



Antimicrobial Activity of Honey on Some Bacterial Isolates from Selected Brands of Sachet Water Sold within Port Harcourt, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author VNA designed the study. Author OAO performed the statistical analysis and wrote the protocol. Author CAA wrote the first draft of the manuscript. Author DUA managed the analyses of the study. Author CPA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Background: The present study was carried out to assess the antimicrobial effect of honey on bacterial isolates from sachet water sold within Eligbolo Community in Port Harcourt, Nigeria.

Methodology: Five brands of sachet water commonly consumed by the people living in Eligbolo Community of Port Harcourt, Nigeria were purchased from different Vendors in the community. Nutrient and MacConkey agar plates were used for culturing of water samples using spread plate method. Ten-fold serial dilution and Most Probable Number (MPN) were among the methods used and the samples analyzed were according to standard procedures. Natural honey purchased from Ogbokolo in Benue State, Nigeria was used for susceptibility testing. Quality control, ant inhibition and water test methods were performed using the honey to confirm its originality before use. Antimicrobial sensitivity testing was done using the agar well diffusion method.

Results: Results obtained showed the bacterial isolated from the 5 sachet brands of water. These include *Bacillus species* 5 (62.5%), *Enterococcus faecalis* 1 (12.5%), *Staphylococcus epidermidis* 1 (12.5%), and *Escherichia coli* 1 (12.5%). All of the 5 sachet water samples analyzed failed to meet the WHO drinking water standard of zero coliform per 100 ml making them unsuitable for

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human consumption. Faecal coliform was isolated from sample C indicating faecal contamination of the drinking water. The sensitivity of the isolates to the honey sample showed higher zone of inhibition compared to the standard antibiotic used as control. *Staphylococcus epidermidis* showed the highest zone of inhibition (39 mm), followed by *Escherichia coli* (37 mm), *Bacillus species* (35 mm) and *Enterococcus faecalis* (32 mm) respectively.

Conclusion: The results revealed that honey has a broad antimicrobial spectrum against Gram positive and Gram negative bacteria and could provide alternative agent to overcome the problem of increasingly bacteria resistance to synthetic antimicrobial agents. It is therefore, recommended that further work should be encouraged for the extraction of the crude components of honey and their use for antibiotic production.

Keywords: Honey; antimicrobial agents; sensitivity; most probable number; sachet; serial dilution.

1. 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 [1]. This type of bacterial resistance to the antimicrobial agent poses a very serious threat to public health and for all kinds of antibiotics, including the major last-resort drugs, the frequencies of resistance is increasing worldwide [2,3]. Therefore, alternative antimicrobial strategies are urgently needed and thus, this situation has led to a re-evaluation of the therapeutic use of ancient remedies, such as plants and plant-base products, including honey [4,5].

Honey has been identified and exploited as one of the popular natural antimicrobial substance.

The western honey bee or European honey bee (*Apis mellifera*) is the most common of the 7 -12 species of honey bees worldwide [6,7]. Honey is the natural sweet substance produced by bees from nectar or the secretions of plants [8]. The medicinal properties of honey have been known since ancient times and have been described as the nectar of life [9].

In most ancient cultures honey has been used for both nutritional and medical purposes. The belief that honey is a nutrient, a drug and an ointment has been carried into our days, and thus an alternative medicine branch called "apitherapy" has been developed in recent years, offering treatment based on honey and other bee products against many diseases including bacterial infections. Honey has been reported to have an inhibitor effect on around 60 species of bacteria, including aerobes and anaerobes, Gram-positive and Gram-negative [10].

Currently, many researchers have reported antibacterial activity of honey and found that natural unheated honey has some broad-spectrum antibacterial activity when tested against pathogenic bacteria, oral bacteria as well as food spoilage bacteria [11]. Natural honey exhibits bactericidal activity against many organisms including *Salmonella*, *Shigella*, *Escherichia coli*, *Helicobacter pylori* and others [12].

Access to safe drinking water continues to be a global concern and thereby continues to receive attention. The stringent requirements of safe drinking water adding to its scarcity shows that in most cases the problem is not availability of water but inability to obtain quality water [13]. Due to the shortfall in the provision of adequate safe drinking water for the populace, the private sector, although for profit purposes have been of increasing significance in the effort to supply the populace with adequate and safe drinking water. They provide alternative to the erratic municipal pipe borne drinking water supply system in the form of packaged water commonly known as "Sachet water" [14].

