Antibacterial Effect of Honey on Staphylococci Species Isolated from Animals and Humans

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Abstract. The inhibitory and bactericide effect of 4 sort of honey (forest honey, poly-flower honey, linden tree honey and acacia honey) on some staphylococci species (Staphylococcus aureus 4 strains, Staphylococcus intermedius, Staphylococcus xilosus, Staphylococcus hominis, Staphylococcus chromogenes and Staphylococcus sciuri) isolated from humans and animals was tested in this experiment. For the tests the following methods were used: difusimethrical method in Müeller-Hinton agar, dilution methods in liquid medium in order to determinate M.I.C. and M.B.C. Species S. aureus, S. xilosus and S. intermedius were sensitive to all the honey types tested, the highest inhibitory diameter areas (30-40 mm) being obtained for forest honey followed by poly-flower honey (maximum 30 mm). S. hominis, S. chromogenes and S. sciuri were not as sensitive to honey activity, obtaining reduced inhibition areas. Staphylococci species used in the experiment were sensitive to ampicillin and ceftiofur but resistant to penicillin, enrofloxacin, tetraciclin, gentamicin and kanamicyn. The results obtained are suggesting that forest honey and poly-flower honey honey had good antibacterial effect on the species tested, similar to the best antibiotics.

Key words: antibacterial effect, honey, staphylococci

INTRODUCTION

Starting from the XVIIth B.C. honey was used in therapy as an aliment and drug. Data from the greek-roman antiquity are mentioning the use of honey in medicine creating a real mythology due to it’s multiple healing properties in digestive, liver, cardio-vasculary, respiratory, infectious, nutrition and skin diseases.

Nowadays is known that bacteria have the genetic ability to gain and transmit the resistance to the substances used as therapeutical agents (Nascimento et al. 2000; Levy and Marshall 2004). The develop of bacteria multiple resistance to current drugs is limiting their efficiency causing major insucses in infections treatment (Hancock, 2005). As an example is mentioned staphylococci resistance to meticillin, ciprofloxacycin, eritromycyn, clindamycyn, gentamicyn, trimetoprim/sulfamethoxazole, linezolid and vancomycin (Norrby et al.,2005; Styers et al., 2006; Fong, 2008).

Considering the microorganism resistance and a possible lack of new antimicrobial drugs, is required beside their rational utilization the discover and develop of new therapies able to block bacteria resistance mechanism conducing to the disease control, treatment and eradication. According to the WHO, of naturist therapy approximately 80% of the world population has benefits (WHO, 2002).
With no underestimation of the antibiotics, honey and bee products, mostly the one used in natural therapy are filling the preventive and curative methods applied currently.

Considering that honey proved to have antibacterial effect and that many bacterial strains developed multiple resistances to antibiotics and chemoterapics used in therapy, in our study we aimed to evaluate the inhibitory and bactericide effect of honey on staphylococci species isolated from humans and animals.

MATERIALS AND METHODS

The researches were made during 2008-2009 within the Microbiology Laboratory of the Faculty of Veterinary Medicine in the University of Agricultural Sciences and Veterinary Medicine Cluj-Napoca.

The biologic material used in the experiment was represented by 4 sorts of honey (forest honey, poly-flower honey, linden tree honey and acacia honey) obtained from Deva area, honey having parameters accepted by the European Union proved by products analysis certificate. 6 species of staphylococci were also used (Staphylococcus aureus, Staphylococcus intermedius, Staphylococcus xilosus, Staphylococcus hominis, Staphylococcus chromogenes and Staphylococcus sciuri): 4 strains of Staphylococcus aureus had human origin obtained from the Microbiology Department within the Medical III Clinic Cluj-Napoca. The other strains were isolated from cats and dogs with skin lesions presented to the Microbiology Laboratory of the Faculty of Veterinary Medicine Cluj-Napoca.

The methods for the determination of the inhibitory effect of 4 sorts of honey were realized using the difusimethric method of testing germs sensibility to antibiotics and chemoterapics and establishing the minimum inhibitory concentration (M.I.C.) using dilutions technique.

The difusimethric technique was realized using as culture medium Müeller-Hinton agar in Petri dish of 9 cm diameter while the tested strains were cultivated for 24 hours in broth obtaining a suspension in salina with optical density of 0,5 on McFarland scale. The plates were flooded with the bacterial strain, dried and placed on the plate surface using a radial model with paper discs with honey above.

