In Vitro Activity of Natural Honey Alone and In Combination with Certain Types of Antibiotics Against Some Pathogenic Bacteria

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ABSTRACT

The composition of honey, which is a supersaturated sugar solution made from plant nectar and influenced by its geographic and floral origins, influences its health-promoting effects. The aim of this study was to characterize the antibacterial activity of honey against Gram-positive Staphylococcus aureus, the combined antibacterial activity of a mixture of traditional antibiotic Amoxicillin and honey. The front-label descriptors were used to classify a total of 3 commercial kinds of honey. Quality (Hydroxymethylfurfural), color (color intensity, pH, TSS, viscosity, water activity), bioactive composition (phenolic, flavonoid, and carotenoid content), and antibacterial activity (MIC50) of honey were all analyzed. Our in vitro data show that Honey and Amoxicillin have a lot of promise against Staphylococcus aureus, The current study demonstrates that Honey and Amoxicillin have synergistic effects against all strains of Staphylococcus aureus that have been tested and that any combination of Honey reduced the MIC of Amoxicillin by half. When using Amoxicillin and Honey together rather than Amoxicillin or Honey alone, there is a synergistic effect seen in the time-killing curves of certain staphylococcus aureus strains.

INTRODUCTION

The risks posed by bacteria are reduced by antimicrobial medications used to treat infectious disorders, but over time, the proliferation of resistant infections gradually reduces the efficacy of these medications (Alqurashi et al., 2013). Bacterial resistance to antibiotics, including the main last-resort medications, poses a substantial threat to public health (Levy & Marshall, 2004; Mandal et al., 2009). Overuse of antibiotics, particularly in emerging and disadvantaged nations, has the potential to breed community resistance and make the elimination of infectious illnesses exceedingly challenging (Patel & Chauhan, 2016).

Scientists are therefore interested in the development of drugs from naturally occurring substances that have antibacterial properties in order to look for novel antimicrobial agents. Due to these circumstances, it was necessary to reconsider the medicinal value of traditional treatments like honey (Bagde et al., 2013; Mandal et al., 2010).
Since ancient times, honey has been used as both conventional and alternative medicine. Honey is a wonderful functional food with a wealth of nutrients. It possesses a wide range of advantageous biological properties, including antibacterial, antioxidant, anti-browning, angiotensin-converting enzyme (ACE) inhibitory, anti-inflammatory, antiparasitic, and immunosuppressive properties (Chang et al., 2011; León-Ruiz et al., 2013). Recent studies have attributed its therapeutic benefits to the treatment of burns, the healing of infected and chronic wounds, skin ulcers, eye conditions, asthma, gastrointestinal disorders, and it is an anticancer (López-Lázaro, 2007), antimitogenic, antiproliferative, hepatoprotective, and hypoglycemic properties (Al-Waili et al., 2011). About 75% of honey is made up of fructose and glucose, with small amounts of sucrose and a few polysaccharide sugars (Alqarni et al., 2014). However, it also considerably benefits from the presence of minerals, proteins, phenolic compounds, and other minor components (Moniruzzaman et al., 2013). The biological activity and composition of honey vary depending on its botanical source and region (Alzahrani et al., 2012). The key factors influencing honey's antibacterial action are its physical characteristics (osmosis and acidity) and chemical composition (Weston, 2000). Hydrogen peroxide is the major component in honey that contributes to its antibacterial properties.

Honey contains glucose, which is oxidized by the enzyme glucose oxidase to produce hydrogen peroxide. Floral nectar is the source of glucose and other carbohydrates, and honey bee glands release glucose oxidase (León-Ruiz et al., 2013). Due to the high sugar content in the honey, this enzyme is inactive until the honey is diluted (Weston, 2000). In addition to hydrogen peroxide, honey also contains additional compounds that are referred to as "non-peroxide" components that support its antibacterial properties. Like lysozyme (Cliver & Snowdown, 1996), flavonoids (flavones, flavonols, flavanones, and dihydroflavonols), other phenolic compounds (cinnamic acids and their esters), methylglyoxal, and bee peptides, these molecules are proteinaceous in origin (Israili, 2014). The botanical origin, type, and geographic location of a honey all affect its antibacterial action (León-Ruiz et al., 2013). A wide variety of bacterial species are inhibited by honey. It has been documented that honey has an antagonistic impact on almost 60 bacterial species, including aerobes, anaerobes, Gram positives, and Gram-negatives (Hannan et al., 2004).

