Original Research Article

Determination of Antimicrobial Properties of Raw and Commercial Honey against Methicillin Resistant *Staphylococcus aureus*

ABSTRACT

Background: Honey has powerful antibacterial properties, and in contrast to antibiotics, it has a wide range of action, has no side effects, is non-toxic and has no problems like developing resistance.This study aimed to find out the antimicrobial properties of raw and commercial honey against Methicillin resistant *Staphylococcus aureus* and ATCC *Staphylococcus aureus.***Methodology:** Four different types of raw honey were collected from different flowers. Three different commercial honey were also collected from nearby grocery shops. Five*Staphylococcus aureus* samples were cultured in Mueller Hinton agar which is collected from the microbiology laboratory of BIHS General hospital.Then we proceed agar well diffusion method to determine the antimicrobial properties of raw and commercial honey.**Result:** The presence of antimicrobial activity is indicated by the absence of bacterial growth directly below the test sample. From this study we can see an inhibition zone around honey well. The zone was not clear but from that we get to know that honey can prevent Methicillin resistant *Staphylococcus aureus* and the ATCC strain of it. **Conclusion:** The present investigation shows that bioactive constituents from raw and commercial honey have antimicrobial activity against Methicillin resistant *Staphylococcus aureus* and ATCC *Staphylococcus aureus*, however, can be further explored as an alternative anti-staphylococcal agent.

Keywords: Honey, *Staphylococcus aureus,* Methicillin, ATCC.

1. INTRODUCTION

Honey, one of the major bee products, is a sweet viscous natural fluid made from the nectar of plants. Honey was defined as "the sweet substances produced by honeybees from the nectar of blossoms or from secretions on living plants, which the bees collect, transform and store in honey combs" [1]. Honey has been used as a medicine since ancient times, mainly for the treatment of skin wounds, burns, ulcers, ocular infections, sore throat and digital dermatitis [2]. The healing capacity of honey is strongly influenced by both physical and chemical properties of this food [3]. The capability of honey to kill microorganisms has been attributed to its high osmotic effect, high acidic nature, hydrogen peroxide concentration and its phytochemical nature [4].

Staphylococcus aureus is a Gram-positive bacterium widely distributed throughout the world. Nowadays, this microorganism is one of the main causes of infections related to hospital care [5]. This is favored by the fact that this species is found in both the skin and mucous membranes of humans, which allows its penetration into the patient's bloodstream through surgical wounds, as well as through direct or indirect contact with medical personnel with a contaminated object, or with another patient [6]. Furthermore, this microorganism is also an important foodborne pathogen responsible for several outbreaks [7]. Gram-positive organisms have a thicker peptidoglycan cell wall compared with gram-negative bacteria. It is a 20 to 80 nm thick polymer while the peptidoglycan layer of the gram-negative cell wall is 2 to 3 nm thick and covered with an outer lipid bilayer membrane. Gram-positive bacteria are bacteria classified by the color they turn in the staining method. Hans Christian Gram developed the staining method in 1884. The staining method uses crystal violet dye, which is retained by the thick peptidoglycan cell wall found in gram-positive organisms. Gram-positive bacteria comprise of cocci, bacilli, or branching filaments [8].

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases [9]. However, emergence and dissemination of multidrug resistant (MDR)strain e.g. MRSA in pathogenic bacteria have become a significant public health threat as there are fewer, or even sometimes no, effective antimicrobial agents available for the infection caused by pathogenic bacteria [10,11]. The importance of honey for human use is described in several classical texts of ancient Greece, such as Homer's Iliad and the Odyssey, and in the philosophical texts of Plato,

Aristotle, and others. The use of honey in therapy is described in 5000-old Egyptian writings: Papyrus Ebers is full of praises of the curative properties of honey. Honey has been used in Ayurvedic medicine in India for at least 4000 years. The therapeutic use of honey in wound healing is recorded on a Sumerian clay tablet [12,13].

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 decreased. This type of bacterial resistance to the antimicrobial agents constitutes a very serious threat to public health and all kinds of antibiotics, including the major last-resort drugs, as the frequencies of resistance are increased worldwide. Honey inhibits the growth of pathogenic bacteria such as *E. coli*, *S. aureus*, *Salmonella* spp., *Shigella* spp., *B.subtilis*and*V.cholera* and is superior to several well-known antibiotics[14,15]. The aim of the present study was to collect raw and commercial honey samples from Finnish supermarkets in order to investigate their antimicrobial activity against important human pathogens like *Staphylococcus aureus* and the ATCC strain of it.

2. METHODOLOGY

This is an experimental study. The laboratory work was done in the Department of Microbiology at Bangladesh University of Health Sciences (BUHS). The study period was December 2021 to June 2022.

