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Efficacy of Nigerian Local Honey on Pseudomonas Aeruginosa from Infected Wound among Genders in Federal Medical Center, Bayelsa

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Abstract : Honey, a natural product can be used as a sweetening and as well as an antimicrobial agent in most parts of the world and it is generally believed that it is a wound healer. This work was carried out between February and April 2022 to assess the antimicrobial effectiveness of Nigerian honey on the *Pseudomonas aeruginosa* associated with the wound infection. The Infected wound samples collected from fifty (50) patients from Federal Medical Centre, Yenagoa were cultured on Nutrient agar and further confirmed on Cetrimide agar and characterized by standard microbiology techniques. Undiluted isolated Pseudomonas aeruginosa strains standardized by 0.5 Mac Farland standard turbidity solution was tested different concentrations (20%, 40%, 60%, 80%, 100%) of Nigeria locally produced honey purchased from Tombia main market, Bayelsa State using cork borer agar diffusion method; this was incubated for 24 hrs for the expression of the zone of inhibition. Findings showed that out of 50(100%) wound samples examined, 15 (30%) were positive for *Pseudomonas aeruginosa*. From the statistical calculation, observation showed no significant difference in the frequency of *Pseudomonas aeruginosa* among genders at p=0.955 and age group examined at p value of 0.08. Likewise, the combination statistical analysis for both gender and age had no difference significantly in the prevalence of the isolated strains of Pseudomonas organism at P value of 0.27. Of all the concentrations of honey used against these strains, there was no zone of inhibition to be measured amounting to lack of potency of this product on *Pseudomonas aeruginosa*. In conclusion, locally made honey in the Niger Delta region of Nigeria is not effective against *Pseudomonas aeruginosa* associated with wound infection in this region therefore it should not be used as a wound healer due to its ineffectiveness. Further research should be done by carrying out the phyto-chemical analysis of Nigeria locally produced honey especially in the Niger Delta region of Nigeria to confirm its authenticity and the use of spectrophotometer machine for analyzing the effectiveness of honey could be significant.

Keywords: Antimicrobial activity, Nigeria honey, *Pseudomonas aeruginosa*; wound infection; gender based prevalence;

Introduction

The healing effects of some natural locally produced juices from green leaves, healing paraffins, medicinal roots and honey comb cannot be overestimated in Africa countries especially in Nigeria. Eighty percent of Nigerians especially the elderly prefer to use these herbal products for various diseases as healing power, ranging from diabetes mellitus, malarial fever, cough, break in the skin, hypertension ie high blood pressure, yellow fever (viral infections), bone diseases such fractures, dislocations, leukaemia ie cancer of the bone marrow and many others to mention a few. It is a general believe in Nigeria among the populace that these products are far better in healing various diseases than modern antibiotics even though the exact dosage of these products are not fully understood by the local practitioners. Despite their under exposure to the actual dosage to be administered on each disease or infection, the ignorantly applied dosages are still working for them and they so much believe in the healing ability of these products Zainol *et al*., 2013. These sets of traditional practitioners got their knowledge from their forefathers according to them as children and took over after the death of their fathers or grandfathers; it is very rare to get people trained from outside their extended family lineage. Different herbs can be brought and cooked together to cure different infection and ailments; this is seriously working for them. Honey, being a natural product, obtained from the nectar and sweetening portion of plants by an insect, honeybees, *Apis melifera* Jiang *et al*. (2014), can be further formulated by traditional medicine practitioners for more potency on infections such as wounds. Honey is universally used for the cure of various diseases by inhabitants of the world, especially in Africa, China and India, even before the modern discoveries of the first antibiotic, Penicillin by Alexander Flemings in 1958 and agents of infections from law of germ theory by Pasteur Louis. This natural product displays its potency among the people of old and they have exploited and explored this vital value, Pimental *et al*., 2013). Honey has demonstrated potency in all categories of diseases include fire burnt and cut skins, many people of old and modern medicine have recommended the potent honey as a replacement to modern antibiotics against wound infections caused by microorganisms especially *Pseudomonas aeruginosa* either from motor accident or as a result of diabetes mellitus infections and they have not reported otherwise to its effectiveness (Henriques *et al*., 2011).

