



# Antibacterial Activity of Bangladeshi Raw and Commercial Honey Against *Staphylococcus aureus*



Abhinandan Chowdhury\*, Sohidul Islam & Ramen Chowdhury

Department of Biochemistry & Microbiology, North South University, Bangladesh

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\*Corresponding author: Abhinandan Chowdhury, North South University, Bashundhara, Dhaka 1229, Bangladesh. Tel: +880-2-55668200; Email: abhinandan.chowdhury@northsouth.edu

## Abstract

Honey has been used as antibacterial agent since ages in the Asian countries. Though many studies have been done on this antimicrobial property of honey, studies on Bangladeshi honey related to such property are rare. Therefore, this study investigates the antibacterial spectrum of raw honey (collected from Sundarbans) and commercial honey from Bangladesh against ten strains of *Staphylococcus aureus* including multidrug resistant *Staphylococcus aureus*. Disk Diffusion method was performed to determine the zone of inhibition of honey as well as antibiotics against each *Staphylococcus aureus* strains. The zone of inhibition for different concentration of honey (25%, 50%, 75% and 100% v/v) were between 7 to 12mm. Out of ten strains, seven exhibited sensitivity to honey and three exhibited resistance. It was also observed that the sensitivity of bacteria to antibiotic (methicillin) increased when antibiotic discs were soaked in honey. Furthermore, it was also found that raw honey (ZOI, 12 mm max.) is more effective than commercial honey (ZOI, 9-10 mm max.) when it comes to inhibiting *Staphylococcus aureus*. These results indicate that Bangladeshi honey especially raw honey can be used for better treatment of *Staphylococcus aureus* infections along with antibiotic.

**Keywords:** Honey; Bangladesh; Antibacterial; *Staphylococcus aureus*; Multidrug resistant

## Introduction

Since the ancient times, honey has been used for both nutritional and therapeutic purposes. However, in the primordial application of honey as a medication, there was no understanding of it having antibacterial properties. Recently, many researchers have reported about broad-spectrum antibacterial activity of honey against various bacterial species. Studies found that honey works well against almost sixty species of bacteria including aerobes and anaerobes as well as gram-positives and gram-negatives [1,2]. This remarkable attribute of honey has been proved to be derived from multiple factors, such as high sugar content, low water content, methylglyoxal, low pH, hydrogen peroxide, bee defensin-1, natural flora and phytochemicals [3]. Moreover, studies have shown that honey hinders the development of biofilms as well as suspend quorum sensing, which diminishes bacterial virulence. Hence, making the bacteria more sensitive to traditional antibiotics which have lost their potency. [4-12].

One of the concerning issues, considering the antibiotic resistant bacteria, is the rise of methicillin-resistant *S. aureus* (MRSA) which is a worldwide problem especially in developing countries like Bangladesh [13]. *Staphylococcus*

*aureus* (*S. aureus*) is frequently found in the nose, respiratory tract, and on the skin and they do not always cause diseases. Nonetheless, pathogenic strains often promote infections by producing potent protein toxins and expressing cell-surface proteins that bind and inactivate antibodies [14]. The evolution of antibiotic resistance does not cause the organism to be more intrinsically virulent than strains of *S. aureus* that have no antibiotic resistance, but resistance does make *S. aureus* infection more difficult to treat with standard types of antibiotics and thus more dangerous [15]. The current situation extremely demands the development of unique antibacterial agents, which provides an opportunity for honey to be re-established as a broad-spectrum antibacterial agent that has less or almost no side effects, and is non-toxic to human tissue. Though honey had been used for treating bacterial infections in Bangladesh, but rarely any studies had been performed on the antibacterial spectrum of Bangladeshi honey.

Therefore, the focus of this research was to report on antibacterial spectrum of Bangladeshi honey against ten strains of *S. aureus* including MRSA. Moreover, the antibacterial efficacy of commercial honeys found in local markets were also investigated and compared with raw honey.

## Methodology

### Honey sample

Raw honey sample from Sundarban, Bangladesh and two others were commercial honey (named C1 and C2), which were produced and manufactured in Bangladesh were selected for the experiment.

### Bacterial sample

The antibacterial properties of three different honey samples were tested against ten *Staphylococcus aureus* isolates. Six of them were isolated from clinical samples and four were isolated from environmental samples (Table 1).

**Table 1:** List of *Staphylococcus aureus* isolates used in this research and their sources.

Sample ID	LAB ID	Sample Source	
1	IIB	Urine of heart patient	Clinical Sample
2	IIIB	Urine of heart patient	
3	VIIIB	Urine of heart patient	
4	845	Pus (Ibn sina hospital)	
5	550	Pus (Ibn sina hospital)	
6	599	Urine (Ibn sina hospital)	
7	EF 1	Waste water (Bashundhara eye hospital)	Environmental Sample
8	EF 2	Waste water (Bashundhara eye hospital)	
9	AM 1	Waste water (Apollo hospital)	
10	AM 2	Waste water (Apollo hospital)	

### Antibiotic

Ten antibiotics i.e. ampicillin, cefotaxime, doxycycline, erythromycin, gentamicin, imipenem, levofloxacin, methicillin, tetracycline and tobramycin were used for susceptibility testing.

