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Antibacterial Activity of Honey Against Methicillin-Resistant *Staphylococcus aureus*

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Abstract: For centuries honey had a valued place in traditional medicine, being used in the treatment of wounds and diseases of the gut. The scientific community has now rekindled interest in the therapeutic use of honey in modern medicine and a number of published reports support its use in certain medical conditions, including burns and wounds. The aim of the present study to the effectiveness of the antimicrobial activity of honey against Methicillin-Resistant *Staphylococcus aureus* (MRSA) and Methicillin-Sensitive *Staphylococcus aureus* (MSSA) isolates collected from various Malaysian hospitals. Thirty isolated of Staphylococcus *aureus* were found to be resistant to routinely used higher antibiotics. Using an agar incorporation technique the sensitivity of these strains to honey was tested by the method of minimum inhibitory concentration. All the tested strains of *Staphylococcus aureus* showed inhibition with honey at concentrations of 25 and 30%. The present study recommended that the multidrug-resistant *Staphylococcus aureus* infection particularly wound and burns honey may be useful for controlling infection.

Key words: Methicillin-Sensitive *Staphylococcus aureus* (MSSA), Methicillin-Resistant *Staphylococcus aureus* (MRSA), Minimum Inhibitory Concentration (MIC), honey, wound infections, Malaysia

INTRODUCTION

Methicillin-Resistant *Staphylococcus aureus* (MRSA) is one of the most significant human pathogens that causes both nosocomial and community-acquired infections worldwide (McDonald, 2006). This dangerous bacterium could also cause a wide range of infectious diseases from mild conditions, such as skin and soft tissue infections, to severe, life-threatening debilitation, such as Toxic Shock Syndrome (TSS) and bacterial endocarditis (Lowy, 1998). MRSA was first detected in the early 1960s, shortly after methicillin (a β-lactam antibiotic) came into clinical usage (Livermore, 2001; Lowy, 1998). However, treatment of these infections has become more difficult since, *S. aureus* has become resistant not only to the regularly used penicillin-related (β-lactams) antibiotics, but also to other various structurally-unrelated antibiotics such as tetracycline, rifampicin and chloramphenicol (Enright *et al*., 2002). Although, honey has historically been known to have antimicrobial activity, to date, few reports have examined such activity against MRSA.

The recent emergence of CA-MRSA combined with its predominant presentation associated with skin and soft tissue infection, the previous study indicating honey as an effective treatment of HA-MRSA-related wound infection, as well as honey's ease of topical application, make the current study timely and of interest to healthcare practitioners involved with wound management. In particular, in burns, honey was found to be effective in preventing the conversion of superficial burns to deep burns, hypertrophic scarring and contractures. So far, the aim of the present study to effectiveness of the antimicrobial activity of honey against Methicillin-Resistant *S. aureus* (MRSA) isolates collected from various hospitals.

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Fig. 1: Inhibition of MRSA growth with 10% concentration of honey on mueller hinton agar

Fig. 2: Inhibition of MRSA growth with 15% concentration of honey on mueller hinton agar

Fig. 3: Inhibition of MRSA growth with 20% concentration of honey on mueller hinton agar

MATERIALS AND METHODS

Bacterial isolates: A total of thirty isolates of *S. aureus* were used in this study. Included were two reference strains of multiple drug resistant *S. aureus* obtained from University Hospital Petaling Jaya. All of them were obtained from different patients visiting Hospital Seremban, Hospital Miri, Sarawak and from the Laboratory Gribbel's Petaling Jaya.

Effect of inhibitory agent (honey) on growth of MRSA isolates and the related sequences: The pine tree honey was investigated for activity on MRSA isolates. Different concentrations of honey (v v^{-1}) were diluted in Mueller-Hinton's medium at 56°C to give final concentrations of 10, 15, 20, 25 and 30% as shown in the Fig. 1-7, which used for MRSA and MSSA. The inhibition effect of honey was studied *in vitro* by the agar dilution method. Bacterial broth culture (5 colonies/10 mL of broth) was incorporated into the Mueller-Hinton agar medium. Different concentrations of honey plates were incubated for 18 h at 37°C. After overnight incubation, the plates were observed for inhibition of growth. The plate of Mueller-Hinton/honey agar medium with the minimum honey concentration that completely inhibited the growth of the isolatewas taken as the Minimum Inhibitory Concentration (MIC) for that isolate (Subrahmanyam and Ugane, 2004). The MIC found to be optimal, as being completely inhibitory to all the strains tested, was taken as the MIC of the honey used for all the isolates.

