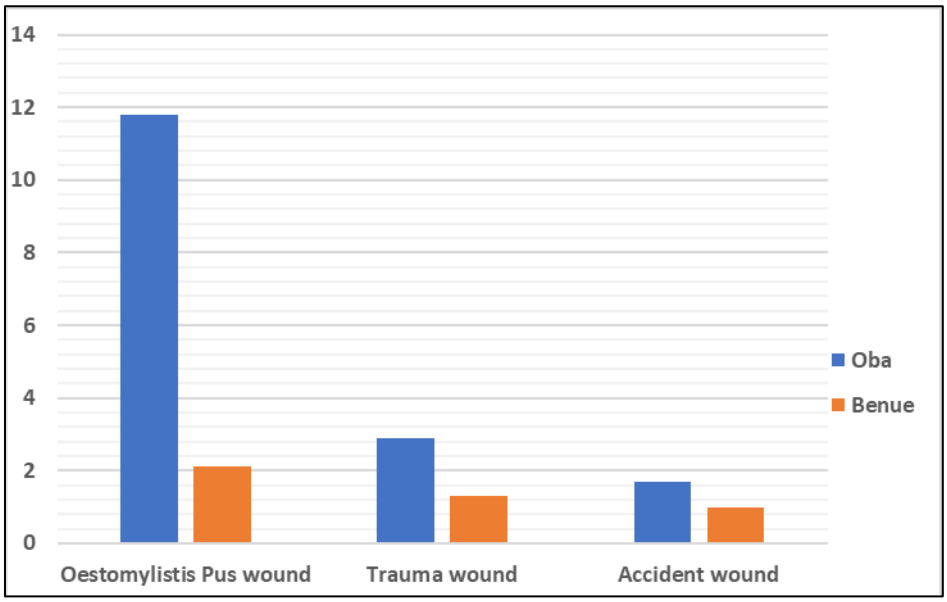

Figure 9 Comparison of the ZID of *Escherichia coli* of the two honeys for the different wound type

Fig 9 - Compares the ZID of *Escherichia coli* for the honey gotten from Oba and that gotten from Benue. When compared across the two honeys, there was statistically significant difference between the two different honeys ($p > 0.05$). $p = 0.146$.

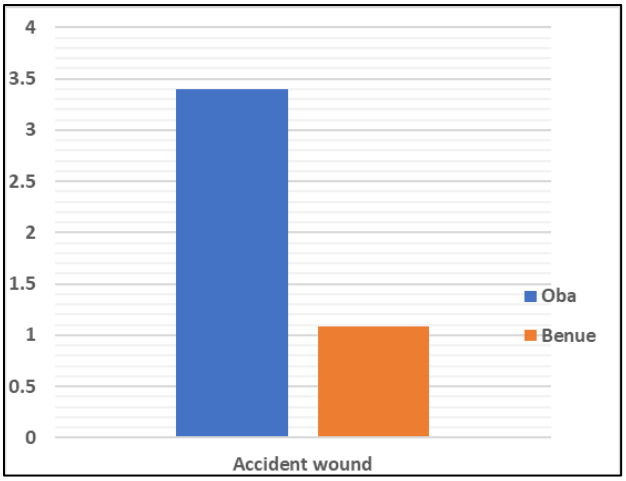

Figure 10 Comparison of the ZID of *Enterobacter spp* of the two honeys for the different wound type

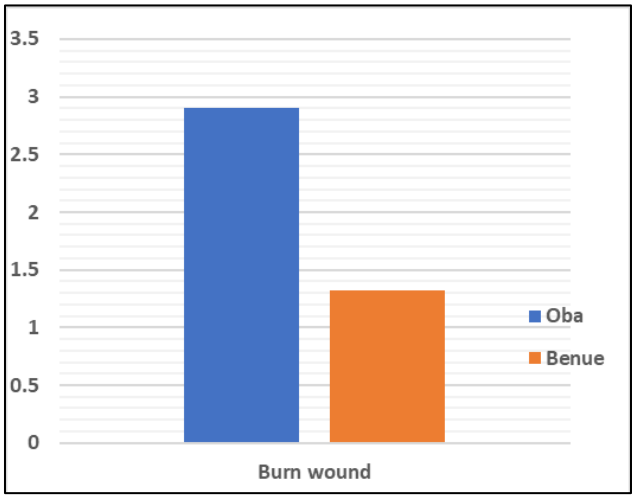

Figure 11 Comparison of the ZID of *Citrobacter koseri* of the two honeys for the different wound type

