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In Vitro Antibacterial Activity of Honey from Wamba, Nasarawa State, Against Clinical Isolates of Escherichia coli and Staphylococcus aureus

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ABSTRACT

Microbial resistance to modern antimicrobial drugs poses a serious threats to the health of individuals worldwide. This study was aimed to exploring honey as a natural alternative to solving microbial resistance to antimicrobial drugs. Honey was used against clinically gotten *Escherichia coli* and *Staphylococcus aureus*. *Escherichia coli* and *Staphylococcus aureus* were reconfirmed by using cultural, morphological and biochemical tests. Agar well diffusion was used to determine the minimum inhibitory concentration (MIC) of honey. The antibacterial activity of various concentration of honey against *E. coli* and *S. aureus* shows that honey inhibited all the test isolates at 100mg/mL, 50.00mg/mL, 25.00mg/mL and 12.50mg/mL against *E. coli.* The mean inhibition zone diameters were 15.00± 0.09 mm at 100.00mg/mL, 9.10± 0. 51mm at 50.00mg/mL, 6.01± 0.11mm at 25.00mg/mL and 4.14± 0.13mm at 12.50mg/mL while for MDR *S. aureus*, the inhibition zone diameters were 18.07± 0.00mm at 100.00mg/mL, 11.20± 0.32mg at 50.00mg/mL and 5.11± 0.07mm at 25.00mg/mL amount of honey. Honey was more active with MIC of 12.50mg/mL against MDR *E coli* and 25.00mg/mL against MDR *S aureus*. The result showed that honey was more effective against *S. aureus* than *E. coli*. The posive control, amoxicillin-clavulanate showed lower MIC values ranging from 1.56-3.12mg/mL against *E. coli* and *S. aureus*. These findings indicate that honey possesses significant antimicrobial properties but its efficacy is lower than conventional antimicrobials. However, honey may serve as a potential alternative or complementary therapeutic agent against resistant bacterial strains.

Keywords: Honey, *Staphylococcus aureus*, *Escherichia coli*, Minimum Inhibitory Concentration (MIC), Antibacterial.

1.0 INTRODUCTION

Microbial resistance to modern antimicrobial drugs is a serious problem worldwide due to the misuse and overuse of drugs. It has a significant consequences and leads to the persistence of infections and increased healthcare costs. It's important to solve this problem by developing new drugs to which microbes have little or no resistance (Khan *et al.,* 2018). The phenomenon of antimicrobial resistance refers to the potential of microorganisms including bacteria, viruses, fungi, and parasites to thrive and continue to grow in the midst of drugs designed to kill them. Infections caused by antimicrobial-resistant organisms are not only difficult to treat, there is also always an increased chance of severe illness and even death due to these infections (Salam *et al.,* 2023). The lack of alternatives to mitigate the antimicrobial resistance is based on the low number of new antibiotics developed and approved over the past decades (Andrei *et al.* 2018). The current problems with conventional antibacterial agents have led to the choice of honey and other natural products by the populace in treatment of bacterial infections (Ogwu and Izah, 2025). Honey is the sweet substance made by bees using nectar from flowers. Honey is made when the nectar and deposit from plant are gathered, modified and stored in the honey comb by honey bees. The definition of honey stipulates a pure product that does not allow for the addition of any other substance such as water or other sweeteners. The flower from which bees gather nectar largely determines the color, flavor and aroma of honey (Yafiana Qaromah *et al.*, 2024). Honey has been known for its antimicrobial properties for thousands of years (Hossain *et al.*, 2022). It is a complex mixture, its chemical composition and biological properties vary according to its geographical and botanical origins (Machado *et al.*, 2018). The effectiveness of honey as an antimicrobial agent is associated with many factors such as low pH, high osmolality, hydrogen peroxide content, presence of methylglyoxal (MGO) and defensin-1(Machado *et al.*, 2018). Furthermore, flavonoids and phenolic compounds contribute to the antibacterial effect of honey (Tan *et al.*, 2009). Honey's antimicrobial activity can vary depending on factors such as flower source, geographic origin and processing methods (Irish *et al.*, 2011). In addition, honey showed no side effects and was inexpensive, which is an additional advantage when used for medicinal purposes. Honey has an important antimicrobial activity and it can be used as an alternative natural agent in a number of medicines (Guruvu *et al.*, 2021). The aim of this study was to determine the

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antimicrobial activity of honey against *Escherichia coli* and *Staphylococcus aureus*.

