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In vitro Evaluation of Antimicrobial Activity of Bee Honey against Gram-Positive Cocci Bacteria Isolated from Clinical Specimen in Shendi Town

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Authors' contributions

This work was carried out in collaboration among all authors. Author AAAA the principal investigator, conceived the idea, conducted laboratory analysis, collected the data, interpreted the data generated and wrote the draft of the manuscript. Author MMA. The principal investigator, conceived the idea, conducted laboratory analysis, collected the data, interpreted the data generated and wrote the draft of the manuscript. Author MBMB the supervisor of the research. Author GMM the co-researcher, contributed to data collection, and laboratory analysis and, interpreted the data generated and wrote the draft of the manuscript. Author BMTG. Provided critical suggestions and comments and edited the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Background: Bacterial resistance is a more serious threat, and herbal medicines have been used as an alternative solution to this problem. Honey has been used to treat bacterial infections for decades.
Methods: This is a prospective cross-sectional study conducted at the Microbiology Laboratory, Faculty of Laboratory Medicine, Shendi, Sudan between October 2021 and November 2021. In this study, 50 different clinical samples were collected from different microbiology laboratories in Shendi City, and different Gram-positive cocci bacteria were isolated and identified by Gram's staining and

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biochemical tests. The susceptibility of Gram-positive bacteria to the bee honey was determined using agar well diffusion technique.

Results: Out of a total of 50 clinical specimens cultured the most frequent isolated bacteria were *Staphylococcus aureus* (46%), *Streptococcus epidermidis* (36%), *Streptococcus viridian's* (10%), *Staphylococcus saprophyticus* (4%), while the least isolated bacteria was *Enterococcus fecalis* (4%). On the other hand, 100% (v/v) honey was more effective than the tested antibiotics, inhibiting 74% of clinical isolates, while 38% of microorganisms were inhibited by only 50% (v/v) honey and 14% of organisms inhibited by 25% (v/v) Honey. Bee honey showed a clear effect on the isolated bacteria.

Conclusion: Based on these results, we can conclude that honey has broad activity against Gram-positive bacteria. Therefore, honey can be considered as a broad-spectrum antibacterial agent. The medicinal use of Sudanese honey and the assumption that the possibility of local production of bioactive honey requires additional investigation.

Keywords: Herbal Medicine; antimicrobial activity; bee honey; positive Cocci bacteria; Shendi Town.

1. INTRODUCTION

Bacterial infection is a major health problem all over the world due to the misuse of broad-spectrum antibiotics. Gram-positive cocci are a large family of gram-positive bacteria, and staphylococci and streptococci are large important genera in this family which are human pathogens. Staphylococci and Streptococci are non motile and non-spore-forming but can be distinguished microscopically from staphylococci in the cluster, and streptococci in chains. Biochemically, staphylococci produce catalase enzyme to degrade hydrogen peroxide, streptococci do not [1]. Both these genera can produce resistance to antibiotics, for example, MRSA and others. Antibiotic-resistant is the ability of the microorganism to resist and tolerate the antibiotic action, and then no effect of antibiotic on the microorganisms [1]. Nutritional and therapeutic bee honey effects have been known for many thousands of years. It has been reported to be effective in several infections. Many previous studies demonstrate that using of honey on infected wounds promote rapid clearance and healing of the tissues, due to the broad-spectrum antimicrobial activity of the bee honey, which is due to many factor and content of various substances(called inhibin because of inhibitory effect) that interfere with microbial growth, this includes inhibitory factors like low pH, high sugar concentration and osmotic effect, presence of bacteriostatic and bacteriocidal substances such as hydrogen peroxide, phenolic acid, and polyphenols, methylglyoxal, flavonoids, antioxidants bee peptides, lysozyme. The composition and physiochemical properties and flavor of honey vary with the floral source and type of nectar used by bees and also with region, climate, and storage condition [2]. The potential effects of selected honey for the therapy of certain diseases have been known for centuries as certain honey was chosen for the treatment of certain ailments; regardless, it was not until newly that the investigation has proved that certain honey possesses unusual antimicrobial effects [3, 4]. And hence have been the choice for wound management. The comprehensive research into the antibacterial properties of Leptospermum honey has led to the approval of licensed products for wound administration in regions including Canada, the USA, Australasia, Europe, and Hong Kong [5]. There is an urgent need for new novel antibacterial agents that could be used against antibiotic-resistant bacteria as well as antioxidants that could protect humans as well as foods from the destructive effects of oxidative stress. Immediate attention is required to back up the currently available antibiotics as multi-drug resistant bacteria become a real threat. Despite the spread of multi-resistant (or pan-resistant) pathogens large pharmaceutical companies continue to decrease their support for antibacterial and antibiotic research and continue with chronic disease therapy (e.g. cardiovascular, CNS, pain, arthritis, and cholesterol-lowering agents), which means that with increased spread and emergence of resistant strains the mortality and morbidity could rise to a maximum level [6,7].

