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In-vitro susceptibility of methicillin-resistant *Stayphylococcus aureus* to honey

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ABSTRACT

Wound infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) is becoming much complicated and costly to treat as antimicrobial resistance is quite common. Twenty five MRSA strains isolated from infected wounds and three ATCC reference strains were evaluated for their susceptibility to locally produced black seed (*Nigella sativa*), beri (*ZiziphusJujuba*) and shain honey (*Plectranthus rugosus wall*) by agar incorporation assay. Medically graded manuka honey (UMF 21^{+}) was included as control. Locally produced black seed honey inhibited all clinical isolates at mean MIC of 5.5% (v/v), whereas manuka honey at mean MIC of 4.4% (v/v). The other two locally produced honey; beri and shain honey inhibited these isolates at 6.4% and 10.4% (v/v) respectively. The result of the study has demonstrated that indigenous black seed honey has comparable antibacterial activity to manuka honey and thus offers a good new addition to the existing honey resource for the treatment of wound infections.

1. Introduction

Wound infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) has been associated with prolonged wound healing time, postoperative complications and mortality [1,2]. MRSA is one of the most frequently isolated bacteria in wound cultures [3]. In Pakistan, the prevalence of MRSA is quite high as compared to other parts of the world [4]. The inadequate progress of antimicrobial research in the recent decades has further worsened condition [5,6]. Therefore it is important to explore new products for treatment of wounds infected with MRSA.

Honey in this context offers multiple advantages over conventional antibiotics and been used as a popular food and alternative therapy for wound infections since antiquity [7]. Besides its potent antimicrobial effect honey also reduce inflammatory process, debride the necrotic tissue and enhance granulation, angiogenesis and epithelialization [8,9]. There have been numerous studies that have shown the effectiveness of honey in treating wound infections of miscellaneous etiology [10–12]. Recently Jull et al. (2015) in his intervention review, comprising of26 clinical trials (total of 3011 participants), concluded that honey is more effective in healing burns of partial thickness and post-operative infected wounds in comparison with conventional treatment [13].

The antimicrobial activity of honey is mainly derived from hydrogen peroxide generated by glucose oxidase (bee origin), its acidity (pH between 3.2 and 4.5), high osmolarity and a variety of non-peroxide factors (plant origin) [14–17]. The water activity (aw) of 'super-saturated' sugar solution in honey is around 0.6, while most bacteria required 0.94 and above. This level is well below the threshold needed for bacterial growth [18]. The osmotic pressure of high sugar content in honey draws water from bacterial cells, consequently this dehydrate and deprive them from the most essential requirement of life [19]. The acidity of honey also contributes to some extent in preventing the growth of many bacteria. Low pH of honey is due to the presence of several different organic acids. The acids are formed in honey from conversion of glucose and water into the gluconic acids and hydrogen peroxide by bee generated glucose oxidase enzyme [20]. The presence of such plethora of substances in one product makes it an ideal therapeutic agent for treatment of wound infections, particularly for MRSA

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infection, which is a local as well as global problem.

Although, honey as an alternative and complementary option has showed wide range of antimicrobial and bactericidal properties [21–23], still a limited range of certified licensed honey are in clinical practice for wound care [24–27]. Additionally, the assumed limitation of accessibility and cost are important factors which prompted us to explore indigenous honey with high medicinal values. Pakistan produces varieties of good quality honey, has been consumed as popular food and alternative therapy for several ailments including infected wounds [28]. There are number of studies conducted in this part of the world which highlighted the physiochemical and antibacterial aspects of indigenous honey against multi-drug resistant pathogens [28–31]. However, we have very little knowledge regarding antibacterial potential of locally produced honey against MRSA isolated from infected wounds. Previously we have screened one hundred honey samples from different geographical areas of Pakistan for their antibacterial activity against blood culture isolate of multidrug resistant *Salmonella typhi* [28]. In the present study minimum inhibitory concentrations (MICs) of three indigenous honey were determined against MRSA (n = 25) and ATCC reference strains (n = 3). Manuka honey which has been already approved as therapeutic agent for infected wounds and burns is used for comparison.

