DETERMINATION OF ANTIBACTERIAL ACTIVITY OF HONEY ON WOUND ISOLATES FROM PATIENTS ATTENDING UNIVERSITY OF BENIN TEACHING HOSPITAL, EDO STATE

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Abstract
Background: Honey is one of the most appreciated and valued natural products introduced since ancient times, as it is used not only as a nutritional product but also in traditional medicine.
Aim: The aim of this study is to determine the antibacterial activity of honey on wound isolates from patients attending University of Benin Teaching Hospital, Edo State.
Materials and Methods: Samples from wound were collected from 300 patients comprising of males (123) and females (177) within the age range of (21 - 50) years old. The samples were processed by culture methods and the isolates obtained were subjected to Gram staining and biochemical tests for identification. Antibacterial activity of honey was determined using standard agar well diffusion technique. A structured questionnaire was administered for data on the socio-demographic characteristics of the patients.
Results: An overall prevalence of bacterial infection 93(31.0%) was recorded. Five bacteria were isolated, namely; *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa, Klebsiella spp* and *Steptococcus pyogenes*; where *Staphylococcus aureus* was the most common organism isolated (25.81%). The higher bacterial growth (16.0%) was recorded in High vaginal swab (HVS) samples with statistical significant difference (P=0.0063). Females had the highest prevalence of (64.52%). Age group (41-45) had the highest prevalence of (61.29%) with a significant difference (p=0.0002). Furthermore, educational and economic status have no statistical association (p>0.05) with the infection. The Gram negative bacteria examined showed *S. aureus* and *E. coli* having the highest prevalence of (27.27%) each in males while *P. aeruginosa* had the highest prevalence rate (30.0%) in female**.** There was decrease in zone of inhibition of honey in a dose dependent manner in all isolates observed when compared to standard antibiotics.
Conclusion: The antibacterial activities of honey against the microorganisms suggest it's used as an alternative therapeutic agent in certain medical conditions, particularly wound infection.
Keywords: Antibacterial activity, Honey, Patients, *S. aureus*, Wound samples

INTRODUCTION

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases. However, as resistant pathogens develop and spread, the effectiveness of the antibiotics is diminished. This type of bacterial resistance to the antimicrobial agents poses a very serious threat to public health and for all kinds of antibiotics including the major last-resort drugs, whereby the frequencies of resistance are increasing worldwide (Levy and Marshall, 2004; Mandal *et al*., 2009).

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This has led to frantic effort in seeking alternative antimicrobial strategies and the re-evaluation of the therapeutic use of ancient remedies such as plants and plant-based products, including honey (Basualdo *et al*., 2007; Mandal *et al*, 2010).

The use of this plant-based products/natural product is becoming a more popular approach in medical treatment. The increase in their popularity is due to their potent activities and generally very low toxicity (Slover *et al*., 2009). According to the world Health Organization (WHO) statistics, up to 80% of the population in some developed countries have used natural product in their primary care (WHO, 2014). Scientists have found out that natural materials are generally more acceptable to consumers and if these natural approaches are effective, this may reduce the reliance on synthetic substance (Slover *et al*., 2009).

Wound infection causes great distress in terms of associated mortality and morbidity, increased length of hospital stays, profound discomfort and significant increase in healthcare cost (Rubin, 2006). Infection in a wound delay healing and may cause wound break down, herniation of the wound and complete wound dehiscence (Rubin, 2006). Honey is one of the naturally available products and is the only concentrated sweetener that can be found in nature. The antibacterial activities of honey were first recognized in 1892, although it has a limited use in modern medicine due to lack of scientific support (Mohapatra *et al*., 2011).

The healing effect of honey could be due to various physical and chemical properties (Snow and Manly-Haris, 2004). It contains 15% - 20% water and 80% -85% sugars. The remainder of the honey is made up of protein, enzyme and nonessential amino acids (Sharp, 2009). Glucose oxidase which changes glucose to gluconolactone and then to hydrogen peroxide is one of the enzymes found in honey. The release of hydrogen peroxide (H_2O_2) is slow and continuous for a constant antibacterial effect by, successfully eliminating microorganism but dilute enough not to damage host tissue (Lusby *et al*., 2002; Olaitan *et al*., 2007).

