Antibacterial Activity of Honey on Staphylococcus aureus, Escherichia coli and Streptococcus pyogenes Isolated from Wounds

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Authors’ contributions

This work was carried out in collaboration among all authors. Author VUU designed the study, wrote the protocol and first draft of the manuscript. Authors MOE and BNU managed the analyses of the study. Authors AN, NOU and SOA managed the literature searches. Author MEK collected sample and managed the analyses too. All authors read and approved the final manuscript.

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ABSTRACT

Aim: To assess the antibacterial activity of crude honey obtained from Ishielu Local Government of Ebonyi State on pathogenic bacterial species (Staphylococcus aureus, Escherichia coli and Streptococcus pyogenes) isolated from wounds.

Study Design: An experimental study which involved a random selection of patients with wound

Place and Duration of Study: Department of Medical Laboratory Science, Ebonyi State University, Abakaliki, Nigeria, between February 2019 and November 2019.

Methodology: A total of 50 samples of wound swabs collected from different sites of open wounds were cultured on blood agar, chocolate agar and MacConkey agar. The crude honey was diluted to concentrations ranging from 20% to 100% and the antibacterial activity was carried out by well diffusion method with augmenitin used as a control.

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**Result:** Out of the 50 samples, 43 showed growth of bacterial species isolated, identified and confirmed using standard bacteriological techniques. *Staphylococcus aureus* (60.5%) was the most frequent isolates, followed by *Escherichia coli* (27.9%) and *Streptococcus pyogenes* (11.6%). All the tested bacterial isolates were susceptible to the honey and the number of isolates as well as the diameter of zone of inhibition was positively linearly correlated with increasing concentration of the honey (p= 0.00). At 100% honey, 22 (22.25±0.46 mm) out of 26 *Staphylococcus aureus* were susceptible as against 8 (4.62±0.31 mm) at 20% honey. The number of *Escherichia coli* inhibited at 20% honey was 2 (1.96±0.04 mm) out of 12 isolated and at 100%, 7 (19.17±0.31 mm) were inhibited. At 20% honey, no *Streptococcus pyogenes* was inhibited and at 100%, 4 (21.84±0.15 mm) out of 5 isolated were inhibited.

**Conclusion:** Locally produced crude honey may be used as a source of an effective antibacterial agent for wound management.

**Keywords:** Antibacterial activity; honey; *Staphylococcus aureus*; *Escherichia coli*; *Streptococcus pyogenes*, wounds.

### 1. INTRODUCTION

Infections and other health related problems caused by bacterial pathogens poses threat to the existence of humans. Investigation of microbial presence in wounds which began in the late 19th century has improved consistently in techniques involved in recovery, identification and enumeration of a number of microbial species [1]. Most wounds support relatively stable polymicrobial communities [1] often without signs of clinical infection [2]. However, potential pathogens may be present and the delicate balance between an uninfected wound and an infected wound depends on the interplay of a complex host and microbial affects [3]. The development of wound infection has deleterious effect on patients by causing increased pain, discomfort, inconveniences and can lead to life threatening conditions or even death. Antibiotics were regarded as a major medical breakthrough in the 20th century due to its efficacy in the treatment of infections. However, the emergence of multiple antibiotic resistance (MAR) pathogenic bacteria strains including *Staphylococcus aureus*, *Escherichia coli*, as well as *Streptococcus pyogenes* implicated in wounds, threatens the futurist potency of formerly efficacious antibiotics in the treatment of wound infection as well as other infections [4,5].

Aside the increased trauma to the patient and the financial burden implicated in the management of wound infection, it is a major concern among healthcare practitioners considering the increase requirement for cost-effective management within the healthcare system [6,7]. Therefore, knowledge of the causative agents of wound infection become imperative for selective therapy and control measures as well as in the formulation of antibiotic policy [8].

With the increased production of more potent antibiotics over the years such as the third and fourth generation of cephalosporin; though not readily available and expensive, the invasion of pathogenic organism is on the rise [9]. As a result, efforts are being made to develop antibacterial agents from natural resources for improved therapeutic effect [10]. Although, synthetic antimicrobial agents have been approved in many countries. Currently, research focused on natural products from animals or plant, which historically have proven to be important in the identification and development of antibacterial agents [11] in overcoming the emergence of antibiotic resistant pathogens.

Honey has been undoubtedly used for wound dressing even before the discovery of bacteria [12]. Gunther [13], described honey as good for all rotten and hollow ulcers. Honey is a natural sweet substance produced by bees, composed mainly of glucose and fructose, as well as other complex carbohydrates, various amino and organic acids, vitamins, minerals and inhibit [14]. The medicinal uses of honey have been reported among the Egyptians, Chinese, Greeks and the Romans, where it is used for the treatment of wounds and disease of the gut [14]. More recently, honey has been reported to have an inhibitory effect on about 60 species of bacteria including aerobes and anaerobes, gram positive and gram negative. This potency of honey is arrogated to its high content of reducing sugars, low pH, low protein content, presence of hydrogen peroxide, high viscosity, low water activity and high osmotic pressure [15].
Hydrogen peroxide produced by the action of glucose-oxidase, is reported by Alanimat et al. [16] as the major antibacterial agents in honey. Antibiotacl activity of honey has also been reported by Velikova et al. [17] and Marcucci et al. [18].