Honey is widely consumed in Sudan as a preventive and curative agent for several human illnesses in addition to its popular usage as food. In recent time new interest in honey has been witnessed mainly due to an increasing concern on the side effect of chemical medicines (3). This situation forced the researcher to search for new antimicrobial substance (4). Therefore, this study

was aimed to determine the antimicrobial Activity of Bee Honey against Gram-Positive Cocci Bacteria Isolated from Clinical specimens in Shendi Town.

2. MATERIALS AND METHODS

2.1 Design of Setting

This study was a prospective cross-sectional and hospital base study conducted from October 2021 and November 2021. Non – probability sampling technique was used to select patients in Shendi hospitals and centers – in Sudan. A total of Fifty samples (n=50) were collected from different hospitals and clinical centers located in Shendi Town, River Nile State, Sudan.

2.2 Specimen Collection

Under the aseptic condition, wound swabs were collected using sterile cotton swabs moistened with sterile normal saline, urine and stool were also collected in sterile screw-capped universal containers.

2.3 Isolation and Identification of Gram's Positive Bacteria

The different clinical samples collected were cultured on cysteine lactose electrolyte deficient (CLED) agar, blood agar, MacConkey agar, and chocolate agar. The plates were observed for any bacterial colonies to grow significantly. The bacteria were well isolated and then identified by colonial morphology, Gram stain, and biochemical tests. The bacteria isolated were identified using Gram's staining and biochemical tests. After identification, pure isolated was obtained and preserved in the refrigerator at 40C.

Preparation of honey concentrations for testing its antimicrobial activity: Honey was diluted into different concentrations as follows: 100%, 50%, and 25%, to be used against the selected organisms.

2.4 Preparation of Bacterial Suspension

Two ml of normal saline was distributed in test tubes and sterilized in an autoclave at 121 oC for 15 mins. A loopful of the purified bacterium was inoculated in sterile normal saline. Inoculum density was compared with 0.5 McFarland standard solution.

2.5 Antimicrobial Activity of Honey against the Clinical Isolates

The antibacterial activity was analyzed by the agar well diffusion technique .on Mueller- Hinton Agar (MHA). The suspension corresponding to 0.5 McFarland Turbidity standards was inoculated by swabbing on MHA, and the wells were made with the help of a sterile cork borer of 5mm diameter. 50 µL of the honey samples were dispensed into the different wells and a vancomycin 30µg disc is placed for comparison. The plates were incubated overnight at 37 °C aerobically after the complete diffusion of honey. After overnight incubation, the zones of inhibition on MHA plates around the wells were observed, and the diameters of the inhibition zones were measured [8].

2.6 Data Collection and analysis

Data were collected from the patients using a structured questionnaire. Data were entered, check, and analyzed using Microsoft Excel 2007 and SPSS (Statistical Package of Social Science) soft program version 11.5. Proportional data were presented as frequencies and percentages.

3. RESULTS

A total of 50 samples from different clinical specimens were collected from a different laboratory in Shendi town and processed during the period from April to August 2021. The study population involved 21(42%) males and 29(58%) females (Table 1). Clinical samples involved in this study were 27 urine samples, 15 wound swabs, 5 oral swabs, and 1 sputum, eye swab, high vaginal swab respectively (Table 2). Out of a total of 50 clinical specimens cultured the most frequent bacteria isolated were *Staphylococcus aureus* (46%), *Streptococcus epidermidis* (36%), *Streptococcus viridian's* (10%), *Staphylococcus saprophyticus* (4%), while the least isolated bacteria was *Enterococcus faecalis* (4%). (Table 3). There were 74% of organisms sensitive to stock honey and only 26% were resistant as shown in (Table 4). 38% of the organisms are sensitive to (50% (v/v) honey and 62% resistant as shown in (Table 5). For the honey with 25% v/v, 14% of organisms were sensitive and show a clear zone of inhibition, while 82 were resistant as shown in Table 6. The results of antimicrobial activity of the honey bees were compared with vancomycin 30µg to evaluate their relative percentage inhibition, the honey bees exhibited

maximum relative percentage inhibition against *S. saprophyticus* and *E. fecalis* (100%) and minimum relative percentage inhibition against *S. viridans* was 40% as described in Table 9.

Table 1. Distribution of clinical specimens according to the gender

Gender	Frequency	Percentage (%)
Male	21	42
Female	29	58
Total	**50**	**100**

Table 2. Distribution of clinical specimen according to its Type

Type of sample	Frequency	Percentage (%)
Urine	27	54
wound swab	15	30
Oral swab	5	10
Sputum	1	2
Eye swab	1	2
High vaginal swab	1	2

Table 3. Frequency and percentage of isolated organisms

Isolate	Frequency	Percentage (%)
S. aureus	23	46
S .epidermidis	18	36
E. fecalis	2	4
S. saprophyticus	2	4
S. viridans	5	10
Total	50	100

Table 4. Show sensitivity of microorganisms to bee honey at concentration 100%

Organisms	Honey 100%	
	Sensitive	Resistant
S. aureus	19	4
S. epidermidis	12	6
S. saprophyticus	2	0
E. fecalis	2	0
S. viridans	2	3
Total	74	26

