UJMR, Vol. 7 No. 2, December, 2022, pp. 19 - 23 ***E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668***

https://doi.org/10.47430/ujmr.2272.003

Received: 13th September, 2022 **Accepted:** 13th October, 2022

Antibacterial Activity of Imported Honey against Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolated from Infected Wounds

[*1]Khadija Muhammad Lawan, [2]Binta Muhammad Aminu, [3]Abdulrazak Muhammad Hussain and [4]Abdulmumin Yunusa

[1]Department of Science Laboratory Technology, Kano University of Science and Technology, Wudil P.M.B 3244, Kano

[2]Department of Microbiology, Bayero University. P.M.B. 3011, Kano

[3]Department of Biological Sciences, Sule Lamido University, P.M.B 048, Kafin Hausa

[4]Department of Biochemistry, Kano University of Science and Technology, Wudil P.M.B 3244, Kano

[*]Correspondence Author: khadijamuhammadlawan@gmail.com, **07039650028**

Abstract

The use of traditional and herbal medicine to treat infections has been in practice since the origin of mankind, and it was the only option for treatment before the era of antibiotics. This research work was aimed at evaluating the antibacterial activity of imported honey against methicillin resistant Staphylococcus aureus isolated from different wound infections.152Samples from different wounds were obtained from patients with infected wounds attending Murtala Muhammad Specialist Hospital and Muhammadu Abdullahi Wase Specialist Hospital, Kano. The isolates were identified and confirmed using biochemical tests. The antibacterial activity of the honey and minimum inhibitory concentration (MIC) against the isolates were determined using agar well diffusion and two-fold dilution method respectively. The results to detect MRSA revealed its incidence as 24.9%. The result of the antibacterial activity of honey revealed activity at all the concentrations with the diameter of zones of inhibition ranging from 8-34mm.The two imported honey used in the study showed varied bacteriostatic activities, and none of the isolates was resistant to the tested honeys. Therefore, the antibacterial activity of honey even at lower strength justify their efficacy in the treatment of wound infection.

Keywords: Honey, Wound infection, Methicillin resistant Staphylococcus aureus, Antibacterial activity.

INTRODUCTION

The use of traditional and herbal medicine to treat infections was practiced since the origin of mankind, and it was the only option to treat before the era of antibiotics (Jawad, 2011).A variety of plants and their extracts have been used for treatment requiring antimicrobial activity, and one of the popular natural antimicrobial substances described in the ancient medicine is honey (Mandal and Mandal, 2011).

Several studies revealed the antibacterial activity of honey against both Gram positive and Gram negative bacteria (Al-waili, 2004; Khadija *et al.*, 2018). 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 (Molan, 1992).

Wound infection could be defined as the presence of pus in a lesion, as well as other general or local features of sepsis including pyrexia, pain and indurations (Shija, 1976).

According to level of contamination, a wound can be classified as clean wound or contaminated wound. Clean wound is made under sterile conditions where there are no organisms present and the skin is likely to heal without complications. Contaminated wound usually result from accidental injury and contain pathogenic organisms and foreign bodies in the wound; Infected wound has pathogenic organisms present and multiplying, exhibiting clinical signs of infection (yellow appearance, soreness, redness, oozing pus) and colonized wound is a chronic situation, containing pathogenic organisms which are difficult to heal (i.e. bedsore) (Shija, 1976).

Methicillin-resistant *Staphylococcus aureus* is a bacterium responsible for several difficult-to-treat infections in humans (Yan *et al.*, 2013).

Methicillin Resistance *Staphylococcus aureus* are any strains of *Staphylococcus aureus* that have developed, through horizontal gene transfer and natural selection, multi-resistance to beta-lactam antibiotics, which include penicillins (methicillin, dicloxacillin, nafcillin, oxacillin,) and cephalosporins (cefuroxime, cephalaxin). MRSA evolved from horizontal gene transfer of the *mecA* gene to at least five distinct *Staphylococcus aureus* lineages (Yan *et al.*, 2013). The aim of this work is to evaluate the antibacterial activity of imported honey against methicillin resistant *Staphylococcus aureus* from wound infections.

MATERIALS AND METHODS

Preparation of honey sample

Two imported honey were obtained from departmental stores andwas placed in a sterile universal container which was handled aseptically and protected from bright light to prevent photo-degradation of the glucose oxidase enzyme. The honey samples were diluted with physiological saline to 100, 75, 50%and 25% (Chauhan *et al.*, 2010). Ciprofloxacin was used as control during Minimum Inhibitory Concentration test.