Water in sachets is readily available and the price is affordable, but there are concerns about its purity. The integrity of the hygienic environment and the conditions where the majority of the water in sachets are produced has also been questioned. Dada [15] also documented the increased microbial contamination of sachet water as it is moved down the distribution line. Studies in Nigeria have documented claims of past outbreaks of water-borne illnesses resulting from the consumption of polluted sachet water, bacterial contamination with organisms such as *Bacillus species*, *Pseudomonas species*, *Klebsiella species*, *Streptococcus species*, alkalinity of the water and presence of chemicals such as aluminum and

fluoride above the recommended rallies [15,16,17].

2. MATERIALS AND METHODS

2.1 Sample Collection

Five brands of sachet water commonly consumed by the people living in Eligbolo Community of Port Harcourt, Nigeria were purchased from different Vendors in the community. Natural honey purchased from Ogbokolo in Benue State, Nigeria was used for susceptibility testing.

2.2 Sample Analysis

Nutrient and MacConkey agar plates were used for culturing of water samples using spread plate method. Sensitivity testing was done on agar medium.

2.2.1 Media preparation

Nutrient agar medium and MacConkey medium were prepared by weighing the agar powder according to the manufacturer's instruction and was dispensed in distilled water. It was mixed thoroughly and sterilized by autoclaving for 15 minutes at 121°C and allowed to cool to 45°C. About 20 ml volume was then aseptically poured into each clean sterile Petri-dish. The plates were allowed to solidify at room temperature and placed upside down to avoid moisture settling on the surface of the medium. The prepared medium was then stored at 4°C in the refrigerator.

2.3 Preparation of Materials Used in the Study

2.3.1 Preparation of peptone water

Peptone water was prepared by weighing according to the manufacturer's instruction and dispensing in distilled water. It was sterilized and autoclaved at 121°C for 15 minutes and adjusted using 0.5 McFarland's standard.

2.3.2 Quality control of honey

The quality control of honey was performed with matchstick dipped into the honey. Then the matchstick was struck. When the matchstick burns, it shows non-adulteration of honey. In the other hand when it fails to light well it is an adulterated honey and not fit for use in the research work [18].

2.3.3 Ants inhibition method

The honey was dropped on the floor to check if it attracts ants or repels them, when the honey was dropped; it repelled ants which showed good quality of the honey.

2.3.4 The water test method

A table spoon of honey was added into a glass cup filled with water to check if it will dissolve immediately in water. Adulterated honey will dissolve and spread around the glass while pure honey on the other hand will settle right at the bottom of the glass cup.

2.4 Methodology

2.4.1 Most Probable Number (MPN)

Measured volumes of water and dilutions of water are added to a series of tubes containing a liquid indicator medium. The indicator medium used is MacConkey broth containing bromocresol purple to indicate a colour change. An inverted durham tube is placed in each tube to detect the presence of gas. The media receiving one or more of the indicator bacteria show growth and a colour change which is absent in those receiving an inoculum of water without indicator bacteria. The following tubes were put up:

- One 50 ml of water to 50 ml of double strength medium
- Five (5 tubes of 10 ml quantities each to 10 ml double strength medium).
- Five (5 ml) tubes of 1 ml quantities each to 5 ml single strength

From the number and distribution of positive and negative reactions, MPN of indicator organisms in the sample may be estimated by reference to statistical tables. The bottles were incubated at 37°C and examined after 24 hours. The probable numbers of coliforms were read from the probability tables of McCrady [19]. After the presumptive test, subcultures were made from all tubes showing acid and gas to fresh tubes of single strength MacConkey medium and incubated at 44°C for 48 hours.

2.4.2 Total heterotrophic plate count

A ten-fold serial dilution was made up to 10⁻¹ dilution, by transferring 1 ml of the sachet water

sample into 9 mls of the peptone water (1:10 dilution). Therefore, an aliquot of 0.1 ml was placed on a sterile Nutrient and MacConkey agar plate and spread evenly using bent glass rod. The plates were incubated at 37°C for 24 hours. Colonies were counted and identified using standard procedures [20].

