In vitro effect of some Indian honeys on Staphylococcus aureus from wounds

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Staphylococcus aureus is the most frequently isolated pathogen from wounds with multiple resistances to antibiotics. Honey has been demonstrated and reported to be effective antibacterial agent on Gram positive and Gram negative organisms. Hence, the present study was conducted to evaluate the in vitro antibacterial effect of Indian honeys on Staphylococcus aureus obtained from wounds. A total of 123 Staphylococcus aureus isolates along with ATCC 25923 were categorized as sensitive, multi drug resistant (MDR) and non-MDR strains. Out of total nine Indian honeys (three each of unifloral, multifloral and branded marketed honey) used, three unifloral and three multifloral honey samples showed antibacterial activity against all the organisms tested by Agar diffusion method but not the branded marketed honeys. The MIC values of all honey samples for all studied Staphylococcus aureus isolates ranged between 5-15% (v/v). Unifloral honey samples showed higher antibacterial activity than multifloral honey. The single sample of Jambhul honey showed the highest activity. Thus, Indian honeys were found to be effective for their antimicrobial activity on sensitive, non-MDR, MDR and ATCC strains of S. aureus.

Keywords: Antibacterial activity, Honey sensitivity, Indian honeys, Multi drug resistance, Staphylococcus aureus

Staphylococcus aureus is the most frequently isolated pathogen from different kind of wounds and poses great difficulty in selecting antimicrobial agents due to emergence of strains with multiple resistances to antibiotics.

The prevalence of multidrug resistant organisms now justifies the interest in re-evaluation of therapeutic use of ancient remedies. Among the different medicinal natural products used in ancient times, honey possesses higher antibacterial activity and has potential to be used as an antibacterial agent for treatment of wound. Honey has been demonstrated and reported in many studies carried out in other countries to be effective antibacterial agent on Gram positive and Gram negative organisms\(^1\). There are very few studies from India\(^2,3\).

Hence, the present study has been undertaken to evaluate the in vitro antibacterial activity of some Indian honeys on isolates of Staphylococcus aureus obtained from different kind of wounds along with standard ATCC strain.

Materials and Methods

A total of 123 Staphylococcus aureus isolates were collected from various clinical wounds. Identification and speciation of each isolate was confirmed by microscopy, culture and biochemical tests\(^4\). Antibiotic sensitivity testing (AST) was carried out for 8 antibiotics by the Kirby Bauer disc diffusion method according to NCCLS guidelines on all isolates and with standard ATCC 25923 strain\(^5\). Hi-media antibiotic discs were used for AST which were Amoxyclav, Ampicillin, Cloxacillin, Penicillin, Ceftazidime, Erythromycin, Oxacillin and Clotrimoxazole. Each organism was further categorized as multidrug resistant (MDR), non-MDR (NMDR) and sensitive (S) according to their antibiotic sensitivity pattern. MDR strains were resistant to all 8 antibiotics; NMDR strains were sensitive to atleast 2 antibiotics and S strains were sensitive to all 8 antibiotics.

Following nine Indian honeys were used and categorized on the basis of the information received from the collection centre regarding their source and area of procurement and it was then confirmed by pollen study in the Botany Section, School of Biotechnology, Vidyapratishthan, Baramati.

Unifloral honey:

1. Mustard (Uttar Pradesh)
2. Eucalyptus (Tamilnadu)
3. Jambhul (Maharashtra)

Multifloral honey:
4. Maharashtra
5. Kashmir
6. Kothimbe

Branded marketed:
7. Khadi
8. Dabur
9. Phondaghat pharmacy

Unifloral and multifloral honeys were procured as raw honey before heat processing from Phondaghat Pharmacy, Mumbai; which is a collection centre for different types of honeys. Branded honeys were obtained from shop counter which were heat processed.

All nine Indian honeys were studied for the following:

(i) Physicochemical properties namely colour, pH, specific gravity, percentage of sucrose, glucose, ash, moisture and total reducing sugars, acidity, fructose/glucose ratio, Fiehe’s test and aniline chloride test were checked as per Agmark standard in Agmark laboratories.

(ii) Sterility testing was done by performing “test of sterility” by Direct Inoculation method as stated in Indian Pharmacopoeia. All unsterile honeys were further sterilized by gamma irradiation at 25 kGy at the ISOMED unit of BARC-Mumbai. All irradiated honey samples were again tested for their sterility and only sterile honey samples were used for antibacterial testing.