Numerous studies have found that honey has antibacterial properties that can combat pathogenic bacteria like Acinetobacter baumannii, Bacillus cereus, B. subtilis, Enterobacter aerogenes, Klebsiella pneumonia, Micrococcus luteus, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella typhi, S. Typhimurium, Shigella flexneri, Shigella Sonne (Al-Nahari et al., 2015; Alqurashi et al., 2013; Deng et al., 2018; Hegazi et al., 2017; Kingsley, 2001; Noori et al., 2013; Nzeako & Hamdi, 2000; Rani et al., 2017; Wasihun & Kasa, 2016).

MATERIALS AND METHODS

1. Three Honey Samples:

Three different feeding botanical sources were used in the experiment: one sample of clover honey, one sample of citrus honey, and one sample of Sider honey. Beekeeping Research Dept, Plant Protection Research Institute, Agriculture Research Centre, Dokki, Giza. Each sample was given a code based on its type either the location or the species of bee that collected the honey or both. The local beekeepers who were keeping native honey bees (Apis mellifera jemenitica) in conventional log hives set in various places provided samples of raw honey. Prior to the experiment, all honey samples were kept in a refrigerator at 4 C. To check the sterility of the honey, a loopful of each sample was placed on nutritional agar (Mulu et al., 2004).

2. Bioactive Composition:
2.1. Total Phenolic Content:

With modifications for honey analysis, the Folin-Ciocalteur technique (Kähkönen et al., 1999) was used to estimate total phenolic content (TPC) in triplicate. Using a UV spectrophotometer, absorbance was measured at 765 nm in comparison to a blank of DI water (Multiskan Go, Thermo Scientific, MA, USA). The results were given in milligrams of gallic acid equivalents (mg GAE/g) per gram of honey.

2.2. Total Flavonoid Content:

According to Wu et al. (2008), total flavonoid content (TFC) was calculated. Thermo Scientific's Multiskan Go, located in Massachusetts, USA, was used to measure the absorbance at 510 nm in comparison to a DI water blank. The findings were represented as micrograms of Catechin Equivalents (CE) per gram of honey (g CE/g).

2.3. Carotenoid Content (β-carotene and lycopene):

A modified version of Ferreira et al., 2009 approach was used to calculate the carotenoid equivalents. Initially, 10 mL of a 6:4 hexane-acetone combination was mixed with 2 mL of each 50% (w/v) honey sample before being instantly sonicated for 10 minutes and filtered (Whatman No. 1 filter paper). With a DI water blank as a reference, absorbance was measured spectrophotometrically using a Novaspec Plus visible spectrophotometer from Amersham Biosciences in the UK. The following equations were used to calculate the concentrations of β-carotene (β-CE) and lycopene (LE), with the results given as milligrams of carotenoid per kilogram of honey (mg β-CE/kg; mg LE/kg).

3. Physical and Chemical Attributes:

3.1. Analysis of Color (Color saturation):

Beretta et al. (2005), technique was used to measure the color intensity (2005). The absorbance of the 50% (w/v) honey samples was measured spectrophotometrically (Multiskan Go, Thermo Scientific, MA, USA) at 450 nm and 720 nm after filtering (Whatman No.1 filter paper). The spectrophotometric absorbance difference at the two wavelengths measured against a DI water blank is reported as the color intensity and is quantified as mAU.

3.2. pH At Room Temperature (22.5 C ± 0.660):

The pH of the undiluted honey samples was measured in triplicate using a pH meter (Mettler Toledo, Port Melbourne, Australia) (Aumeeruddy et al., 2019).