2.4.3 Subculturing of test organism and preparation of the bacterial inoculums

The discrete colonies isolated in pure culture were aseptically picked using wire loop and inoculated into peptone water and adjusted with McFarland's standard. The broth containing the inoculums was left for some minutes on the bench and thereafter seeded on nutrient agar.

2.4.4 Antimicrobial assay using agar well diffusion method

The antimicrobial activity of honey was assayed using agar well diffusion method. A representative colony of each isolate was inoculated into peptone water; thereafter it was seeded onto nutrient agar medium and distributed evenly discarding the excess. The seeded plates were allowed to dry and sterile cork borer were used to bore holes on the agar plates. An aliquot (0.1 ml) of the honey sample was introduced into the holes using a sterile Pasteur pipette while the selected antibiotic discs were aseptically placed on the surface of the agar side by side with the honey. Pre-diffusion was allowed for 15 minutes after which it was incubated for 24 hours at 37°C. After incubation, the plates were observed for inhibition zones. Thereafter, the zone of inhibition was measured in millimeters [21].

2.4.5 Gram staining

A colony of the culture was emulsified in a drop of distilled water on a grease free glass slide. The emulsion was evenly distributed on the surface of the slide as to obtain a thin smear. The smear was allowed to air dry before it was heat fixed by passage over blue bunsen flame. It was then stained with crystal violet, which was allowed to stay for one minute before it was rinsed off with tap water. Lugol's iodine was applied to it and left for one minute and rinsed with tap water. The smear was decolorized with acetone and rinsed off swiftly and it was then counter stained with safranin for 30 seconds, rinsed with tap water and was placed on the rack to dry and viewed under x 100 magnification.

2.5 Biochemical Test

2.5.1 Catalase test

Two drops of 3% hydrogen peroxide were dropped on a clean grease free slide placed inside a petri dish and the test isolate was transferred to one of the drops of hydrogen peroxide on the slide while the other was used as a control. Effervescence shows a positive reaction.

2.5.2 Coagulase test

A drop of distilled water was placed on a slide and with the aid of a wire loop, a colony of the test organism from nutrient agar plate was emulsified in the distilled water to make thick suspension. A loopful of plasma was added to the suspension and mixed gently and thereafter observed for clumping.

2.5.3 Citrate utilization test

Slopes of the Simmon's citrate agar were prepared in bijou bottles as recommended by the manufacturer. Using a sterile straight wire, the test organism was streaked on the slope, thereafter it was incubated at 37°C for 24 hours. Bright blue colour was indicative of positive citrate test.

2.5.4 Indole test

The test organism was inoculated in a bijou bottle containing 3 ml of sterile tryptone water and incubated at 37°C for up to 48 hours. Thereafter, the organism is tested for indole by adding 0.5 ml of Kovac's reagent and examined for a red colour ring on the surface layer within 10 minutes.

2.6 Data Analysis

Percentage occurrence was used to analyze the data and organisms isolated.

3. RESULTS

Table 1 shows the total heterotrophic count (THC) of the various brands of sachet water sold in Port Harcourt. Sample B had the highest number of bacterial count while sample E had the least bacterial count.

Table 2 shows the coliform estimation of the sachet water brands. Sample C had high coliform counts than the rest.

Table 1. Shows the bacteria heterotrophic count of all brands of the sachet water

Sample	Heterotrophic plate count (CFU/ml)
A	3.9×10^2
B	1.03×10^3
C	9.9×10^2
D	8.0×10^2
E	3×10^1

Table 2. Faecal coliform count estimation

Sample	Mean <i>E. coli</i> count/100 ml
A	0
B	0
C	4
D	0
E	0

Table 3 shows the percentage occurrence of bacteria isolated from the sachet waters. Among the organisms present, *Bacillus spp* was the frequently occurring organism in all the water samples.

Table 4 shows the antimicrobial activity of honey and standard antibiotics. *S. epidermidis* showed high zone of inhibition with honey. It was sensitive to gentamycin but resisted to ampicillin/cloxacillin.

4. DISCUSSION

Sample B has the highest heterotrophic count. Followed by sample C, D and A heterotrophic count respectively in the order 103, 99, 80 and 39 with the least being sample B with a HPC of 3 CFU/ml. Among the water samples analyzed,

only sample C revealed the presence of faecal coliforms. Heterotrophic bacteria present in water poses no health risks to humans but a high HPC count is an indicator for ideal condition for bacteria growth. However, HPC alone cannot give an indication on the risk of the infection on the consumption of sachet water.

