The antibacterial activity of honey against methicillin-resistant *Staphylococcus aureus* isolated from pus samples

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ABSTRACT

A total of 300 samples were collected from hospital at Vapi and surrounding regions of Valsad district, Gujarat over a 24-month period to find out the antibacterial activity of honey on methicillin-resistant *Staphylococcus aureus* (MRSA). Of 144 *Staphylococcus aureus* isolates, 36 percent were found to be methicillin resistant. Among the different age groups a maximum of 39 samples were from the male patients, of which 44% isolates belong to the age group between 30 and 50 years, whereas 46% were from the age group between 15 to 30 years in case of female patients (n=13). The antibiotic resistivity of MRSA isolates showed a maximum of 98% to cloxacillin which was followed by other antibiotics in the following order: vancomycin > lincomycin > cefuroxime. The resistance to vancomycin was found to be 61.54%. The isolates were then tested against honey which is the most famous rediscovered remedy that is used to treat infected wounds and promote healing. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of honey were estimated against MRSA which was found to be effective at 30% concentration and above. This suggests the possible role of honey in the prevention of pyogenic infections.

Keywords: MRSA, antibacterial properties, honey, pus samples, minimum inhibitory concentrations, minimum bactericidal concentrations.

INTRODUCTION

The emergence of antibiotic resistance in microorganisms and their spread is threatening the medical community. This is particularly true in the case of *Staphylococcus aureus*. *S. aureus* is the most common cause of nosocomial infection and is of increasing concern because of their tendency to multiple antibiotic resistances which often complicates treatment. Many isolates of *S. aureus* have been found to be resistant to new semi-synthetic β-lactams (methicillin, oxacillin and flucloxacillin) which become known as Methicillin Resistant *Staphylococcus aureus* (MRSA). During the past four decades MRSA has spread throughout the world and has become highly endemic in many geographic areas [1]. MRSA infections are difficult to treat because of their resistance to the commonly used anti staphylococcal antibiotics namely macrolides, tetracycline, aminoglycosides, etc. Some of these MRSA strains are resistant to the most powerful antibiotics like vancomycin [2]. The increase in consumer use of complementary medicines has prompted an increasing interest in traditional and nonconventional medical treatments. One treatment that has

received much interest is honey. Honey is a traditional topical treatment for infected wound and can be effective on antibiotic resistance strains of bacteria. Their antibacterial activity varies with origin and processes. Honey has a long tradition of use within various medical systems [3,4] and over the past decade several research groups have focused their attention to this product [5-8].

Honey is essentially a highly concentrated water solution of two sugars, dextrose and levulose, with small amounts of at least 22 other more complex sugars. Many other substances also occur in honey, but the sugars are by far the major components. The principal physical characteristics and behavior of honey are due to its sugars, but the minor constituents such as flavouring materials, pigments, acids and minerals are largely responsible for the differences among individual honey types [9]. The ability of honey to kill microorganisms has been attributed to its high osmotic effect, high acidic nature, hydrogen peroxide concentration and phytochemical nature, which include its content of tetracycline derivatives, peroxides, amylose, fatty acids, phenols, ascorbic acid, terpenes, benzyl alcohol and benzoic acid [9,10]. However, large variations in the *in vitro* antibacterial activity of various types of honey have been reported and thus hampered its acceptance in modern medicine [11]. The production and type of honey produced by honeybees is dependent on the natural vegetative flowers blooming in different seasons. Thus the flowers from which bees gather nectar to produce honey may contribute to the difference in the antimicrobial activities. The purpose of the present study was therefore to evaluate the antibacterial activity of honey against MRSA from patients suffering from pus infections.

MATERIALS AND METHODS

A total of 52 clinical isolates of MRSA were obtained from patients of different age groups from hospital at Vapi and surrounding regions of Valsad district, Gujarat. Sterile cotton swabs and needle aspiration method were used for collecting samples from different pathological sites. The obtained clinical samples were plated on three different media namely nutrient agar, blood agar and mannitol salt agar and were incubated at 37ºC in an incubator. Identification was done on the basis of morphology, cultural characteristics, biochemical reactions and resistant to oxacillin discs (1µg) using Mueller Hinton agar. The isolates were then tested for antibiotic sensitivity pattern with cloxacillin, cefuroxime, lincomycin, tetracycline, levofloxacin, gentamicin and vancomycin. The zone of inhibition was measured and results were interpreted.

