Short Communication

Comparison of the antimicrobial activity of Manuka honey and native honey against methicillin resistant staphylococci from asymptomatic healthcare workers.

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Abstract

Asymptomatically colonized healthcare workers are the major source of Methicillin-Resistant Staphylococcus aureus (MRSA) in the hospital environment and serve as links in the transmission of MRSA among patients. Honey is known for antimicrobial properties due to various factors including phytochemical components eg: methyl glyoxal (MGO). Hence, the present study was designed to compare the antimicrobial activity of Manuka honey with native honey against methicillin resistant staphylococci from asymptomatic health care workers.

Nasal swabs were collected from anterior nares of 100 healthcare workers from the hospital set up and a total of 36 isolates of staphylococci (36%) were obtained. The study has shown 7% carriage rate of MRSA and 9% MRCoNS among the healthcare workers. Initial screening with agar well diffusion method with Manuka honey showed higher inhibitory activity against all the methicillin resistant staphylococcal isolates giving a zone size of 30 mm at 50% (v/v) compared to native honey which gave zone size of 12 mm for 50% (v/v). Lower MIC of methicillin resistant staphylococci was seen for Manuka honey (6.3% to 12.5%), while the MIC of the native honey was found to be 50% in our study.

The present study shows higher efficacy of Manuka honey compared to native honey against both MRSA and MRCoNS. Manuka honey shows promise as an antibacterial agent for methicillin resistant staphylococci.

Keywords: Methicillin resistant staphylococci, Healthcare workers, Nasal carriage, Manuka honey, MRSA, MRCoNS.

Introduction

Staphylococcus aureus is one of the major human pathogens that can cause community and hospital-acquired (HA) infections [¹,²]. The global emergence of methicillin-resistant Staphylococcus aureus (MRSA) has turned into a serious public health problem. The bacterium is known as the most significant cause of nosocomial infections, which is resistant to different antibacterial classes and has posed a threat to antibiotic therapy [³].

Anterior nares are the best ecological niches for S. aureus [⁴] and S. aureus nasal carriers may transmit the pathogen among patients. It subsequently causes infections in susceptible hosts [⁵]. The colonization of S. aureus in the nose is a cause of subsequent infections [⁶]. Three principles prove that S. aureus is a very important risk factor for becoming infectious in the community and hospitals. 1) the rate of S. aureus infections is much higher in the carriers [⁷]. 2) studies on nasal carriage have shown that people are usually infected with the isolates they carry [⁸]. 3) eradication of S. aureus in carriers following the administration of mupirocin has statistically decreased hospital infections in dialysis patients and those who have undergone surgeries. S. aureus strains containing the pvl gene have the potential to epidemiologically spread in the community [⁹].

The antimicrobial activity of honey is attributed largely to osmolarity, pH, hydrogen peroxide production and the presence of other phytochemical components e.g. methylglyoxal (MGO) (10). Manuka honey which originates from the manuka tree (Leptospermum scoparium) is sold as a therapeutic agent worldwide. The presence of MGO in manuka honey contributes to its uniqueness and has been termed the Unique Manuka Factor (UMF®). The present study aims at evaluating the in vitro antimicrobial property of Manuka honey against MRSA & MRCoNS (Methicillin-Resistant Coagulate Negative Staphylococci) and comparing it with native honey.

Materials and methods

Samples were collected from the anterior nares of health care workers with the help of a sterile cotton swab by swabbing of the
anterior nares of the healthy volunteers. The swabs were rubbed well by rotating five times over the inner wall of the nasal septum and were transported in salt nutrient broth (7.5%) to the laboratory and were processed. Based on the colony morphology and gram staining and mannitol fermentation, the gram-positive cocci in clusters were further identified based on the biochemical methods as per standard protocols.

**Honey samples**

Honey samples used in this study included Manuka honey with UMF® 15+ which was purchased from Australia and native honey collected from bee vendors from Chennai.

**Antibiotic sensitivity testing**

Antibiotic sensitivity testing was carried out by Kirby Bauer (11) disc diffusion method for the following antibiotics- (in µg/disc) ampicillin (10), amikacin (30), chloramphenicol (30), ciprofloxacin (5), co-trimoxazole (25), erythromycin (15), gentamicin (10), linezolid (30), netilmicin (30), norfloxacins (10), ofloxacin (5), rifampicin (5), tetracycline (30) and vancomycin (30).

Screening for methillin resistance was done by cefoxitin disc diffusion method and oxacillin agar screening method as per CLSI guidelines.