However, there are few scientific studies on the antibacterial activity of honey and none on the natural honey from Ebonyi State. Therefore, the current study assesses the antibacterial activity of natural honey from Ebonyi State against *Staphylococcus aureus*, *Escherichia coli* and *Streptococcus pyogenes*.

2. MATERIALS AND METHODS

2.1 Bacterial Species

A culture swab collected from various sites of opened wounds from 50 patients who visits the Federal Teaching Hospital, Abakaliki were used, from which three bacterial species were isolated at the Ultramodern Diagnostic and Research Laboratory of the Department of Medical Laboratory Science, Ebonyi State University. The samples were inoculated on blood agar, chocolate agar and MacConkey agar and the Petri dishes were incubated at 37°C for 24-48 hrs. The isolates were identified based on colony characteristics or morphology after which pure colonies were sub-cultured on blood agar, Mannitol salt agar, MacConkey agar and chocolate agar. Further identification or confirmation of isolates was done by standard bacteriological techniques as described by Cheesbrough [19].

2.2 Honey Samples

Crude honey was obtained from local apiarists in Nkalagu, Ishielu Local Government Area of Ebonyi State. The honey was obtained conventionally by uncapping the comb frame, no diluent or additive was added. The honey sample was stored in a lightless place in a clean and closed polyethylene flasks at 20-25°C until required for analysis. The test samples were prepared by diluting the honey with distilled water to produce honey of various concentration, i.e. 20%, 40%, 60%, 80% and 100%.

2.3 Antibacterial Activity Test

The antibacterial activity of honey against the isolated pathogens was tested using a well diffusion method (Kirby-Bauer’s method). Muller-Hinton agar plates, prepared according to manufacturer’s instruction was used. Each plate was inoculated with each isolate and evenly streaked out. Wells of 6mm in diameter were made on the inoculated plates using a sterile cork-borer. Aseptically, each respective well was filled with different concentration of the honey using a sterile dropper. The plates were then incubated at 37°C for 24 hrs. Same procedure was followed in plates where augmentin disc of 6 mm in diameter (30 ug/disc) was used as a control for antibacterial activity. At the end of incubation, the plates were examined for clear area around the wells, indicating the zone of inhibition. These areas were measured in diameter for each isolate.

2.4 Statistical Analysis

The data obtained were expressed in mean± standard deviation. The degree of zone of inhibition was correlated with the various concentration of test sample and statistically analysed using special package for social science (SPSS) version 20.0 for windows. *p*-value of ≤ 0.05 was considered significant.

3. RESULTS

A total of 50 swab samples (n = 50) were collected from different sites of opened wounds of patients who visited Federal Teaching Hospital, Abakaliki, 43 of the samples had growth, while 7 had no growth. Distribution of bacterial species isolated was as follows: 26 (60.5%) *Staphylococcus aureus*, 12 (27.9%) *Escherichia coli* and 5 (11.6%) *Streptococcus pyogenes* (Fig. 1). The relative susceptibility pattern of the isolated bacterial species to the various concentrations of honey showed thus, at concentration of 20%, 40%, 60%, 80% and 100%, the number of each of the bacterial species isolated were 8 (30.7%), 46 (46.2%), 14 (53.8%), 18 (69.2%) and 22 (84.6%), respectively for *Staphylococcus aureus*, 2 (16.7%), 3 (25.0%), 3 (25.0%), 5 (41.7%) and 7 (58.3%), respectively for *Escherichia coli* and 0 (0.0%), 1 (20.0%), 2 (40.0%), 2 (40.0%) and 4 (80.0%), respectively for *Streptococcus pyogenes* (Table 1).

Table 2 lists the degree of inhibition of isolated bacterial species growth and its correlation with the various concentration of honey. *Staphylococcus aureus* was least inhibited at
20% concentration of honey (4.62 ± 0.31). The highest zone of inhibition was found at 100% honey concentration (22.08 ± 0.54). *Escherichia coli* was least and most inhibited at honey concentration of 20% (1.96 ± 0.04) and 40% (19.17 ± 0.31), respectively and *Streptococcus pyogenes* growth was most inhibited at 100% honey concentration (21.84 ± 0.15) and least at 40% (7.21 ± 0.00). The correlation of the degree of inhibition and the varying concentration of the test sample was found to be statistically significant (p= 0.00).

