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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: In contrast to antibiotics, honey has strong antibacterial characteristics, a broad spectrum of action, no side effects, is non-toxic, and has no issues like building 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 samples were also collected from nearby grocery shops. Five *Staphylococcus aureus* samples were cultured in Mueller Hinton agar which was collected from the microbiology laboratory of BIHS General hospital. Then agar well diffusion method was carried out to determine the antimicrobial properties of raw and commercial honey.

Results: 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 the 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, this can be further explored as an alternative anti-staphylococcal agent.

Keywords: Honey; Staphylococcus aureus; methicillin; ATCC.

1. INTRODUCTION

One of the main products of bees is honey, a sweet, viscous natural liquid produced from plant nectar. According to a definition provided by the University of Illinois, honey is "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]. Since ancient times, honey has been used as a medication, mostly for the treatment of sore throats, burns, ulcers, eye infections, and digital dermatitis [2]. Physical and chemical characteristics of honey have a significant impact on its ability to heal [3]. According to research, honey's high osmotic impact, high acidity, concentration of hydrogen peroxide, and phytochemical origin all contribute to its ability to destroy germs [4].

A common Gram-positive bacterium found all around the world is *Staphylococcus aureus.* These days, this bacterium is one of the major factors in hospital-related infections [5]. This is made possible by the fact that this species can live on human skin and mucous membranes, which allows it to enter a patient's bloodstream through surgical wounds, direct or indirect contact with a contaminated object or another patient, and other means [6]. Additionally, this microbe is a significant foodborne pathogen that has been linked to a number of outbreaks [7]. Gram-positive organisms have a thicker peptidoglycan cell wall compared to 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].

In general, antimicrobial drugs have a critical role in lowering the burden of infectious diseases worldwide [9]. However, due to the fact that there are fewer, or occasionally no, effective antimicrobial treatments available for the infection caused by pathogenic bacteria, the introduction and spread of multidrug resistant (MDR)strains of pathogenic bacteria, such as MRSA, have become a serious concern to public health [10,11]. Several classical Greek works, including Homer's Iliad and Odyssey as well as the philosophical writings of Plato, Aristotle, and others, discuss the significance of honey for human usage. Ancient Egyptian writings from 5000 years ago explain the therapeutic use of honey. 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].

In general, antimicrobial drugs have a critical role in lowering the burden of infectious diseases worldwide. However, the effectiveness of the antibiotics declines when resistant organisms emerge and proliferate. The public health is seriously threatened by this kind of bacterial resistance to antimicrobial agents, and usage of all antibiotics, including the main last-resort medications, is rising globally. Honey inhibits the growth of pathogenic bacteria such as *Escherichia coli*, *Staphylococcus aureus*, *Salmonella* spp., *Shigella* spp., *Bacillus subtilis* and *Vibrio 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 its ATCC strain.

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 tests.

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 isolates 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.

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 Hydrogen Sulfide (H_2S) 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

Antimicrobial susceptibility testing can normally be conducted using one of three basic methods: broth dilution, agar dilution, or disc diffusion. Due to their relative simplicity and ease of testing several antimicrobial drugs on each bacterial isolate, disc diffusion methods have long been a favorite in busy clinical microbiology laboratories. Antibiotic-containing wafers or disks are used in Kirby-Bauer antibiotic testing (also known as KB testing or disk diffusion antibiotic sensitivity testing) to determine which bacteria are sensitive to certain 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. The bacteria are more susceptible to the antibiotic in the disk if there is a greater zone of inhibition surrounding it. For each antibiotic, standard-sized zones of inhibition have been developed, and KB testing are conducted under standardized settings. Based on the dimensions of the zone of inhibition, KB test findings are often classified as sensitive, moderate, or resistant. The bacterium is regarded as sensitive to the antibiotic if the observed zone of inhibition is larger than or equal to the size of the standard zone. In contrast, the microorganism is regarded as resistant if the observed zone of inhibition is lower than the accepted size.