Aerobism, gram negativity with non-sporulation displayed by the *Pseudomonas aeruginosa* has been documented by many researchers as the major contributor in wound infections, the organism can survive harsh environmental conditions, an ubiquitous, with the ability to cause different infections ranging from urinary tract infection, otitis media, osteomylitis and can cause opportunistic infections among the catheter users, hospital workers and wound infections Alhazmi (2014), drug users with injections, immune-compromised people such as tuberculosis, human immunodeficiency virus, elderly and children. The mechanism of *Pseudomonas aeruginosa* has many phases as the organism can produce various toxins such pili, enterotoxins, lipopolysaccharides which are good pyrogens that can Alhazmi (2014) penetrate the blood vessel causing sepcaemia as a result of bacteremia by the microorganism being an invasive one. (Doring *et al*., 2014). *Pseudomonas aeruginosa* can resist various antibiotics and its actions leading to multidrug resistance; just like the work done in southwest of Nigeria by Ojo *et al*, (2020) percentage prevalence of *Pseudomonas aeruginosa* in respiratory diseases as 38.8 and Cohen and Prince, (2013) stated that the infection caused by this microorganism has a tremendous challenge in the medical field due to its resistance genes which give the organism the ability to withstand many antibiotics and pose difficulty in the treatment. Honey has varying moisture content which range from 14 to 20% with other components such as disaccharides sugars; their colours can vary from faint yellow, lipton colour, brownish red; honey can as well contain some alkaloids such as Flavonoids which could help contribute to its antimicrobial activities Albaridi, 2019; Zainol *et al*., 2013. All these components can as well attract microbial degradation and spoilage. Number of authors have reported through antibiogram and animal experimentation on the healing efficacy of honey as a result of its composition and have

proved its potency on many microbial lives. The commonly availability of this honey in local and modern setting in the environments and its affordable prices could be an attractive factor to clinicians and modern medicine to digress into the use of honey to cure major infections caused by many consortium of microorganisms most especially *Pseudomonas aeruginosa*, the common etiological agent of the wound infection, instead of our modern days antibiotics. The aim of this investigation was to evaluate the antimicrobial potency of Bayelsa State locally produced honey on *Pseudomonas aureginosa* strains causing wound infections.

Materials and Methods

Study Area

Bayelsa state is proud to imhabit 1,704,515 people which include males, females and children fro the result of 2006 census; a state situated at Niger Delta region of Nigeria home Federal Medical Center Yenagoa, a 800 bedded hospital with categories of competent medical personnel from various fields such as orthopaedic, gynecology, paediatric, accident and emergency, uitra sound, medical laboratory science units. All inhabitants of Bayelsa and diaspora visit this hospital for one health challenge or the other and the hospital also serve as where private clinics around the city of Yenagoa refer their patients with health complications to for effective medical attention and treatment. Bayelsa state is surrounded by river and the mode of habitation is linear settlement as a result of river water that has taken most parts of the state. Most educated citizens in this state are civil servants, there are no modern industries and the major occupation is fishing and farming which engage majority of women.

Clearance for Ethical Request

Ethical clearance was sought for and obtained from the special committee in possession in ethical unit of the study hospital

Sample Size

Taro Yamane formula was used in the calculation of sample size

Sample Collection Processing

Collection of Test Samples

Wound samples collected from fifty 50 (23 males, 2 7females) patients with wound infections from Federal Medical Centre, Yenagoa, medical mi c robiology unit were plated on nutrient agar and cetrimide and Cetri mide agar, a selective medium and kept in the incubator for 24hours at 37oc. The pure isolates were further characterized and identified using gram staining, biochemical test such as oxidase, citrate, lactose fermentation and indole test.

Collection of the Honey

The honey used in this study was purchased from a Southern Ijaw local producer at General Tombia market unit an d the producers assured of the authenticity of the product. The product was collected with carefulnesss under sterile environmental condition and kept sterile from microbes at ambient tem perature prior to use at medical microbiology diagnostic laboratory, Niger Delta University. Honey used in this study is a product of Southern Ijaw, Bayelsa state, the Niger Delta region of Nigeria

Antimicrobial Susceptibility Testing Using Honey

Cork borer agar diffusion (kirby Bauer's well diffusion) method was invented on Mueller Hinton medium at different concentrations (20%, 40%, 60%, 80%,100%) of honey with sterile distilled water to evaluate the antimicrobial activity honey against *Pseudomonas aeruginosa*. The standardized microbial suspension of test organism *Pseudomonas aeruginosa* strains were first flooded on the petri dishes containing sterile solidified Mueller Hinton agar using sterile swab stick by spread plate method. Holes were bored on the 30ml Mueller Hinton agar containing petri dishes and the bottom of the holes sealed with few drops of molten agar to avoid spilling of the test honey underground, before filling the holes with the test concentrations of 20, 40, 60, 80, and 100 with sterile injection syringe. The inoculated petri dishes were kept in the incubator for incubation for 24hours, 37Oc for clear, circular zone of inhibition to be measured the next day with the aid of measuring rule.