### Media preparation

Mannitol salt agar (Oxoid, Product ID# CM0085) and Mueller-Hinton agar (Oxoid, Product ID# CM0337) was prepared according to standard procedure [16,17]. Phosphate Buffer Saline (PBS) was prepared comprised of the following: 8 g of NaCl, 0.2 g of KCl, 1.44 g of Na<sub>2</sub>HPO<sub>4</sub> and 0.24 g of KH<sub>2</sub>PO<sub>4</sub>. These amounts were added to the required volume of distilled water (1 liter). The pH was adjusted to 7.4 with HCl and then autoclaved [18].

### Isolation and identification of *Staphylococcus aureus*

For all the ten *S. aureus* strains, a tenfold serial dilution in PBS was performed. 100 µl of bacterial suspension was placed in the center of the Mannitol Salt Agar (MSA) plate using pipette. The sample was spread evenly over the surface of agar using the sterile cotton swab. All the plates were then incubated at 37°C for 24 hours. Several *Staphylococcus* species other than

*S. aureus* are mannitol positive and produce yellow colonies surrounded by yellow zones on this medium (e.g. *S. capitis*, *S. xylosus*, *S. cohnii*, *S. sciuri*, *S. simulans*, and other species) [19]. Therefore, further biochemical tests were performed for the identification of *S. aureus* such as, catalase and coagulase test. After biochemical identification, *S. aureus* isolated were sub-cultured on MSA plate for later use.

### Disc diffusion test

Bacterial suspensions were prepared in PBS from fresh culture of *S. aureus*. The suspensions were adjusted to 2 McFarland standard. 2 McFarland standard was prepared by mixing 0.2 ml of 1.0% barium chloride dihydrate (BaCl<sub>2</sub>•2H<sub>2</sub>O), with 9.8 ml of 1% sulfuric acid (H<sub>2</sub>SO<sub>4</sub>) [20]. Muller Hinton agar plates were inoculated by immersing sterile cotton swabs in 100µl bacterial suspensions on entire surface of the plate. 5mm diameter discs were cut from filter paper using punch machine and autoclaved. Different concentrations (25%, 50%, 75% and 100% v/v) of all three honey samples were prepared and the discs were soaked in it. The discs were then applied on the inoculated agar plate. Plates containing the discs were incubated at 37°C for 24 h. Zones of inhibition were measured by using ruler and the diameter of zones was recorded.

To compare efficacy between methicillin and methicillin soaked in honey, similar procedure was followed. Methicillin discs were soaked in 100% honey sample and were put on inoculated agar plates. Plates were incubated at 37°C for 24 h. The zone of inhibition of methicillin discs containing honey were measured and compared with zone of inhibition of methicillin discs.

Antibiotic susceptibility tests were also performed by the Kirby-Bauer method [17]. The antibiotic discs were applied directly on the inoculated plate to find out antibiotic susceptibility of the isolates. All the plates were incubated at 37°C for 24 h. Zone of inhibition was measured by using ruler and the diameter of zones was recorded.

## Results

### Antibiotic resistance profile of the isolates

From the antibiotic susceptibility testing it was found that, among ten *S. aureus* strains, five were resistant and five were intermediate susceptible to methicillin and erythromycin (Table 2). All the strains were resistant to ampicillin and nine were cefotaxime resistant. Most of the strains showed sensitivity towards doxycycline, gentamicin, imipenem, levofloxacin, tetracycline and tobramycin. The strain *S. aureus* 599 showed resistance to six antibiotics and intermediate susceptible to two antibiotics. All the test organisms were multidrug resistant. No single strain showed resistance to tobramycin.

### Synergistic effect of methicillin and honey

For this test, antibacterial effect of methicillin discs were compared with methicillin discs soaked in honey (Figure 1).

Among ten *S. aureus* strains, three strains were not susceptible to honey soaked methicillin discs, which were EF 1, AM 1 and AM 2. For rest of the isolates, honey soaked methicillin discs exhibited greater zone of inhibition than methicillin discs alone. Furthermore, raw honey soaked methicillin showed

greater zone of inhibition than C1 honey soaked methicillin or C2 honey soaked methicillin discs. One of the strains, *S. aureus* 599, which was resistant to six antibiotics including methicillin, showed greater zone of inhibition in presence of both raw and commercial honey-soaked methicillin.