Honey was used such as (no diluted) and a dilution of 1/1. After the distribution of honey samples, they were placed in thermostat and incubated at 37°C ± 1 for 24 hours and interpreted by measuring the diameters of inhibition areas. By the same technique the test of the bacterial species sensibility was realized for the following antibiotics: penicillin, enrofloxacin, tetracicline, gentamicin, ampicillin, ceftiofur and kanamycin.

Minimum inhibitory concentration (M.I.C.) was determinate using honey dilution method achieving dilutions in tubes with broth: 1/1, 1/4, 1/16, 1/32 and 1/64. For each dilution tube, 24 h bacterial culture was sowed in quantity of 0,1 ml. The tubes such prepared were placed in thermostat at 37°C and interpreted at 24 respectively 48 hours. MIC was represented by the lowest honey dilution that inhibits the culture development.

Minimum bactericide concentration (M.B.C.) for honey was realized using the same dilution technique for each bacterial specie using the dilutions of 1/1, 1/4, 1/16, 1/32 and 1/64. From these dilutions 1 ml sample was sowed in broth and placed in the thermostat at 37°C and interpreted at 24 hours observing the development or turbidity absence in liquid culture medium. For bactericide effect the culture presented no development while for bacteriostatic effect the culture developed.
RESULTS AND DISCUSSIONS

After the determination of the antibacterial effect of 4 sort of honey on 6 staphylococci species using the difusimethrical method, we observed that forest honey had the best inhibitory effect both such as and diluted (Table 1, Chart 1). The best inhibitory effect was observed for \textit{S. xilosus} with an inhibition diameter of 40 mm for honey such as and 30 mm for diluted honey. On the strains of \textit{S. aureus}, forest honey had a similar effect with an average of the diameter area of 30 mm for no diluted honey and 20 mm for diluted honey (Fig. 1). On the strains of \textit{S. intermedius} the inhibitory effect was reduced, observing considerable differences between the diluted and no diluted honey, with diameters of 16 and respectively 7 mm. Strains of \textit{S. hominis}, \textit{S. chromogenes} and \textit{S. sciuri} were not that sensitive for forest honey observing inhibition areas of maximum 7 mm.

The inhibitory effect of poly-flower honey proved using the difusimethric method was similar to forest honey observing diameters of the inhibition areas smaller on staphylococci compared to forest honey. For this product \textit{S. xilosus} was the most sensitive both for honey such as and diluted with inhibition areas of 30 mm (Fig. 2). \textit{S. aureus} was the most sensitive to no diluted honey obtaining inhibition areas of 30 mm compared to 16 mm registered in case of the dilution. The following specie as sensibility for poly-flower honey was \textit{S. intermedius} where inhibition areas of 14 mm for honey such as and 12 for diluted honey were observed. \textit{S. hominis}, \textit{S. chromogenes} and \textit{S. sciuri} presented reduced diameter areas, maximum diameter being of 6 mm, both for honey such as and diluted (Chart 2).

The tests on linden tree honey for staphylococci revealed that \textit{S.xilosus} was the most sensitive specie both diluted and no diluted, followed by \textit{S.aureus}. The diameters of the inhibition areas produced by linden thee honey on \textit{S.intermedius} were of 8 mm both for diluted and no diluted honey. Species \textit{S. hominis}, \textit{S. chromogenes} and \textit{S. sciuri} produced the largest inhibition areas for \textit{S. hominis} with no diluted honey (9 mm) while for the other 2 species the effect was hardly observed (5 mm). On \textit{S. sciuri}, diluted linden thee honey had no inhibitory effect.

<table>
<thead>
<tr>
<th>Staphylococci species</th>
<th>Forest honey</th>
<th>Poly-flower honey</th>
<th>Linden thee honey</th>
<th>Acacia honey</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
<td>1/1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>\textit{S. aureus} – 4 strains (diameter average)</td>
<td>30</td>
<td>20</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>\textit{S. intermedius}</td>
<td>16</td>
<td>7</td>
<td>14</td>
<td>12</td>
</tr>
<tr>
<td>\textit{S. xilosus}</td>
<td>40</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td>\textit{S. hominis}</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>\textit{S. chromogenes}</td>
<td>7</td>
<td>7</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>\textit{S. sciuri}</td>
<td>5</td>
<td>0</td>
<td>5</td>
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</tr>
</tbody>
</table>

Acacia honey determined inhibition areas for \textit{S.xilosus} of 30 mm, identical with the areas observed for poly-flower honey. For \textit{S. aureus} the inhibitory effect was good determining inhibition areas of 26 mm for no diluted honey and 22 mm for diluted honey.
Regarding the other staphylococci species the inhibitory effect was reduced, observing for *S. intermedius* inhibition diameters of 10 mm, for *S. hominis* of 7 mm, respectively 6 mm, for *S. chromogenes* of 10 mm, respectively 7 mm while for *S. sciuri* just for no diluted honey we observed a diameter of inhibition area of 7 mm.