Statistical Analysis

SPSS IBM 2019 for chi-square analysis was employed in the statistical calculation in this

investigation and the significant difference among various experiments conducted was based on P values < 0.05, any greater numbers were considered significant

Results

Table 1: Distribution of *Pseudomonas aeruginosa* test isolate by gender

	Gender		
Presence of the isolate	Female	Male	Total
Negative	19 (70.4%)	16 (69.6%)	35 (70.0%)
Positive	8 (29.6%)	7 (30.4%)	15 (30.0%)
Total	27 (54.0%)	23 (46.0%)	50 (100%)

Table 2: Chi-square analysis of the prevalence of *Pseudomonas aeruginosa* in male and female (Sex)

Observed (O)	Expected (E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
11	11.08	-0.0800	0.0064	0.0006
10	9.91	0.0900	0.0081	0.0008
8	7.91	0.0900	0.0081	0.0010
7	7.08	-0.0800	0.0064	0.0009
				Total, $X^2_m = 0.0033$

Null Hypothesis : The distribution of *Pseudomonas aeruginosa* is independent of gender (M/F)
Alternative: Hypothesis: The prevalence of *Pseudomonas aeruginosa* is dependent of gender (M/F)
Degree of Freedom : (nrow – 1) x (ncol -) = (2-1) – (2-1) = 1
Measured Chi-square(X^2_m) value = 0.0033
Critical Chi-square(X^2_c) value = 3.841
P – value : 0.955,
Confidence level : 95%
Conclusion : Since Measured chi-square value is less than critical chi-square Value, and measured p-value (0.955) > 0.05 null hypothesis is accepted: no significant differenc e in male and female prevalence of *Pseudomonas aeruginosa*

Table 3: Distribution of *Pseudomonas aeruginosa strains* isolated by the age range

	(%)	(%)	Age Rage	(%)	
	(%)				
The Presence of isolate	<35	35 – 44	45 - 54	55 and above	Total
Negative	6 (17.1)	11 (31.4)	12 (34.2)	6 (17.1)	35 (70.0)
Positive	4 (26.67)	2 (13.33)	4 (26.67)	5 (33.33)	15 (30.0)
Total	10 (20.0)	13 (26.0)	16 (32.0)	11 (22.0)	50 (100)

Table 4: Chi-square analysis of the prevalence of *Pseudomonas aeruginosa* amongst study age group

Obsereved (O)	Expected (E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
2	3.50	-1.50	2.25	0.64
8	5.83	2.17	4.69	0.80
9	7.58	1.42	2.01	0.26
2	4.08	-2.08	4.34	1.06
4	2.50	1.50	2.25	0.90
2	4.17	-2.17	4.69	1.13

4	5.42	-1.42	2.01	0.37
5	2.92	2.08	4.34	1.49
				Total, X^2_m = 6.66

Null Hypothesis : The prevalence of *Pseudomonas aeruginosa* is independent of age group
Alternative: Hypothesis: The prevalence of *Pseudomonas aeruginosa* is dependent on age group
Degree of Freedom : (no of row – 1) x (ncol -) = (2-1) – (4-1) = 3
Measured Chi-square(X^2_m) value = 6.66
Critical Chi-square(X^2_c) value = 7.81
P – value : 0.08,
Confidence level : 95%
Conclusion : Since Measured chi-square value is less than critical chi-square Value, and measured p-value (0.08) > 0.05 null hypothesis is accepted

Table 5: Distribution of *Pseudomonas aeruginosa* strains isolated by gender and age range

Sex	Age Range	Negative	Positive	Total
F	<35	6 (66.7%)	3 (33.3%)	9 (18.0%)
	35 – 44	6 (75%)	2 (25%)	8 (16.0%)
	45 – 54	7 (77.8%)	2 (22.2%)	9 (18.0%)
	55 and above	0 (0%)	1 (100%)	1 (2.0%)
M	<35	0 (0%)	1 (100%)	1 (2.0%)
	35 – 44	5 (100%)	0 (0%)	5 (10.0%)
	45 – 54	5 (71.4%)	2 (28.6%)	7 (14.0%)
	55 and above	6 (60.0%)	4 (40.0%)	10 (20.0%)
	Total	35 (70.0%)	15 (30.0%)	50 (100%)

Table 6: The Chi-square analysis of the prevalence of *Pseudomonas aeruginosa* amongst study gender and age group

Obsereved (O)	Expected (E)	(O-E)	$(O-E)^2$	$(O-E)^2/E$
2	2.92	-0.92	0.85	0.29
3	2.08	0.92	0.85	0.41
4	3.50	0.50	0.25	0.07
2	2.50	-0.50	0.25	0.10
5	4.08	0.92	0.85	0.21
2	2.92	-0.92	0.85	0.29
0	0.58	-0.58	0.34	0.58
1	0.42	0.58	0.34	0.80
0	0.58	-0.58	0.34	0.58
1	0.42	0.58	0.34	0.80
4	2.33	1.67	2.79	1.20
0	1.67	-1.67	2.79	1.67
4	3.50	0.50	0.25	0.07
2	2.50	-0.50	0.25	0.10
2	3.50	-1.50	2.25	0.64
4	2.50	1.50	2.25	0.90
				Total, X^2_m = 8.71