2.0 MATERIALS AND METHODS

2.1 Study Area

This study was conducted in Microbiology Laboratory, Department of Microbiology, Faculty of Natural and Applied Science in Nasarawa State University, Keffi, Nasarawa State, Nigeria.

2.2. Study Design

Agar well diffusion assays was used to evaluate the antibacterial activity of honey. This method involves inoculating a bacterial culture onto an agar plate and introducing test sample into the well. Wells were cut into the agar plates using a sterile cork borer and different concentrations of the test samples were placed in each well and incubated. After incubation, zones of inhibition which are the areas where bacterial growth is absent was measured around the wells indicating the antimicrobial activity.

2.3 Sample Collection

The raw honey was obtained aseptically from a bee farm located in Wamba Local Government Area of Nasarawa State. It was transferred to sterile containers aseptically and kept at 4°C until use. *Staphylococcus aureus* and *Escherichia coli* isolated were obtained from Federal Medical Center Keffi and were confirmed in Microbiology Laboratory, Department of Microbiology, Faculty of Natural and Applied Science in Nasarawa State University Keffi for further analysis. (Clinical and Laboratory Standards Institute, 2020).

2.4 Reconfirmation of *Staphylococcus aureus* and *Escherichia coli*

Clinically obtained *E. coli* and *S. aureus* were reconfirmed by using cultural, morphological and biochemical tests. Gram staining, microscopy and biochemical tests were carried out to reconfirm the test isolates. *E. coli* and *S. aureus* were prepared in nutrient broth at 37°C for 24 hours then the inoculum was adjusted to 0.5 McFarland standard (1.5x 10^8 CFU/mL) using sterile saline (0.85% NaCl) and was mixed thoroughly to create a homogeneous suspension. The turbidity was confirmed with a spectrophotometer (OD600 ≈ 0.08–0.1) and used within 15 minutes of preparation. (Clinical and Laboratory Standards Institute, 2020).

2.5 Preparation of Honey Solution

A stock solution of honey was prepared at a concentration of 100.00mg/mL. Different concentrations of honey were obtained using a two-fold serial dilution technique. Briefly, 1 mL of the stock solution from 100.00mg/mL was transferred into a test tube containing 1 mL of sterile distilled water and mixed thoroughly to obtain a concentration of 50.00mg/mL. Subsequently, 1 mL of the 50.00mg/mL solution was transferred into another tube containing 1 mL of sterile distilled water to obtain 25.00mg/mL. The procedure was repeated successively to obtain concentrations of 12.50mg/mL**,** 6.25mg/mL**,** and 3.125 mg/mL. The prepared concentrations were used for the antimicrobial susceptibility testing of the bacterial isolates (Kadeřábková *et al.,* 2024). The solution was filter-sterilized through a sterile (0.22µm) membrane to ensure sterility (Clinical and Laboratory Standards Institute, 2020). Heating honey >60°C may degrade antimicrobial compounds (Molan, 1992).

2.6 Antibacterial activity of Honey

Agar well diffusion method was performed using Kirby-Bauer method on pure cultures of *S. aureus* and *E. coli* and zone diameters was measured as described by Clinical and Laboratory Standards Institute (2023). Inoculum was swabbed evenly on Mueller-Hinton agar (MHA) plates, 3 rotations and 60̊ apart. Using a sterile cork borer, a well (6 mm diameter) was created in the agar and the plates were pre-dried for 30 minutes at 37°C. Each well was filled with 10µL of 100.00mg/mL, 50.00mg/mL, 25.00 mg/mL, 12.50 mg/mL, 6.25 mg/mL, 3.12 mg/mL and 1.56 mg/mL of honey solution at different concentrations and incubated at 37°C in ambient air for 24 hours. The zone of inhibition was measured (mm) using callipers and the diameter measured was interpreted in accordance with the susceptibility break point of Clinical and Laboratory Standard Institute (Clinical and Laboratory Standards Institute, 2023).