![Chart 1. Graphical representation of the inhibition diameter areas obtained with 4 sorts of no diluted honey on staphylococci](image)

*Chart 1.* Graphical representation of the inhibition diameter areas obtained with 4 sorts of no diluted honey on staphylococci

*S. aureus* tested strains proved to be sensitive to all honey sorts used in the experiment but the highest inhibition areas were obtained for forest honey, both diluted and no diluted.

*S. intermedius* presented low sensibility to the honey tested, obtaining the highest inhibition areas also for no diluted forest honey, the strain being considered as having intermediary sensibility if compared to the inhibition areas obtained to most of the antibiotics.

*S. xilosus* tested strain was the most sensitive to the activity of diluted and no diluted honey, the highest inhibition areas being obtained for forest honey, where the culture developed just at the side of the plate due to the presence of the substances with antibacterial effect from honey (Fig. 2).

![Chart 2. Graphical representation of the inhibition diameter areas obtained with 4 sorts of 1/1 diluted honey on staphylococci](image)

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*S. xilosus* tested strain was the most sensitive to the activity of diluted and no diluted honey, the highest inhibition areas being obtained for forest honey, where the culture developed just at the side of the plate due to the presence of the substances with antibacterial effect from honey (Fig. 2).
**S. hominis** strain proved to be resistant to all honey sort resulting reduced inhibition areas of maximum 7 mm for linden tree honey.

**S. chromogenes** had reduced sensibility to honey activity, the highest inhibition area being observed for no diluted acacia honey (10 mm) while for no diluted poly-flower honey the inhibition of the bacterial development was absent.

Aldo for **S. sciuri** the inhibition areas were reduced, in the plate we observed that most of the bacterial lown is inhibit by the presence of honey with colonies developed to the margin of the honey disk.

In order to appreciate the inhibitory effect of 4 honey sort for each staphylococci specie, antibiograma were realized using the most frequent antibiotics in the treatment of the bacteria. The staphylococci tested in the experiment had various behaviours for antibiotic type, ampicillin and ceftiofur producing the highest inhibition area diameter (Table 2). **S. aureus**, **S. intermedius** and **S. chromogenes** were sensitive to Ampicillin, while **S. xilosus**, **S. hominis** and **S. sciuri** were sensitive to ceftiofur. For the other antibiotics, penicillin, enrofloxacin, tetraciclin, gentamicin and kanamicyn, inhibition areas were satisfying but only framed in the cathegory of intermediar sensible or resistant according to the producer indicatives.

![Fig. 1. Antibiogram realized with such as (no diluted) and diluted honey for *S. aureus*](image1)

![Fig. 2. Antibiogram realized with such as (no diluted) and diluted honey for *S. xilosus*](image2)

**Table 2**

<table>
<thead>
<tr>
<th>Staphylococci species</th>
<th>Antibiotics</th>
<th>Penicillin</th>
<th>Enrofloxacin</th>
<th>Tetraciclin</th>
<th>Gentamicin</th>
<th>Ampicillin</th>
<th>Ceftiofur</th>
<th>Kanamicyn</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td></td>
<td>18</td>
<td>20</td>
<td>0</td>
<td>12</td>
<td>22</td>
<td>16</td>
<td>12</td>
</tr>
<tr>
<td><em>S. intermedius</em></td>
<td></td>
<td>22</td>
<td>16</td>
<td>0</td>
<td>18</td>
<td>22</td>
<td>16</td>
<td>18</td>
</tr>
<tr>
<td><em>S. xilosus</em></td>
<td></td>
<td>16</td>
<td>16</td>
<td>10</td>
<td>16</td>
<td>16</td>
<td>22</td>
<td>14</td>
</tr>
<tr>
<td><em>S. hominis</em></td>
<td></td>
<td>0</td>
<td>14</td>
<td>0</td>
<td>14</td>
<td>0</td>
<td>20</td>
<td>0</td>
</tr>
<tr>
<td><em>S. chromogenes</em></td>
<td></td>
<td>18</td>
<td>13</td>
<td>20</td>
<td>14</td>
<td>24</td>
<td>18</td>
<td>17</td>
</tr>
<tr>
<td><em>S. sciuri</em></td>
<td></td>
<td>10</td>
<td>17</td>
<td>17</td>
<td>16</td>
<td>10</td>
<td>20</td>
<td>14</td>
</tr>
</tbody>
</table>