3.3. Total Soluble Solids:

A portable digital refractometer (Opti Brix 54, Bellingham = Stanley, Kent, United Kingdom) that was adapted to employ 50% honey dilutions in order to meet required specifications was used to measure the total soluble solids (TSS) (Chan et al., 2017). The results of the measurements are given as Brix and were performed in triplicate.

3.4. Viscosity:

Using a viscometer (Smart Series, Fungilab, Barcelona, Spain) and an R6 spindle at 5, 10, or 20 rpm, depending on the sample's percent torque, the viscosity of the undiluted honey samples (Pa s) was calculated (Yücel & Sultanog, 2013).

3.5. Water Activities:

At room temperature (24.6 C ± 0.702), the Water activity of the undiluted honey samples was measured in accordance with the manufacturer's instructions using a Water activity measuring device (LabSwift-aw, Novasina, Switzerland).

4. Bacterial Isolates and Media:

The bacterial strains utilized in this study came from the Microbiological Laboratory in September 2021, Faculty of Science's Department of Micro. Gram-positive Staphylococcus aureus along with Gram-negative Escherichia coli. These bacteria were grown on nutrient agar slants at 4 C and identified using conventional bacteriological methods in accordance with Harley (2004). The experiment employed nutrient agar and nutrient broth that was made in accordance with the manufacturer's instructions by HiMedia Laboratories Pvt. Ltd. in India.

Creation of Honey Concentrations:

To investigate the antibacterial
activity, two distinct concentrations of each honey sample were created using sterile distilled water. Shimadzu Corporation’s electric balance was used to weigh ten grams of each honey into a 50 mL beaker (Karter Scientific, USA), to which 16 mL and 10 mL of water were added to create concentrations of 80% and 60% (w/v), respectively. The formula $C_1 V_1 = C_2 V_2$ was used to determine the amounts of water and honey needed for various concentrations.

The MICs of Amoxycillin 20% on MRSA were determined using an agar dilution method. Petri plates of Brain Heart Infusion (BHI) agar containing various concentrations of Amoxycillin were inoculated with 24 hrs culture of bacterial strain in triplicates, was individually spread on the surface of the solid wed agar plates. Test and control plates were then incubated at 35°C.

Antibacterial activity testing using a good diffusion method by using an agar well diffusion experiment, the antibacterial activity of honey samples at different concentrations was investigated. Bacterial isolates were put overnight in a shaking incubator (Sheldon Manufacturing, Inc. USA) at 37°C after being inoculated in 10 mL of nutritional broth. The preparation of nutrient agar plates was done according to the manufacturer’s instructions. Using the distal end of a sterile Pasteur pipette, five wells, each measuring 6 mm in diameter, were created in each nutritional agar plate. Each bacterial suspension (10^8 colony forming units (CFU)/mL) was distributed on an individual agar plate using sterile cotton wool before creating wells. Each honey sample was divided into 100 microliters and placed into a different well on the nutrient agar plate. These Petri plates were incubated aerobically for 24 hours at 37°C. Following Barry and Thorsberry's instructions, the diameter of the zone of inhibition surrounding the well's outer surface was measured (1985).

**Calculation of the Minimum Inhibitory Concentration:**

2.5 (MIC) With slight adjustments, the technique of (Wasihun & Kasa, 2016) was used to calculate the MIC of a honey sample. In a platform, eight spotless test tubes (13X100 mm) were arranged. In order to prepare the test tubes for serial dilution, the nutrient broth was made in accordance with the manufacturer’s instructions. One test tube received 2 mL of 100% pure honey as a positive control, whereas another test tube just received 2 mL of nutritional broth without any bacterial suspension (negative control). The next six test tubes received progressive dilutions of the honey sample with 2 mL of nutritional broth, resulting in concentrations of 80%, 60%, 40%, 20%, 10%, and 5% (v/v) respectively. Negative control tubes were not infected. Instead, each tube received a 20 mL dose of bacterial suspension (10^8 CFU/mL) before being incubated at 37°C for 24 hours. Each sample of honey was subjected to the whole procedure in three copies against bacterial strain. Visual checks were performed on the MIC to check for growth (turbidity).