Bioassay

Wound swab collection

Wound swab was collected according to the procedure of Cheesbrough, (2006). A sterile swab stick was used to collect cells or pus from a superficial wound site. From deeper wounds, aspirations of fluid into a syringe were collected with the help of health personnel in Murtala Muhammad specialist Hospital and Muhammadu Abdullahi Wase Hospital, Kano.

The wound samples were categorized into surgical wounds, Burn, Diabetic foot ulcer, Osteomyelitis, Abscess, and Laceration wounds respectively.

Isolation and identification of test isolates

Wound swabs and aspirates collected were inoculated onto the surface of Mannitol Salt Agar plate using streak plate technique (Cheesbrough, 2006).

This was followed by incubation at 37°C for 20hours aerobically. After 24 hours of incubation, the cultured plates that yield growth were considered and the characteristics of such colonies were used to identify the organisms. These organisms were then subjected to biochemical tests (catalase, citrate, urease, coagulase, and gram staining) as described by Cheesbrough (2006).

Standardization of inoculum

Using sterile inoculation wire loop, 3colonies from an overnight culture of the test organism was transferred into a tube of saline until the turbidity of the suspension matched the turbidity of 0.5 McFarland Standard (0.5ml of a 1.175% (w/v) barium chloride dehydrate solution to 99.5ml of 1% (v/v) sulphuric acid) as described by the National Committee for Clinical Laboratory Standard (NCCLS, 2008).

Detection of methicillin-resistant *Staphylococcus aureus* (MRSA) strains

All the *Staphylococcus aureus* isolates identified were subjected to antibiotic susceptibility test by disc diffusion method. In this technique, antibiotic discs were placed onto plates of Mueller Hinton agar that have already been inoculated with the sample strain and the antibiotics (oxacillin, and cefoxitin). The plates were then incubated overnight at 37°C. Following incubation, the zones of inhibition surrounding the various antibiotic discs were measured and compared with Clinical and Laboratory Standard Institute guidelines (2017). Isolates resistant to either oxacillin, cefoxitin or both were considered to be MRSA strains.

Antimicrobial assay/sensitivity for Honey

Antimicrobial susceptibility test was done using the agar well diffusion method (Wasihun and Kasa, 2016). Mueller-Hinton agar was prepared according to the manufacturer's instructions. After solidification, the Mueller-Hinton Agar was inoculated with standardized inoculums under aseptic conditions. Four wells of 6mm in diameter was punched in the agar medium with sterile cork borer and filled with Honey. The honey sample was diluted with physiological saline to 25, 50, 75%respectively and then the non-diluted honey (100%) (Chauhan *et al.*, 2010). The plates were allowed to stand for 15 minutes on a Table to allow pre diffusion of the Honey. The plates were then incubated at 37°C for 24hours. The antibacterial activity was assessed by measuring the diameter of the zones of inhibition using Vernier callipers.

Determination of minimum inhibitoryconcentration (MIC) of honey

The minimum inhibitory concentration of the honey was determined using broth tube dilution method according to Kacaniova *et al.* (2011) procedure. Briefly, ten sterile test tubes were placed in rack, labelled each 1 through 8. Honey control tube (HC) and growth control tube (GC) were used as a quality control. One ml of freshly prepared Mueller-Hinton broth was added to each tube, sterilized and cooled. Then one ml of honey solution 50% was added to test tube number 1 and Honey Control with a sterile micropipette and tips. Then two-fold serial dilution was performed by transferring 1 ml of 25% honey into the second tube with separate sterile micropipette and tips for homogenization. After a thorough mixing, 1 ml was transferred with another sterile micropipette from tube 2 and tube 3. These procedures were repeated until eighth tube

with a dilution of 1:128 was reached and finally 1 ml was taken and discarded from tube 8. The growth control tube received no honey which served as a growth control with the exception of the honey control tube.

Except the honey control tube, each tube was inoculated with 0.1 ml of the standardized inoculum. The tubes were incubated at 37°C for 24 hours and observed for the least concentration without turbidity (Cheesbrough, 2006). Minimum Inhibitory Concentration was recorded as lowest concentration of the honey inhibiting the visible growth of the bacteria.

RESULTS

The result of the prevalence of *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* from different wound infection is presented in Table 1. Among the 101 *Staphylococcus aureus* isolated from surgical wound 54(27.9%) were found to be Methicillin Resistant, 20(22.9%) from burn wound are MRSA, 1(3.5%) from diabetic foot ulcers, 0(0%) from osteomyelitis, 8(42.1%) from abscesses and 2(22.2%) from laceration wound. Therefore, out of the 152 strain of *staphylococcus aureus* isolated 85(24.9%) were found to be Methicillin Resistant *Staphylococcus aureus*.