2.1 Site of Sampling

Raw honey sample: 4 raw honey samples were collected from 3 different districts of Bangladesh. One from Sundarban, one from Ishwardi and two from Magura district.

Commercial honey sample: 3 commercial honey samples were collected from 3 nearby grocery shops.

2.2 Isolation and identification of Bacterial Strains

5 Methicillin resistant *Staphylococcus aureus* and ATCC *Staphylococcus aureus* isolates were obtained from BIHS General Hospital in Dhaka city. After that identification was carried out by cultural characteristics, colony morphology of gram stain and biochemical test.

2.3 Biochemical Characterization of Isolates

In the identification of bacteria, biochemical characteristics are more significant than morphological characteristics. The biochemical characteristics of the selected bacterial isolate also have been explored in the following studies.

a. Catalase test

This test demonstrates the presence of catalase, an enzyme that catalyzes the release of oxygen from hydrogen peroxide.

Catalase enzyme

$2H_2O_2$ ▼ $2H_2 + 2O_2$

A drop of 3% hydrogen peroxide is placed on a glass slide. A colony was transferred to the slide by a loop. By observing the evolution of oxygen bubbles, a positive catalase test is confirmed.

b. Citrate Utilization Test

The Citrate utilization test detects the ability of an organism to use citrate as a carbon and energy source, alkaline carbonates and bicarbonates are produced ultimately. In addition, ammonium hydroxide is produced when the ammonium salts in the medium are used as the sole nitrogen source. Bacterial colonies were picked up from a straight wire and inoculated into the slope of Simmon's citrate agar slant and inoculated overnight at 37°c. The color of the medium change from green to blue indicated positive result and no color change indicated a negative result.

c. Triple Sugar Iron Agar Test

Triple Sugar Iron Test (TSI) detects bacterial ability to ferment Lactose, Sucrose, and Glucose and their ability to produce H_2 S. TSI medium contains 3 sugars: 1% lactose, 1% sucrose, and 0.1% glucose. When glucose is used, the agar turns yellow (acid). After continued incubation due to decrease in glucose concentration, the slant turns alkaline (Red), with butt remaining acid (Yellow) which results in an alkaline over acid reaction (K/A). When lactose and /or sucrose are used, the entire slant will turn yellow (acid) giving an acid over acid reaction (A/A). If these two sugars are not used, the entire slant appears red (alkaline), yielding an alkaline over alkaline reaction (K/K). Bacterial colony is stabbed and streaked on a TSI agar slant and incubated for 18-24hrs at 37ºC.

2.4 Antibiotic Sensitivity Test on selected *Staphylococcus aureus* samples by using kirbybauer disk diffusion method

There are generally three basic approaches to performing antimicrobial susceptibility tests, namely, broth dilution, agar dilution and disc diffusion methods. Disc diffusion methods have always

enjoyed great popularity in busy clinical microbiology laboratories because of their relative simplicity and ability to easily test multiple antimicrobial agents on each bacterial isolate. Kirby-Bauer antibiotic testing (also called KB testing or disk diffusion antibiotic sensitivity testing) uses antibiotic-containing wafers or disks to test whether particular bacteria are susceptible to specific antibiotics. First, a pure culture of bacteria is isolated from the patient. Then, a known quantity of bacteria is grown overnight on agar (solid growth media) plates in the presence of a thin wafer that contains a known amount of a relevant antibiotic. If the bacteria are susceptible to the particular antibiotic from a wafer, an area of clear media where bacteria are not able to grow surrounds the wafer, which is known as the zone of inhibition. A larger zone of inhibition around an antibiotic-containing disk indicates that the bacteria are more sensitive to the antibiotic in the disk. KB tests are performed under standardized conditions and standard-sized zones of inhibition have been established for each antibiotic. KB test results are usually reported as sensitive, intermediate, or resistant, based on the size of the zone of inhibition. If the observed zone of inhibition is greater than or equal to the size of the standard zone, the microorganism is considered to be sensitive to the antibiotic. Conversely, if the observed zone of inhibition is smaller than the standard size, the microorganism is considered to be resistant.

2.5 Determination of antimicrobial activity of raw and commercial honey by using agar well diffusion method

Agar well diffusion method is widely used to evaluate the antimicrobial activity of different kind of extracts (honey, plants). A hole with a diameter of 6 to 8 mm was punched aseptically with a sterile cork borer and a volume (100 µL) of the antimicrobial agent or extract solution at desired concentration was introduced into the well. Then, agar plates were incubated under suitable conditions depending upon the test microorganism. The antimicrobial agent diffuses in the agar medium and inhibits the growth of the microbial strain tested [41]. In our study, we notice a hazy inhibition zone around the well.