The world Health Organization (WHO) has described alternative medicine as a cheap way to achieve health care coverage of the world's population and has encouraged the rational use of plant-based alternative medicine (Zhang, 2000).

MATERIALS AND METHODS

Study Area

This study was conducted at University of Benin Teaching Hospital, Benin City, Edo State, Nigeria. The hospital is located in the south-south geographical zone on latitude 6.3903^0N and longitude 5.6118^0E of the ancient city of Benin. Benin City is the capital of Edo state, located between latitude 6°44'N and $6^0$21'N and longitude $5^0$35'E and 5°44' E. The area is about 1125 km^2 and situated on fairly flat land, about 8.5km above sea level. The state is situated in the low rain forest area of Nigerian and has two seasons namely rainy and dry. Edo state consists of a population of five (5) million (Edo State Ministry of Land and Survey, 2009).

Study Population

The study population consists of male and female patients with wound attending University of Benin Teaching Hospital, Edo state. A structured questionnaire was administered to each patient after proper notification and information on the nature of the research, risk involved, benefits as well as confidentiality. The ethical clearance for this study was obtained from the Edo State Ministry of Health. Informed Consent was obtained from each participant prior to collection of samples.

Sample collection and processing

Pus samples were collected from 300 patients with sterile cotton swabs. The swab was gently rolled over the surface of the wound approximately 5 times focusing on area where there was evidence of pus or inflamed tissue.

Following the manufacturer's instructions, the samples were immediately cultured on Nutrient agar (NA), Mannitol salt agar (MSA), and MacConkey agar plates for the isolation of viable bacteria. After inoculation, plates were kept at 37°C for 24 - 48h according to the methods of (Nur *et al*., 2017 and Talukder *et al*., 2019). The colonial morphology of the various isolates was read and recorded. All isolates were further identified using standard microbiological techniques such as Gram staining, Catalase, Coagulase, Citrate and Oxidase tests (Cheesebough, 2006).

The honey was obtained from local commercial producers in Ovia North East local government area of Edo state. It was examined for any diluents or additives and had not been heated (Molan, 2011). Hundred percent pure honeys (100% v/v) were obtained after filtration using sterile gauze. To get 1 ml of 20%, 40%, 60% and 80% concentrated honey solution (v/v); 0.20 ml, 0.40ml, 0.60ml and 0.80ml of honey was diluted in 0.80ml, 0.60ml, 0.40ml and 0.20ml distilled water constitutively following the method of (*Mama et al*., 2019).

Antibacterial activity of honey

The agar well diffusion technique was used to screen for antibacterial activity of honey. The standard inoculums (0.5 MacFarland Standard) were introduced into the surface of the Mueller Hinton agar plate and a sterile glass was used for even distribution over the media. Five wells were made using a sterile cork borer and each well was filled with different concentrations (20, 40, 60 and 100v/v) of the honey. The plates were incubated at 37 °C for 24hrs to 48 hours and observed for zone of inhibitions. This in-vitro experiment was compared with the use of 50mg/ml Gentamicin (Micro lab) as control. The antibacterial activity was expressed as the mean diameter of inhibition zone (mm) produced by the honey (Ali *et al*., 2017).

Statistical Analysis

Graph pad prism 8.0.2 from California, USA was used to analyze data. ANOVA was used when comparing different concentration of honey. Frequency was obtained when determining the prevalence. Significance level for the differences was set at $p<0.05$.

RESULTS

Out of the 300 wound samples examined, 31.0% prevalence was found to have significant growth using standard bacteriological procedure. Five bacterial isolates of medical importance were identified; *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella spp* and *Steptococcus pyogenes*. The most common organism isolated was *Staphylococcus aureus* (25.81%), followed closely by *Pseudomonas aeruginosa* (22.58%) (Fig 1). The highest prevalence of wound infection was found in HVS 48(16.0%), followed by Ear and Nose 23(7.66%) (Table 1).

Table 2 shows the age related prevalence examined, 123 were males of which 33(35.48%) were infected and 177 females with infection rate of 60(64.52%). Thus females were more exposed than males (OR=1.263; 95% Cl=0.7892, 2.040; P=0.3961).