(iii) The antibacterial activity of all honeys was determined by using standard microbiological methods. Primary screening of in vitro antibacterial activity of each honey was carried out by performing Agar well diffusion method by selecting nine random isolates each of sensitive, non-MDR, MDR along with one S.aureus ATCC 25923 strain. Size of zone of inhibition of each honey to all organisms were measured and compared as shown in Table 1.

The six honey samples which showed zone of inhibition by Agar diffusion method were further tested for their minimum inhibitory concentration by Agar dilution method for all test organisms (123). The range of honey dilutions for Agar dilution method was decided from the inhibition zone size obtained from Agar diffusion.

Results

All 123 Staphylococcus aureus isolates along with ATCC 25923 strains were categorized on the basis of AST results as:
- MDR – 66 (54)
- Non-MDR – 48 (39)
- Sensitive – 9 (7) + 1 ATCC strain.

All nine Indian honey samples were found to be up to the Agmark standard. The pH of all honey samples was found in the range of 3.2 to 4.5. Among all nine honey samples only Jambhul honey passed the test for sterility. Gamma irradiation at 25 kGy was an effective method for the sterilization of remaining 8 honey samples as it does not affect any of physicochemical and antibacterial activity of honey.

Three unifloral and three multifloral honey samples showed antibacterial activity against all the organisms tested by the Agar diffusion method. But all three honeys procured from the local market failed to show any antibacterial activity against any of the organisms tested. Hence 66.6% of honey samples showed antibacterial activity to all the organisms tested.

The inhibition zone size was maximum by standard ATCC strains, followed by sensitive, non-MDR and MDR isolates of S.aureus towards various concentrations of honey. The maximum zone of inhibition was seen at 50% (v/v) concentration of honey towards all the organisms tested as shown in Table 1.

Pair-wise comparison between sensitive, non-MDR and MDR strains of S. aureus studied for antibacterial activity of Indian honeys using Agar diffusion method in terms of diameter of zone of inhibition was significantly different (P<0.05) between sensitive-NMDR, sensitive-MDR and NMDR-MDR for S. aureus.

The MIC values of all honey samples for standard ATCC, sensitive, non–MDR and MDR isolates of S. aureus were ranged between 5-15% (v/v) (Table 2).

On comparison of concentration of honey needed for inhibition of growth among sensitive, NMDR and
MDR strains of *S. aureus*, it was observed that the association between three types of strains of *S. aureus* and the concentration of honey needed for inhibition was statistically significant (*P* <0.05) indicating that the sensitive, NMDR and MDR strains required significantly different concentrations of honey for their inhibition. Comparing the antibacterial activity exhibited by six Indian honeys showed that all the 6 honeys had different levels of antibacterial activity against the different organisms tested.

Unifloral honey samples showed higher antibacterial activity than multifloral honey. Jambluh honey showed the highest activity among the 3 Unifloral honey samples followed by Eucalyptus and Mustard honey. Among all 3 Multifloral honey samples tested, Maharashtra honey was more effective followed by Kashmir and Kothimbe honey respectively (Table 2).

### Discussion

Despite recent advances in antimicrobial therapy, several types of wounds still show resistance to routine wound treatment. The emergence of resistant microbial strains with multiple patterns reduced the efficacy of conventional therapies. Indiscriminate and irrational use of antibiotics is primarily responsible for the emergence and spread of resistance in almost all the microorganisms.

In the present study, 54% isolates were MDR followed by 39% non-MDR and only 7% sensitive strains. The maximum proportion of *S. aureus* strains...
showed resistance to all the 8 antibiotics tested. Similar observations are reported by Chambers.

Hence, the relentless emergences of antibiotic resistant strains of pathogens often with multiple antibiotic resistance lead to the need to find alternative. This has forced the re-evaluation of traditional remedies in the search of appropriate antimicrobial agents.

Manuka, Kanuka, and Pasture honey are known to have antibacterial activity. Not much work has been done on in vitro antibacterial activity of various Indian honeys except Subramanyam et al. has reported in vivo effectiveness of Indian Jambhul honey in his various publications.

Hence, till date, Indian honey has been used mostly as home remedy. Due to lack of adequate scientific research and documentation, the medicinal properties of Indian honeys still remain mostly in dark.

The present study included 3 unifloral, 3 multifloral and 3 marketed processed honeys.