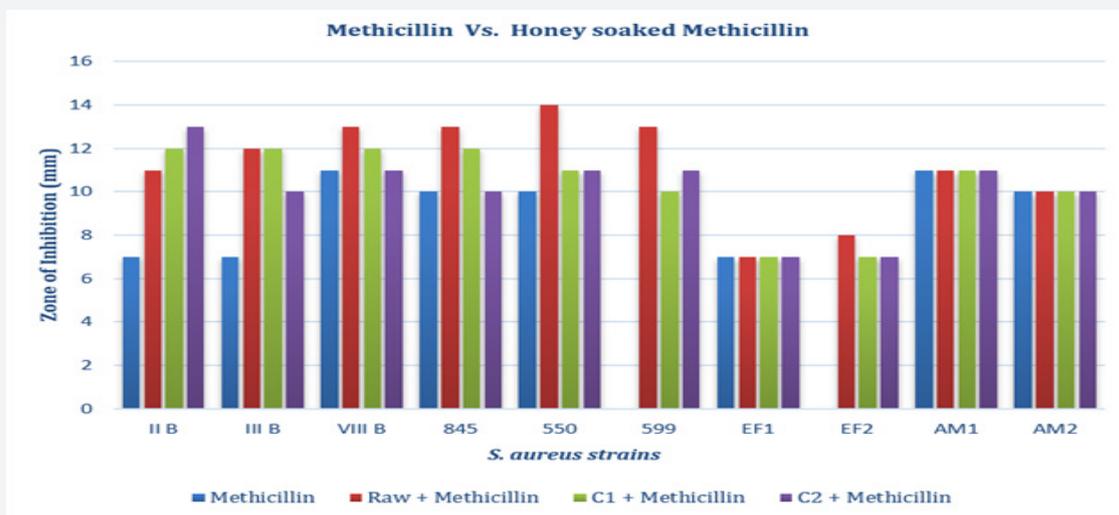


Figure 1: Methicillin vs. honey containing methicillin: A comparative graph.

### Susceptibility profile against different concentrations of honey

The strains, EF1, AM1 and AM2 were not susceptible to any concentration of three different types of honey samples (Figure 2). Strain III B showed no inhibition at 25% concentration of raw honey and 25%, 50% and 75% v/v concentration of C2 honey. Similarly, strain VIII B showed no zone of inhibition at 25%, 50% and 75% v/v concentration of C1 honey. The strains 845 and

550 showed no inhibition at 25% concentration of C1 honey. At 25% v/v concentration of C2 honey, no inhibition zone was seen by strain 845 and 599. Also, EF 2 showed no zone of inhibition at 25% v/v concentration of raw, C1 and C2 honey. The highest zone of inhibition was shown by raw honey against strains 845 and 550 which was 12mm while the lowest zone of inhibition was 7mm. The highest zone of inhibition exhibited by C1 and C2 honeys was 10mm.

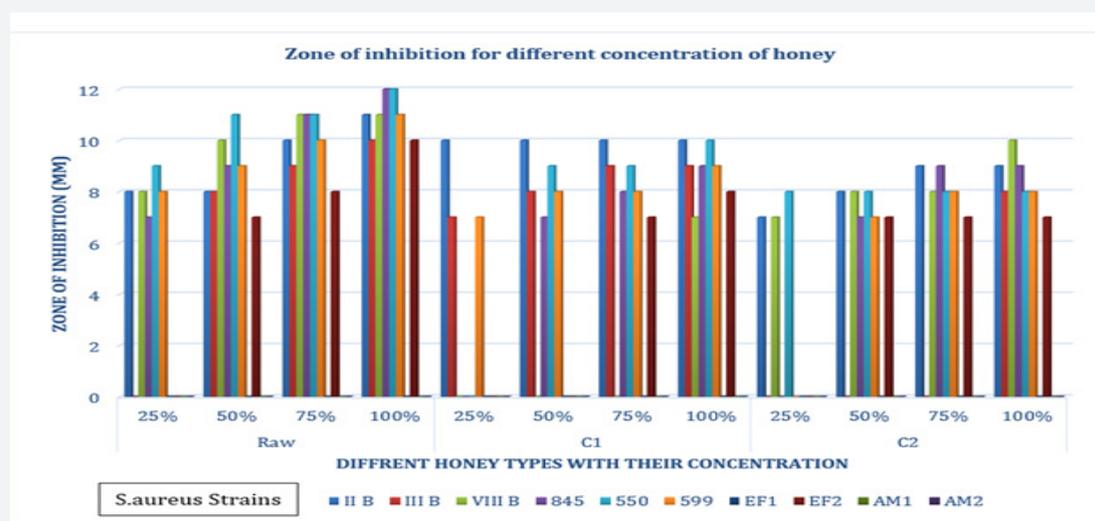


Figure 2: Graph showing zone of inhibition for different concentration of honey against *S. aureus* strains.