**Statistical Evaluation:**

The average of three replicates standard deviation was used to quantify all antibacterial activity data in terms of ZOI average zone of inhibition (mean ±SD ).

The Statistics 8.1 program was used to do the analysis. To compare all pairwise means, Tukey's Honest Significant Difference (HSD) test was used. At a p-value of 0.05, differences between means were deemed statistically significant. In order to determine the degree of similarity between the tested honey samples based on their botanical origin, the cluster analysis of five honey samples at 80% and 50% concentrations against five bacterial strains was carried out. The honey sample comparisons were performed using ZOI mean values. Using Euclidean distances and Ward's Linkage technique, the dendrogram was created. Software called Past v.3.12 was used to do discriminant analysis.

**Assessment of Killing Curve:**

Selected strains of Staphylococcus aureus isolates were grown overnight in BHI broth medium. 0.2 ml of inoculums was
added to 20 ml Nutrient broth flasks containing antibacterial agent 4 MIC for amoxicillin, (1MIC) for natural extracts, and (1/2MIC of honey plus 2MIC of amoxicillin) for combination. Flasks were then incubated at 35°C without shaking. After 2 hrs the flasks were strongly agitated and a 0.1ml sample was diluted and immediately plated; the Flasks were immediately returned to incubation. (Flandrois et al., 1988).

**Bacterial Count:**

Bacterial counts were performed on Plat count agar after dilution, ranging from 10-1 to 10-6, in sterile saline solution, 0.1ml sample of each dilution was plated into agar medium, and counts were made in triplicate. Colonies were counted after 48 hrs of growth at 35°C. Means were calculated from plates with counts ranging from 20 to 300 CFU/0.1ml (Flandrois et al., 1988).

### RESULTS

Determination of antibacterial activities of amoxicillin µg/ml against Gram-positive Staphylococcus aureus, according to the present results (Table 1) Mean time-growth rate of Staphylococcus aureus exhibit 6.01 log10 CFU/ 0.1ml of nutrient broth medium after 24 hrs of incubation period at 37°C, the time-killing curve of selected strains studied with amoxicillin exhibit 3.75 log10 CFU/0.1ml at 24 hrs of the incubation period.

**Table 1:** Minimal inhibitory concentration (MIC) of amoxicillin on MRSA.

<table>
<thead>
<tr>
<th>Amoxicillin Concentrations</th>
<th>0.4 µg/ml</th>
<th>0.8 µg/ml</th>
<th>2 µg/ml</th>
<th>4 µg/ml</th>
<th>8 µg/ml</th>
<th>16 µg/ml</th>
<th>32 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of susceptible strain</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>11</td>
<td>20</td>
<td>42</td>
<td>50</td>
</tr>
<tr>
<td>% No. 0f susceptible strain</td>
<td>4 %</td>
<td>8 %</td>
<td>16 %</td>
<td>22 %</td>
<td>40 %</td>
<td>84 %</td>
<td>100 %</td>
</tr>
</tbody>
</table>

MIC = Minimal inhibitory concentration. MICns = Number of strains inhibited with (MIC)

Determination of antibacterial activities of honey against Staphylococcus aureus isolates, Minimal inhibitory concentration (MIC) of clover honey (CLO) on Staphylococcus aureus ranged between 35 mg/ml and 50 mg/ml, while MIC of citrus honey (CIT) on Staphylococcus aureus ranged between 35 and 50 mg/ml and MIC of sider honey (SID) on Staphylococcus aureus ranged between 20 and 35 mg/ml. (Table 2).