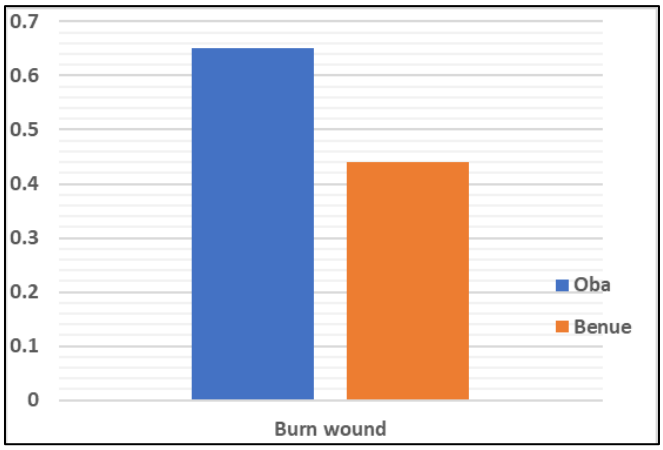

Figure 12 Comparison of the ZID of *Serratia marcescens* of the two honeys for the different wound type

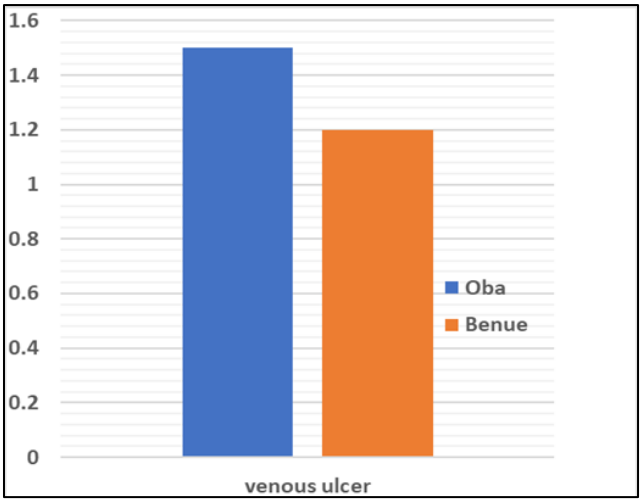

Figure 13 Comparison of the ZID of *Klebsiella pneumoniae* of the two honeys for the different wound type

Figures 10–13 Compare the zones of inhibition (ZID) of *Enterobacter spp*., *Citrobacter koseri*, *Serratia marcescens*, and *Klebsiella pneumoniae* for honey samples obtained from Oba and Benue. In each case, a statistically significant difference was observed between the two honeys ($p > 0.05$), with p-values of 0.081, 0.095, 0.110, and 0.074, respectively.

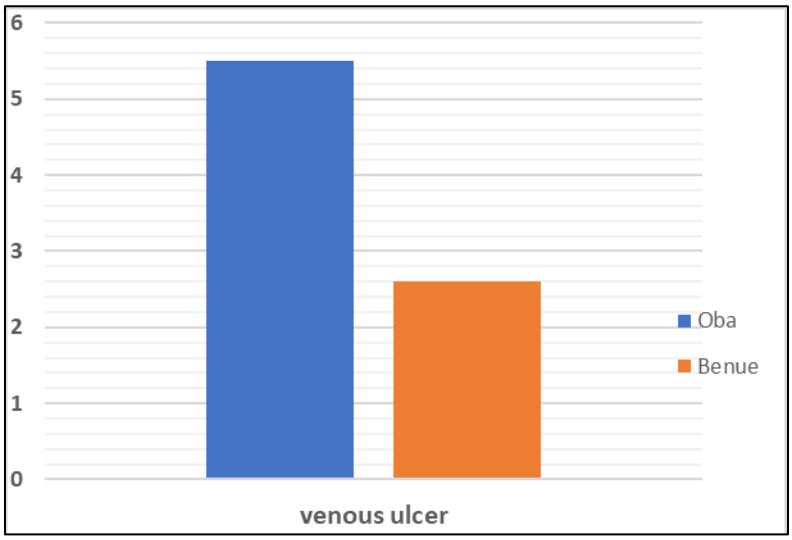

Figure 14 Comparison of the ZID of *Staphylococcus aureus* of the two honeys for the different wound type

Fig 14, compares the ZID of *Staphylococcus aureus* for the honey gotten from Oba and that gotten from Benue. When compared across the two honeys, there was statistically significant difference between the two different honeys ($p > 0.05$). $p = 0.125$.