2.6 Statistical Analysis

Statistical analysis was done on an MS Windows-based PC computer. The data were first keyed

into a Microsoft Excel spreadsheet and then analyzed by Statistical Package for the Social Sciences (SPSS).

2.7 Ethical consideration

This study was approved by the ethical committee of Bangladesh University of Health Sciences (BUHS).

3. RESULTS

3.1 Biochemical test results for *Staphylococcus aureus*

Test	Result
Catalase	+ve
Coagulase	+ve
Urease	+ve
H2S	-ve
Citrate	+ve

Table 1: Biochemical tests for *Staphylococcus aureus*

3.2 Antibiotic Sensitivity Test on selected *Staphylococcus aureus* samples by kirbybauer disk diffusion test

	Zone of inhibition							
Organism	FOX	CM	CHL	VA	CAZ	GEN	AZM	DO

ID								
ATCC-01	18	12	27	17	21	15	18	16
S-01	24	27	31	20	11	27	24	28
S-02	22	28	30	21	22	28	22	29
S-03	22	21	27	20	17	21	22	16
S-04	23	24	33	19	21	24	23	26
S-05	**24**	**28**	**25**	**22**	**23**	**29**	**24**	**28**

Table 2: Inhibition zone of antibiotics on *Staphylococcus aureus* samples

3.3 Antibiotic Sensitivity Test on selected Methicillin resistant *Staphylococcus aureus* samples by raw and commercial honey

Organism name	Raw honey	Commercial honey
ATCC	Moderately sensitive	Moderately sensitive
MRSA-01	Moderately sensitive	Slightly sensitive

MRSA-02	Moderately sensitive	Slightly sensitive
MRSA-03	Moderately sensitive	Slightly sensitive
MRSA-04	Moderately sensitive	Slightly sensitive
MRSA-05	Moderately sensitive	Slightly sensitive

Table 3: Inhibition zone of antibiotics on Methicillin resistant *Staphylococcus aureus* samples

3.4 Antimicrobial Activity of Raw Honey

Out of four types of honey, all of the four honeys showed antibacterial activity. Agar well diffusion of raw honey against Methicillin resistant *Staphylococcus aureus* and ATCC *Staphylococcus aureus* showed a hazy zone of inhibition. But from the study we get to know that raw honey can inhibit specific bacterial strain like *Staphylococcus aureus*.

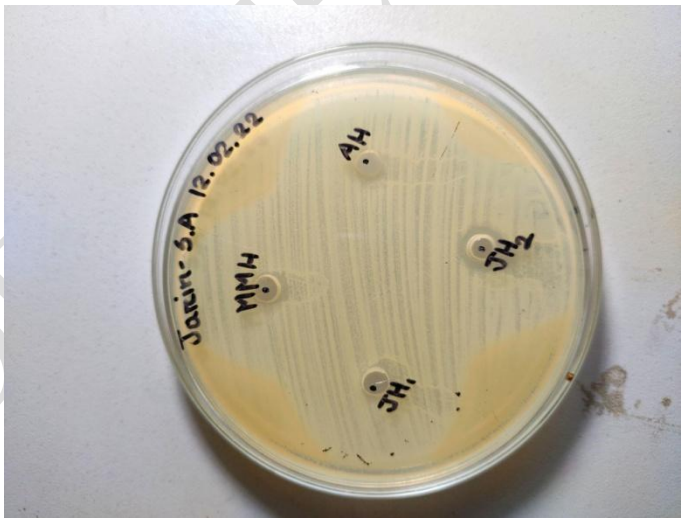

Figure 1: Raw honey on Methicillin resistant *Staphylococcus aureus* cultured plate

Figure 2: Raw honey on ATCC *Staphylococcus aureus* cultured plate

3.5 Antimicrobial Activity of Commercial Honey

Out of three types of commercial honey, all of them showed antimicrobial activity. Agar well diffusion of commercial honey against Methicillin resistant *Staphylococcus aureus* and ATCC *Staphylococcus aureus* showed a hazy zone of inhibition. But compared to raw honey commercial honey showed less inhibition zone.