**Table 2:** Determination of minimal inhibitory concentration (MIC) of honey type on Staphylococcus aureus strains.

<table>
<thead>
<tr>
<th>Clover honey (CLO)</th>
<th>Concentration</th>
<th>20 mg/ml</th>
<th>35 mg/ml</th>
<th>50 mg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of susceptible strain</td>
<td>23</td>
<td>38</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>(%) susceptible strain</td>
<td>46%</td>
<td>76%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Citrus (CIT)</td>
<td>20 mg/ml</td>
<td>35 mg/ml</td>
<td>50 mg/ml</td>
<td></td>
</tr>
<tr>
<td>No. of susceptible strain</td>
<td>27</td>
<td>36</td>
<td>50</td>
<td></td>
</tr>
<tr>
<td>(%) of susceptible strain</td>
<td>54%</td>
<td>72%</td>
<td>100%</td>
<td></td>
</tr>
<tr>
<td>Sider (SID)</td>
<td>20 mg/ml</td>
<td>35 mg/ml</td>
<td>50 mg/ml</td>
<td></td>
</tr>
<tr>
<td>No. of susceptible strain</td>
<td>37</td>
<td>50</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(%) of susceptible strain</td>
<td>74%</td>
<td>100%</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

MIC = Minimal inhibitory concentration, MICns = Number of strains inhibited with minimal inhibitory concentration
Determination of antibacterial activities of amoxicillin in combination with different types of honey against Staphylococcus aureus isolates. Minimal inhibitory concentrations of amoxicillin µg/ml plus clover honey (CLO) mg/ml on Staphylococcus aureus ranged between 0.2 µg/ml of amoxicillin plus 25 mg/ml of CLO and 16 µg/ml of amoxicillin plus 25 mg/ml of CLO, MIC of amoxicillin µg/ml plus citrus honey (CIT) mg/ml on Staphylococcus aureus ranged between 0.2 µg/ml of amoxicillin plus 25 mg/ml of CIT and 16 µg/ml of amoxicillin plus 25 mg/ml of CIT. Moreover, the MIC of amoxicillin µg/ml plus sider honey (SID) mg/ml on Staphylococcus aureus ranged between 0.2 µg/ml of amoxicillin plus 17.5 mg/ml of SID and 16 µg/ml of amoxicillin plus 17.5 mg/ml of SID (Table 3).

**Table 3: Determination of minimal inhibitory concentration (MIC) of Amoxicillin µg/ml in combination with different honey type mg/ml on Staphylococcus aureus.**

<table>
<thead>
<tr>
<th>Amoxicillin plus CLO</th>
<th>0.2 µg/ml</th>
<th>0.4 µg/ml</th>
<th>1µg/ml</th>
<th>2 µg/ml</th>
<th>4 µg/ml</th>
<th>8 µg/ml</th>
<th>16 µg/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>25 mg/ml</td>
<td>25mg/ml</td>
<td>25mg/ml</td>
<td>25mg/ml</td>
<td>25mg/ml</td>
<td>25mg/ml</td>
<td>25mg/ml</td>
<td>25mg/ml</td>
</tr>
<tr>
<td>No. of susceptible strain inhibited with MIC</td>
<td>2</td>
<td>3</td>
<td>2</td>
<td>4</td>
<td>12</td>
<td>16</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>4%</td>
<td>6%</td>
<td>4%</td>
<td>8%</td>
<td>24%</td>
<td>32%</td>
<td>22%</td>
</tr>
<tr>
<td>Amoxicillin plus CIT</td>
<td>0.2 µg/ml</td>
<td>0.4 µg/ml</td>
<td>1µg/ml</td>
<td>2 µg/ml</td>
<td>4 µg/ml</td>
<td>8 µg/ml</td>
<td>16 µg/ml</td>
</tr>
<tr>
<td>25 mg/ml</td>
<td>25mg/ml</td>
<td>25mg/ml</td>
<td>25mg/ml</td>
<td>25mg/ml</td>
<td>25mg/ml</td>
<td>25mg/ml</td>
<td>25mg/ml</td>
</tr>
<tr>
<td>No. of susceptible strain inhibited with MIC</td>
<td>3</td>
<td>3</td>
<td>5</td>
<td>4</td>
<td>14</td>
<td>15</td>
<td>6</td>
</tr>
<tr>
<td></td>
<td>6 %</td>
<td>6%</td>
<td>10%</td>
<td>8%</td>
<td>28%</td>
<td>30%</td>
<td>12%</td>
</tr>
<tr>
<td>Amoxicillin plus SID</td>
<td>0.2 µg/ml</td>
<td>0.4 µg/ml</td>
<td>1µg/ml</td>
<td>2 µg/ml</td>
<td>4 µg/ml</td>
<td>8 µg/ml</td>
<td>16 µg/ml</td>
</tr>
<tr>
<td>17.5 mg/ml</td>
<td>17.5 mg/ml</td>
<td>17.5mg/ml</td>
<td>17.5mg/ml</td>
<td>17.5mg/ml</td>
<td>17.5mg/ml</td>
<td>17.5mg/ml</td>
<td>17.5mg/ml</td>
</tr>
<tr>
<td>No. of susceptible strain inhibited with MIC</td>
<td>3</td>
<td>4</td>
<td>5</td>
<td>5</td>
<td>6</td>
<td>17</td>
<td>10</td>
</tr>
<tr>
<td>(%) of susceptible strain inhibited with MIC</td>
<td>6%</td>
<td>8%</td>
<td>10%</td>
<td>10%</td>
<td>12%</td>
<td>34%</td>
<td>20%</td>
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</tbody>
</table>

