Original Research Article

Isolation of pathogenic microorganisms from burn patient and in vitro determination of antibacterial activity of honey against antibiotic resistance isolates

Ifra Tun Nur*, Tahmina Jahan, Sharmin Akter

Department of Microbiology, Stamford University Bangladesh, Siddeswari Road, Dhaka, Bangladesh

Received: 13 May 2020
Revised: 13 June 2020
Accepted: 01 July 2020

*Correspondence:
Ifra Tun Nur,
E-mail: tun.ifra@yahoo.com

ABSTRACT

Background: Honey is a natural therapeutic agent which manifest antimicrobial activity against a wide range of bacteria. Therefore, the current study was designed to isolate pathogenic bacteria from burn wound and also to determine the anti-bacterial traits of natural and processed honey against infectious agents.

Methods: Wound samples were collected from burn unit of Dhaka Medical College Hospital and conventional cultural methods were applied to identify pathogenic microorganisms. A total of six samples including three each of natural and processed honey were tested for the determination of antimicrobial activity by agar well diffusion method.

Results: Among ten wound samples highest load of total viable bacteria was recorded up to 3.7×10⁶ cfu/ml. The maximum load of *Pseudomonas* spp. and *Staphylococcus* spp. were found up to 1.6×10⁴ cfu/ml and 8.7×10⁴ cfu/ml respectively. Significant in vitro antimicrobial activity was found in all the samples. Natural honey showed a little bit more efficacy than processed honey. The samples exhibited antibacterial traits against *Staphylococcus aureus* with a wide zone of inhibition and moderate zone of inhibition against *Pseudomonas* spp, when they are subjected to 100% concentrated honey. *E. coli* and *Klebsiella* spp. were remained to be unaffected at 75% and 50% concentrated honey, while *S. aureus* and *Pseudomonas* spp. were found to be sensitive at those concentrations.

Conclusions: The in vitro efficacy of different types of honey tested against the bacteria dependent on the type of honey and the concentration at which it was administered. In our study 100% concentrated honey was more efficient in inhibiting all the tested isolates.

Keywords: Burn wound, Pathogen, Honey, Antibiotic resistance, Anti-bacterial activity

INTRODUCTION

Burn patient especially with first degree or second degree injuries, are frequently exposed to microbial infection.1-3 Burn wound infection accelerated by opportunistic pathogen or exogenous infectious agent which acquired through exposure to the hospital environment, hospital personnel or medical devices.4-9 Any bacterium could be a likely pathogen in burn wounds considering the extent and depth of the injury; however, coagulase-negative *Staphylococi, Staphylococcus aureus* and *Enterococcus* spp. have been reported to be the most common gram positive pathogens, and *Pseudomonas aeruginosa, Escherichia coli, Klebsiella pneumoniae* and *Acinetobacter* spp. are the most common gram negative microorganisms.7,10-13 Physical condition of host and virulence factors of microbial flora enhances the risk of disease progression.14,15 Unfortunately resistant pathogens are continue to develop and spread in the environment and as a result effectiveness of antibiotics is being diminished day by day. Moreover, vigorous bacterial resistance was reported against the latest
Honey is the natural sugary substance collected and stored by honey bees from flower. The honey has been used from ancient times as a method of accelerating wound healing, and the potential of honey to assist with wound healing has been demonstrated repeatedly. There are many reports in the clinical literature of honey being used with success in treatment of a wide range of burn wound infection. It inhibits a broad spectrum of bacterial species. Honey is gaining acceptance as an agent for the treatment of ulcers, bed sores and other skin infections resulting from burns and wounds because the antibacterial properties of honey speed up the growth of new tissue to heal the wound. However, one of the most important properties seems to be its antibacterial action. High sugar concentration and low pH of honey is very effective to prevent microbial growth. Besides honey absorbs water out from the environment and as a consequence’s bacteria dehydrated. Previous studies revealed that the honey exhibit effectiveness against methicillin-resistant S. aureus (MRSA), beta hemolytic Streptococci and vancomycin resistant Enterococci (VRE).

