Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)

Volume 6 Issue 1 January 2023

Research Article

Isolation and Identification of Common Food-borne Pathogens from Honey and Determination of Antimicrobial Activity of Honey in Greater Dhaka Region, Bangladesh

Mahmudul Hasan Masud[1]*, Md Rezaul Alam[1], Md Aoulad Hosen[2], Md Shajadur Rahman[1], Nusrat Jahan[1], Md Hasibul Hasan[3], Raisa Rafia[2], Sohel Miah[2], Mohammad Shariful Islam[4] and Nazmi Ara Rumi[2]

[1]*Department of Microbiology, Gono Bishwabidyalay, Savar, Dhaka, Bangladesh*

[2]*Department of Microbiology, Hajee Mohammad Danesh Science and Technology University, Dinajpur, Bangladesh*

[3]*Faculty of Veterinary, Animal and Biomedical Sciences, Sylhet Agricultural University, Bangladesh*

[4]*Department of Microbiology, Jagannath University, Dhaka, Bangladesh*

***Corresponding Author:** Mahmudul Hasan Masud, Department of Microbiology, Gono Bishwabidyalay, Savar, Dhaka, Bangladesh.

DOI: 10.31080/ASMI.2022.06.1184

Received: November 26, 2022

Published: December 25, 2022

Abstract

The world's oldest medical texts have described the medicinal value of honey, and they also note that it contains antibacterial and wound-healing properties. Although honey has antibacterial properties, different microbes can contaminate it at different phases of production. Therefore, this research aimed to identify and antimicrobial activity of foodborne pathogens isolated from raw and processed honey from different supper shops in Dhaka. In this study total of 6 honey samples were collected from different sources, including three raw and three processed samples. Out of 3 processed honey samples, no pathogenic bacteria was detected, whereas *Escherichia coli* and *Pseudomonas* spp. were detected from a raw honey sample from samples 2 and 3, located in Gazipur and Pollibidduth. All isolates were confirmed by using cultural and A set of biochemical tests. Additionally, five indicator isolates, such as *E. coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Pseudomonas* spp., and *Candida albicans*, were selected for the antimicrobial activity of honey. In this study, Ten commercially available antibiotic discs were applied for the zone of inhibition against test organisms and isolated bacteria. Different concentrations of raw and processed honey were applied for the growth of inhibition of indicator strain. Out of 6 samples, processed sample 1 had the highest zone of inhibition (23mm) and was sensitive to *Candida albicans,* followed by *Pseudomonas* spp.*,* (21mm) and *Bacillus cereus* (18mm), respectively. In the case of raw sample 1, *E. coli* gives the highest sensitivity (20mm), whereas *Candida albicans* give a (19 mm) zone. The results indicate that the quality of processed honey is better than raw honey. Based on the result of this research, it is concluded that the antimicrobial activity of honey is comparatively good, along with commercial antibiotics. Natural honey and processed honey can be used to treat several infections.

Keywords: Antibacterial Activity; Food Borne Pathogen; Honey; Medicinal Property; Wound Healing Property

Abbreviations

EMB: Eosin Methylene Blue Agar; MSA: Mannitol Salt Agar; MR: Methyl Red; VP: Voges-Proskauer; TSI: Triple Sugar Iron; MIU: Motility Indole Urease

Introduction

Honey is a traditional cure for infected wounds that has recently been "rediscovered" by the medical community, particularly when standard modern medicinal treatments have failed. Ancient Sumerian tablets from around 2100–2000 B.C. highlight honey's medicinal and topical uses. When speaking about the many types of honey, Aristotle (384-322 BC) mentioned that pale honey was "excellent as a balm for painful eyes and bruises." The antibacterial action of manuka honey against harmful microorganisms such as *S. aureus* and *Helicobacter pylori* makes this honey a good functional food for treating wounds or stomach ulcers [1]. Honey is used to treat ulcers, bed sores, and burn and wound-related skin infections. Honey's healing benefits can be attributed to its antibacterial activity, ability to maintain a moist wound environment that promotes healing, and high viscosity, which provides a protective barrier against infection. Honey's antimicrobial qualities expedite wound healing by promoting the creation of new tissue. It has been demonstrated that mudhoney and manuka honey have *in vivo* action and can cure ulcers, wounds, and burns [2]. Honey can improve healing in infected wounds that don't respond to traditional treatment, such as medicines and antiseptics, including methicillin-resistant *S. aureus* wounds.