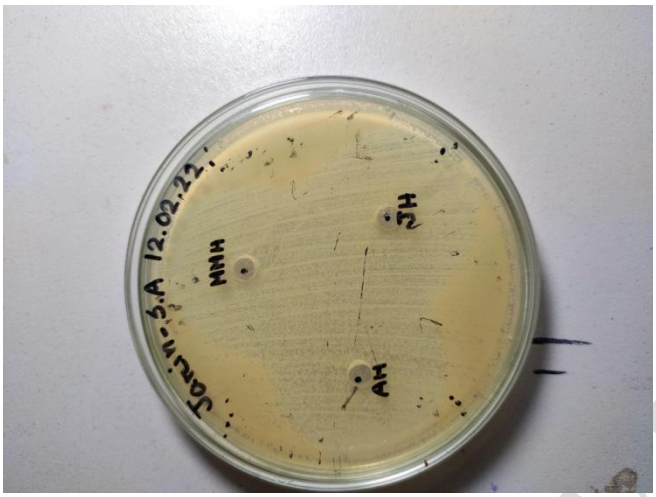

Figure 3: Commercial honey on Methicillin resistant *Staphylococcus aureus* cultured plate

Figure 4: Commercial honey on ATCC *Staphylococcus aureus* cultured plate

4. DISCUSSION

Honey has been used as a medicine since ancient times, mainly for the treatment of skin wounds, burns, ulcers, ocular infections, sore throat and digital dermatitis, among others [17,18]. The healing capacity of honey is strongly influenced by both physical and chemical properties of this food [19], which are also related to botanical source, honey bee's metabolism, as well as environmental, seasonal and climatic conditions. Apart from healing, honey has been also employed as an excellent preservative for other food commodities, due to its antimicrobial activity [20,21].Honey has formerly been shown to have wound healing and antimicrobial properties, but this is dependent on the type of honey, geographical location and flower from which the final product is obtained [22].It is well established that honey prevents a broad spectrum of bacterial species. More recently, honey has been reported to have an inhibitory effect to around 60 species of bacteria including aerobes and anaerobes, Gram positives and Gram negatives [23]. There are many reports of bactericidal as well as bacteriostatic activity of honey and the antibacterial properties of honey may be especially useful against bacteria, which have developed resistance to many antibiotics [24]. Honey comprises hydrogen-peroxide, flavonoids and phenolic acids and many other unidentified properties. The chemical composition of honey is said to comprise of seven tetracycline, fatty acids, lipids, amylase, ascorbic acid, peroxidase and fructose all of which are attributed to its antimicrobial activity together with high osmolality, low pH (3.6- 3.7), content of phenol (inhibin), peroxidase, glucose and fructose in honey and the presence of tetracycline derivatives of fatty acids [25,26].

Antimicrobial agents are essentially important in reducing the global burden of infectious diseases [27].With the irrational and excessive use of antibiotics in underdeveloped and developing countries the developed resistance may spread in the community making the strains as superbugs causing difficulties in eradication [28]. As a result, the effectiveness of the antibiotics is diminished [29].Therefore, the need for novel alternative antimicrobial strategies has renewed interest in natural products like turmeric, honey, ginger etc., exhibiting antibacterial properties. This situation has led to a re-evaluation of the therapeutic use of ancient remedies including honey [30-32]. Honey has well established function as an effective antibacterial agent with a broad spectrum of activity against Gram-positive and Gram-negative bacteria [33-35]. The application of honey can promote the healing of infected wounds that do not respond to the conventional therapy, i.e., antibiotics and antiseptics [36].

Disease causing bacteria have always been considered a major cause of morbidity and mortality in humans. The appearance of resistant microorganisms paved the way to the occurrence of infections that are only treated by a limited number of antimicrobial agents. Currently, many researchers have reported the 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. According to Nzeako and Hamdi in their studies of six commercial honeys found that inhibition of *S. aureus* which was consistent with the findings of present study [16]. The present study was undertaken to compare the inhibitory effect of raw and commercial honey against *Staphylococcus aureus*. It shows that there was significant antibacterial effect by the honey. Honey is a gift to the medical field that has multiple beneficial effects including the antibacterial activity. Moreover, study showed that some multidrug-resistant bacteria were sensitive to Bangladeshi honeys. Therefore, these honeys could be used as potential alternative therapy against bacteria. Further studies into the composition and stability of the active constituents of these honeys are warranted. Hence, it was concluded that raw and commercial honey can be used in treating human and plant diseases and it is a potential source of novel substances for future drug discovery.

5. Conclusion

Raw honey has much better inhibitory effect than commercial honey against Methicillin resistant *Staphylococcus aureus*. So honey can be a good source of antibiotic. Now our main concern is how we can make new antibiotics from honey. In the present study, we tried to focus more on whether honey can be used for treating Staphylococcal infections or not. We came to the conclusion that Honey, the nature blessed and environmental friendly product may be elaborately used in future with some more molecular studies on its method of action as an antimicrobial agent. This study represented that the raw and commercial honey have promising antibacterial activity against Methicillin resistant *Staphylococcus aureus* and ATCC *Staphylococcus aureus.*

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