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 [14]. 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).

3. RESULTS

3.1 Biochemical Test Results for *Staphylococcus aureus*

Table 1. Biochemical tests for *Staphylococcus aureus*

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

To determine if the organism is *Staphylococcus aureus* or not we need to perform some biochemical test. The results of those tests are shown in the table. **Catalase test**: In the table we can see that the result of catalase test is positive. When we perform the test we can see the oxygen bubble on the glass slide so we can say that the catalase test is positive for the organism. **Coagulase test:** Then we perform the coagulase test to confirm the organism. During the coagulase test we can clearly see the clotting of plasma in the test tube. This indicates the positive result of the test and confirm that the organism is *Staphylococcus aureus*. **Urease test:** When we perform urease test for the organisms we found some of them positive. The indication of positive result is the change of color of broth. For *Staphylococcus aureus* the color of the broth turn pink from yellow**. Hydrogen sulfide test**: In this test indication of positive result is the black precipitate in the test tube. The color of media will also change into black from yellow. **Citrate utilization test**: Citrate agar is used to complete the test. The positive indication for this test is the color changing pattern. In this study we saw that the color of citrate agar turned blue from green. From this we can be sure that the organism is *Staphylococcus aureus*.

3.2 Antibiotic Sensitivity Test on Selected *Staphylococcus aureus* Samples by Kirby-bauer Disk Diffusion Test

From Table 2 we saw that, Kirby-Bauer disk diffusion susceptibility test was done to determine the sensitivity or resistance of pathogenic aerobic and facultative anaerobic bacteria.

3.3 Antibiotic Sensitivity Test on Selected Methicillin Resistant *Staphylococcus aureus* Samples by Raw and Commercial Honey

From the Table 3 we can see when we use raw and commercial honey for the ATCC strain of *Staphylococcus aureus* it shows moderate stage of sensitivity. That means raw and commercial honey can inhibit the growth of *Staphylococcus aureus* but not fully. It shows an inhibition zone around the honey well which is not very clear. On the other hand, when we use raw and commercial honey for other five strains of *Staphylococcus aureus* raw honey shows better efficiency than commercial honey. We can see a clear inhibition zone around honey well for raw honey. That's why we can say that the raw honey is inhibiting the growth of *staphylococcus aureus* moderately. But when we use commercial honey as an antimicrobial agent it can slightly inhibit the growth of microorganisms. That means we can see a zone of inhibition around the honey well but the zone is not very clear.

3.4 Antimicrobial Activity of Raw Honey

Out of four types of honey, all of the four honey samples 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 a specific bacterial strain like *Staphylococcus aureus*.

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.

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

	Zone of inhibition							
Organism ID	**FOX**	**CM**	**CHL**	**VA**	**CAZ**	**GEN**	**AZM**	**DO**
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 3. Inhibition zone of antibiotics on Methicillin resistant *Staphylococcus aureus* samples

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

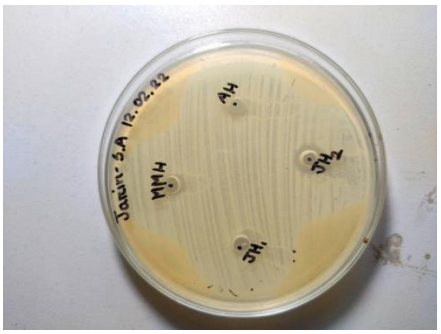

Fig. 1. Raw honey on Methicillin resistant *Staphylococcus aureus* cultured plate

Fig. 2. Raw honey on ATCC *Staphylococcus aureus* cultured plate

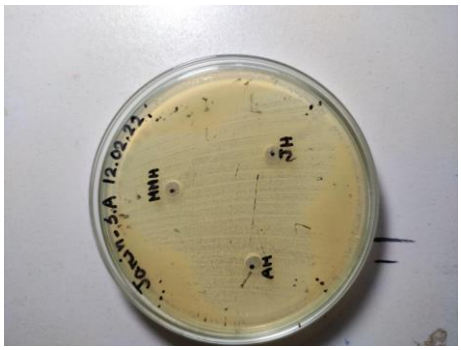

Fig. 3. Commercial honey on Methicillin resistant *Staphylococcus aureus* cultured plate

