ORIGINAL ARTICLE

Antibacterial Profile of Honey on *Staphylococcus Aureus* Isolated from Surgical Wound in Awomamma General Hospital

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ABSTRACT

Antibacterial profile of Honey on *Staphylococcus aureus* isolated from surgical wound was studied. A total of thirty (30) samples swab were samples processed, *S. aureus* was isolated from 15 samples. The isolates were tested for the antibacterial profile of honey obtained from three (3) various company labeled Honey A, Honey B and Honey C. The samples were inoculated into mannitol salt agar which is a selective medium for *S. aureus.* It was observed that the average of a diameter of inhibition zones of 15*S. aureus* isolates due to Honey A, Honey B and Honey C were found to be 15 mm, 10 mm and 22 mm respectively. Hence, honey obtained from Honey C was found to be the most effective against *S. aureus* isolates from infected surgical wounds. Antibiotic sensitivity test of the isolated *S. aureus* was also performed using the antibiotic Tetracyclin (30 mcg), Ciprofloxacin (5mcg), Vancomycin (30 mcg), Methicillin (5 mcg), and Co-trimoxazole (25 mcg). The most effective antibiotic against *S. aureus* tested was found to be Vancomycin with 100% efficacy. The effectiveness of the honey sample against the antibiotic-resistant organisms helps to treat the open wounds due to burning, abrasions, incision infected with *S. aureus.* Honey C might be beneficial for the treatment of surgical wounds infected by *S. aureus* to get a better healing.

Key words: Antibacterial Profile, Honey, *Staphylococcus Aureus*, Surgical wound

INTRODUCTION

A surgical wound is primarily a cut or incision in the skin which is usually made by a scalpel during surgery. It could be the result of a drain placed during surgery. Surgical wounds are greatly in various sizes. They are usually closed with sutures, but are sometimes left open to heal[1].

Surgical wounds can be grouped into one of four classes. These classes depend on how contaminated or clean the wound is, the risk of infection, and where the wound is located on the body[2].

Class I: These are considered clean wounds. They show no signs of infection or inflammation. They often involve the eye, skin, or vascular system.

Class II: These wounds are considered clean-contaminated. Although the wound may not show signs of infection, it is at an increased risk of becoming infected because of its location. For example, surgical wounds in the gastrointestinal tract may be at a high risk of becoming infected.

Class III: A surgical wound in which an outside object has come into contact with the skin has a high risk of infection

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DOI: 10.33309/2639-8893.040105

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and is considered a contaminated wound. For example, a gunshot wound may contaminate the skin around where the surgical repair occurs.

Class IV: This class of wound is considered dirty-contaminated. These include wounds that have been exposed to fecal material[3, 4].

Surgical wounds are formed when a surgeon makes an incision or cut with a surgical instrument called a scalpel. A wide variety of medical circumstances require surgery. The size of a wound depends on the type of procedure and location on the body[5].

Surgical wound infection is a fearful complication of surgery, and the capability to identify risk factors for patients can be useful for managing patient expectations as well as maximizing good clinical outcomes. Surgical wound infection is a big problem in surgery, in spite of great advances in surgical methods, modern technologies in the operating room, and preemptive measures such as perioperative intravenous antibiotics and preoperative skin antisepsis. This tends to elevate a patient's risk of morbidity and mortality and can have harmful economic effects[6].

In fact, surgical wound infection results to big discomfort linked with mortality and morbidity, as well as increased period of hospital stays, great discomfort and significant elevation in healthcare expenses. Surgical wound infection may cause delay in healing and may result to wound break down, herniation of the wound and complete wound dehiscence(8). Hence, the knowledge of the causative agents of surgical wound infection may be beneficial in the control of surgical wound infection and selection of empiric antimicrobial therapy as an infection control techniques. Some aerobic pathogens like *Staphylococcus aureus*, *Pseudomonas aeruginosa,* and beta haemolytic *Streptococci* have been commonly reported as the cause of delay surgical wound recovery[7].

On the other hand, honey is a thick, sweet fluid produced by bees from plant nectars. It is mainly used as a sweetener in food, however it should be avoided in infants[8].

Most chemicals in honey might kill some bacteria and fungi. When it is applied to the skin, honey might serve as a barrier to moisture and keep skin from sticking to wound dressings. This might also provide nutrients and chemicals that speed wound healing. Though, honey can become contaminated with germs during production[9].