Multidrug resistance bacterial strains become apparent because of over and non-selective use of antibiotics especially methicillin-resistance Staphylococcus aureus which is the major contributor of skin infections. To get over this global challenge, like plants and plant-based products such as honey have currently get more attention. Considering all these fact, we designed the study to investigate the antimicrobial traits of honey against the isolate collected from burn wounds.

**METHODS**

**Sampling from burn wound**

Taken consent from the patient of burn unit of Dhaka Medical College Hospital (within April 2019 to July 2019) surface swabs were collected from burn wounds after the removal of dressings and topical antimicrobial agents and cleansing of the wound surface with 70% alcohol. Specimen was collected on sterile cotton swab by rotating with sufficient pressure. Samples were homogenized in 4 ml sterile saline.

**Microbiological and biochemical analysis**

Samples were immediately cultured on NA (nutrient agar), Mannitol salt agar (MSA), MacConkey agar and PA (Pseudomonas agar) plates for the isolation of Total viable bacteria, Staphylococcus spp., coliform group bacteria and Pseudomonas spp. respectively. After inoculation, plates were kept at 37°C for 24-48h. A series of several biochemical tests were performed following the standard protocol to identify the bacteria isolated from the wound samples.

**Determination of antimicrobial susceptibility**

The standard agar disc diffusion method known as the Kirby-Bauer method was applied. A suspension of the test organisms were prepared by adjusting the turbidity of the broth in phosphate buffer saline by comparing with McFarland 0.5 solutions. Each bacterial was prepared on Muller Hinton agar plates by sterile cotton swab. Commercially available antimicrobial discs (Oxoid, Hampshire, UK) were applied aseptically (neomycin 10 µg, chloramphenicol 10 µg, polymyxin B 30 µg, ofloxacin 5 µg, amoxicillin 10 µg, ciprofloxacin 5 µg, cefpodoxime 30 µg, nalidixic acid 30 µg, imipenem 10 µg, tetracycline 30 µg) on the surface of the inoculated plates at appropriate spatial arrangement by means of a sterile needle. Susceptibility to the specific antibiotic was interpreted by the presence of clear zone around the disc.

**Honey sampling**

Three kinds of natural honey (honey from Khalisha tree, Poshur tree and Gewa tree) were collected from beekeepers of the Sundarbon zone and three types of processed honey of different brands were collected from super shop by using purposive sampling technique. Honey was collected in sterile screwed cups/culture bottle.

**Preparation of honey solutions**

Hundred percent pure honey (100% v/v) was obtained after filtration using sterile gauze. To get 1 ml of 75%, 50% and 25% concentrated honey solution (v/v); 0.75 ml, 0.5 ml and 0.25 ml distilled water constitutively. For processed honey same methods are followed.

**Determination of antimicrobial efficiency of honey**

The antimicrobial activity both natural and processed honey samples were performed by agar well diffusion method. At first, the inoculum (with standard turbidity compared to that of the McFarland standard of 0.5) of each of the test bacteria; i.e., Pseudomonas spp., Klebsiella sp., Staphylococcus sp. and E. coli was prepared and by using sterile cotton swab uniform lawns were produced on MHA. Wells were then made spanning the MHA by means of sterile cork borer. 100 µl of honey with the concentration of 75%, 50%, and 25% was added to the wells in the plate. Plates were incubated at 37°C for 12 h. The mean diameters of inhibition zones were measured in mm, and the results were recorded. A positive control well was equally filled with vancomycin 30µg, while sterile distilled water used as negative control.
RESULTS

Prevalence of microorganisms in burn wound samples

Out of 10 samples, 8 were found to be hugely populated with bacteria ranging from $10^3$-$10^5$ CFU/ml, among which almost all were found to harbor Pseudomonas spp. in the range of $(10^1$-$10^5$ CFU/ml) and S. aureus $(10^2$-$10^6$ CFU/ml). Among the enteric bacteria, Klebsiella spp. was found to prevail among 6 samples in the range of $(10^3$-$10^4$ CFU/ml) and a comparative lower frequency was observed in case of E. coli (in 2 samples) (Table 1).