RESULTS AND DISCUSSION

Susceptibility of *S. aureus* to honey: The Minimum Inhibitory Concentration (MIC) values started with five strains of MRSA and five strain of MSSA were found to be remarkably consistent as shown in Table 1 and 2.

The results of the study clearly show that honey has the potential to be used as an antibacterial agent to prevent and control infection with *S. aureus*. The use of natural products to enhance wound healing is a common practice in many parts of the world. Honey consists of a supersaturated solution of sugars and has a low pH

Table 1: Minimum inhibitory concentration (v v^{-1}) of MRSA strains

Concentration of honey on mueller hinton agar	10%	15%	20%	25%	30%
Number and percentage of MRSA strains inhibited	0% (all plates have growth)	0% (all plates have growth)	4 plates have no growth (80%)	5 plates have no growth (100%)	5 plates have no growth (100%)

Table 2: Minimum inhibitory concentration (v v^{-1}) of MSSA strain

Concentration of honey on mueller hinton agar	10%	15%	20%	25%	30%
Number and percentage of MSSA strains inhibited	0% (all plates have growth)	0% (all plates have growth)	2 plates have no growth (40%)	4 plates have no growth (80%)	5 plates have no growth (100%)

Fig. 4: Inhibition of growth of MRSA with 25 and 30% concentration of honey on mueller hinton agar

Fig. 5: Inhibition of MSSA growth with 10% concentration of honey on mueller hinton agar

between 3.2 and 4.5. This, together with honey's high osmolarity and the presence of hydrogen peroxide, reduces bacterial growth at the wound site. When, used as a wound dressing, honey has been reported to provide an ideal environment for the rapid tissue repair, regeneration and re-modeling that are essential for growth of the wound bed. *S. aureus* is the most frequently isolated wound pathogen and it is becoming increasingly resistant to antibiotics. Honey has been reported to be effective in eradicating antibiotic-resistant bacteria, in particular, MRSA. In this study, *S. aureus* was inhibited at concentrations of 30%, which was much higher than the percentage reported in other studies (Cooper *et al*., 2002). In clinical studies, it was observed that subsequent culture of wounds infected with *S. aureus* after treatment with honey made the wounds sterile. The MIC value (20%) obtained in this study demonstrate that the honey of median levels of potency were significantly effective in inhibiting MRSA and non-MRSA in *in vitro* tests. *S. aureus* is the most osmotolerant bacterium capable of causing wound infection (Chirife *et al*., 1983), honey at least 10 times dilute prevented growth. Honey achieved equivalent inhibitory effects at concentrations 6 and 3 times more dilute. The mode of action of honey has not yet been fully elucidated, but osmolarity, acidity, hydrogen peroxide generation and phytochemical components are considered important. In undiluted honey, the osmolarity and acidity undoubtedly limit bacterial growth. When, honey is diluted, a bee-derived enzyme (glucose oxidase) present in the honey is activated and catalyses the slow generation of hydrogen peroxide, which inhibits bacterial growth (White *et al*., 1963). In practice, when undiluted honey is applied to wounds, it is diluted by exudates and its antimicrobial activity at low concentrations is therefore, crucial. For clinical use, the selection of honeys with high levels of antibacterial activity is indicated to maximize therapeutic effects. Thus, the MIC value (20%) determined with the MRSA and the MSSA strains in this study indicate that there is not much difference in sensitivity to honey