Additionally, it applies to skin transplants and infected skin graft donor sites successfully [3]. Additionally, darker honey had more antioxidants. Catalase did not diminish the antibacterial action of the darker-colored test honey, indicating that non-peroxide components such as antioxidants may contribute to suppressing the growth of some foodborne infections [4]. Due to its high osmolality, acidity, and hydrogen peroxide (H_2O_2) content, honey's positive effects can be related to its antibacterial and anti-inflammatory qualities. Honey's antibacterial ingredient is hydrogen peroxide, whose concentration depends on inhibin and glucose oxidase levels [5]. *Clostridium perfringens* produce enterotoxin [6]. Disc diffusion is a qualitative test for antimicrobial susceptibility. This investigation aimed to evaluate the effectiveness of various kinds of honey in inhibiting the growth of *E. coli*, *Salmonella* spp., *Shigella* spp., *Listeria monocytogenes*, *S. aureus*, and *Bacillus cereus*. Additionally, the research aimed to isolate and identify the foodborne pathogens from raw and processed honey and also evaluate the antimicrobial activity of honey.

Materials and Methods

Study area and design settling

This investigation was carried out from September 2015 to March 2016 with the goals of isolating common food-borne pathogens from honey and evaluating the antibacterial activity of honey gathered from different areas of greater Dhaka. All microbiological analysis was carried out in the microbiology laboratory of Gono Bishwabidyalay, Savar, Dhaka, Bangladesh. A total of 6 raw and processed honey samples were collected from Mirpur, Gazipur, Pallibittut, and Savar, in Bangladesh. Raw honey samples were collected from a local market, and processed honey was collected from shops.

Confirmatory test of natural (raw) honey and sample preparation

A glass of warm water was filled, and a tablespoon of honey was added. This made it easier to determine if it dissolved in the water. The majority of raw honey congeals and sinks as a solid lump or stays adhered to the spoon as a lump. To see if there was any additional water in the honey that would have stopped it from burning, a candle wick dipped in honey was lit on fire [7]. Solutions of honey were prepared immediately before testing by diluting honey to the required concentration. Serial dilution (10^{-1} to 10^{-3}) was prepared to dilute honey into the water.

Isolation and Identification of Pathogenic isolates

All diluted honey (dilution 10^{-1} to 10^{-3}) was spread over the Nutrient and MacConkey agar media by spread plate technique. All culture plates were incubated at 37°C overnight. Additionally, for confirmation of positive bacterial growth, EMB, MSA, and Cetrimide agar were used as selective agar media (HI media, India). All culture plates were then subcultured for pure isolation and incubated at 37°C for 24 hours. A group of morphological and biochemical tests was applied, such as microscopic examination (Gram-staining), Catalase, MR-VP, TSI, Indole, MIU, and Citrate utilization. All tests were performed by conventional methods [8].

Antibiotic sensitivity test

The antimicrobial susceptibility profiles of the isolates were determined using the standard Kirby-Bauer disk diffusion method

following the recommendations of the National Committee for Clinical Laboratory Standards [9]. The Mueller-Hinton medium was utilized to conduct the antimicrobial susceptibility testing. All isolates were tested for sensitivities to 6 (Chloramphenicol (25 µg), Fluconazole (10 µg), streptomycin (10 µg), Erythromycin (15 µg), Nystatin (30 µg), Cephalexin (10 µg) of routine and practical antibiotics.