There was statistically significant association between some of the Socio-economic factors and the infection (P< 0.05). For age distribution, the age group 41 – 45 years had a higher prevalence of 61.29% followed by age group 36 -40 years with 35.48%, the difference was statistically significant (P = 0.0002). The patients that have secondary education were more infected (64.52%), although this was significantly not associated (P = 0.0746) with bacterial infection. There was a significant difference in self-employed patients and bacterial infection (P< 0.05). Subsequently, there was no significant association between economic status and prevalence of bacterial infection in this study (P= 0.6575) (Table 3).

Among the isolates examined, *S. aureus* and *E. coli* showed the highest prevalence of 9(27.27%) each in males while *P.aeruginosa* had the highest prevalence rate (30.0%) in female (Table 4). The zone of inhibition of honey in a dose dependent manner in all isolates was observed. The zone of inhibition of different concentrations of honey shows that there was significant difference ($p<0.001$).

Generally, the susceptibility profile ranged from poor to high depending on the isolates (Table 5).

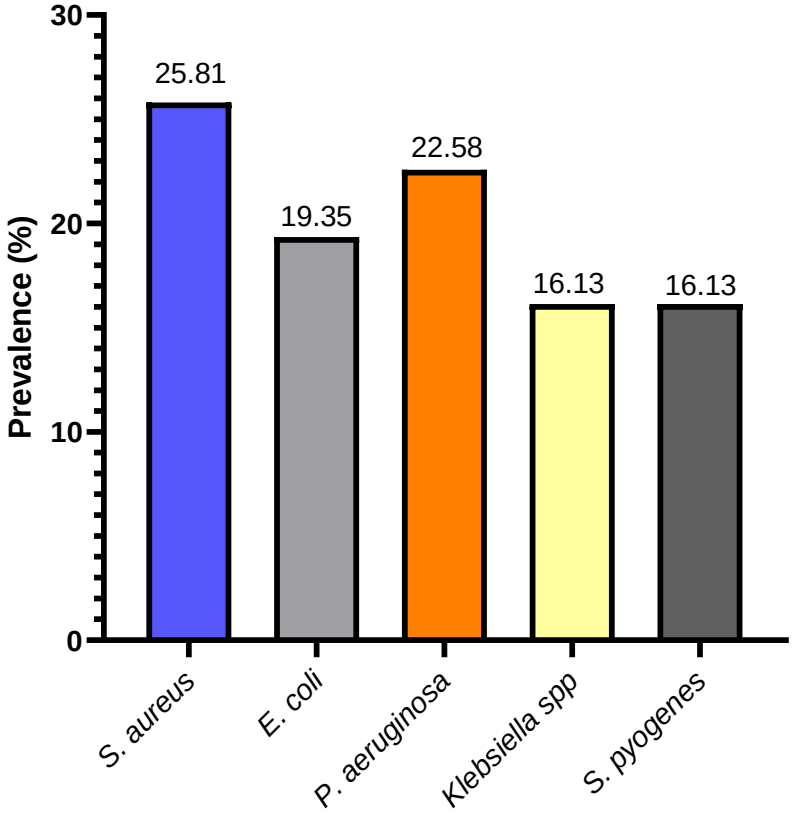

Figure 1: Prevalence of Bacteria isolates from infected patients in the study

Table 1: Frequency of Bacterial Isolates from wound samples

Samples	Number Examined	Number of bacterial Isolated	Prevalence (%)	P-value
Leg	100	22	7.33	0.0063
HVS	100	48	16	
Ear and Nose	100	23	7.66	

Table 2: Prevalence of bacterial infection in relation to gender of the patients

Gender	Number Examined	Number Infected (%)	OR	95% CL	P value
Male	123	33 (35.48)	1.263	0.7892 - 2.040	0.3961
Female	177	60 (64.52)			
Total	300	93 (31.00)			

Table 3: Socio-demographic factors associated with bacterial infection among the study Subjects.