Honey is commonly used for wound healing, burns, swelling and sores inside the mouth, and cough. It is also used for many other conditions but there is no good scientific evidence to support most of these other uses[10].

More recently, honey have been reported to have effects on burns. Applying honey preparations directly to burns seems to improve healing. Similarly, taking a small amount of honey by mouth atbedtime seems to decrease coughing spells in children aged 2 years and older. Honey seems to be at least as effective as the cough drugs. Also, applying dressings containing honey to diabetic foot ulcers appears to reduce healing time and prevent the need for drugs especially antibiotics[11].

The medicinal benefits of honey have gained significant interest as more medical professionals and scientific researchers acknowledge its antibacterial activities.

It has been reported that commercial honey had antimicrobial effects against *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* [12].

The antibacterial efficacies of honey is mainly due to inhibines found in honey. These inhibines are hydrogen peroxide, flavonoids, and phenolic acids, as well as many other unidentified inhibines. The inhibines could result to shrinkage disruption of the bacterial cell wall due to osmotic effect of the sugar content; induction of an unfavourable environment with low water activity, hence inhibiting bacterial growth; and a low pH of 3.6 and the fermentation of honey, producing alcohol[13]. The present study tends to determine the antibacterial profile of honey on *Staphyococcus aureus* isolated from surgical wound.

MATERIALS AND METHODS

The samples used in this work was collected from the medical Microbiology department of General Hospital Awo Omamma in Imo state.

Source of Honey

The honey used was obtained from local commercial producers in Owerri. It did not contain any diluents or additives and had not been heated.

Collection of Samples

Samples from surgical wounds mostly pus samples were collected using sterile swab sticks and were preserved in normal saline to preserve the viability of the organisms.

Media Preparations

The solid components of the media were dissolved in a conical flask according to the manufacturer's instructions. The flasks were closed with cotton plug and covered with Aluminium foil, placed into an autoclave and sterilized at 121^0C for 15mins. After sterilization, the medium were cooled to 45^0C, the cotton plug were removed and the mouth of the flask were flamed over a Bunsen burner in other to ensure sterility, and the medium were poured into sterile, empty petri dishes (15-20ml into each petri dish). The petri dishes were kept horizontally until the medium are completely solidified, then they were turned upside down and stacked for storage.

The plates were labelled according to the medium and also a sterility test was performed on them by incubating some plates at 37^0C for 24hrs and after which they were examined.

Microbiological Analysis of Samples

Sample each were inoculated on the agar media using the streaking method of inoculation on the surface of sterile solidified plates of freshly prepared molten Nutrient agar and Mannitol salt agar after which the media were incubated for 24 hrs at 37^0C for bacterial isolation.

After the incubation period, the plates were observed and colonies counted, and the discrete colonies were sub-cultured into a freshly prepared Nutrient agar plate to get a pure culture. The sub-cultured plates were incubated for 24 hrs, and examined for pure culture. The pure culture growth were used for gram staining, motility test and biochemical characterization of the organisms like Oxidase tests, Citrate utilization test, Indole test, Methyl-red test, Voges proskaeur test, Coagulase test, Sugar fermentation and Catalase test. A stock culture was prepared using a bijou bottle: this stock culture was used in storing the organisms for further characterization by standard techniques.

Antibacterial Sensitivity Test

The antibacterial activity of honey collected from two different honey dealers at Imo State against *Staphylococcus aureus* was tested in-vitro using well diffusion method (Kirby Bauer's method). The test materials were prepared by diluting each honey in sterilized double distilled water at different dilution (concentration) 20%, 40%, 60% and 80% also net honey (100%). Muller Hinton agar plates were prepared and each plate was properly inoculated with each test organism using streaking method with the help of a sterile wire loop. Wells were made using a sterile cork borer and each well was filled with different concentrations of the honey. The plates were incubated at 37^0C for 24hrs and observed for zone of inhibitions. This in-vitro experiment was compared with the use of a sensitivity disc (Augumentine) which served as a control.

RESULTS

Analysis of the *Staphylococcus Aureus* Isolate

A total of thirty (30) samples swab were samples processed, *S. aureus* was isolated from 15 samples. The isolates were tested for the antibacterial profile of honey obtained from three (3) different company labeled Honey A, Honey B and Honey C. The antibacterial profile of the honey samples against *S. aureus* isolates in the study is presented in Table.