4. Discussion

This study demonstrated that honey, particularly raw and unprocessed honey from Oba Nsukka and Benue, possesses significant antibacterial activity. This finding aligns with the work of Irish and Carter [39], who reported that the inherent antibacterial properties of honey are attributed to factors such as low water content, high osmolarity, and the presence of hydrogen peroxide, bee defensin-1, and phenolic compounds. Similarly, Cianciosi and colleagues also confirmed the role of these compounds in honey's antimicrobial efficacy [40].

Honey from Oba, Nsukka (Enugu State), has previously been shown to inhibit both Gram-positive and Gram-negative bacteria [35]. Ogundele et al., further emphasized its high nutritional, antioxidant, and medicinal value, recommending honey as a potential natural alternative to antibiotics [36]. The present findings in our study further reinforce these earlier studies, as the honey samples tested were effective against wound pathogens. A total of 128 bacterial isolates

were recovered from wounds, with *Pseudomonas aeruginosa* being the most prevalent (58 isolates, 45.3%), followed by Proteus vulgaris (12 isolates), Enterobacter spp. (12 isolates), and *Escherichia coli* (11 isolates). Honey demonstrated antibacterial activity against these pathogens, in agreement with studies by Kwakman and Zaat [41] and Mandal and Mandal [20], who highlighted honey's efficacy against wound isolates. Clinical trials further support this role; honey has been shown to reduce wound size and bacterial load in diabetic foot ulcers [42], treat infected wounds in pediatric patients [43], and accelerate healing of chronic wounds such as bedsores and venous ulcers [44].

The mechanisms of action of honey in wound healing are multifactorial. Honey inhibits a wide range of bacteria, including resistant strains such as MRSA [45], stimulates immune responses by enhancing cytokine and growth factor production [24], reduces inflammation, promotes angiogenesis, and increases collagen deposition [22]. These combined effects explain its ability to both control infection and promote tissue repair. When comparing the antimicrobial efficacy of Oba Nsukka honey with that of Benue honey, this study found Oba honey to be significantly more effective against wound isolates. The difference may be linked to variations in honey sourcing: Nsukka honey is obtained from the core of the comb, while Benue honey is collected from comb droplets [34]. This observation further supports the claim that honey's antibacterial potency depends on its type, geographical origin, climatic conditions, and processing [24, 41]. Similarly, Olawuyi et al., [46] reported that honey samples from different Nigerian agro-ecological zones varied in antimicrobial activity due to geographical and climatic influences. A report by Ajobiewe et al., [47] who accessed the effect on honey from three different regions in Nigeria further showed that honey proves to very effective against wounds but with no statistical mean difference among the three variants of honey obtained. In our study, statistical analysis shows a statistically significant difference from the different honey obtained and further confirmed the superiority of Oba Nsukka honey. Statistically significant differences were observed in Oba Nsukka honey, revealing its high efficacy and effectiveness compared to Benue honey across various wound isolates ($p < 0.05$). This finding is consistent with the report of Ogundele et al.,[36] who emphasized the therapeutic value of Oba Nsukka honey in wound management.

5. Conclusion

This study investigating the antibacterial activity of honey on bacterial isolates from wounds highlights its significant potential as a natural therapeutic agent for wound care. The findings showed that *Pseudomonas aeruginosa* was the most frequently isolated organism, likely due to a local outbreak in the study area. A total of 18 significant bacterial isolates were identified, all of which were sensitive to both varieties of honey at 100% concentration. Notably, Oba Nsukka honey produced larger zones of inhibition across all concentrations compared to Benue honey, demonstrating greater antibacterial efficacy.

These results are consistent with previous studies that confirmed honey's broad-spectrum antibacterial activity and its effectiveness in wound healing. Its therapeutic effects are attributed to multiple factors, including its antibacterial, anti-inflammatory, antioxidant, and immunomodulatory properties. Honey has proven particularly effective in treating infected and chronic wounds, with clinical studies demonstrating its ability to reduce bacterial load, promote tissue repair, and support healing.

Based on these findings, prior to the use of honey as an alternative remedy, clinicians and healthcare providers are advised to consult with the medical laboratory to inform optimal application of honey in wound care, while regulatory agencies should ensure the availability of pure, undiluted honey for clinical use. Honey represents a natural, cost-effective, and safe complementary therapy to standard wound care practices, warranting further research into its mechanisms of action and broader clinical applications.

Compliance with ethical standards

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Disclosure of conflict of interest

We declare no conflict of interest.

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