Drug-resistance traits of the isolates

Out of 10 common antibiotics, amoxicillin, tetracycline and chloramphenicol were found to be effective against E. coli isolates. Imipenem, tetracycline and chloramphenicol were found to be effective against Klebsiella spp. Imipenem and cefpodoxime were found effective against Pseudomonas spp. and ciprofloxacin, tetracycline and ofloxacin were found to be effective against S. aureus (Table 2).

Bacteriostatic/bactericidal efficacy of natural honey and processed honey

Table 3 and 4 demonstrate the inhibitory action of three natural honey and three processed honey on the tested bacterial strains. Different types of honey possess different efficacies and mechanisms against the same type of bacteria. 100% concentrated honey samples exhibited best results against almost all isolates. 100% Khalisha flower honey showed its highest antibacterial activity against S. aureus (38 mm) and Pseudomonas spp. (25 mm).

Table 1: Bacterial load (CFU/ml) in burn wound samples.

| Sample | Total viable bacteria (CFU/ml) | Pseudomonas spp. (CFU/ml) | S. aureus (CFU/ml) | E. coli (CFU/ml) | Klebsiella spp. (CFU/ml) |
|--------|-------------------------------|---------------------------|-------------------|-----------------|-------------------------|
| 01     | 2.13x10^6                    | 6.8x10^3                  | 1.12x10^5         | 0               | 6.7x10^3                |
| 02     | 1.48x10^6                    | 8.9x10^4                  | 1.8x10^4          | 0               | 6x10^3                  |
| 03     | 2.33x10^6                    | 6.3x10^4                  | 4.5x10^4          | 0               | 0                       |
| 04     | 3.19x10^6                    | 1.32x10^5                 | 2.7x10^4          | 0               | 1.7x10^4                |
| 05     | 1.40x10^6                    | 7.9x10^4                  | 2.8x10^4          | 4x10^3          | 0                       |
| 06     | 9.8x10^3                     | 2.5x10^5                  | 3.1x10^3          | 0               | 0                       |
| 07     | 3.65x10^6                    | 7.7x10^4                  | 4.3x10^4          | 0               | 6.5x10^4                |
| 08     | 7.6x10^3                     | 1.6x10^5                  | 9x10^3            | 0               | 0                       |
| 09     | 4.24x10^6                    | 1.43x10^5                 | 8.7x10^4          | 0               | 3.4x10^4                |
| 10     | 1.54x10^6                    | 1.21x10^5                 | 3.3x10^3          | 7x10^3          | 9x10^3                  |

All the experiments have been performed three times and one reproducible data has given.

Table 2: Antimicrobial susceptibility pattern of different pathogenic isolates in the burn wound sample.

| Organisms antibiotics | E. coli (n=2) | Klebsiella spp. (n=6) | Pseudomonas spp. (n=10) | Staphylococcus spp. (n=10) |
|-----------------------|---------------|-----------------------|-------------------------|---------------------------|
|                       | R (%)         | S (%)                 | R (%)                   | S (%)                     | R (%)       | S (%)       |
| CIP (5 μg)            | 67            | 33                    | 98                      | 2                         | 70          | 30          | 40          | 60          |
| CPD (30 μg)           | 80            | 20                    | 100                     | 0                         | 34          | 66          | ND          | ND          |
| AMO (10 μ)            | 33            | 67                    | 87                      | 12                        | 80          | 20          | 100         | 1           |
| IPM (30 μg)           | 90            | 10                    | 0                       | 100                       | 20          | 80          | ND          | ND          |
| N (10 μg)             | 73            | 27                    | 60                      | 40                        | ND          | ND          | ND          | ND          |
| CHL (10 μg)           | 45            | 55                    | 24                      | 76                        | 66          | 34          | ND          | ND          |
| TE (30 μg)            | 20            | 80                    | 18                      | 82                        | ND          | ND          | 30          | 70          |
| PB (30 μg)            | 80            | 20                    | ND                      | ND                        | 40          | 60          | ND          | ND          |
| NA (30 μg)            | 80            | 20                    | 75                      | 25                        | 40          | 60          | ND          | ND          |
| OFL (5 μg)            | 70            | 30                    | ND                      | ND                        | ND          | ND          | 22          | 78          |