Parameters	Number Examined	Number Infected (%)	P value
Age group			
21- 25	30	0 (0)	0.0002
26 – 30	27	3(3.22)	
31 – 35	12	0 (0)	
36 – 40	90	33 (35.48)	
41 – 45	132	57 (61.29)	
46 – 50	9	0 (0)	
Educational status			
Primary School	102	30 (32.26)	0.0746
Secondary School	156	60 (64.52)	
NCE	3	0 (0)	
OND	3	0 (0)	
HND	12	0 (0)	
BSc	24	3 (3.22)	
Economic status			
Middle	129	36 (38.71)	0.6575
Below Middle class	132	42 (45.16)	
Poor	39	15 (16.13)	

Table 4: Distribution of the Isolates according to gender

Organisms	Male (%)	Female (%)	Total (%)
S. aureus	9 (27.27)	15 (25.00)	24 (25.8)
E. coli	9 (27.27)	9 (15.00)	18 (19.35)
P. aeruginosa	3 (9.09)	18 (30.0)	21 (22.58)
Klebsiella spp	6 (18.18)	9 (15.00)	15 (16.13)
S. pyogenes	6 (18.18)	9 (15.00)	15 (16.13)
Total	33 (35.5)	60 (64.5)	93 (100)

Table 5: Antibacterial Activity of honey on Bacteria Isolates Based on Concentration

Organisms	Control (Standard drug)	100% Honey	80% Honey	60% Honey	40% Honey	20% Honey	f value	p value
S. aureus	26.75±0.3134	20.75±0.3134	16.88±0.479^{ab}	14.13±0.350^{abc}	7.5±0.654^{abcd}	1.875±0.58^{abcde}	368	<0.0001
E. coli	25.63±0.46	12±0.327^{a}	9.125±0.35^{ab}	5.875±0.22^{abc}	3.625±0.26^{abcd}	0.625±0.26^{abcde}	746.2	<0.0001
P. aeruginosa	29±0.422	18±0.378^{a}	12.75±0.25^{ab}	7.75±0.313^{abc}	4.875±0.22^{abcd}	0.5±0.267^{abcde}	1044	<0.0001
Klebsiellaspp	26.25±0.366	15.63±0.375^{a}	6.625±0.263^{ab}	1±0.378^{abc}	0±0.00	0±0.00	1405	<0.0001
S. pyogenes	24.38±0.92	9.125±0.295^{a}	5.5±0.267^{ab}	2.625±0.263^{abc}	0±0.00	0±0.00	472	<0.0001

DISCUSSION

The search for an effective drug against wound infections have been a major problem in the field of medicine for a long period of time and the problem complicated more recently because of the increased in antimicrobial resistance (Levy and Marshall, 2004). The results from this study showed an overall prevalence of 93(31.0%) bacterial infection in wound samples among patients attending University of Benin Teaching Hospital, Edo state. This prevalence is differed from the 64.8% of bacterial contamination in wound, reported by Egbe *et al*., 2011 in Benin City and 78.9% by Valarmathi *et al*., 2013 in Colombia. This disparity in the prevalence of wound infections observed in the different studies may be attributed to the cost in treatment that is burdensome and not only for patients but also their families (Shang *et al*., 2015). The most common isolated microorganism from this study was *Staphylococcus aureus*, followed by *P. aeruginosa* and *Escherichia coli*, as previously demonstrated by other authors (Guan, 2021; Puca *et al*., 2021). They reported the frequency of hospital based infection to be Staphylococci (39.6%), *Staphylococcus aureus* (12.3%), *Klebsiella pneumoniae* (8.2%), *Escherichia coli* (5.7%) and *Pseudomonas aeruginosa* (3.8%). In a similar study in India, five microorganisms, namely, *S. aureus, P. aeruginosa, Klebsiella* species, *E. coli*, and *Enterobacter* species were reported to cause approximately 80% of infections (Jones *et al*., 2011) which is in partial agreement with results observed in this study. However, there was association between the type of wound and the type of micro-organism isolated, it is important to note that pus from traumatic wounds yielded significant bacterial growth and were thus deemed to indicate infection (Jones *et al*., 2011). Contrary to this study, two previous studies also done in Nigeria had associated specific micro-organisms with particular wound types (Otokunefor and Datubo-Brown, 1990; Okesola and Kehinde, 2008). *S. aureus* is a Gram-positive bacterium which is a major pathogen implicated in skin infections such as impetigo, furuncles, boils, sties, pustules, burns, and others. Antibiotic-resistant strains of *S. aureus* are the major cause of infections especially in a hospital setting (Mudey *et al*., 2010). Strains of *S. aureus* that were fully sensitive to penicillin now developed resistance to methicillin, and other latest ones resort antibiotics (Naik and Teclu, 2010).