Time-killing curve of selected Staphylococcus aureus strains affected with CLO exhibit zero CFU after 24 hrs of incubation period at 37°C, of CIT exhibit zero CFU after 20 hrs of incubation period at 37°C. However, SID did not exhibit zeros CFU/0.1ml after 24 hrs of incubation period at 37°C. (Table 4 & Fig. 1)

Time-killing curve of selected Staphylococcus aureus strains affected with a combination of CLO plus amoxicillin exhibit zero CFU after 20 hrs of incubation period at 37°C, while a combination of CIT plus amoxicillin exhibit zero CFU after 14 hrs of incubation period at 37°C. However, a combination of SID plus amoxicillin exhibit zeros CFU/0.1ml after 10 hrs of incubation period at 37°C. Mean of the time-killing rate of selected strains of Staphylococcus aureus affected with a combination of amoxicillin plus sider honey (SID) exhibit 2.75 log10 CFU/0.1ml after 8 hrs and zero CFU/0.1ml after 10 hrs of incubation period at 37°C. Mean of the time-killing rate of selected strains of Staphylococcus aureus affected with a combination of amoxicillin plus citrus honey (CIT) exhibit 3.95 log10 CFU after 12 hrs and zero CFU after 14 hrs of incubation period at 37°C. Mean of the time-killing rate of selected strains of staphylococcus aureus
affected with a combination of amoxicillin plus CLO exhibit 3.18 log10 CFU after 18 hrs and zero CFU/0.1ml after 18 hrs of incubation period at 37°C. (Table 5 & Fig. 2)

The present study shows that honey produces a synergistic effect with amoxicillin against Staphylococcus aureus strains, while the MIC of amoxicillin in combination with any honey was decreased by half. There is a synergy effect observed in the time-killing curve of selected Staphylococcus aureus strains where amoxicillin is combined with SID than amoxicillin or SID alone.

Table 4: Time-killing rate (Log10CFU/0.1ml) of Staphylococcus aureus affected with Amoxicillin µg/ml and different honey types mg/ml.