Controls: Negative control (sterile water) and positive control (30µg amoxicillin-clavulanate) were used.

Each experiment was conducted three times (in triplicate) and the results were presented as the average (mean) along with the measure of variability (standard deviation, SD) around that average.

2.7Determination of Minimum Inhibitory Concentration (MIC) of Honey and Amoxicillin-Clavulanate using the Agar Dilution Method

Minimum inhibitory concentration (MIC) of honey and amoxicillin-clavulanate were determined using agar dilution method by modification of the method described by Clinical and Laboratory Standards Institute (2023). Using a 2-fold serial dilution, a stock solution of honey and amoxicillin-clavulanate were prepared individually at the highest concentration of 50mg/mL. Sterile molten Muller-Hinton Agar (MHA) was prepared and allowed to cool to about 45 ^{0}C. The prepared stock solutions were mixed thoroughly with molten Muller-Hinton Agar (MHA) to obtain a final concentration of 50mg/mL and mixtures were pour into sterile petri dishes aseptically. The same procedure was used to obtain the following concentrations: 25.00, 12.50, 6.25, 3.12 and 1.56 mg/mL. Subsequently, 10µL of the standardized *E. coli* and *S. aureus* isolates were inoculated in the MHA plates containing different concentrations of the honey and amoxicillin-clavulanate were incubated at 37 ^{0}C for 24 hours, the lowest concentration of the extract that inhibited the visible growth was read as the MICs (Clinical and Laboratory Standards Institute, 2023).

3.0 RESULT

Clinically obtained *E. coli* and *S. aureus* were reconfirmed by carrying out cultural, morphological and biochemical test and the results is as shown in Table 1. The antibacterial activity of the various concentration of honey against *E. coli* and *S. aureus* as shown in Table 2 showed that honey exhibits significant antibacterial activity against *E. coli* and *S. aureus* as the concentration increases but progressively decreased at lower concentrations. The antibacterial activity of honey against *E. coli* showed inhibition zones at 100.00mg/mL, 50.00 mg/mL, 25.00 mg/mL and 12.50 mg/mL while *S. aureus* showed inhibition zone at 100.00 mg/mL, 50.00 mg/mL and 25.00 mg/mL. The minimum inhibitory concentration (MIC) of honey against *E. coli* is 12.50mg/mL and *S. aureus* is 25.00mg/mL as shown in table 3.

Table 1: Cultural, Morphological and Biochemical characteristics of the Test Organisms

Test Organism	Cultural Characteristics	Biochemical Characteristics										
		Gram Stain	Morphology	VP	OXI	MOT	CAT	CIT	COA	MR	IND	UR
E. coli	Bright Pinkish colonies on MCA and Metallic sheen colonies on EMB	-	Rod	-	-	+	+	-	-	+	+	-
S. aureus	Golden yellowish on MSA	+	Cocci	+	-	+	+	-	+	+	-	-

MSA = Mannitol Salt Agar; MCA = MacConkey Agar; EMB = Eosin Methylene Blue; MOT = Motility; CAT = Catalase; MR= Methyl Red; UR = Urease; CIT = Citrate; COA= Coagulase VP =Voges Proskauer, IND = Indole, - = Negative; + = Positive

Table 2: Antibacterial Activity of honey against both MDR *E. coli and S. aureus*

Concentration of Honey (mg/mL)	Inhibition Zone Diameter (mm) of the honey against	
	MDR *E. coli*	*S. aureus*
100	15.00± 0.09	18.07± 0.00
50.00	9.10± 0. 51	11.20± 0.32
25.00	6.01± 0.11	5.11± 0.07
12.50	4.14± 0.13	0.00± 0.00
6.25	0.00± 0.00	0.00± 0.00
3.13	0.00± 0.00	0.00± 0.00