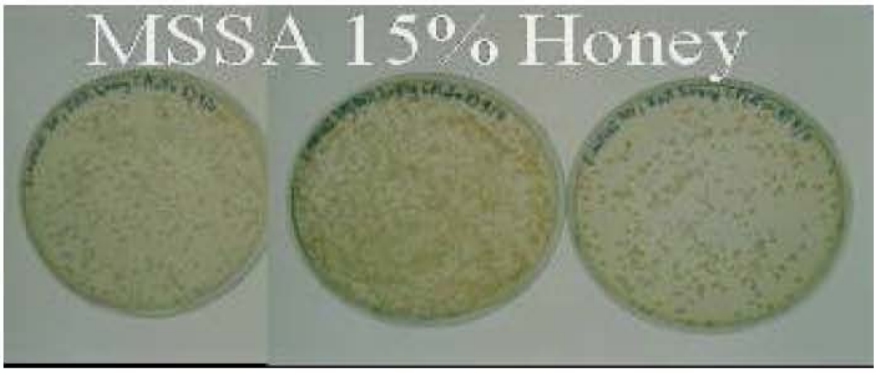

Fig. 6: Inhibition of MSSA growth with 15% concentration of honey on mueller hinton agar

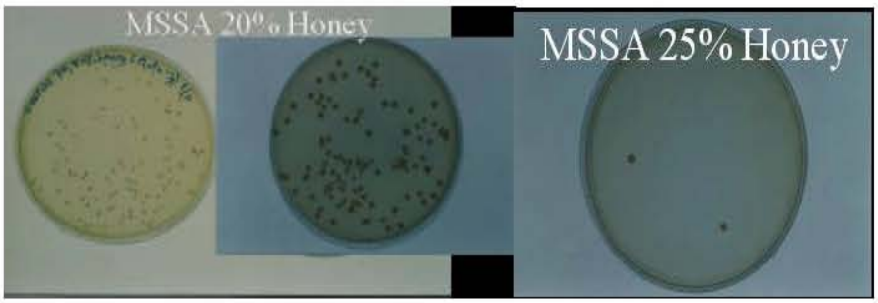

Fig. 7: Inhibition of growth of MRSA with 20 and 25% concentration of honey on mueller hinton agar

between methicillin-sensitive and methicillin-resistant staphylocoocci. Hence, honey has potential in the decontamination of wounds colonized by antibiotic resistant strains of bacteria and non-resistant strains. Generally, *in vitro* tests provide only an indication of the dilution capacity of an antimicrobial agent and do not assure that such potency will persist *in vivo*. Daily topical application of honey to infected wounds, however, has been reported to achieve wound sterility within 7-10 days. The findings of this study, together with three previous studies (Cooper and Molan, 1999; Cooper *et al*., 1999, 2002) show that honey offers promise as an effective wound antiseptic, with broad spectrum antimicrobial activity. Unlike, the use of antibiotics in treating wounds, laboratory evaluation of susceptibility to honey would not be necessary before the commencement of treatment. Also, honey does not adversely affect human tissue unlike other topical antimicrobial agents (Ward and Saffle, 1995).

Not only has it the potential to limit the growth of wound pathogens, but there is evidence that honey has the potential to promote healing (Molan, 1999; Tonks *et al*., 2001). No other antimicrobial agent possesses these characteristics. Honey is effective even when, it is diluted by burn wound exudates. In burns honey's antibacterial and anti-inflammatory properties allow a moist healing environment to be maintained that protects the wounds from deterioration and fibrosis. In a systematic review and meta-analysis assessing the rates of wound healing and the presence of bacteria in wounds in four studies using honey, including three studies on burns by the present research, honey was found to be beneficial (Molan, 2001). The development of honey in the form of a soft gel that can be easy modified and to conform to any shape will further increase the practicality of use with medical devices beyond that with the honey-impregnated dressings currently available. It remains for further clinical evaluation to be tried.

CONCLUSION

The present study demonstrated that *in vitro*, these natural products had an antimicrobial activity against the MRSA organisms tested. Further studies are now required to demonstrate the mechanism and components of such activity and whether this antimicrobial activity has any clinical application for the treatment of MRSA particularly skin and wound infections.

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