Amongst the documented Indian studies regarding antibacterial activity of honey, sterility of honey sample were not considered except by Subramaniam et al. who used Jambhul honey which he found sterile. This coincides with present results as Jambhul honey was the only honey found to be sterile among all other honey samples.

All unifloral and multifloral honey samples showed antibacterial activity against all the organisms studied except 3 honey samples procured from local market which failed to show any antimicrobial activity against any of the organisms tested. This finding corroborates that of Postmes et al. who reported supermarketed honey had no antibacterial activity.

The maximum zone of inhibition was shown by 50% (v/v) concentration of all the 6 honey samples on all organisms tested. The antibacterial activity of honey was higher at higher concentrations. This finding is consistent with that reported by Bannur et al. and Subramanyam et al. from India.

### Table 2—Number of isolates showing MIC of six honeys by Agar dilution method

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Type of honey</th>
<th>Concentration (% v/v) of honey</th>
<th>No of isolates showing MIC of honey</th>
<th>No inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>5</td>
<td>10</td>
<td>15</td>
</tr>
<tr>
<td>S.aureus</td>
<td>Mustard</td>
<td>1(100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>ATCC 25923</td>
<td>Mustard</td>
<td>1(100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Eucalyptus</td>
<td>1(100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Jambhul</td>
<td>1(100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Maharashra</td>
<td>1(100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Kashmir</td>
<td>1(100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Kothimbe</td>
<td>1(100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>S.aureus</td>
<td>Mustard</td>
<td>7(78)</td>
<td>2(22)</td>
<td>-</td>
</tr>
<tr>
<td>sensitive</td>
<td>Eucalyptus</td>
<td>7(78)</td>
<td>2(22)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Jambhul</td>
<td>9(100)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Maharashra</td>
<td>5(56)</td>
<td>4(44)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Kashmir</td>
<td>4(44)</td>
<td>5(56)</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Kothimbe</td>
<td>4(44)</td>
<td>2(22)</td>
<td>3(33)</td>
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<tr>
<td>S.aureus</td>
<td>Mustard</td>
<td>42(88)</td>
<td>6(13)</td>
<td>-</td>
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<tr>
<td>Non-MDR</td>
<td>Eucalyptus</td>
<td>45(94)</td>
<td>3(6)</td>
<td>-</td>
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<td></td>
<td>Jambhul</td>
<td>48(100)</td>
<td>-</td>
<td>-</td>
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<td></td>
<td>Maharashra</td>
<td>38(80)</td>
<td>10(21)</td>
<td>-</td>
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<td></td>
<td>Kashmir</td>
<td>35(73)</td>
<td>7(15)</td>
<td>6(13)</td>
</tr>
<tr>
<td></td>
<td>Kothimbe</td>
<td>37(77)</td>
<td>3(6)</td>
<td>8(17)</td>
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<tr>
<td>S.aureus</td>
<td>Mustard</td>
<td>37(50)</td>
<td>23(35)</td>
<td>6(9)</td>
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<td>Eucalyptus</td>
<td>49(74)</td>
<td>17(26)</td>
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<td>Jambhul</td>
<td>57(86)</td>
<td>9(14)</td>
<td>-</td>
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<td>41(62)</td>
<td>8(12)</td>
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<tr>
<td></td>
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<td>37(56)</td>
<td>16(24)</td>
</tr>
<tr>
<td></td>
<td>Kothimbe</td>
<td>9(14)</td>
<td>33(50)</td>
<td>24(36)</td>
</tr>
</tbody>
</table>

( ) = %
In the present study MIC values for *S. aureus* ranged from 5-15% (v/v) over the honey samples. *S. aureus*, a species that has developed resistance to many antibiotics and has become the predominant agent of wound sepsis is very susceptible to the antibacterial activity of honey. More or less similar sensitivity of MDR, non-MDR and sensitive isolates to honey was observed.

Comparing the antibacterial activity exhibited by 6 honey samples, it was observed that each honey has different levels of antibacterial activity against the different organisms tested. These findings are in accordance with others reporting that the antibacterial activity differs with each honey.

It was observed from the present study that unifloral honey samples showed higher antibacterial activity than multifloral honey samples. Amongst them Jambhul honey showed the highest antibacterial activity.

Therefore for clinical use, the selection of honey with high levels of antibacterial activity is important to maximize therapeutic effects.

Hence, it can be concluded that different types of Indian honeys are potent antibacterial agents against ATCC, sensitive, Non MDR and MDR strains of *Staphylococcus aureus*.

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**References**