Null Hypothesis : The prevalence of *Pseudomonas aeroginosa* is independent of gender and age group

Alternative: Hypothesis: The prevalence of *Pseudomonas aeroginosa* is dependent of gender and age group
Degree of Freedom : (nrow – 1) x (ncol -) = (8-1) – (2-1) = 7
Measured Chi-square(X^2_m) value = 8.71
Critical Chi-square(X^2_c) value = 14.06
P – value : 0.27,
Confidence level : 95%
Conclusion : Since Measured chi-square value is less than critical chi-square Value, and measured p-value (0.27) > 0.05 null hypothesis is accepted

Table 7: The Antibacterial Effect of Honey on *Pseudomonas aeruginosa* isolates

Number of positive sample	Presence of isolate (*Pseudomonas aeroginosa*)	20%	60%	80%	100%
1	+	0	0	0	0
2	+	0	0	0	0
3	+	0	0	0	0
4	+	0	0	0	0
5	+	0	0	0	0
6	+	0	0	0	0
7	+	0	0	0	0
8	+	0	0	0	0
9	+	0	0	0	0
10	+	0	0	0	0
11	+	0	0	0	0
12	+	0	0	0	0
13	+	0	0	0	0
14	+	0	0	0	0
15	+	0	0	0	0

Discussion

In the investigation of the antimicrobial efficacy of honey to isolates strains of *Pseudomonas aeruginosa*, females were slightly more susceptible to infection caused by *P. aeruginosa* (53.3%) compared to their male counterparts (46.7%), although the relationship was not statistically significant (P-value>0.05). A similar finding was reported by Alhamzi (2014), who noted that *P. aerugnosa* was a common cause of health-care associated infections irrespective of the age group. A possible explanation for this is that the organism is a common contaminant of catheters, surgical equipments and burn wounds. It does can establish an infection in a susceptible host irrespective of the sex and age of the patient.

The present study revealed that patients aged 55 years and above were more commonly infected with *P. aeruginosa* (33.33%) than other age groups although the results were not statistically significant (P-value>0.05). Our finding is in agreement with the work of Odumosu *et al*. (2012) and Abdulmutallib *et al*. (2019) who reported that infection with *P. aeruginosa* was more common in older patients than in younger ones; impaired immunity could be the cause. Moreover, older people are more likely to be on invasive surgical and chemotherapeutic treatments for cancer and diabetes; these could be established factors for infection with this organism.

The findings in this investigation revealed that among the study females subjects, *P. aeruginosa* was more commonly isolated among patients aged 55 and above (100%) while among males, *P.*

aeruginosa cause infection in subjects younger than 35 years, although the results were not statistically correlated (P-value>0.05). Our finding is in agreement with the work of Whitney *et al*. (2014) and Abdulmutallib *et al*. (2019) who reported similar findings. A possible reason for this could be that older subjects are at risk to chronic and metabolic diseases which may render them susceptible to infections caused by *P. aeruginosa.*

The present study showed that local honey being distributed in this Bayelsa state has no antimicrobial effects on *P. aeruginosa isolates* in this study population as expressed by the absence of the zone of inhibition on the agar plates. Our findings are in disagreement with the works of various researchers (Agbagwa & Frank-Peterside, 2010; Falegan & Amidobu, 2015; John-Isa *et al.*, 2019; Akinduti *et al*., 2020) who noted significant antimicrobial effects of honey against isolates of *P. aeruginosa.* This disparity may have been due to the type of honey used for the study, as most studies have utilized Manuka honey, which is not available in the Niger Delta region. Also, the study design may have contributed to these differences. Methods for detecting the antimicrobial effect of honey extracts such as spectrophotometric analysis for bacterial growth are more sensitive.

Conclusion

The findings of this study have shown that honey that are currently produced for sale in major markets and shops in Bayelsa state, the Niger Delta region of Nigeria have failed in its expected contribution in the antimicrobial effectiveness world of anti-infection drugs on *P. aeruginosa* strains isolated from infected wound with microorganisms in this study population.

Recommendation

The honey produced in Bayelsa state is not potent hence not useful in the treatment of wound infection caused by *Pseudomonas aeruginosa.* Other sources of honey from another part of Nigeria or in diaspora can be employed, but the phyto- chemical analysis of the honey should be carried out before its antimicrobial effectiveness is tested against *Pseudomonas aeruginosa* isolates from infected wound.

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Competing interest: none

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