The results from this study showed that females had a higher prevalence (64.52%) of wound bacterial infection compared to males (35.48%). This is consistent with the reports of Pondei *et al*. (2013) that reported a prevalence rate of males (39%) and females (48%). This could be attributed to the fact that there are sex-specific differences in the ability to detect pathogens, as females have higher expression of pathogen-associated molecular pattern receptors compared to males (Sabra and Craig, 2015). Our findings showed that age specific prevalence rate was greater for age 41- 45 years old (61.29%), followed by 36 -40 years (35.48%), and there was significant association between age and wound infection (P = 0.0002). This agrees with the study done in the Niger Delta region (Egbe *et al*., 2011) that reported a lower prevalence of wound infections among age group of <5 years old (20.0%) and higher among the age group of 36-40 years old (77.5%) with *Staphylococcus aureus* being the most prevalent etiologic agent (21.5%). Ideally, the age of a patient seems likely to have a bearing on wound infection and healing (Egbe *et al*., 2011).

In this study, there was no significant association between isolates distribution and educational status of patients. The prevalence of 60 (64.52%) was found among secondary status holders followed by primary 30 (32.26%). However there were paucity of literature on the relationship between infection and educational status of patients.

There is strong evidence of a social gradient in most health outcomes whereby the poorest in society experience greater levels of illness and premature death than those further up the socioeconomic scale (Wilkinson and Marmot, 2003). This study reveals patients belonging to the middle and lower than middle socioeconomic class having high prevalence rate. This is in contrast with the work of (Boglione and Dodaro, 2022). However, the results obtained in this study could be due to the fact that in developing countries, most persons who could afford hospital bills are either in the middle class or just below the middle class.

It was also observed that the antimicrobial activity of honey extract increased with increasing concentration of the extracts. The 100% and 80% concentrated honey showed a higher level of effectiveness compared to 60%, 40% and 20% concentrated honey which expressed a lesser antimicrobial activity on the wound isolates. Besides H_2O_2, an endogenous enzyme glucose oxidase, produced by honey also has antimicrobial activity (Mohapatra *et al*., 2011). The bactericidal effect of honey is reported to be dependent on concentration of honey used and the nature of the bacteria (Wilkinson and Cavanagh, 2005). The result of antibacterial activity of honey from this study against bacterial isolates shows that honey had the highest minimum inhibitory concentration (MIC) against *S. aureus* which is in conformity with the reports of (Molan, 2011) that *S. aureus* is one of the bacterial species most susceptible to the antibacterial activity of honey. These might be due to the osmotic effect, the effect of pH and the sensitivity of these organisms to hydrogen peroxide which represented an ‘inhibine, factor in honey (Baltrusaityte *et al*., 2007). But contrary to that of Abd-el *et al*. (2007) who showed that honey has a greater inhibitory effect on isolated gram-negative bacteria (*P. aeruginosa, Enterobacter spp*. and *Klebsiella*) when compared to Gram positive. Also El-Sukhon *et al*. (1994) showed gram negative bacteria to be more sensitive to action of honey than Gram-positive bacteria.

Furthermore, Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects (Mohapatra *et al*., 2011). Therefore, it has been shown that the antimicrobial activity of honey may range from concentrations <3% to 50% and higher (Wilkinson and Cavanagh, 2005).

CONCLUSION

The overall prevalence of 31.0% of wound infection among patients attending University of Benin Teaching Hospital, Edo state is a risk of wound bacterial infection with increase in age. High vagina swab is the most prevalent specimen with bacterial infections. Honey showed antibacterial activity on a few isolates only. These findings contribute to our understanding of the diverse interactions between natural compounds, antibiotics, and bacterial isolates, shedding light on potential avenues for alternative wound infection treatments.

RECOMMENDATION

Honey was found to have a significant effect on wound infection of the study patients in this study. Honey can be safely recommended as an alternative natural antibiotic, mostly in cases where conventional antibiotics are not readily available, which exerts a stimulating effect on macrophages to release mediators needed for tissue healing and reducing microbial infections.

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