S - susceptibility, R - resistance, ND - not done, (CIP - ciprofloxacin, CPD - cefpodoxime, AMO - amoxicillin, IMP -imipenem, N - neomycin, CHL - chloramphenicol, PB - polymyxin B, NA - Nalidixic acids, OFL - ofloxacin, TE -tetracycline.)
Table 3: Anti-bacterial activity of natural honey against burn wound isolates.

| Raw honey                        | Zone of inhibition in diameter (mm) |   |   |   |
|----------------------------------|------------------------------------|---|---|---|
|                                  | E. coli (n=2)                      | Klebsiella spp. (n=6) | Staphylococcus spp. (n=10) | Pseudomonas spp. (n=10) |
| Khalisha flower honey            |                                     |   |   |   |
| 100% concentrated                | 14                                  | 15 | 38 | 25 |
| Khalisha flower honey            |                                     |   |   |   |
| 75% concentrated                 | 0                                   | 0  | 22 | 14 |
| Khalisha flower honey            |                                     |   |   |   |
| 50% concentrated                 | 0                                   | 0  | 15 | 0  |
| Poshur flower honey              |                                     |   |   |   |
| 100% concentrated                | 16                                  | 8  | 42 | 23 |
| Poshur flower honey              |                                     |   |   |   |
| 75% concentrated                 | 0                                   | 0  | 21 | 12 |
| Poshur flower honey              |                                     |   |   |   |
| 50% concentrated                 | 0                                   | 0  | 16 | 8  |
| Gewa flower honey                |                                     |   |   |   |
| 100% concentrated                | 15                                  | 13 | 36 | 18 |
| Gewa flower honey                |                                     |   |   |   |
| 75% concentrated                 | 0                                   | 0  | 23 | 16 |
| Gewa flower honey                |                                     |   |   |   |
| 50% concentrated                 | 0                                   | 0  | 14 | 0  |

All the experiments have been performed three times and one reproducible data has given.

Table 4: Anti-bacterial activity of processed honey against burn wound isolates.

| Processed honey | Zone of inhibition in diameter (mm) |   |   |   |
|-----------------|------------------------------------|---|---|---|
|                 | E. coli (n=2)                      | Klebsiella spp. (n=6) | Staphylococcus spp. (n=10) | Pseudomonas spp. (n=10) |
| Sample 1        |                                     |   |   |   |
| 100% concentrated| 12                                  | 15 | 32 | 18 |
| Sample 1        |                                     |   |   |   |
| 75% concentrated| 9                                   | 0  | 24 | 10 |
| Sample 1        |                                     |   |   |   |
| 50% concentrated| 0                                   | 0  | 13 | 0  |
| Sample 2        |                                     |   |   |   |
| 100% concentrated| 9                                   | 8  | 37 | 17 |
| Sample 2        |                                     |   |   |   |
| 75% concentrated| 0                                   | 0  | 21 | 9  |
| Sample 2        |                                     |   |   |   |
| 50% concentrated| 0                                   | 0  | 12 | 0  |
| Sample 3        |                                     |   |   |   |
| 100% concentrated| 11                                 | 10 | 40 | 13 |
| Sample 3        |                                     |   |   |   |
| 75% concentrated| 0                                   | 0  | 26 | 11 |
| Sample 3        |                                     |   |   |   |
| 50% concentrated| 0                                   | 0  | 16 | 0  |

All the experiments have been performed three times and one reproducible data has given.