The result of the antibiotic confirmatory test to detect Methicillin Resistant *Staphylococcus aureus* (MRSA) as presented in Table 2.

The result of the antibacterial activity of the imported honey as well as that of the antibiotic ciprofloxacin at four different concentrations (100, 75, 50 and 25%) against the test bacteria is presented in Table 3. Methicillin Resistant *Staphylococcus aureus* (MRSA) against the two tested honey samples revealed highest zone of inhibition in Imported A honey (22.3±3.7mm) at 25% concentration and the least zone diameter (21.5±4.3) was observed in Imported B honey at 70% concentration.

The result of the minimum inhibitory concentration of Imported A, Imported B as well as that of the test antibiotic ciprofloxacin is presented in Table 4

For Imported A honey it shows MIC of 25% (v/v), While Imported B honey maintained MIC of 50% (v/v) against the tested bacteria.

Table 1: Prevalence of *Staphylococcus aureus* and Methicillin Resistant *Staphylococcus aureus* from Different wounds

Wounds	Number examined	Number of *S. aureus* Isolated (%)	Number of MRSA Detected (%)
SURGICAL WOUNDS	193	101	54 (27.9)
BURN	87	30	20 (22.9)
DIABETIC FOOT ULCER	28	3	1 (3.5)
OSTEOMYELLITIS	6	3	0 (0)
ABSCESS	19	11	8 (42.1)
LACERATION	9	4	2 (22.2)
TOTAL	342	152	85(24.9)

Table 2: Antibiotic susceptibility profile showing diameter of inhibition (mm) for *Staphylococcus aureus*

Antibiotics tested	NIT	Mean diameter(mm)	Range diameter (mm)	NS	NR
Cefoxitin (30ug)	152	19±2.3	13-21	101	51
Oxacillin (1ug)	152	8.3±1.8	8-10	118	34
TOTAL	304			219	85

Key: ± = Mean ± standard deviation CLSI break points for resistance Cefoxitin (30ug) = ≤21mm, Oxacillin (1ug) = ≤10m, NIT = **No of isolate tested,** NS = No. Sensitive, NR = No. Resistant

Table 3: Antibacterial Activity of Imported Honey showing Diameter of Inhibition (mm) Against Methicillin Resistant *staphylococcus aureus*

HONEY		IMPORTED (A)		IMPORTED (B)		CIPROFLOXACIN	
(%) CONC.	NT	S	R	S	R	S	R
25%	85	76	9	58	27	66	19
50%	85	69	16	70	15	73	12
75%	85	54	31	44	41	76	9
100%	85	80	5	66	19	70	15
TOTAL	340	279(82.0)	61(17.9)	238(70)	102(30)	285(83.8)	55(16.1)

KEY: NT= Number of isolate tested S=Sensitive R=Resistance Figures in parenthesis are percentage

Table 4 Minimum Inhibitory Concentration of Honey

Test organisms	Imported A HONEY (A) % (v/v)	Imported B HONEY % (v/v)	CIPROFLOXACIN 5ug % (v/v)
MRSA	25	50	12.5

DISCUSSION

Similar prevalence rate of MRSA (24%) was observed in another study conducted in 2007 to determine prevalence of antimicrobial resistance among pathogenic bacteria isolated from three major hospitals in Khartoum (Al-Sadig, 2007). More so, the results of surveillance studies carried out by Kesah and other researchers (Kesah *et al.,* 2003) in some parts of Africa (Lagos-Nigeria; Cameroon, Kenya and Algeria) and Malta between 1996 and 1997 revealed rates of 21 – 30% MRSA prevalence among the participating countries of the sub-Saharan region (Nigeria, Cameroon and Kenya) while that of North Africa (Algeria) and Malta presented lower rates of below 10%. However, results of similar studies carried out at different locations in Nigeria which include: Ilorin, (Taiwo *et al.,* 2004) Calabar, (Azeez *et al.,* 2008) Jos, (Ikeh, 2003 with Olayinka *et al.,* 2005) revealed higher MRSA prevalence rates of 34.7%, 36.4%, 43% and 49.1% respectively.

The finding of this study is in accordance with the CLSI guidelines where the zone of inhibition that shows resistance for *Staphylococcus aureus* has average mean of (19±2.3mm) for cefoxitin and (8.3±1.8mm) for oxacillin respectively.

