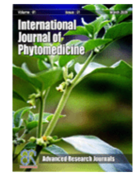
International Journal of Phytomedicine 5 (2013) 262-266
http://www.arjournals.org/index.php/ijpm/index

Short Communication



ISSN: 0975-0185

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 staphylococcus.

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. i) the rate of *S. aureus* infections is much higher in the carriers [7]. ii) studies on nasal carriage have shown that people are usually infected with the isolates they carry [8]. iii) 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 Coagulase 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), norfloxacin (10), ofloxacin (5), rifampicin (5), tetracycline (30) and vancomycin (30).
Screening for methicillin 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 detectecd 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-

Target Gene	Oligonucleotide (primer) Sequence	ProductSize
mecA Forward	F: 5' – TGC TAT CCA CCC TCA AAC AGG – 3'	286 bp
mecA Reverse	R: 5' – AAC GTT GTA ACC ACC CCA AGA –3'	
femA Forward	F: 5' – AAA AAA GCA CAT AAC AAG CG – 3'	132bp
femA Reverse	R: 5' – GAT AAA GAA GAA ACC AGC AG – 3'	
pvl Forward	F: 5' – ATC ATT AGG TAA AAT GTC TGG ACA TGA TCC A – 3'	433bp
pvl Reverse	R: 5' – GCA TCA AST GTA TTG GAT AGC AAA AGC – 3'	

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 $MgCl_2$}, 200mM dNTP 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 microtitre 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 37ºC for 24 hrs. The MBC was read as the least concentration showing no growth on MHA plates.

Results

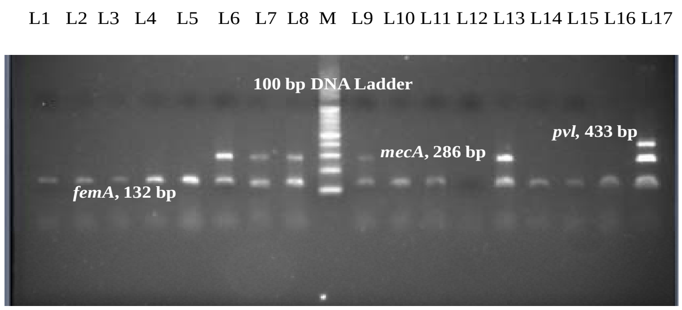
Out of 100 sample collected from the anterior nares of healthcare workers from the hospital set up, a total of 36 isolates of staphylococci (36%) were obtained. 12/36 were *S.aureus* and 24/36 were found to be coagulase negative staphylococci (CoNS).

Screening for methicillin resistance: Phenotypic Method

Screening for methicillin 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 methicillin 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 *femA* gene. 4/36 (11%) showed the presence of *pvl* genes- 3 of which were MRSA and 1 was MSSA (Figure 1).



L1-MSSA, L2-MSSA, L3-MSSA, L4-MSSA, L5-MSSA, L6-MRSA, L7-MRSA, L8- MRSA, M- 100bp DNA Ladder, L9- MRSA, L10-MSSA, L11-MSSA, L12- MSCoNS, L13-MRSA, L14-MSSA, L15-MSSA, L16-MSSA,

Figure 1. Gel picture showing *mecA*, *femA* and *pvl* genes

Antibiotic sensitivity testing

The most effective drugs were linezolid and vancomycin to which isolates showed 100% sensitivity. *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 occured. 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%) *S.aureus* were found to harbour *mecA* gene confirming them as MRSA. 9/24 (37%) CoNS were found to harbour *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 *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

Manuka honey **Native honey**

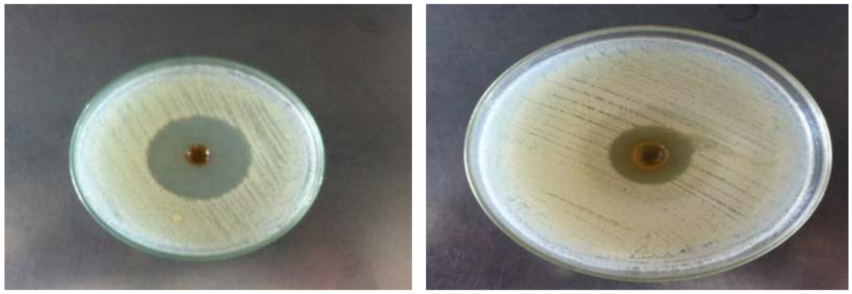


Figure 2.Agar well diffusion method (50% v/v)

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) acqusition 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) interpretion of the findings to bring out its significance, c) drafting of the manuscript and its revision to bring out critically important intellectual content.

Acknowledgement

We are grateful to all the health care workers who participated in this study. We acknowledge the help of Ms. Summera Rafiq, Head of the Department of Microbiology JBAS College for Women for helping us get Manuka honey from Australia and towards resources and facilities provided for this research.

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