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amoxicillin-clavulanate	24.01± 1.11	22.61± 1.87

Table 3: Minimum Inhibitory Concentration of honey solution against both MDR *E. coli and S. aureus*

Extract	Minimum Inhibitory Concentration (mg/mL) of the extract	
	E. coli	***S. aureus***
Honey	12.50	25.0
Amoxicillin-clavulanate	1.56	3.12

4.0 DISCUSSION

Honey is more than a natural sweetener, it is a powerful and excellent antimicrobial agent against a wide range of microorganisms (Rana *et al.*, 2025). The antimicrobial activity of honey is attributed to its low pH, phenolic acids levels, flavonoids, osmotic effects due to high sugar content and hydrogen peroxide production (Rana *et al.*, 2025). These combined mechanisms contribute to its effectiveness against resistant strains and reduced likelihood of resistance development (Rana *et al.*, 2025). The inhibition zone diameters of 15.00mm for *E. coli* and 18.07mm for *S. aureus* at 100mg/mL observed in this study fall within the range reported by David *et al., (*2024). The reported ranges are 4.8-12.9mm for *S. aureus* and 5.0-11.8mm for *E. coli* depending on the concentration. Studies by Jacinta *et al., (*2023) also revealed that honey demonstrated antibacterial effect against *S. aureus* with inhibition zone ranging from 10 - 21mm and *E. coli* with inhibition zone ranging from 10-18mm while the result from this study showed that honey had antimicrobial activity against *E. coil* with inhibition zone ranging from 4.14-15mm and up to 18.07mm for *S. aureus*. This confirms that the result from this study is consistent with established outcomes. *Staphylococcus aureus* which is a Gram-positive bacteria showed greater susceptibility compared to Gram-negative *Escherichia coli*, this difference may be due to the presence of an outer membrane found in Gram-negative bacteria which limits the penetration of antimicrobial agents (Ghai, 2023). Honey demonstrated MIC values of 12.50mg/mL against *E coli* and 25.0 mg/mL against *S. aureus*. It is in agreement with the study published in Scientific Reports by Luca *et al.,* (2025) which documented a wide range of MIC values for *S. aureus* and *E. coli*, emphasizing that antibacterial potency varies with honey composition. Even though honey showed significant antibacterial activity, the positive control, Amoxicillin-clavulanate showed MIC values ranging from 1.56-3.12mg/mL against both *E. coli and S. aureus* indicated that conventional antimicrobial remain potent over honey (Ogwu & Izah, 2025).

4.1. CONCLUSION

This study investigated the antibacterial activity of honey against *Escherichia coli* and *Staphylococcus aureus* at different concentrations. Honey has shown a higher antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* with larger inhibition zones observed at different concentrations highlighting the importance of dosage in maximizing the antimicrobial efficacy of honey. The result also showed that honey was more effective against Gram-positive bacteria than Gram-negative bacteria. The ability of honey to inhibit *Escherichia coli* and *Staphylococcus aureus* highlights its potential as a broad-spectrum antimicrobial agent and its ability to be used as an alternative or complementary antimicrobial agents due to the continue rise in antimicrobial resistance. Its antibacterial effects are attributed to multiple factors, including high osmolarity, low pH, hydrogen peroxide production, and bioactive phytochemicals, making it less likely for bacteria to develop resistance. Therefore, demonstrating antibacterial activity against *S. aureus* and *E. coli* supports the potential use of honey in wound care, infection management, and the development of novel antimicrobial therapies. Amoxicillin-clavulanate (standard antibiotic control) which served as a positive showed the strongest antibacterial activity against *Escherichia coli* and *Staphylococcus aureus* than honey. This revealed that conventional antimicrobial remain potent and are more effective even at lower concentration. Further studies should be carried to determine the optimal therapeutic concentrations, isolate and characterize the active compounds in honey, access the clinical efficacy and safety through in vivo studies and evaluate synergistic effects with antimicrobial.

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