75% and 50% concentrated forms of this honey have no anti-bacterial activity against E. coli and Klebsiella spp. but a significant zone of inhibition recorded against S. aureus in its all three concentration. In case of Poshur flower honey and Gewa flower honey a wide clear zone of inhibition was observed against S. aureus (42 mm and 36 mm respectively). Other three isolates also exhibited remarkable zone of inhibition when exposed to 100% concentrated honey. However, 75% and 50% concentrated honey had no antibacterial activity on E. coli and Klebsiella spp. and limited activity on Pseudomonas spp. Moderate zone of inhibition was reported in case of S. aureus. On the other hand, all the three processed samples were able to effectively inhibit the growth of S. aureus and Pseudomonas spp. Unlike natural honey, 100% concentrated processed honey reveal a clear zone of inhibition against S. aureus and
Pseudomonas spp., E. coli and Klebsiella spp. were remained unaffected at 75% and 50% concentration of honey.

DISCUSSION

Effective drug against wound infections have been a problem in the field of medicine for a long time and nowadays antimicrobial resistance increases which leads, to a continued search for new agents. Broad spectrum antibacterial activity of honey against gram positive and negative bacteria had reported earlier. Floral honey has expressed efficacy against S. aureus, E. coli and Klebsiella spp. which can be vary from more than 100 folds, depending on its geographical, seasonal and botanical source as well as harvesting, processing and storage conditions. Honey contains sugar mainly (glucose, fructose, sucrose) in high concentration up to 82%, H₂O₂, phenolic compounds, phytochemical components such as methylglyoxal and a wide range of minerals those are effective for the treatment of infections, burns, wounds. Hydrogen peroxide is the major contributor to the antimicrobial activity of honey, and the different concentrations of this compound in different honeys result in their varying antimicrobial effects. Therefore, it has been shown that the antimicrobial activity of honey may range from concentrations <3% to 50% and higher. Our present study exhibited that 100% concentrated honey showed the higher effectiveness, on the contrary 75% and 50% concentration of honey expressed less activity. Besides H₂O₂, an endogenous enzyme glucose oxidase, produced by honey has also antimicrobial activity. The bactericidal effect of honey is reported to be dependent on concentration of honey used and the nature of the bacteria. The antibacterial property of honey is also derived from the osmotic effect of its high sugar content and low moisture content, along with its acidic properties derived from the osmotic effect of its high sugar content.

In current study, we observed that compare to the antibiotics, honey has the better antimicrobial activity against pathogenic isolates and some studies proved that honey has a potential role in the decontamination of wound-infecting antibiotic-resistant strains of bacteria like MRSA. This evidence supports the existing local traditional practice of using honey to treat wound infections.

CONCLUSION

Currently, the emerging antimicrobial resistance trends are a serious challenge to limiting virulence properties of burn wound bacterial pathogens. Therefore, honey is very promising natural antimicrobial agent. In our current investigation both natural and processed honey proved their efficiency against burn wound infectious agent whereas commercial antibiotics found less functional. From ancient period to till date honey act as a healing agent so it could definitely be listed as a potential therapeutic agent.

ACKNOWLEDGEMENTS

Authors would like to thank Microbiology Laboratory, Stamford University Bangladesh for laboratory facilities.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