### Discussion

Previous research showed that Manuka honey (from the tree *Leptospermum scoparium*, found in Australia and New Zealand)

can inhibit growth of methicillin-resistant *Staphylococcus aureus*(MRSA) [3,4,21-23]. This study was undertaken to investigate the antibacterial activity of Bangladeshi honey. Three

different types of honey were used in this study. One of them was raw honey collected from Sundarban, Khulna and other two were commercially produced honey in Bangladesh obtained from local market. These honey samples were tested against

certain *Staphylococcus aureus* isolates. All the *Staphylococcus aureus* strains used in this experiment were found out to be multidrug resistant. Most of the strains showed resistance to ampicillin, cefotaxime, erythromycin and methicillin (Table 2).

**Table 2:** Antibiotic susceptibility pattern of ten different *Staphylococcus aureus* isolates (R- Resistant, I.S.- Intermediate susceptible, S- Susceptible).

Antibiotics	<i>Staphylococcus aureus</i> strains									
	II B	III B	VIII B	845	550	599	EF 1	EF 2	AM 1	AM 2
Ampicillin	R	R	R	R	R	R	R	R	R	R
Cefotaxime	R	R	R	R	R	I.S.	R	R	R	R
Doxycycline	S	S	S	S	S	R	S	S	S	S
Erythromycin	R	R	R	I.S.	R	R	I.S.	I.S.	I.S.	I.S.
Gentamicin	S	S	S	S	S	I.S.	S	S	S	S
Imipenem	S	S	S	S	S	R	S	S	S	S
Levofloxacin	S	S	S	R	R	S	S	S	S	S
Methicillin	R	R	I.S.	I.S.	I.S.	R	R	R	I.S.	I.S.
Tetracycline	S	S	S	S	S	R	S	S	S	S
Tobramycin	S	S	S	S	S	S	S	S	S	S

From the observed data, it was found that Bangladeshi honey (both raw and commercial) not only showed bactericidal effect to methicillin resistant *Staphylococcus aureus* but also showed inhibitory effect to multidrug resistant *Staphylococcus aureus*. However, three among ten *S. aureus* strains used in this study exhibited resistance to honey. The antibacterial activity of honey is highly variable and can be changed over time. Even, honeys produced in one location at one time could vary in antibacterial activity [24]. It was prominent from our result that at 100% concentration all three honey samples showed maximum inhibitions and the bactericidal effect decreased when the concentrations of honey were reduced. However, honey could still retain its bactericidal activity at 50% v/v and 75% v/v concentration. Yet considerable amount of inhibition to most of the isolates was noticed even at 25 % v/v.

Previously some researchers demonstrated that at low concentration such as 25% v/v concentration of Costa Rican honey can inhibit *Staphylococcus aureus* growth [25]. Present study showed that not all study strains of *Staphylococcus aureus* were sensitive to 25% v/v concentration of Bangladeshi honey samples. The possible reason could be the variation of antibacterial factors present in honey region to region. Another reason could be, not all bacterial strains of similar species show sensitivity or resistance in similar manner. A previous study reported that medical-grade manuka honey (Medihoney) when used together with rifampicin, not only improved the efficacy of the antibiotic but also inhibit methicillin-resistant *Staphylococcus aureus*(MRSA) and rifampicin-resistant *S. aureus* in vitro [24,26].

In the present experiment, raw honey showed greater zone of inhibition when compared to commercial honeys when applied

with antibiotic (methicillin) or used as a sole agent. To find out if honey showed a synergistic effect when applied with methicillin, antibiotic discs were soaked in 100% honey solution and the results showed that the efficacy of methicillin increased when it was soaked in honey. Increased sensitivity to both antibiotics and Bangladeshi honeys were demonstrated in this study when they are used in combination, even when strains are clinically resistant to a particular antibiotic (methicillin); however, this depends on the antibiotic and on the *S. aureus* strain. This result clearly showed that honey can be used with a combination of antibiotics to increase its (antibiotic) efficacy.

### Conclusion

Combinational treatment of honey and common antibiotics may offer a wide range of advantages including synergistic enhancement of the antibacterial activity, reduction of the effective dose of the antibiotic, and reduction of the risk of antibiotic resistance. It can also be suggested observing the results that raw honey should be used rather than commercial ones in *S. aureus* related infections. Nonetheless, only three types of honey were investigated in this study, while more diverse location and origin based honey is needed to be investigated in order to enhance knowledge on bactericidal activity of Bangladeshi honey. Based on the current study, it can be concluded that Bangladeshi raw honey has the potential to be an export quality honey, similar to Medihoney, if a broad range of analyses on both nutritional and bactericidal properties are carried out. Last but not the least can open a new door to novel drug development.

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### Conflict of Interest

Authors declare no conflict of interest.

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