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

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INTRODUCTION

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. Samples from different wounds were obtained from patients with infected wounds attending Murtala Muhammad Specialist Hospital and Muhammad 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.

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. Samples from different wounds were obtained from patients with infected wounds attending Murtala Muhammad Specialist Hospital and Muhammad 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 for treatment 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-walli, 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, cephalexin). 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 and was 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 Muhammad 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 24 hours aerobically. After 24 hours of incubation, the cultured plates that yielded 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, 3 colonies 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 sterile broth was transferred with another sterile micropipette and tips for each plate). Then one ml of honey solution 50% was added to each tube, sterilized and cooled.

Determination of minimum inhibitory concentration (MIC) of honey
The minimum inhibitory concentration of the honey was determined using broth tube dilution method according to Kacanikova 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.

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

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

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

<table>
<thead>
<tr>
<th>Antibiotics tested</th>
<th>NIT</th>
<th>Mean diameter(mm)</th>
<th>Range diameter (mm)</th>
<th>NS</th>
<th>NR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cefoxitin (30ug)</td>
<td>152</td>
<td>19±2.3</td>
<td>13-21</td>
<td>101</td>
<td>51</td>
</tr>
<tr>
<td>Oxacillin (1ug)</td>
<td>152</td>
<td>8.3±1.8</td>
<td>8-10</td>
<td>118</td>
<td>34</td>
</tr>
<tr>
<td>TOTAL</td>
<td>304</td>
<td>219</td>
<td>85</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Key: ± = Mean ± standard deviation CLSI break points for resistance Cefoxitin (30ug) = ≤21mm, Oxacillin (1ug) = ≤10mm, 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*

<table>
<thead>
<tr>
<th>HONEY</th>
<th>IMPORTED (A)</th>
<th>IMPORTED (B)</th>
<th>CIPROFLOXACIN</th>
</tr>
</thead>
<tbody>
<tr>
<td>(%) CONC.</td>
<td>S</td>
<td>R</td>
<td>S</td>
</tr>
<tr>
<td>25%</td>
<td>85</td>
<td>76</td>
<td>9</td>
</tr>
<tr>
<td>50%</td>
<td>85</td>
<td>69</td>
<td>16</td>
</tr>
<tr>
<td>75%</td>
<td>85</td>
<td>54</td>
<td>31</td>
</tr>
<tr>
<td>100%</td>
<td>85</td>
<td>80</td>
<td>5</td>
</tr>
<tr>
<td>TOTAL</td>
<td>340</td>
<td>279(82.0)</td>
<td>61(17.9)</td>
</tr>
</tbody>
</table>

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


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**Table 4 Minimum Inhibitory Concentration of Honey**

<table>
<thead>
<tr>
<th>Test organisms</th>
<th>Imported A HONEY (A) % (v/v)</th>
<th>Imported B HONEY % (v/v)</th>
<th>CIPROFLOXACIN 5ug % (v/v)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MRSA</td>
<td>25</td>
<td>50</td>
<td>12.5</td>
</tr>
</tbody>
</table>

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