REFERENCES

1. Dai T, Huang YY Sharma SK, Hashmi JT, Kurup DB, Hamblin MR. Topical antimicrobials for burn wound infections. Recent Pat Anti-infect Drug Discov. 2010;5(2):124-51.
2. Ozbalkan Z, Aslar AK, Yildiz Y, Aksaray S. Investigation of the course of pro inflammatory and anti-inflammatory cytokines after burn sepsis. Int J Clin Pract. 2004;58(2):125-9.
3. Alum SMS, Kalam MA, Munna MS, Munshi SK, Noor R. Isolation of pathogenic microorganisms from burn patients admitted in Dhaka Medical College and Hospital and demonstration of their drug resistance traits. Asian Pac J Trop Dis. 2014;4(5):402-7.
4. Abreu AC, Tavares RR, Borges A, Mergulhao F, Simoes M. Current and emergent strategies for disinfection of hospital environments. J Anti-micro Chemother. 2013;68(12):2718-32.
5. Taneja N, Chari P, Singh M, Singh G, Biswal M, Sharma M. Evolution of bacterial flora in burn wounds: key role of environmental disinfection in control of infection. Int J Burns Trauma. 2013;3(2):102-7.
6. Danzmann L, Gastmeier P, Schwab F, Vonberg RP. Health care workers causing large nosocomial outbreaks: a systematic review. BMC Infect Dis. 2013;13:98.
7. Bayram Y, Parlak M, Aypak C, Bayram I. Three years review of bacteriological profile and antimicrobial of burn wound isolates in Van, Turkey. Int J Med Sci. 2013;10(1):19-23.
8. Askarian M, Hosseini RS, Kheirandish P, Assadian O. Incidence and outcome of nosocomial infections in female burn patients in Shiraz, Iran. Am J Infect Control. 2004;32:23-6.
9. Chim H, Tan BH, Song C. Five years review of infections in a burn intensive care unit: high incidence of Acinetobacter baumannii in a tropical climate. Burns. 2007;33(8):1086-14.
10. Church D, Elsayed S, Reid O, Winston B, Lindsay R. Burn wound infections. Clin Microbiol Rev. 2006;19(2):403-34.
11. Bayram Y, Parlak M, Aypak C, Bayram I. Three years review of bacteriological profile and antimicrobial of burn wound isolates in Van, Turkey. Int J Med Sci. 2013;10(1):19-23.
12. Wilbennmeyer LA, Kealey GP, Latenser BA, Diekman DJ, Williams IM, Coffman SL, et al.
Emergence of the USA300 strain of methicillin-resistant *Staphylococcus aureus* in a burn-trauma unit. J Burn Care Res. 2008;29:790-7.

13. Macedo DJL, Santos JB. Bacterial and fungal colonization of burn wounds. Mem Inst Oswaldo Cruz. 2005;100(5):535-9.

14. Ramakrishnan MK, Sankar J, Venkatraman J, Ramesh J. Infections in burn patients: experience in a tertiary care hospital. Burns. 2006;32(5):594-6.

15. Sharma BR, Harish D, Singh VP, Bangar S. Septicemia as a cause of death in burns: an autopsy study. Burns. 2006;32(5):545-9.

16. Levy SB, Marshall B. Anti-bacterial resistance worldwide: causes, challenges and responses. Nat Med. 2004;10:122-9.

17. Basualdo C, Sgroy V, Finola MS, Juan M. Comparison of the antibacterial activity of honey from different provenance against bacteria usually isolated from skin wounds. Vet Microb. 2007;124:375-81.

18. Vallianou N, Gounari P, Skourtis A, Panagos J, Kazazis C. Honey and its anti-inflammatory, antibacterial, and antioxidant properties. Gen Med. 2004;2(1):132-7.

19. Berg VAJ, Worm VDE, Ufford VHC, Halke SB, Hoekstra MJ, Beukelman CJ. An *in vitro* examination of the antioxidant and anti-inflammatory properties of buckwheat honey. J Wound Care. 2008;17:172-8.

20. Molan PC. The evidence supporting the use of honey as a wound dressing. Int J Low Extreme Wounds. 2006;5:40-54.

21. Simon A, Traynor K, Santos K, Blaser G, Bode U, Molan P. Medical honey for wound care - still the ‘Latest Resort’. Evid Based Complement Alternat. 2009;6(2):165-73.

22. Lusby PE, Coombes AL, Wilkinson JM. Bactericidal activity of different honeys against pathogenic bacteria. Arch Med Res. 2005;36:464-7.

23. Cooper RA, Molan PC, Harding KG. Honey and gram-positive cocci of clinical significance in wounds. J Appl Microbiol. 2002;93:857-63.

24. Lusby PE, Coombes A, Wilkinson JM. Honey: a potent agent for wound healing. J Wound Ostomy Continence Nurs. 2002;29:295-300.