### Fig. 1. Frequency of isolated bacterial species

**Table 1. Number of isolates inhibited at different concentration of honey**

<table>
<thead>
<tr>
<th>Organism isolated</th>
<th>Number (%) of strains inhibited at different honey concentration</th>
<th>r-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20%</td>
<td>40%</td>
<td>60%</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>8 (30.7)</td>
<td>12 (46.2)</td>
<td>14 (53.8)</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>2 (16.7)</td>
<td>3 (25.0)</td>
<td>3 (25.0)</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>0 (0.0)</td>
<td>1 (20.0)</td>
<td>2 (40.0)</td>
</tr>
</tbody>
</table>

1Staphylococcus 2Escherichia 3Streptococcus

**Table 2. Inhibitory efficacy of honey at different concentration**

<table>
<thead>
<tr>
<th>Organism isolated</th>
<th>Number (%) of strains inhibited at different honey concentration</th>
<th>r-value</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20%</td>
<td>40%</td>
<td>60%</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>4.62 ± 0.31</td>
<td>8.86 ± 0.40</td>
<td>12.42 ± 0.42</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>1.96 ± 0.04</td>
<td>3.32 ± 0.11</td>
<td>7.46 ± 0.08</td>
</tr>
<tr>
<td><em>S. pyogenes</em></td>
<td>0.00 ± 0.00</td>
<td>7.21 ± 0.22</td>
<td>11.23 ± 0.22</td>
</tr>
</tbody>
</table>

1Staphylococcus 2Escherichia 3Streptococcus
4. DISCUSSION

The study which was carried out to assess the antibacterial activity of honey from Ishielu Local Government Area of Ebonyi State on some pathogenic microorganisms (Staphylococcus aureus, Escherichia coli and Streptococcus pyogenes) isolated from wounds, confirmed the antibacterial efficacy of honey against the isolated bacterial species. The study also showed Staphylococcus aureus to be the most predominant bacterial species isolated from infected wounds, which is consistent with the study of Aynalem et al. [20], Surajit et al. [21] and Okeke et al. [22]. However, this study varied with the report of Farrag et al. [23] who reported Pseudomonas species as the most common isolates. The 60.5% prevalence of Staphylococcus aureus found in this study was similar to the 69.7% reported by Kibret and Abera [24] and 65.5% reported by Garba et al. [25]. The prevalence was higher than the 28.2% reported by Giacometti et al. [26] and the 25.1% and 25.0% reported by Shittu et al. [8] and Ohalete et al. [27], respectively. This difference may be attributed to difference in hospital facility and management as less pathogenic organism would be isolated from wounds of patients treated in an improved facility.

Recent research showed that honey has an antibacterial effect on pathogenic bacteria of the gastrointestinal tract, urinary tract as well as wound infection [11]. In this study, honey was able to inhibit the growth of the isolated bacterial species and degree or zone of inhibition was found to be positively linearly correlated with the concentration of the honey as increase in concentration of the honey sample showed a corresponding increase in its inhibitory potency. This property of honey may be arrogated to the high osmotic pressure exerted by the sugar content [15]. In all the isolates, the highest number of isolated bacterial species as well as zone of inhibition was recorded at a honey concentration of 100%, while the lowest number of isolated bacterial species, as well as the zone of inhibition was recorded at honey concentration of 20%. Although, at the lowest concentration, no Streptococcus pyogenes was inhibited. Studies which support these findings are those of Reham in 2016 [28], reported a moderate susceptibility of the isolated bacterial to honey at 100% concentration and resistance to honey at 25%. Honey also successfully inhibited the growth rate of pathogenic microorganism isolated from urine sample of patients with urinary tract infection [30].

The antibacterial efficacy of honey is enhanced by the presence of hydrogen peroxide, an important enzyme with antibacterial activity, which is also an oxidizing and sanitizing agent [31]. Other enzymes produced in honey include glucose oxidase, though not activated in undiluted honey [32] but activated when honey is diluted where it react with the endogenous glucose to produce hydrogen peroxide. Hydrogen peroxide has been reported to linearly correlate with the antibacterial activity of honey [33]. Honey is also rich in phenolic compounds which might contribute to its antibacterial activity. These compounds, regarded as non peroxide constituents of honey [34] along with flavonoids have been reported to enhance the antibacterial activity of honey [34].

Honey exhibits other pharmaceutical functions besides antibacterial activity. At a concentration of about 0.1%, honey enhances the proliferation of peripheral lymphocytes in cell cultures [35]. Even though tested honey shows antibacterial effect, literature has shown that not all honey samples have the same degree of antibacterial activity against the same type of bacteria. This is due to differences in osmolarity, viscosity, hydrogen peroxide content, as well as protein content [36].

5. CONCLUSION

This study has confirmed the antibacterial effect of crude honey on common pathogenic bacteria species isolated from wound infections as a promising relief to the condition of antibiotic resistance encounter in clinics. The susceptibility pattern of the predominant isolates; S. aureus, E. coli and S. pyogenes were found to positively linearly correlate with varying concentration of honey. Therefore the use of honey as a non-toxic and cheap natural antibacterial agent should be globalized after being subjected to pharmaceutical standardization and further clinical trials.

CONSENT

It is not applicable.
ETHICAL APPROVAL

All authors hereby declare that all experiments were examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES


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