Antimicrobial properties of honey

Indicator strain

To determine the antimicrobial activity of various bacterial isolates from different kinds of honey, five microorganisms, including four bacteria and one yeast strain, were used as indicator strains. *E. coli, S. aureus, Bacillus cereus, Pseudomonas* spp., and *Candida albicans* were used in this experiment. All test isolates were collected from the Department of Microbiology, Gono Bishwabidyalay, Savar, Dhaka, Bangladesh. All test isolates were collected from different diseased patients.

Overnight incubations were performed at 37°C with each bacterial indicator strain. To obtain the inoculum for the yeast strain, the yeast was allowed to grow on 3% malt extract (BD, Sparks, MD, USA) combined with 1.5% (w/v) agar at 30° C for thirty days. After being harvested, the yeast cells were suspended in sterile deionized water that had 0.01% (v/v) of Tween 80 solution added to it (Fisher Scientific, Hampton, NH, USA).

Antimicrobial activity tests of honey

Four concentrations of honey were used, including control, dilution 10^{-1}, 10^{-2}, and 10^{-3}. 1 ml of honey is diluted in 9 ml of distilled water. All agar plates were inoculated using the sterile method. Horizontally, vertically, and around the plate's edge, agar was streaked to ensure heavy growth. The plates were then allowed to dry for approximately 5 minutes. Honey samples of different concentrations were applied to soak sterile discs. Honey discs were placed on an agar plate with sterilized forceps. All plates were incubated at 37°C for 24 hours. Using the good diffusion method, the antimicrobial activity of various honey solutions was tested against *E. coli*, *S. aureus*, *Bacillus cereus*, *Pseudomonas* spp., and *Candida albicans* [10]. On Tryptic Soy Agar plates, 100 microliters of diluted sample were put into wells (8 mm in diameter). The five indicator strains were added to the plates and inoculated into the molten TSA. Due to the unexpected way the size (diameter) of the honey samples' "inhibitory zone" was measured, the antimicrobial activity was judged by giving a range of activity from + (lowest activity) to +++ (highest activity) for each sample.

Results and Discussion

Isolation and identification of food-borne pathogens

In this research, six honey samples were collected from different super shops. Out of 6 samples, 3 were raw honey samples, and the remaining 3 were processed samples. *E. coli* and *Pseudomonas* spp. were identified from 2 raw honey samples. 3×10^{-1} CFU/ml was found in raw sample 2, and 5×10^{-1} CFU/ml was found in sample 3 (Table 1).

On different selective media, *E. coli* and *Pseudomonas* spp. grow in ways that are different from one another. *E. coli* produces

Sampling sources	Positive/Negative	Number of Colonies (CFU/ml)	Microscopic Characteristics	Isolates
Raw Sample-01 (Mirpur)	Negative	-	-	-
Raw Sample-02 (Gazipur) (Dilution 10^{-1})	Positive	3×10^{-1}	Gram-negative rod	*E. coli*
Raw Sample-03 (Pallibiddut) (Dilution 10^{-1})	Positive	5×10^{-1}	Gram-negative rod	*Pseudomonas* spp.
Processed Sample-01(D-honey)	Negative	-	-	-
Processed Sample-02 (A-honey)	Negative	-	-	-
Processed Sample-03 (P-honey)	Negative	-	-	-

Table 1: Gram staining and biochemical characterization of isolates.

green colonies with a metallic sheen on *EMB* agar (Figure 1) while *Pseudomonas* spp. produce yellow colonies on Cetrimide agar. Microscopically, *E. coli* is pink, rod-shaped, singular, pair, or short chain. Biochemical tests result showed in table 2. This result indicates less secondary contamination during processing, packing, or intentional adulteration. So, the honey samples were good in quality for consumption as food. This can not cause any food-borne disease.