<table>
<thead>
<tr>
<th>Hour</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
<th>F-test</th>
<th>L.S.D.</th>
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<tbody>
<tr>
<td>2</td>
<td>6.4</td>
<td>6.4</td>
<td>6.4</td>
<td>6.4</td>
<td>6.4</td>
<td>**</td>
<td>0.051</td>
</tr>
<tr>
<td>4</td>
<td>7.4</td>
<td>7.4</td>
<td>5.59</td>
<td>6.51</td>
<td>6.12</td>
<td>**</td>
<td>0.054</td>
</tr>
<tr>
<td>6</td>
<td>8.18</td>
<td>8</td>
<td>5.31</td>
<td>6.51</td>
<td>5.236</td>
<td>**</td>
<td>0.067</td>
</tr>
<tr>
<td>8</td>
<td>8.36</td>
<td>8.1</td>
<td>4.51</td>
<td>6.72</td>
<td>5.216</td>
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<td>0.032</td>
</tr>
<tr>
<td>10</td>
<td>8.34</td>
<td>7.12</td>
<td>4.44</td>
<td>4.45</td>
<td>5.203</td>
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<td>0.041</td>
</tr>
<tr>
<td>12</td>
<td>8.41</td>
<td>7.01</td>
<td>4.48</td>
<td>4.12</td>
<td>4.97</td>
<td>**</td>
<td>0.049</td>
</tr>
<tr>
<td>14</td>
<td>8.22</td>
<td>6.69</td>
<td>4.11</td>
<td>4.25</td>
<td>4.94</td>
<td>**</td>
<td>0.088</td>
</tr>
<tr>
<td>16</td>
<td>7.44</td>
<td>5.42</td>
<td>4.14</td>
<td>4.2</td>
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<td>**</td>
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</tr>
<tr>
<td>18</td>
<td>7.31</td>
<td>5.49</td>
<td>3.49</td>
<td>4.27</td>
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</tr>
<tr>
<td>20</td>
<td>6.3</td>
<td>4.41</td>
<td>3.2</td>
<td>0</td>
<td>4.19</td>
<td>**</td>
<td>0.087</td>
</tr>
<tr>
<td>24</td>
<td>6.01</td>
<td>3.75</td>
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<td>0</td>
<td>4.091</td>
<td>**</td>
<td>0.59</td>
</tr>
</tbody>
</table>

M1 = mean population (Log10 CFU/0.1ml) of Staphylococcus aureus without any antibacterial agent (control). M2 = mean population (Log10 CFU/0.1ml) of Staphylococcus aureus inhibited with Amoxicillin.M3 = mean population (Log10 CFU/0.1ml) of Staphylococcus aureus inhibited with clover honey (CLO). M4 = mean population (Log10 CFU/0.1ml) of Staphylococcus aureus inhibited with citrus (CIT). M5 = mean population (Log10 CFU/0.1ml) of Staphylococcus aureus inhibited with sider, (SID). ** = Highly significant at 0.1 level of probability, L.S.D. = Least significant difference.

Table 5: Time-killing rate (Log10CFU/0.1ml) of Staphylococcus aureus affected with Amoxicillin µg/ml.

<table>
<thead>
<tr>
<th>Hour</th>
<th>M1</th>
<th>M2</th>
<th>M3</th>
<th>M4</th>
<th>M5</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
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<td>6.4</td>
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M1 = mean population (Log10 CFU/0.1ml) of Staphylococcus aureus without any antibacterial agent (control). M2 = mean population (Log10 CFU/0.1ml) of Staphylococcus aureus inhibited with Amoxicillin. M6 = mean population (Log10 CFU/0.1ml) of Staphylococcus aureus inhibited with Amoxicillin plus CLO. M7 = mean population (Log10 CFU/0.1ml) of Staphylococcus aureus inhibited with Amoxicillin plus CIT. M8 = mean population (Log10 CFU/0.1ml) of Staphylococcus aureus inhibited with Amoxicillin plus SID. ** = Highly significant at 0.1 level of probability. L.S.D. = Least significant difference.
**Fig. 1:** Time-killing curve of Staphylococcus aureus affected with Amoxicillin μg/ml and different types of honey mg/ml.

M1= mean population (Log10 CFU/0.1ml) of Staphylococcus aureus without any antibacterial agent (control). M2= mean population (Log10 CFU/0.1ml) of MRSA inhibited with Amoxicillin. M3= mean population (Log10 CFU/0.1ml) of Staphylococcus aureus inhibited with clover honey (CLO). M4= mean population (Log10 CFU/0.1ml) of Staphylococcus aureus inhibited with citrus (CIT). M5= mean population (Log10 CFU/0.1ml) of Staphylococcus aureus inhibited with sider (SID). **= Highly significant at 0.1 level of probability, L.S.D. = Least significant difference.

**Fig. 2:** Time-killing curve of Staphylococcus aureus affected with Amoxicillin μg/ml in combination with different types of honey.

