Original Research Article

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

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 samples of different types were collected from different microbiology laboratories in Shendi City and identified by Gram staining and biochemical tests. The susceptibility of Gram-positive bacteria to honey was determined. The susceptibility of gram-positive bacteria to the bee honey was determined. **Result:**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 and14% of organismsinhibited 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, BeeHoney,Positive Cocci Bacteria.

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 nonmotile 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~~bactericidal 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]**.

MATERIALS AND METHODS

Design of setting

This was a prospective cross-sectional and hospital base study conducted during the period from Aprilto August 2021.Non –probabilitysampling 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 centersare located in Shendi locality, River Nile State, Sudan.

Specimen collection

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

Cultivation of the specimensand interpretation of culture growth

Different types of culture media (CLED agar, Blood agar, Macconckey Agar, and Chocolate blood Agar) were used for the identification and isolation ofclinical isolates.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.After the identification of isolates, pure culture inoculated with organisms incubated for 24hrs and preserved in the refrigerator at 4^{0}C~~C~~4 $^{\circ}$C.

Preparation of serial dilution of honey For Testing the Antimicrobial activity

Honey was diluted into different concentrations as follows: 100%, 50%, and 25%, to be used against the selected organisms.

Preparation of bacterial suspension

Clinical isolates were isolated from different samples and subcultured.Two ~~ml~~ mLof normal saline was distributed in test tubes and sterilized in an autoclave at 121~~o~~°C for 15 mins. A loopful of the purified bacterium was inoculated in sterile normal saline. Inoculum density was compared with McFarland standard solution.

Testing of bee honey for antimicrobial activity against standard organisms and clinical isolates

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 three wells were made with the help of a sterile cork borer of 5mm diameter. ~~50~~ FiftyμL of three honey samples were dispensed in three different wells and a vancomycin disc is placed for comparison. The plates were incubated overnight at ~~37~~ 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]**.

Data collection and analysis

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

Scientific & Ethical considerations:

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

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, one Sputum and one Eye swab,

and only one high vaginal swab **(Table 2)**.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%).**(Table 3)**.There were 74% of organisms sensitive to stock honey and only 26% were resistant as shown in **(Table 4).** There were 38% of organisms sensitive to (50% ~~(~~v/v) honey and 62% resistant as shown in **(Table 5)**. There were 14% of organisms sensitive to this dilution (25% ~~(~~v/v) honey, while 82 were resistant as shown in **(Table 6)**.The results of antimicrobial activity of the honey bees were compared with vancomycin 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%)**(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~~ 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:Sshow 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
Percentage	**74%**	**26%**

Table-5:show 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
Percentage	**38%**	**62%**

Table-6:show 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
Percentage	**14%**	**82%**

Table-7:show Show the sensitivity of isolated microorganisms to vancomycin.

Organisms	***Sensitive***	***Resistant***
S. aureus	8	15
S. epidermidis	9	9

S. saprophyticus	0	2
E. fecalis	1	1
S. viridans	5	0
Percentage	**46%**	**54%**

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

Organisms	***Vancomycin***	***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~~ Show comparison of sensitivity between microorganisms to vancomycin and different concentrations of bee honey (Percentage).

Organisms	***Vncomycin Sensitivity%***	***Honey 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%

DISCUSSION:

Antimicrobial-~~resistant~~ resistanceis 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~~ hydrogen

peroxide, and factors like ~~Acidic~~ acidic ~~PH~~pH, ~~Hygroscopic~~ hygroscopic properties, and other factors. In this study, we teste~~d~~ 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* 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 of honey that shows the highest activity against ~~the~~ most bacteria, and when it is decreased the effect decrease~~s~~, which agrees with the study of Al-Hasani in Iraq**[16]**. Also agree with a study done by Basualdo and her colleagues that shows that undiluted honey has a high~~er~~ 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.

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.*~~the~~ The antibacterial activity of Sudanese honey varies as some were found to be bactericidal and others were bacteriostatic.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers

of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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