Fig. 4. Commercial honey on ATCC *Staphylococcus aureus* cultured plate

4. DISCUSSION

“Since ancient times, honey has been used as a medicine, mostly to treat skin wounds, burns, ulcers, eye infections, sore throats, and digital dermatitis, among other conditions” [16,17,18]. “The physical and chemical characteristics of honey, which are also connected to its botanical source, the metabolism of honey bees, as well as environmental, seasonal, and climatic factors, have a significant impact on its ability to heal” [19]. “Due to its antibacterial properties, honey has been used as an effective preservative for various food products in addition to healing” [20,21]. “Honey has been demonstrated to have antibacterial and wound-healing qualities in the past, however this depends on the type of honey, area, and flower from which the final product is made” [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]. “Hydrogen peroxide, flavonoids, phenolic acids, as well as numerous other undiscovered substances, are all present in honey. According to reports, honey has a chemical makeup that includes seven tetracycline’s, fatty acids, lipids, amylase, ascorbic acid, peroxidase, and fructose, all of which are thought to contribute to its antimicrobial activity. It also has a high osmolality, low pH (3.6–3.7), and a high content of phenol (inhibin)” [25,26].

“In general, antimicrobial medicines have a critical role in lowering the burden of infectious diseases worldwide” [27]. “Due to the illogical and excessive use of antibiotics in undeveloped and underdeveloped nations, the superbug-causing strains may become established and difficult to eradicate” [28]. The result is a reduction in the antibiotics' efficacy [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]. Applying honey to infected wounds that don't heal after receiving traditional treatment, such as antibiotics and antiseptics, can speed up the healing process [36]. Human illness and mortality have long been attributed in large part to disease-causing microbes. A small number of antimicrobial medicines can only effectively cure certain infections as a result of the emergence of resistant microorganisms. Many scientists have studied honey's antibacterial properties and discovered that natural, unheated honey exhibits some broad-spectrum antibacterial action when tested against pathogenic bacteria, oral bacteria, and bacteria that cause food to spoil. According to Nzeako and Hamdi in their studies of six commercial honey types found that inhibition of *S. aureus* which was consistent with the findings of present study.

The majority of antibiotics, including those that are penicillinase-resistant like methicillin, have been known to develop resistance in *Staphylococcus aureus*. In contrast to the incidence of 66.7% reported in the current study, a study conducted in the USA identified lower rates of 20.6% MRSA, 10% in, and 21.7% in; greater incidences of 45% and 58.2% MRSA have been reported by Eagye et al. and Keith et al. In China, a greater incidence of (63.4%) was also found, which is similar to the findings of the current study. Another study discovered that all MRSA strains were (100%) sensitive to vancomycin and amikacin, with (90%) gentamicin and (90%) ciprofloxacin following closely behind. This data may be significant clinically in hospital antibiotic policy guidelines [37]. “Both MRSA and MSSA isolates were sensitive to honey. But MRSA were resistant to all antimicrobials tested except linezolid where as MSSA were sensitive to all except penicillin”. [38]. One study “in Malaysia, Tualang honey exhibited variable activities against different microorganisms, but they were within the same range as those for manuka honey. This result suggests that tualang honey could potentially be used as an alternative therapeutic agent against certain microorganisms, particularly A. baumannii and S. maltophilia” [35]. 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 honey types. Therefore, these types of honey could be used as a potential alternative therapy against bacteria. Further studies into the composition and stability of the active constituents of the honey 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

Compared to commercial honey, raw honey has a substantially stronger inhibitory impact on Methicillin-resistant *Staphylococcus aureus*. Consequently, honey may be a useful 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.*

ETHICAL APPROVAL

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

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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