25. Rani GN, Budumuru R, Bandaru NR. Anti-microbial Activity of Honey with Special Reference to Methicillin Resistant *Staphylococcus aureus* (MRSA) and Methicillin Sensitive *Staphylococcus aureus* (MSSA). J Clin Diagn Res. 2017;11(8):5-8.

26. Kacaniya M, Vukovic N, Bobkova A, Fikselova M, Rovna K, Hascik P, et al. Anti-microbial and anti-radical activity of Slovakian honeydew honey samples. J Microbiol Biotechnol Food Sci. 2011;1(3):354-60.

27. Nur IT, Baishnab R, Tethee NS. Microbiological quality analysis of domestic water collected from the slum area’s people in Dhaka city. Stamford J Microbiol. 2017;7(1):19-22.

28. Talukder M, Nur IT. Microbiological analysis of commonly used toothpaste samples in Bangladesh. Stamford J Microbiol. 2019;8(1):38-40.

29. Sharmin M, Nur IT, Acharjee M, Munshi SK, Noor R. Microbiological Profiling and demonstration of *in vitro* Anti-bacterial Traits of the Major Oral Herbal Medicines Used in Dhaka Metropolis. Springer Plus. 2014;3:739.

30. Almasaudi SB, Nahari AAM, Abd ESM, Ghany E, Barbour E, Muhayawi SMA, et al. Anti-microbial effect of different types of honey on *Staphylococcus aureus*. Saudi J Biol Sci. 2017;24(6):1255-61.

31. Mama M, Teshome T, Detamo J. Anti-bacterial Activity of Honey against Methicillin-Resistant *Staphylococcus aureus*: A Laboratory-Based Experimental Study. Int J Microbiol. 2019:9:7686130.

32. Sule AM, Thanni LOA, Odu SOA, Olusanya O. Bacterial pathogens associated with infected wounds in Ogun State University Teaching Hospital, Sagamu, Nigeria. Af J Clin Experimental Microbiology. 2002;3(1):13-6.

33. Pyrzynska K, Biesaga M. Analysis of phenolic acids and flavonoids in honey. Trens Anal Chem. 2009:28:893-902.

34. Mandal MD, Mandal S. Honey: its medicinal property and antibacterial activity. Asian Pac J Trop Biomed. 2011;1(2):154-60.

35. Mavric E, Wittmann S, Barth G, Henle T. Identification and quantification of methylglyoxal as the dominant antibacterial constituent of manuka (*Leptospermum scoparium*) honeys from New Zealand. Mol Nutr Food Res. 2008;52(4):483-9.

36. Mohapatra DP, Thakur V, Brar SK. Anti-bacterial efficacy of raw and processed honey. Biotechnol Res Int. 2011:917505.

37. Wilkinson JM, Cavanagh HM. Anti-bacterial activity of 13 honeys against *Escherichia coli* and *Pseudomonas aeruginosa*. J Med Food. 2005;8:100-3.

38. Badawy OFH, Shafii SSA, Tharwat EE, Kamal AM. Antibacterial activity of bee honey and its therapeutic usefulness against *Escherichia coli* O157:H7 and *Salmonella typhimurium* infection. Rev Sci Technol Int Epiz. 2004;23:1011-122.

39. Maddocks SE, Jenkins RE, Rowlands RS, Purdy KJ, Cooper RA. Manuka honey inhibits adhesion and invasion of medically important wound bacteria in vitro. Future Microbiol. 2013;8(12):1523-36.

40. George NM, Cutting KF. Anti-bacterial Honey (Medi-honey): in vitro activity against clinical isolates of MRSA, VRE, and other multi-resistant Gram-negative organisms including *Pseudomonas aeruginosa*. Wounds. 2007;19(9):231-6.

Cite this article as: Nur IT, Jahan T, Akter S. Isolation of pathogenic microorganisms from burn patient and *in vitro* determination of antibacterial activity of honey against antibiotic resistance isolates. Int J Sci Rep 2020;6(8):310-5.