The present study showed varying degree of *in vitro* growth inhibition activity of the two imported honeys against the tested organism. In this research MRSA was reported to be the susceptible to all the honey samples tested, this confirms the report by Chauhan and co-workers that the most susceptible gram-positive bacteria to honey is MRSA followed by other gram-negative bacteria (Chauhan *et al.,* 2010 and Kwakwam *et al.,* 2011). Also El-Sukhon *et al,* (1994) showed gram positive bacteria to be more sensitive to action of honey than Gram-negative bacteria. The MIC value (12.5-50% v/v) in this study indicated that all tested honeys have potential bacteriostatic activities against MRSA. This was similar to other studies conducted elsewhere (14.8-50% v/v) (Allen *et al.,* 2000; Getaneh *et al.,* 2013; Ahmed *et al.,* 2014). This result was supported by the study finding of Kingsley (12.5-50% v/v) who conducted study on the use of honey in the treatment of infected wound (Kingsley, 2001).

Mullai and Menon (2007) tested the antibacterial effect of different types of honey against 150 strains of methicillin resistant *Staphylococcus aureus* isolated from otitis media, diabetic foot ulcers and burns wound and they obtained MIC of 20% (v/v), 11% (v/v) and 20% (v/v) from Manuka, Khadikraft and Heather honey respectively.

CONCLUSION

This study revealed higher prevalence of bacterial strain of Methicillin-resistant *Staphylococcus aureus* (MRSA) from wound infection (24.9%), which clearly indicates increase in drug resistance. This therefore necessitates the need for alternative therapy. The study also revealed the excellent antibacterial activity of imported honey against clinical isolates of Methicillin-resistant *Staphylococcus aureus* (MRSA) from infected wound.

REFERENCES

Ahmed, M., Sahile, S., Subramanian, C. (2014) Evaluation of antibacterial potential of honey against some common human pathogens in North Gondar zone of Ethiopia. *International Journal of Pure and Applied Zoology 2(4)*:286-295

Al- Waili, N. S. (2004) Investigating the antimicrobial activity of natural honey and its effects on the pathogenic bacterial infections of surgical wound and conjunctiva, *Journal of Medicinal Food*, 7 (2), pp, 210-222. https://doi.org/10.1089/1096620041224139

Allen, K., Hutchinson, G., and Molan, P. (2000). The potential for using honey to treat wounds infected with MRSA and VRE. In: First world healing congress, Melbourne.

Alsadig, M. A. (2007) "Resistance of Pathogenic Bacteria to Antibiotics in Use in Hospitals and Community," Council of Biological Sciences, New Technologies and Environment Tropical Medicine Programmers' Khartoum, Sudan Academy of Sciences, SAS.

Azeez, O.A., Utsalo, S.J., and Epoke, J. (2008) Distribution and antibiotic susceptibility pattern of methicillin resistant *Staphylococcus aureus*isolates in a University Teaching Hospital in Nigeria. *Sahel Medical Journal*. 11(4): 142-147. https://doi.org/10.4314/smj2.v11i4.12989

Chauhan, A., Pandey, V., Chacko, K.M. and R.K. Khandal (2010). Antibacterial activity of raw and processed honey. *Electronic Journal of Biology 5(3): 58-66*

Cheesbrough, M. (2006). District Laboratory Practice in tropical countries.2 U.K. Cambridge University Press. 80-85 https://doi.org/10.1017/CBO9780511543470

Cheesbrough, M. (2006). District Laboratory Practice in tropical countries.2 U.K. Cambridge University Press. 62-70 https://doi.org/10.1017/CBO9780511543470

Clinical and Laboratory Standards Institute CLSI. 2017. *Performance Standards for Antimicrobial Susceptibility Testing*. 27th edition. CLSI supplement. M100-S19. CLSI, Wayne, PA.

El-Sukhon, S.N., Abu-Harfeil, N., and Sallal, A.K. (1994). Effects of honey on Bacterial Growth and Spore Germination. *Journal of Food Protection*. 57(10): 918-920. https://doi.org/10.4315/0362-028X-57.10.918

Getaneh, A., Belyhun, Y., Moges, F., Anagaw, B., Destaw, B., and Unakal, C. (2013) In vitro assessment of the antimicrobial effect of Ethiopian multi-flora honey on methicillin resistant *Staphylococcus aureus*. *Journal of Current Research Review*, 5(11):64–72.