**Molecular detection of mecA, femA and pvl genes**

MRSA isolates were detected by multiplex PCR using mecA and femA by the method of Kondo et al., 2007 (12) and Berger et al., 1989 (13) along with the detection of pvl gene by the method of Lina et al., 1999 (14). The following were the primer sequences used in the study-

<table>
<thead>
<tr>
<th>Target Gene</th>
<th>Oligonucleotide (primer) Sequence</th>
<th>Product Size</th>
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<tbody>
<tr>
<td>mecA Forward</td>
<td>F: 5’ – TGC TAT CCA CCC TCA AAC AGG – 3’</td>
<td>286 bp</td>
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<tr>
<td>mecA Reverse</td>
<td>R: 5’ – AAC GTT GTA ACC ACC CCA AGA – 3’</td>
<td>132 bp</td>
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<tr>
<td>femA Forward</td>
<td>F: 5’ – AAA AAA GCA CAT AAC AAG CG – 3’</td>
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<tr>
<td>femA Reverse</td>
<td>R: 5’ – GAT AAA GAA GAA ACC AGC AG – 3’</td>
<td></td>
</tr>
<tr>
<td>pvl Forward</td>
<td>F: 5’ – ATC ATT AGG TAA AAT GTC TGG ACA TGA TTC A – 3’</td>
<td>433 bp</td>
</tr>
<tr>
<td>pvl Reverse</td>
<td>R: 5’ – GCA TCA AST GTA TTG GAT AGC AAA AGC – 3’</td>
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PCR was performed in a 25µl reaction with 10X standard PCR buffer (100 mM Tris-HCl pH 8.3, 500 mM KCl; 1.5 mM MgCl₂), 200mM NTP mix (Sigma), 25pmol of each primer (Sigma), 2.5U of Taq DNA polymerase and 1µL template DNA. Amplification was performed with initial denaturation at 94°C for 5 min, followed by denaturation at 94°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 5 mints. The PCR products were analyzed in a 2% agarose gel in 1xTBE buffer. Ethidium bromide stained DNA amplicons were visualized using a gel imaging system.

**Antimicrobial activity of Manuka honey and Native honey**

**Agar well diffusion assay**

A screening assay using agar well diffusion was carried out by the method of Somal et al., (1994) (15). Standard suspension of the isolates was made and the turbidity was matched to McFarland standard 0.5. The inoculum was spread evenly on the Mueller Hinton agar surface using a sterile cotton swab and was allowed to dry for 5-10 mints. After inoculation, 8.2 mm diameter wells were cut into the surface of the agar using a sterile cork borer. Eighty µl of test honey was added to the well and the plates were incubated at 37°C for 24 hrs.

**Minimum Inhibitory Concentration (MIC) of Manuka Honey and Native honey**

A serial double dilution of honey was prepared aseptically for use in MIC assay from 50% to 0.02% v/v in Mueller-Hinton broth. From the 50% (v/v) honey solution, 12 serial 1:1 dilutions were made, resulting in final concentrations of- 50%, 25%, 12.5%, 6.3%, 3.1%, 1.6%, 0.8%, 0.4%, 0.2%, 0.1%, 0.05%, and 0.025% and were referred to as ‘test honey’. MIC was performed in sterile 96 well microtiter plates as per the method of Orla Sherlok et al., (2010) (16). The MIC was determined as the lowest concentration of honey inhibiting visible growth of each isolate.

Minimum Bactericidal Concentration (MBC) was determined by taking a loopful from each test well (from the broth MIC assay) that showed no apparent growth and were spot inoculated onto Mueller Hinton agar (MHA) and the plates were incubated at 37°C for 24 hrs. The MBC was read as the least concentration showing no growth on MHA plates.

**Results**

Out of 100 sample collected from the anterior nares of healthcare workers from the hospital set up, a total of 36 isolates of staphylococci (36%) were obtained. 12/36 were S.aureus and 24/36 were found to be coagulase negative staphylococci (CoNS).

**Screening for methillin resistance: Phenotypic Method**

Screening for methillin resistance by cefoxitin disc diffusion method and oxacillin agar screening method showed that 7/12 (58%) S.aureus and 9/24 (37%) CoNS were found to be methillin resistant.

**Genotypic Method**

A total of 7/12 (58%) and 9/24 (37%) staphylococci were found to harbour mecA gene confirming them as MRSA and MRCoNS. The results correlated with the findings of the phenotypic methods. 12/36 (33%) staphylococci were found to be positive for the
presence of \textit{femA} gene. 4/36 (11\%) showed the presence of \textit{pvl} genes- 3 of which were MRSA and 1 was MSSA (Figure 1).