The honey sample used in this study was collected from Pardeshi babul and Neem forest of Kachchh and Banaskantha districts commercially known as Dhanvantari. The MIC of the honey was determined using the broth dilution method in sterile microtiter plates with lids. Fifty percent (w/v) stock solution of honey was prepared by weighing 10 g of the honey and bringing the volume up to 20 ml using Mueller Hinton broth. Further dilutions were done to obtain honey concentrations of 5, 10, 20, 30, 40 and 50%. The lowest concentration of honey that prevented the growth of each microorganism, as detected by lack of visual turbidity compared to a negative control, was recorded as the MIC. All honey solutions were freshly prepared before each assay. MBC was determined by taking a loopful of the culture medium from each test well (from the broth MIC assay) that showed no apparent growth and sub-culturing on fresh BA plates. After incubation at 37ºC for 24 h, the MBC was read as the least concentration showing no growth on the blood agar (BA) plates. The MBC was therefore the lowest concentration of honey required to produce sterile culture [12].

RESULTS AND DISCUSSION

Out of a total of 144 *Staphylococcus aureus* isolates, 52 numbers were found to be methicillin resistant, samples collected for the study were from different pathological sites. It was observed that majority of the MRSA obtained were from the male patients (75%), where 49% of the isolates

collected were from wounds followed by ear (36%), knee (8%) and waist (5%). Persons of the age group between 30 to 50 years seemed to be more prone to *Staphylococcus aureus* infection as compared with those younger to and older to 30 and 50 years of age respectively. In case of female patients (25%) majority of the isolates obtained were from the ear (46%) followed by wound (31%), knee (8%) and other pathological sites (15%) where maximum number of isolates were obtained from the age group between 15 to 30 years (Table 1 and 2).

Table 1. Percentage of isolates from different pathological sites.

Pathological site	Percentage of isolates	
	Male	Female
Ear	36	46
Wound	49	31
Knee	8	8
Leg	2	0
Waist	5	0
Other	0	15

Table 2. Age-wise distribution of MRSA.

Age group	MRSA (%)	
	Male	Female
0-1 year	0	0
1-5 years	0	0
5-15 years	2	8
15-30 years	41	46
30-50 years	44	38
50-70 years	5	8
more than 70 years	8	0

In our study, the prevalence of MRSA was found to be 36% of the investigated *S. aureus* isolates. In India, a higher prevalence rate (54.9%) of MRSA was recorded [13]. In comparison with our results higher prevalence rate of MRSA were recorded in other previous studies [14,15]. According to the gender, our data revealed that the prevalence of MRSA significantly differ between both the sexes. Person in the age group between 30 to 50 years of age seemed to be *S. aureus* carriers more than those younger than 30 years. This finding is in contrast with those obtained by other investigators who reported that MRSA groups did not significantly differ between the sexes [16].

The MRSA isolates subjected to antibiotic sensitivity pattern in the present study reveals that the isolates have also developed resistance to other antibiotics tested. The MRSA of clinical isolates showed a maximum of 98% resistance to cloxacillin and 50% of the isolates were found to be sensitive to tetracycline (Table 3). It is realizable that in the present study, 61.54% of the isolates showed resistance to vancomycin. MRSA are pathogenic due to their biochemical machinery which enable them to quickly colonize on host tissue and protect themselves from host defense mechanism and the distribution of MRSA is worldwide [17]. In this investigation, the resistant rate to different antibiotics among MRSA strains was higher than those sensitive to methicillin and this phenomenon was reported elsewhere [18].

Table 3. Resistogram (%) of MRSA.

Name of antibiotics	Sensitivity (S)	Intermediate (I)	Resistance (R)
Cloxacillin	2	0	98
Cefuroxime	23.08	19.23	57.69
Vancomycin	13.46	25	61.54
Lincomycin	23.08	17.31	59.61
Levofloxacin	44.23	9.62	46.15
Gentamicin	46.15	13.46	40.39
Tetracycline	50	9.62	40.38

Table 4. Evaluation of the Minimum Inhibitory Concentration (MIC) of honey against MRSA.

Honey concentration (%)	MRSA
5	R
10	R
20	R
30	S
40	S
50	S

The MIC for the MRSA strain is 30% in case of majority of bacterial species (Table 4). The MBC for the isolates showing 99.9% reduction in growth in presence of honey was also found to be 30%. Molan demonstrated the activity of honey against *S. aureus*, MRSA and *Pseudomonas* spp. It completely inhibits major wound infection pathogens including *S. pyogenus* and *S. aureus* [19].

The results of our study are consistent with the above study. Growth retardation and inhibition on *B. cereus* and *S. aureus* were observed at concentrations of 10% [20]. In contrast to this report the honey used in the present study could inhibit the test organisms at concentration of 30%. This might be due to the differences in the species of bees, which in turn results in difference in the production and type of honey [21] and the differences in the test methods and test organisms.

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