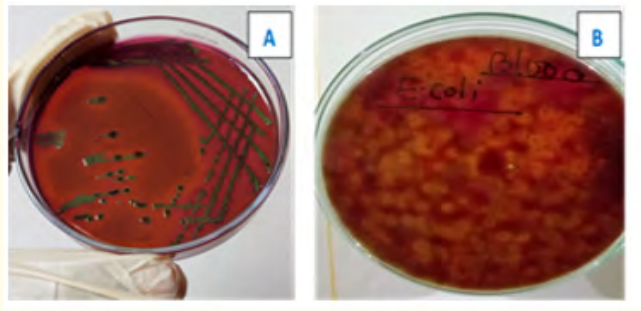

Figure 1: *E. coli* on EMB agar (A), Blood agar (B).

Name of the tests / Name of the isolates	Catalase	MR	VP	Indole	Citrate Utilization	MIU	TSI			Interpretation
							Slant	Butt	H_2S	
Isolate-1 Raw sample 2	+	+	-	+	-	+	Y	Y	-	*E. coli*
Isolate-2 Raw sample 3	+	-	-	-	+	+	R	R	-	*Pseudomonas spp.*

Table 2: Results of biochemical tests of isolates Note: [Y= Yellow; R= Red].

Table 3 represented the highest antimicrobial activity observed in processed honey samples against *Candida albicans*, followed by *Pseudomonas* spp., whereas, *Bacillus cereus* showed the lowest activity. *Candida albicans* and *E. coli* have the highest activity in the raw honey sample, followed by *S. aureus*.

Sampling sources	Indicator strains	Antimicrobial activity
Raw honey sample	*E. coli*	+++
	Staphylococcus aureus	++
	Bacillus cereus	-
	Pseudomonas spp.	-
	Candida albicans	+++
Processed honey sample	*E. coli*	-
	Staphylococcus aureus	-
	Bacillus cereus	+
	Pseudomonas spp.	++
	Candida albicans	+++

Table 3: Antimicrobial activity of honey against test isolates.
+++= Highest Activity, += Lowest Activity, -= No Activity.

Table 4 represented that the control or direct honey samples have greater effectiveness against common test pathogens. Diluted honey samples were not proven effective against these pathogens. Out of six samples, the processed sample 1 had the highest zone of inhibition (23 mm) and was sensitive to *Candida albicans* followed by *Pseudomonas* spp., (21 mm) Figure 2, *Bacillus cereus* (18 mm) respectively. In the case of raw sample 1, *E. coli* gives the highest sensitivity (20 mm), whereas *Candida albicans* give a (19 mm) zone.

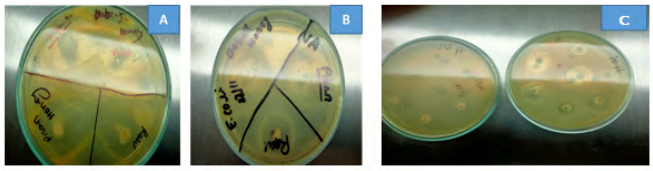

Figure 2: Antimicrobial activity of *Pseudomonas* spp. and *E. coli* against honey (A, B) and commercial antibiotics (C).