Other test aids at detection of certain indicator organism to confirm the microbiological quality of the water. All samples passed the HPC test based on the US EPA and UK standards, which is below 500 CFU/ml and 100 CPU/ml respectively except sample B which failed the UK standard which is above 100 CPU/ml [22].

The brands A, B, D and E passed the tests. Their values fall within the acceptable limits for faecal coliform and 1 CFU/ml for total coliform) used in interpreting the test [23]; they are thus safe for drinking. However, brand C did not pass the test with 4 CFU/ml count for *Escherichia coli*, an indication of faecal contamination of the drinking water which could either be due to unsatisfactory treatment of source water or exogenous introduction during production. Enterotoxigenic *E. coli* is associated with the probable fatal diarrhea; an illness which is an important aspect of drinking water quality [24,25].

The predominant bacteria among the five sachet water are the *Bacillus species* with the highest frequency of occurrence 5(62.5%) while the others have similar percentage incidence of 1(12.5%). All the identified isolates have been isolated previously [26]. The presence of *Bacillus species* in sachet water could be as a result of contamination from poor staff handling during processing of the water sample. *Bacillus species*

Table 3. Percentage occurrence of bacteria isolated from different sachet water samples

Isolated organisms	Sample					Occurrence %
	A	B	C	D	E	
<i>Bacillus species</i>	+	+	+	+	+	5(62.5)
<i>Escherichia coli</i>	-	-	+	-	-	1(12.5)
<i>Enterococcus faecalis</i>	-	+	-	-	-	1(12.5)
<i>Staphylococcus epidermidis</i>	-	-	-	+	-	1(12.5)

Table 4. Antimicrobial activity of honey and standard antibiotics

Test organisms	Inhibition zone (diameter in mm)		CN	AMP
	Honey (100% concentration)			
<i>Bacillus species</i>	35		28	R
<i>Escherichia coli</i>	37		30	R
<i>Staphylococcus epidermidis</i>	39		25	R
<i>Enterococcus faecalis</i>	32		27	R

Key: CN- Gentamycin; AMP- Ampicillin/Cloxacillin; R- Resistance; MM- Millimeter

produces enterotoxin which could be deadly when ingested into the body [27]. *E. coli*, *E. faecalis* and *S. epidermidis* are commensals and opportunistic pathogens and their presence may be as a result of improper handling, processing, purification procedures and non-hygienic handling after production. Water with such bacteria are not safe for human consumption hence, the water source should be re-examined by the NAFDAC [24].

The result of the antimicrobial effect of honey on the bacterial isolates using 100% concentration of honey inhibited the growth of all the isolates; gentamycin equally inhibited the growth of all isolates. However, honey showed a higher sensitivity compared to Gentamycin. *S. epidermidis* had the highest zone of inhibition (39 mm) followed by *E. coli* (37 mm). Further study by Basualdo et al. [4] revealed similar results with *E. coli*, *Bacillus species*, *E. faecalis* and *S. species* showing significant antimicrobial activity with the zones of inhibition. The significant activity may be due to the property of honey which has higher level of hydrogen peroxide along with osmolarity.

Mohapatre et al. [25] also reported that honey was effective against both Gram-positive and Gram-negative bacteria. This is similar to the results obtained in this study. Up till now, there is not enough information of bacterial resistance to honey. This is likely due to the component composition of honey, which causes the individual components to act either individually or in synergy to prevent [28].

5. CONCLUSION

The proof of honey as a broad antibacterial spectrum is evident in the results obtained in this study. The result suggests that honey has the potential of a useful antibiotic on bacteria isolates which can cause infections. The production of antibiotics from this extract would be beneficial to our society as they could be used for the management of ailment caused by these pathogens. Based on this study, it is recommended that further work should be encouraged for the extraction of the crude components of honey and the production of antibiotic from this gift of nature. And to reduce the consumption of contaminated sachet water, further investigation or assessment of sachet water is recommended. Therefore, all water that fails NAFDAC and WHO regulations should be retreated before they are released to the public for human consumption.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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