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Figure 1. Gel picture showing \textit{mecA}, \textit{femA} and \textit{pvl} genes

**Antibiotic sensitivity testing**

The most effective drugs were linezolid and vancomycin to which isolates showed 100\% sensitivity. \textit{S. aureus} isolates were found to be highly resistant to ampicillin (92\%) followed by ofloxacin (83\%), norfloxacin (75\%), netilmicin (66\%), erythromycin (58\%), chloramphenicol and co-trimoxazole (50\%), ciprofloxacin and amikacin (42\%), gentamicin and tetracycline (33\%) and rifampicin (25\%).

CoNS isolates were found to be highly resistant to ampicillin (96\%) followed by ofloxacin (79\%), norfloxacin (71\%), netilmicin and erythromycin (62\%), co-trimoxazole (45\%), ciprofloxacin and chloramphenicol (42\%), amikacin (37\%), tetracycline and gentamicin (33\%) and rifampicin (29\%).

**Antibacterial activity of Manuka Honey and Native honey**

Manuka honey showed high inhibitory activity against all the methicillin resistant staphylococcal isolates giving a zone size of more than 30 mm at 50\% (v/v) by agar well diffusion method, while for native honey, the zone size was found to be 12 mm for 50\% (v/v). The MIC and MBC of Manuka honey and native honey were found to be same. The MIC of Manuka honey against methicillin resistant staphylococci was found to be in the range of 6.3\% to 12.5\%, while the MIC of the native honey was found to be 50\% (v/v) (Figure 2).

**Discussion**

Hospitals worldwide are increasingly concerned about MRSA. Recently, the threat of community-associated MRSA (CA-MRSA) has been associated with young and healthy people without traditional risk factors. CA-MRSA has started to spread from the community into hospitals, where outbreaks have occurred. Since health-care workers are at the interface between hospitals, long-term care facilities and nursing homes on the one hand and the community on the other, they may serve as reservoirs, vectors, or victims of MRSA cross-transmission. (17)

In our study, a total of 7/12 (58\%) \textit{S. aureus} were found to harbour \textit{mecA} gene confirming them as MRSA. 9/24 (37\%) CoNS were found to harbour \textit{mecA} gene confirming them as MRCoNS. CA-MRSA isolates commonly possess genes for the Panton-Valentine Leukocidin (PVL) toxin, which are rarely identified in HA-MRSA isolates (18). In our study, 4/36 (11\%) of staphylococcal isolates showed the presence of \textit{pvl} genes 3 of which were from MRSA and 1 was from MSSA.

The frequency of antimicrobial resistance amongst staphylococci towards all kinds of antibiotics including the major and last resort drugs is increasing worldwide and poses a very serious threat to
public health. Therefore, alternative antimicrobial strategies are urgently needed leading to re-evaluation of the therapeutic use of plant-based products, including honey (19).

The major honey in medical use today-Manuka honey, is available in various licensed dressings and is sourced from the New Zealand Manuka tree *Leptospermum scoparium*. Manuka honey has broad-spectrum antibacterial activity (20) and is known to be effective against antibiotic resistant pathogens. In our study, initial screening with agar well diffusion method with Manuka honey showed higher inhibitory activity against all the methicillin resistant staphylococcal isolates giving a zone size of 30 mm at 50%( v/v) compared to native honey which gave zone size of 12 mm for 50% (v/v). The MIC assay showed lower MIC of methicillin resistant staphylococci for Manuka honey which was in the range of 6.3% to 12.5% , while the MIC of the native honey was found to be 50% in our study. The higher inhibitory activity of Manuka honey against methicillin resistant staphylococci compared to native honey collected from Chennai is probably due to the presence of MGO which is unique in Manuka honey.

**Conclusion**

The present study shows the higher efficacy of Manuka honey compared to native honey against both MRSA and MRCoNS. As the study has shown 7% carriage rate of MRSA and 9% MRCoNS among the healthcare workers, there is a risk of colonization and infection by methicillin resistant staphylococci in community and hospitals. Manuka honey shows promise as an antibacterial agent for methicillin resistant staphylococcus and a potential therapeutic agent. To the best of our knowledge, this is the first study in evaluating antibacterial activity of Manuka honey against carrier isolates of staphylococci to be done in South India, Chennai.

**Authors Contribution**

1. SK.Jasmine Shahina : a)Design of study, b) acquisition of data, c) analysis and interpretation of data.
2. Dr.Padma Krishnan : a)Conception of study design and coordination for smooth execution of the study, b) interpretation of the findings to bring out its significance, c) drafting of the manuscript and its revision to bring out critically important intellectual content.

**Acknowledgement**

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**References**