After the tests for staphylococci with usual antibiotics used in therapy we observed that most of the tested strains are resistant to these antibiotics, except for ampicillin with
sensibility for *S. aureus*, *S. intermedius* and *S. chromogenes* or ceftiofur with sensibility for *S. xilosus*, *S. hominis* and *S. sciuri*.

After the determination of the minimum inhibitory concentration (M.I.C.) using dilution methods in broth we observed that forest honey inhibit the development of *S. intermedius* and *S. chromogenes* even for the final dilution - 1/64, corresponding to a concentration of 1,5% honey. *S. xilosus* was inhibit to the dilution of 1/16 corresponding to a concentration of 5,5%. *S. hominis* and *S. sciuri* were inhibit to the dilution of ¼, representing a concentration of 25%. *S. aureus* strain in broth was inhibited just for the concentration of 50% forest honey in the sample.

For poly-flower honey, the results obtained in determining M.I.C. were identical for *S. aureus*, *S. intermedius*, *S. xilosus* and *S. hominis* where up to the dilution of 1/16 – the concentration of 5,5%, the development of the culture was inhibit (Table 2.4.). *S. sciuri* did not develop even for the dilution of 1/62 corresponding to the concentration of 1,5%. Specie *S. chromogenes* was less sensitive to honey in the culture medium developing for the dilution of 1/16.

Minimum inhibitory concentration was established using dilution method with linden tree honey for *S. xilosus*, *S. hominis* and *S. sciuri* at the dilution of 1/16, corresponding to the concentration of 5,5% honey in broth. For the other species linden tree honey inhibitory effect was reduced, M.I.C being established at the dilution of 1/4, representing 25% honey concentration in broth.

The results obtained for M.I.C. using acacia honey on staphylococci revealed that *S. xilosus* was the most sensitive, being inhibit for the dilution of 1/64 (1,5% honey in culture medium). *S. intermedius* was the following bacteria that presented sensibility to the activity of acacia honey, being inhibit to the dilution of 1/16 (5,5% honey). *S. hominis*, *S. chromogenes* and *S. sciuri* had M.I.C. established for the dilution of 1/4, unable to develop as long as the honey concentration was of 25%. *S. aureus* strain tested was inhibited in broth just at the concentration of 50% honey.

Of all the honey sort tested using the dilution test in broth (M.I.C.), forest honey had the best efficiency being inhibit for two staphylococci species even for the concentration of 1,5%. Poly-flower honey and acacia honey had similar behavior but the inhibitory effect for the concentration of 1,5% only appeared for one staphylococcus specie. Linden tree honey had more reduced inhibitory effect on the staphylococci species tested, active up to the dilution of 1/16, representing 5,5% honey concentration in the medium. After the determination of M.B.C. we observed a bactericide effect for all staphylococci species tested using all sort of honey at the dilution of 1/1 and 1/4, representing a bactericide effect at the concentration of 50% and 25% honey in the culture medium.

Considering the results and that the most of bacterial species tested proved to be resistant to antibiotics, this study fulfill its aim observing that antibacterial effect of honey is similar or even better, recommending the use of honey in the bacterial wounds produced by the germs from genus *Staphylococcus*. Another aspect revealed is that similar to the antibiotics, honey has an inhibitory effect reduced or increased corelated to the bacterial strain and the honey concentration used in the experiment. Based on these results we are recommending the utilization of honey in the situations where the application can be topic because is a natural product preventing the residuum accumulation in the environment. We also recommend before the use of honey in therapy a test regarding the bacterial strain sensibility to honey and it’s use only in the situations when *in vitro* sensibility is observed. Another recommendation would be the use of honey associated in wounds treatment along antibiotics decreasing this way the risk of antibioresistance appearence.
CONCLUSIONS

After the determination of antibacterial activity of honey sorts on several staphylococci species we concluded that honey can be used as natural antibiotic in infectious diseases especially of staphylococci origin. According to the results obtained, it was proved that forest honey and poly-flower honey tested had antibacterial effect on the bacterial species tested similar to the best antibiotics.

REFERENCES