Sampling sources	Different concentrations of honey	*E. coli*	*Staphylococcus aureus*	*Bacillus cereus*	*Pseudomonas* spp.	*Candida albicans*
		Zone size/ mm	Zone size/mm	Zone size/ mm	Zone size/mm	Zone size/mm
Raw Sample-01 (Mirpur)	Control	20 (S)	12 (R)	5 (R)	15 (I)	19 (S)
	Dilution 10^{-1}	10 (R)	10 (R)	2 (R)	2 (R)	5 (R)
	Dilution 10^{-2}	9 (R)	-	-	-	-
	Dilution10^{-3}	-	-	-	-	-
Raw Sample-02 (Gazipur)	Control	5 (R)	17 (S)	14 (I)	12	16 (I)
	Dilution 10^{-1}	3 (R)	12	-	1 (R)	4 (R)
	Dilution 10^{-2}	-	7 (R)	-	-	-
	Dilution10^{-3}	-	-	-	-	-
Raw Sample-03 (Pallibiddut)	Control	4 (R)	12 (R)	12	16 (I)	18 (S)
	Dilution 10^{-1}	-	7 (R)	7 (R)	4 (R)	7 (R)
	Dilution 10^{-2}	-	-	-	-	2 (R)
	Dilution10^{-3}	-	-	-	-	-
Processed Sample-01 (D-honey)	Control	15 (I)	2 (R)	18 (S)	21 (S)	23 (S)
	Dilution 10^{-1}	10 (R)	-	-	12 (R)	15(I)
	Dilution 10^{-2}	7 (R)	-	-	2(R)	1 (R)
	Dilution10^{-3}	3 (R)	-	-	-	-
Processed Sample-02 (A-honey)	Control	6 (R)	15 (I)	3 (R)	18 (S)	20 (S)
	Dilution 10^{-1}	3 (R)	10 (S)	1 (R)	2 (R)	7 (R)
	Dilution 10^{-2}	-	-	-	-	-
	Dilution10^{-3}	-	-	-	-	-
Processed Sample-03 (P-honey)	Control	10 (R)	15 (I)	3 (R)	14 (I)	11(R)
	Dilution 10^{-1}	10 (R)	1 (R)	-	-	-
	Dilution 10^{-2}	-	-	-	-	-
	Dilution10^{-3}	-	-	-	-	-

Table 4: Result of the antimicrobial effect of honey.

Note: S = Sensitive, R = Resistance, I = Intermediate, (-) = No zone.

Among all the bacterial indicator strains tested, *Bacillus cereus* and *Candida albicans* had the highest susceptibility 80% against commercial antibiotics, whereas honey gives 25%. *Pseudomonas* spp. showed the second highest susceptibility 60%, whereas honey shows all indicator microorganisms tested, 25% respectively. *E. coli* and *S. aureus* indicate 40% susceptibility against commercial antibiotics, whereas honey shows 25% (Table 5).

Organisms	Therapeutic Agents		Therapeutic Agents		Percent of Sensitivity (%)	
E. coli	Antibiotics	Zone of Inhibition (mm)	Honey	Zone of Inhibition (mm)	Antibiotics	Honey
	Chloramphenicol	0 (R)	Control	20 (S)	40%	25%
	Cephalexin	30 (S)	Dilution 10^{-1}	10 (R)		
	Ampicillin	0 (R)	Dilution 10^{-2}	9 (R)		
	Erythromycin	0 (R)	Dilution 10^{-3}	0 (R)		
	Streptomycin	25 (S)	-	-		
Staphylococcus aureus	Chloramphenicol	5 (R)	Control	17 (S)	40%	25%
	Cephalexin	24 (S)	Dilution 10^{-1}	12 (R)		
	Ampicillin	0 (R)	Dilution 10^{-2}	7 (R)		
	Erythromycin	4 (R)	Dilution 10^{-3}	0 (R)		
	Streptomycin	31 (S)	-	-		
Bacillus cereus	Chloramphenicol	34 (S)	Control	17 (S)	80%	25%
	Cephalexin	32 (S)	Dilution 10^{-1}	2 (R)		
	Ampicillin	5 (R)	Dilution 10^{-2}	0 (R		
	Erythromycin	20 (S)	Dilution 10^{-3}	0 (R)		
	Streptomycin	30 (S)	-	-		
Pseudomonas spp.	Chloramphenicol	15 (I)	Control	18 (S)	60%	25%
	Ampicillin	18 (S)	Dilution 10^{-1}	2 (R)		
	Erythromycin	19 (S)	Dilution 10^{-2}	0 (R)		
	Streptomycin	0 (R)	Dilution10^{-3}	0 (R)		
	Cephalexin	18 (S)	-	-		
Candida albicans	Ketoconazole	22 (S)	Control	19 (S)	80%	25%
	Clotrimazole	24 (S)	Dilution 10^{-1}	5 (R)		
	Nystatin	14 (I)	Dilution 10^{-2}	0 (R)		
	Miconazole	24 (S)	Dilution10^{-3}	0 (R)		
	Fluconazole	29 (S)	-	-		

Table 5: Comparison between the effectiveness of Honey (Sample-1) and Antibiotics.