In the present study, the means of a diameter of inhibition zones of 15 *S. aureus* isolates due to Honey A, Honey Band Honey B were found to be 15 mm, 10 mm and 22 mm respectively. Thus, honey obtained from Honey C was found to be the most effective against *S. aureus* isolates from infected surgical wounds.

Table 1. Physiological Properties of Isolated Bacteria.

	Bacteriological tests			**Biochemical tests**										**Probable organism**
Isolates	**Gram reaction test**	**Cellular arrangement**	**Motility test**	**Catalase test**	**Citrate test**	**Indole test**	**Oxidase test**	**Coagulase test**	**Voges Proskuer test**	**Methyl red test**	**Glucose test**	**Lactose test**	**Sucrose test**	
1	+	cocci	-	+	+	-	-	+	+	+	+	+	+	*Staphycoccus aureus.*

Key Positive= + **Negative= -**

Table 2. Inhibition Pattern of S. Aureus Isolates By Crude Honey Samples.

Zone of inhibition (diameter in mm) due to honey obtained from Isolated S. aureus HONEY AHONEY B HONEY C			
1	17	9	25
2	19	12	20
3	13	10	23
4	16	9	21
5	14	8	22
6	13	8	24
7	15	12	20
8	17	10	21
9	18	12	20
10	14	10	23
11	15	9	20
12	14	10	26
13	15	10	22
14	12	9	20
15	14	12	23
Mean	15	10	22

Table 3. Antibiotic sensitivity pattern of S. aureus isolates.

Antibiotics Used	**Sensitive**		**Intermediate**		**Resistant**	
	Number	**Percentage**	**Number**	**Percentage**	**Number**	**Percentage**
Methicillin	10	50	5	25	5	25
Tetracylin	16	80	-	-	4	20
Ciprofloxaxin	14	70	2	10	4	20
Vancomycin	20	100	0	0	0	0
Co-trimazole	4	20	8	8	8	40

DISCUSSION

A surgical wound is usually **a cut or incision in the skin** that is usually made by a scalpel during surgery. It can also be the result of a drain placed during surgery. Surgical wounds different in size. They are usually closed with sutures, but are sometimes left open to heal.

In this study, the means of a diameter of inhibition zones of 15*S. aureus* isolates due to Honey A, Honey B and Honey C were found to be 15 mm, 10 mm and 22 mm respectively. Thus, Honey C was found to be the most effective against *S. aureus* isolates from infected surgical wounds[14].

The outcome of the study is also supported by the inhibition of the growth of *S. aureus, Escherichia coli, Proteus mirabilis,* and *Pseudomonas* [15, 16]. The past studies done for the antibacterial activity of honey were not found to be correlated with the bee-species, but with the type of honey sample and with the plant source from which nectar has been collected. According to work of [17, 18], most honey samples at a concentration of above 50% were found to have an inhibitory effect on *S. aureus*. Antibiotic sensitivity test of the isolated *S. aureus* was also performed using the antibiotic Methicillin (5 mcg), Tetracyclin (30 mcg), Ciprofloxacin (5mcg), Vancomycin (30 mcg) and Co-trimoxazole (25 mcg). The most effective antibiotic against *S. aureus* tested and was found to be Vancomycin with 100% efficacy. 57.9% of the isolates were sensitive to Tetracyclin, 42.1% of the isolates were sensitive to Ciprofloxacin, 34.2% were sensitive to Methicillin, and only 15.8% were sensitive to Co-trimoxazole. The antibiotic sensitivity pattern of the isolated *S. aureus* showed that most of the isolates were sensitive to vancomycin and tetracycline, but with other antibiotics, a considerable number of the isolates were resistant too [19]. The effectiveness of the honey sample against the antibiotic-resistant organisms helps to treat the surgical wounds infected with *S. aureus*[20, 21]

CONCLUSION

The effectiveness of the honey sample against the antibiotic-resistant organisms helps to treat the surgical wounds infected with *S. aureus*. Honey obtained from Honey C might be useful for the local application in the treatment of wounds infected by *S. aureus* to get a better results

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How to cite this article: Treasure NO, Johnkennedy N, Chinyere O. Antibacterial Profile of Honey on Staphylococcus Aureus Isolated from Surgical Wound in Awomamma General Hospital. J Pathol Infect Dis 2021;4(1):22-26.
DOI: 10.33309/2639-8893.040105