M1= mean population (Log10 CFU/0.1ml) of Staphylococcus aureus without any antibacterial agent (control). M2= mean population (Log10 CFU/0.1ml) of Staphylococcus aureus inhibited with Amoxicillin. M6= mean population (Log10 CFU/0.1ml) of Staphylococcus aureus inhibited with Amoxicillin plus CHE. M7= mean population (Log10 CFU/0.1ml) of Staphylococcus aureus inhibited with Amoxicillin plus CME. M8= mean population (Log10 CFU/0.1ml) of Staphylococcus aureus inhibited with Amoxicillin plus CAE.

**DISCUSSION**

To combat resistant bacteria internationally, there is now a significant effort being made to create new antibiotics or alter existing ones. Antibiotic resistance develops when bacteria can resist the effects of medications by neutralizing them, pumping them outside of the cell, or changing the exterior structure of the bacterium in a way that prevents the drugs from adhering to it (Silhavy et al., 2010). Because of the widespread and indiscriminate use of antibiotics to treat bacterial infections, resistant strains have emerged and spread, which has caused a change in the antibiotic treatment regimen to second- or third-line
medications, which are frequently more expensive and have more adverse effects (Brook et al., 2000).

Based on the fact that certain studies have looked at how crude plant extracts might speed up the action of antibiotics. Gentamicin (GM) and chloramphenicol (CM) were found by Darwish et al. (Darwish et al., 2002) to be more effective against S. using a few Jordanian plant materials to combat aureus. Combination treatment is frequently used because invasive Staphylococcus aureus infections are often resistant to antibiotic monotherapy. Additionally, many skilled medical professionals start with combination therapy for invasive S in critically unwell patients. infection with aureus.

Honey and Amoxicillin were tested in vitro as a promising novel combination against Staphylococcus aureus (El-Safey & Salah, 2011). According to data from the antimicrobial assay and the killing-curve method, Amoxicillin has a broad spectrum of antimicrobial activity against most aerobic Gram-positive and Gram-negative bacteria (Goau-Brissonnrière et al., 2011).

Honey has bactericidal activity against Staphylococcus aureus isolates. The study of the combination of Honey and Amoxicillin against Staphylococcus aureus has indicated that the inhibitory concentration at (50 mg/ml), and 0.032 µg/ml respectively are sufficient to prevent Staphylococcus aureus growth. However, there are currently no data on the in vitro activity of Amoxicillin in combination with Honey. Selected strains of Staphylococcus aureus were also tested at Amoxicillin concentrations of 0.016 (1/2 MIC) and 25 mg/ml honey to research the in vitro death of the bacteria with medication combinations. For this investigation, synergy was defined as a 2 log10 reduction in CFU following a 24-hour incubation period compared to the presence of a single, more active drug. When CFU/0.1ml was 2 log10 greater following incubation with the combination than with the single, more active drug (Bergen et al., 2011).

The current study demonstrates that Honey and Amoxicillin have synergistic effects against all strains of Staphylococcus aureus that have been tested and that any combination of Honey reduced the MIC of Amoxicillin by half. When using Amoxicillin and Honey together rather than Amoxicillin or Honey alone, there is a synergistic effect seen in the time-killing curves of certain staphylococcus aureus strains. The results of the current study thus imply that the antibacterial chemicals found in Honey were responsible for the synergy of Amoxicillin.

The Honey types employed in this study may be a source of non-antibiotic medications that can help antibiotics work more effectively against infections caused by Staphylococcus aureus. Before that, Honey may be helpful at least for topical application due to Staphylococcus aureus infection. An explanation of the mechanism of action of the compounds must be followed by in vivo experiments to assess the applicability and dose of such compounds in combination therapy.

Conclusion

Our in vitro data show that Honey and Amoxicillin have a lot of promise against Staphylococcus aureus.

REFERENCES


In Vitro Activity of Natural Honey Alone and In Combination with Certain Types of Antibiotics

against multi drug resistant strains of Typhoidal salmonellae (Doctoral dissertation, Ph. D. thesis, Department of Microbiology, University of Health Science, Lahore, Pakistan).