Ikeh, E., I. (2003) Methicillin - Resistant *Staphylococcus aureus*(MRSA) at Jos teaching hospital. *African Journal of clinical Explanation on Microbiology*. 4(1): 52 - 55. https://doi.org/10.4314/ajcem.v4i1.7324

Jawad M (2011) Antimicrobial effect of bee honey on some pathogenic bacteria isolated from infected wounds in comparison to commonly used antibiotics. *Journal of Basrah Research (Science) 37(4)*:78–83

Kacaniova, M., Vukovic, N., Bobkova, A., Fikselova, M., Rovna, K., Hascik, P. (2011) Antimicrobial and antiradical activity of Slovakian honeydew honey samples. *Journal of Microbiology, Biotechnology and Food Science 1(3)*:354–360

Kesah, c., Rdjeb, B., Odugbemi, T. (2003). Prevalence of methicillin - resistant *Staphylococcus aureus*in eight African hospitals and Malta. *Clinical Microbiology Infections*. 9 (2) 153 - 156. https://doi.org/10.1046/j.1469-0691.2003.00531.x

Khadija, M.L., Binta, M.A and AbdulrazakM.H. (2018). Antibacterial Activity of Local Honey against Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolated from Wound Infection *Bayero Journal of laboratory science* 3(1): 294-301

Kingsley, A. (2001). The use of honey in the treatment of infected wound. *Britain Journal of Nursing 10(22):* S3-6, S18, S20 https://doi.org/10.12968/bjon.2001.10.Sup5.12323

Kwakman, P.H.S..TeVelde, A.A., De Boer, L.,Vandenbroucke-Grauls, C.M.J.E.,Zaat, S.A.J. (2011) Two major medicinal honeys have different mechanisms of bactericidal activity. *PLoS ONE* **2011**, https://doi.org/10.1371/journal.pone.0017709

Mandal MD and Mandal S (2011) Honey: its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine* 1(2):154–160. https://doi.org/10.1016/S2221-1691(11)60016-6

Minisha, D., M.&Shyamapada, M. (2011). Honey: its medicinal property and antibacterial activity. *Asian Pacific Journal of Tropical Biomedicine*. pp. 154-160. https://doi.org/10.1016/S2221-1691(11)60016-6

Molan, P. C. (1992b) The antibacterial activity of honey. 2. Variation in the potency of the antibacterial activity, *Bee World, 73(2)*, pp, 59-76. https://doi.org/10.1080/0005772X.1992.11099118

Mullai, V. and Menon, T. 2007. Bactericidal Activity of Different Types of Honey against Clinical and Environmental Isolates of *Pseudomonas aeruginosa*. *Journal Alternative Complement Medicine*, 13: 439-442. https://doi.org/10.1089/acm.2007.6366

National Committee for Clinical and Laboratory Standard Institute Guidelines 2008. Performance Standards For Antimicrobial Susceptibility Testing; 2008; M100 S 14;Vol.24 No.2

Olayinka, B.O., Olayinka, A.T., Onaolapo, J.A., Olurinola, P.F. Pattern of resistance to vancomycin and other antimicrobial agents in *Staphylococcal* isolates in a university teaching hospital *African Journal of Clinical Explanation on Microbiology*. 2005; 6(1): 21 – 27. https://doi.org/10.4314/ajcem.v6i1.7395

Shija, J.K. (1973) The incidence and pattern of sepsis Among General Surgical in-patients at Muhimbili Hospital, Dar Es salaam. A Preliminary report, *East African Medical Journal* (1976) 53(3) 153-159

Taiwo, S.S., Onile B.A., Akanbi, A.A. (2004) Methicillin - resistant *Staphylococcus aureus*(MRSA) isolates in Nigeria. *African Journal of Clinical Explanation on Microbiology*:5(2): 189 - 197. https://doi.org/10.4314/ajcem.v5i2.7376

Wasihun, A.G and Kasa, G,B. (2016): Evaluation of Antibacterial activity of Honey against Multidrug Resistant Bacteria inAyder Referral and Teaching Hospital, Northern Ethiopia *Springer Plus* 5: 845: 1-8. https://doi.org/10.1186/s40064-016-2493-x

Yan, M., Pamp, S.J., Fukuyama, J., Hwang, P.H., Cho, D.Y., Holmes, S., Relman, D.A., (2013). "Nasal microenvironments and interspecific interactions influence nasal microbiota complexity and *S. aureus* carriage". *Cell Host Microbe. S (6): 631-40. https://doi.org/10.1016/j.chom.2013.11.005*