Note: S= Sensitive; R= Resistance; - = No zone.

In this research, both honey samples showed the antimicrobial activity of some test organisms, such *as E. coli*, *S. aureus, Bacillus cereus, Pseudomonas* spp., and *Candida albicans. Candida albicans* and *Bacillus cereus* showed the highest antimicrobial activity against raw and processed honey. The antimicrobial activity of *Candida albicans* and *E. coli* is represented in figure 3 (A, B).

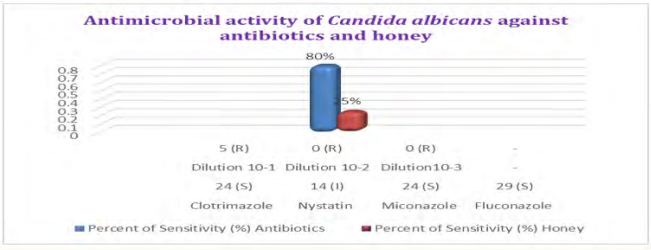

Figure 3 (A): Antimicrobial activity of *Candida albicans* against commercial antibiotics and honey.

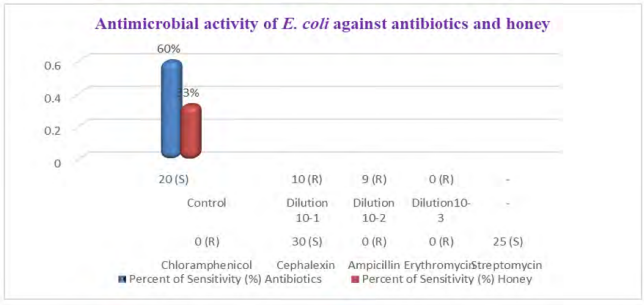

Figure 3 (B): Antimicrobial activity of *E. coli* against commercial antibiotics and honey.

Numerous studies indicate honey's antibacterial properties as one of its mostwell-established bioactivities [11].Multiple processes influence the development and survival of microorganisms when honey is present [12]. Honey is an antibacterial powerhouse due to its low pH, high sugar concentration, high osmolality, and antibacterial compounds, including hydrogen peroxide and polyphenols [13]. Natural, unprocessed honey is not sterile [14]. Several investigations have shown the isolation of microbes from honey samples produced in different geographic regions [15]. Depending on the sample and its freshness, the number of microorganisms in honey varied from 0 to several thousand colony-forming units (CFUs) per gram [15]. Sinacori., *et al*. showed a low bacterial burden in 33 of 38 southern Italian honey samples [15]. Fernández., *et al*. studied at the microbiological quality of honey from Argentina [14]. Their results were similar to those of other studies [14,15] conducted in Argentina and other places worldwide. Bacteria are also found in honey from bees that don't have stingers [16].

Food-borne diseases are common in Bangladesh as Bangladesh is a developing country with a vast population. People, especially those living below the poverty level, get sick from consuming contaminated food by food-borne pathogens. Food-borne pathogens were not founded in this research. We also found some effective antimicrobial activity of honey against common bacterial species. Therefore, honey can be used as a clinical application as a therapeutic agent.

Conclusion

In this research, it has been found that *E. coli* and *Pseudomonas* spp. were identified by using cultural and biochemical tests from honey samples from different sources in Dhaka city. The microbiological characteristics of honey from the greater Dhaka, Bangladesh region were determined to provide information on their level and prevalence depending on the source. The finding of this study demonstrated that raw and processed honey is active against indicator strains. The study also indicates that good hygienic practice was maintained during processing and the presence of components inhibiting growth. I have not found any pathogenic spore-forming or anaerobic bacteria that can cause food poisoning in my study. The quality of raw and processed honey was almost the same, although some raw honey gave intermediate results. It is concluded that honey can be used as good medicine and clinical applications for several infectious diseases due to its antimicrobial activities.