2. Materials and methods

2.1. Bacterial strains

Twenty-five wound culture isolates of MRSA were evaluated for their susceptibility to honeys. *Pseudomonas aeruginosa* (ATCC 27853), *Escherichia coli* (ATCC 25922) and *Staphylococcus aureus* (ATCC 25923) were also evaluated for their sensitivity to honey. The bacterial isolates and reference strains were obtained from Armed Force Institute of Pathology (AFIP), Rawalpindi, Pakistan. The MRSA isolates were re-identified and susceptibility tested at the Department of Microbiology, University of Health Sciences using the Kirby-Bauer method with oxacillin discs (1 μg) supplemented with 4% NaCl and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines [32]. Bacterial strains were stored in Microbanks tubes, which consist of vials containing porous beads that serve as carriers to support bacterial strains for long term storage. Before use, the strains were sub-cultured and retested for characteristic features. Working cultures were maintained on tryptic soya agar (TSA) slants at 2–8 °C for up to 2 weeks.

2.2. Honey samples

Manuka honey (Unique Manuka factor-21^{+}] was purchased from market. Locally produced black seed, beri and shain honey were obtained directly from bee keepers from different geographical areas of Pakistan. The black seed honey sample was collected from Islamabad district, whereas, beri and shain were collected from Bannu and Swat districts, respectively. The floral source of honey was determined on the basis of color, flavor, aroma of each honey, season, geographical areas and presence of flowering plants available for bees [33]. They were kept in amber colored bottles at room temperature. The honey samples which showed growth on blood culture medium were treated with gamma irradiation from Pakistan Radiation Services (PARAS), Lahore, Pakistan at 25 kGy for 10 h. This method of sterilization does not affect the antibacterial activity of honey [34].

2.3. Agar dilution assay

The method was adopted from French et al. (2005) study which evaluated the susceptibility of coagulase negative staphylococci to manuka honey [35]. A stock solution comprising of 20% (v/v) and 50% (v/v) of honey in sterile deionized water was prepared. Out of this stock, 1% incremental dilutions were prepared, up to the level of 20% in a final volume of 20 ml of Mueller Hinton agar (double strength) (Oxoid Ltd, UK). To achieve uniform homogenization, honey was kept at 50 °C, vortex vigorously and dispense into petri dishes (Greiner bio-one, Austria). The agar plates were allowed to cool and dry for 15 min. A 0.5McFarland's standard was adjusted from five well separated colonies of clinical isolates and ATCC strains from overnight blood agar. The poured petri dishes were inoculated (3 μl) with multi-point inoculator (Mast Diagnostic, UK) and incubated at 37 °C for 18 h s. The MICs were calculated as the lowest concentration of honey able to completely prevent the visible bacterial growth in tested media and the experiment was carried out in three sets [36].

2.4. Data analysis

IBM Statistical Package for Social Sciences (SPSS 21.0) was used for data analysis. The mean values of MICs of tested honey against twenty five clinical isolates of MRSA and standard deviation of mean values were calculated. Kruskal-Wallis test was applied to detect differences of mean MICs among tested honey samples. The results were considered to be significant at $p < 0.05$.

3. Results

The MICs values of the tested honey against different isolates of MRSA were almost same (Table 1). The mean MIC for manuka honey against all clinical isolates of MRSA was 4.4% (v/v), whereas the mean MIC values for black seed, beri and shain honey were 5.5,6.4 and 10.4% (v/v) respectively (Fig. 1). The manuka honey showed better antibacterial activity against all tested clinical isolates and ATCC reference strains as compared to indigenous honey (Table 1). There is slight variation of MIC of tested honey against MRSA and ATCC 25923*S.aureus*. Among ATCC reference strains, the *S. aureus* was most sensitive, whereas *E. coli* was found least sensitive (Table 1). There was significant difference in mean MIC of tested honey against MRSA (Kruskal-Wallis test, $p = 0.000$).

4. Discussion

Wounds infected with MRSA are an urgent problem in community, nursing homes and hospitals [37–39]. Unsuccessful attempts to eradicate MRSA from wound infections with conventional antibiotics increase the suffering of patients, increase their hospital stay, increased the risk of cross infection and subsequently increase in the prevalence of hospital acquired infection [40]. Honey dressing is increasingly being used for wound infections with great success because of its multiple benefits over conventional therapy. Honey with established antibacterial activity like manuka honey (UMF 10^{+} and above) has been recommended for the treatment of infected wounds and burns [41]. Therefore we choose manuka honey with UMF 21^{+} for comparison with locally produced honey.

Manuka honey has shown better antibacterial activity (4.4%) as compared to locally produced black seed (5.5%), beri (6.4%) and shain (10.4%) honey against twenty five clinical isolates of MRSA (Table 1). However, the difference of MIC of manuka and black seed honey is almost one percent dilution, which may not be clinically significant.

Therefore, black seed honey which is easily available in Pakistan and in comparison to manuka honey is quite affordable to local population can be used for skin infections and burns. At the same