Table 5. Show sensitivity of microorganisms to bee honey at concentration 50%

Organisms	**Honey *50%***	
	Sensitive	***Resistant***
S .aureus	11	12
S. epidermidis	7	11
S. saprophyticus	1	1
E. fecalis	0	2
S. viridans	0	5
Total	38	62

Table 6. Show sensitivity of microorganisms to bee honey at concentration 25%

Organisms	Honey 25%	
	Sensitive	Resistant
S. aureus	7	16
S. epidermidis	0	18
S. saprophyticus	2	0
E. fecalis	0	2
S. viridans	0	5
Total	14	82

Table 7. Show the sensitivity of isolated microorganisms to vancomycin 30µg

Organisms	Sensitive %	Resistant%
S. aureus	8	15
S. epidermidis	9	9
S. saprophyticus	0	2
E. fecalis	1	1
S. viridans	5	0
Total	46	54

Table 8. Show comparison of sensitivity between microorganisms to vancomycin and different concentrations of bee honey (Frequency)

Organisms	Vancomycin 30µg	Honey		
		Sensitivity to 100%	Sensitivity to 50%	Sensitivity to 25%
S.aureus	8	19	11	7
S.epidermidis	9	12	7	0
S.saprophyticus	0	2	1	1
E.fecalis	1	2	0	0
S.viridans	5	2	0	0
Total	23	37	19	8

Table 9. Show a comparison of sensitivity between microorganisms to vancomycin and different concentrations of bee honey (Percentage)

		Honey Sensitivity %		
Organisms	Vancomycin 30µg Sensitivity%	100	50	25
S. aureus	35	83	48	30
S. epidermidis	50	67	39	0
S. saprophyticus	0	100	50	50
E. fecalis	50	100	0	0
S. viridans	100	40	0	0

4. DISCUSSION

Antimicrobial-resistant is the greatest problem in the world that results from misused antimicrobial agents. The emergence of resistant strains of pathogenic bacteria to the most effective antibiotic made us shift to the use of herbal medicine which can contribute to resolving this problem. The antimicrobial application of honey has been demonstrated by several studies. Honey has been used in food preservation since ancient times [9,10]. Moreover, the increased resistance of bacteria to antimicrobial agents is deriving researchers and industrialists to look for a means of control of bacterial resistance [11]. The use of honey as a traditional remedy for microbial infections dates back to ancient times. Bee honey was widely used as an antimicrobial agent mainly due to the presence of (inhibit) which includes its chemical composition of phenolic compound, methylglyoxal, Hydrogen peroxide, and factors like Acidic pH, Hygroscopic properties, and other factors. In this study, we test bee honey at different concentrations of 100%,50%, and 25% against gram-positive cocci bacteria isolated from the different clinical specimens using the agar well diffusion method. Bee honey shows the highest activity against *S. fecalis* and *S. saprophyticus* followed by *S. aureus* and *S. epidermidis* with the lowest activity against *S. viridans.* This has disagreed with a study done by Leyva-Jimenez and his colleagues reported that *S. fecalis* was the most resistant bacteria to honey followed by *S. aureus* [12]. *S. saprophyticus* and *S. aureus* were inhibited at all honey concentrations, *S. epidermidis* was inhibited at concentrations 100% and 50%, *E. fecalis* and *S. vridins* were inhibited only at concentrations of 100%. French and his colleagues found that bee honey had an inhibitory effect against coagulase-negative staphylococci [13]. As reported by Jeani was found that *S. viridans* was inhibited by wild honey [14]. Also in our study, bee honey shows an inhibitory effect against vancomycin-resistant *S. aureus* (VRSA) that disagrees with the study of Molanaei and his colleagues who found that two strains of VRSA show no sensitivity to bee honey [15]. In our study, 100% is the best concentration that shows the highest activity against the most bacteria, and when it is decreased the effect decrease, which agrees with the study of Al-Hasani in Iraq [16]. Also agree with a study done by Basualdo and her colleagues that shows undiluted honey has a high antibacterial effect than diluted one [17]. Also in our study, *S. viridans* show the highest sensitivity to vancomycin and *S. saprophyticus* show the lowest sensitivity.

5. CONCLUSION

It was concluded that; bee honey; possesses antimicrobial activities, but with varying degrees of effectiveness. Honey was the most potential antibacterial agent against *S. saprophyticus* and *E. faecalis* followed by *S. aureus* and *S. epidermidis* and the lowest activity against *S. viridans.* Antibacterial activity of Sudanese honey varies as some were found to be bactericidal and others were bacteriostatic.

6. RECOMMENDATION

Based on our findings we recommend that to apply the bee honey as an herbal medication in cases of antimicrobial-resistant infections or patients with an antimicrobial contraindication. Further studies are also recommended.

ETHICAL CONSIDERATIONS

Permission was issued by the College of Ethical Committee, Shendi University, and the ethical committee of the hospital. Volunteers were informed and had got all the information about the research study.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

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COMPETING INTERESTS

Authors have declared that they have no known competing financial interests or non-financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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