Acknowledgments

The authors want to thank the Department of Microbiology, Gono Bishwabidyalay, for providing the indicator strains and laboratory facilities.

Conflict of Interests

The authors have no conflict of interest.

Funding

Other sites received no funding, and a departmental research budget supported comprehensive research.

Authors Contribution

Mahmudul Hasan Masud designed, conceived, labwork and wrote the manuscript. Md. Aoulad Hosen checked the plagiarism and grammatical errors. Md. Rezaul Alam supervised and edited

the manuscript. Mohammad Shariful Islam and Md. Hasibul Hasan formatting references. Md. Shajadur Rahman, Raisa Rafia and Sohel Miah prepared the tables and graph. Nazmi Ara Rumi formatted and revised the manuscript according to journal guidelines. Finally, all authors involved in this research read and approved the manuscript for publication.

Bibliography

1. French VM., *et al*. "The antibacterial activity of honey against coagulase-negative staphylococci". *Journal of Antimicrobial Chemotherapy* 56 (2005): 228-231.

2. Shields CJ. "Towards a new standard of perioperative fluid management". *Therapeutics and Clinical Risk Management* 4.2 (2008): 569.

3. Ahmed AkJ., *et al*. "Honey-medicated dressing: transformation of an ancient remedy into modern therapy". *Annals of Plastic Surgery* 50.2 (2003): 143-148.

4. Cooper RA., *et al*. "The sensitivity to honey of Gram-positive cocci of clinical significance isolated from wounds". *Journal of Applied Microbiology* 93.5 (2002): 857-863.

5. Noori AL., *et al*. "Differences in composition of honey samples and their impact on the antimicrobial activities against drug multiresistant bacteria and pathogenic fungi". *Archives of Medical Research* 44.4 (2013): 307-316.

6. Jay JM., *et al*. "Food poisoning caused by gram-positive sporeforming bacteria". *Modern Food Microbiology* (2005): 567-590.

7. Molan PC. "The evidence and the rationale for the use of honey as a wound dressing". *Wound Practice and Research: Journal of the Australian Wound Management Association* 19.4 (2011).

8. Purkayastha M., *et al*. "Cultural and biochemical characterization of sheep Escherichia coli isolated from in and around Bau campus". *Bangladesh Journal of Veterinary Medicine* 8.1 (2010): 51-55.

9. Clinical and Laboratory Standards Institute. "Performance standards for antimicrobial susceptibility testing". CLSI supplement M100. (2017).

10. Cappuccino J., *et al*. Microbiology: A Laboratory Manual (7th Edition) April (2004).

11. Albaridi NA., *et al*. "Antibacterial potency of honey". *International Journal of Microbiology* 2019 (2019).

12. Bucekova M., *et al*. "Demanding new honey qualitative standard based on antibacterial activity". *Foods* 9.9 (2020): 1263.

13. Zulkhairi A., *et al*. "Probiotic properties of Bacillus strains isolated from stingless bee (*Heterotrigona itama*) honey collected across Malaysia". *International Journal of Environmental Research and Public Health* 17.1 (2020): 278.

14. Fernández LA., *et al*. "Microbiological quality of honey from the Pampas Region (Argentina) throughout the extraction process". *Revista Argentina De Microbiología* 49.1 (2017): 55-61.

15. Sinacori M., *et al*. "Cultivable microorganisms associated with honeys of different geographical and botanical origin". *Food Microbiology* 38 (2014): 284-294.

16. Lee H., *et al*. "Antimicrobial activity of bacterial isolates from different floral sources of honey". *International Journal of Food Microbiology* 126.1-2 (2008): 240-244.