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Original Research Paper

ANTIMICROBIAL EFFECT OF HONEY ON MRSA ISOLATED FROM PUS SAMPLES

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ABSTRACT

The genus *Staphylococcus* is widely distributed in nature. *S. aureus* is a normal flora of skin and nasal passages. In present study methicillin resistance *S. aureus* was isolated and their antibiotic susceptibility test was done. A total of 64 staphylococci were isolated from 120 clinical specimens of pus samples. Among them 24 isolates were coagulase positive *S. aureus* and 40 isolates were methicillin resistance. Antimicrobial susceptibility testing revealed that methicillin resistance strain shows significantly resistance to other commonly used antibiotics like tetracycline and vancomycin. All the isolates were sensitive to ampicillin. Antimicrobial effect of honey on MRSA shows that maximum isolates were inhibited at 10% (v v^{-1}) concentration of honey.

Keywords: *Staphylococcus aureus*, MRSA, Antibiotics, Honey, Antibacterial property, Minimum Inhibitory Concentration.

INTRODUCTION

Staphylococcus aureus is a bacteria causing hospital acquired infection. The staphylococci species which are most frequently associated with human infection are *S. aureus, S. epidermidis* and *S. saprophyticus*. The genus of Staphylococcus is Staphylococcaceae, in which 47 species and 21 subspecies of staphylococci was recognized (Prax M., 2013). Staphylococcus species are Gram positive, non-motile, non-sporing cocci occurring singly, in pairs and in irregular clusters with opaque colonies (Vernozy *et al*., 2000). They had intrinsic ability to ferment carbohydrate. They ferment mannitol sugar and produce golden yellow colored colonies that turn Mannitol Salt Agar (MSA) yellow. The organism possesses the ability to produce deoxyribonuclease (DNase) and catalase enzymes and coagulase proteins, (clumping factor) used for their identification. The optimum growth temperature is 30°C-37°C. (P.R. Murray *et al.*, 2003). *S. aureus* has the ability to cause wide range of disease. It get colonised in skin, skin glands and mucous membrane that causes infections both in human and animals (Aklilu *et al*., 2010). It has ability to cause wide variety of diseases which includes superficial dermatological diseases as well as potentially fatal systemic debilitations (Moran *et al*., 2005). It has ability to cause large amount of infections and diseases which include postsurgical wound infections, some invasive infections such a bacteremia and septicemia, acute endocarditis and osteomyelitis, pneumonia, myocarditis, UTI and other soft tissue infections (STIs) (Graffunder & Venezia., 2002).

In 1960 the emergence of Methicillin-resistance Staphylococcus aureus (MRSA) came into existence & became a major problem in hospitals. (T.V., Rao & Babin D., 2013). Methicillin-resistant Staphylococcus aureus (MRSA) can resist large group of "Beta-Lactams" antibiotics. The Penicillins and the Cephalosporins class of antibiotics are most common among them. Due to this multi drug resistance property, and so it is called as a "super bug". Methicillin sensitive &

Methicillin resistant strain can also be found as normal commensals on skin & nasopharynx. Transmission of MRSA is mainly through direct contact with infected patients or through the touch of health care workers (Aseel Abdulsattar H A and Modak M S., 2015). As the prevalence of this pathogen increases, the prevention of its transmission has become important. Thus, Attempts for eradicating the carriage of MRSA is being carried out from either patients or medical staff that has been colonised by this organism. (Tanusri B *et al.,* 2013). MRSA is now endemic in India. The incidence of MRSA is 50% in South India whereas it is 25% in western part of the India. (Joshi S *et al*., 2013). Approx. 35% of hospital strains of *S. aureus* in USA are methicillin resistant & other penicillin antibiotics. (Batabyal B *et al*., 2012).

Due to development of multi drug resistivity, MRSA infections are difficult to treat. Hence various natural products can be used to treat MRSA infections. One of such natural compound that can be used is honey. Honey possesses various antimicrobial compounds that inhibit the growth of *S. aureus*. Honey contains glucose oxidase enzyme that has ability to form hydrogen peroxide which catalyzes conversion of glucose into oxygen and water. Hydrogen peroxide produces the free hydroxyl radicals that result in destruction of membrane lipids, DNA, and other vital cell components (Brydzynski *et al.*, 2011).

In present study 63 Staphylococcus isolates were identified from different clinical samples (pus from different anatomical sites, urine from infected patients and blood of indoor patients). Out of this 63 isolates about 40 isolates were Methicillin resistance *Staphylococcus aureus.* The antimicrobial activity of honey was checked against this MRSA isolates.

MATERIALS AND METHODS

Study Design

It was hospital based study.

Place of Study

KBS Commerce & NATRAJ Professional Sciences Collage, Vapi.

Sample Size

Total of 120 pus samples were collected from different anatomical sites.

Sample Collection

Aseptically pus samples were collected by sterile swab sticks from different anatomical sites such as ears, nasal, throat, molar sinuses, abscesses and wounds. Pus samples of chronic infections such as lungs and muscles abscesses were collected from operation-theater of Jeevandeep Surgical Hospital and Haria L. G. Rotary Hospital, Vapi, India. Blood and urine samples were also collected from hospital at Vapi. Special care was taken to avoid contamination with commensal organisms from skin while collecting samples from abscesses, wounds or other sites.

Bacterial Isolates

A total of 70 *Staphylococcus* isolates were obtained from clinical samples (blood, urine & throat swab, wound, nasal swab & ear swab) from in patients & out patients. Among this 63 isolates were *S. aureus.*

Isolation and Identification

The obtained samples were first plated on nutrient agar medium and incubated for 24 hours at 37°C. Small isolated colonies from nutrient agar plates were proceed for gram staining. Only those samples showing violet colored Gram positive cocci in grapes like clusters in preliminary Grams staining were processed further by culturing on Mannitol Salt Agar and Blood Agar and conformed for *Staphylococcus aureus* on the basis of morphological and colony characteristics. Catalase tube test, coagulase test, and various biochemical tests such as Methyl Red, Vogus Prosker, Indole, Citrate, and carbohydrate fermentation like Mannitol, Glucose, Lactose and Sucrose were used for the identification of *S. aureus*.

Antimicrobial Susceptibility Test

The antibiotic resistance profile was determined by Kirby-Buyer's disc diffusion technique on Muller Hinton agar plates. The suspensions of each isolates were prepared in Normal Saline. The isolates were then swabbed onto Muller Hinton Agar before the application of antimicrobial discs. The isolates were tested against a panel of fifteen

(15) antibiotics with the following concentration: Methicillin (5 μg), Vancomycin (30μg), Cefotaxime (30 μg), Erythromycin (15 μg), Ciprofloxacin (5 μg), Nalidixic acid (30 μg), Tetracyclin (30 μg), Ampicillin (10 μg), Kanamycin (30 μg), Levofloxacin (5 μg), Clindamycin (2 μg), Penicillin G (10 μg), Chloramphenicol (30 μg), Pipracillin (100 μg) and Piperacillin/Tazobactam (100 μg). Zones (ZI) of inhibition were measured and recorded after 24 hours of incubation at 37℃, and were interpreted according to the guidelines of the CLSI (Clinical Laboratory Standards Institute). The isolates that were resistant to methicillin (ZI $\leq$ 9mm) were regarded as methicillin-resistance *Staphylococcus aureus* (MRSA) while those with zone of inhibition (ZI $\geq$ 9mm) were termed susceptible.

Effect of Honey on the Growth of MRSA Isolates

The honey used in this study was collected from Karapur village, Goa and was investigated for antimicrobial activity on MRSA isolates. The MIC of honey was determined using dilution method (Nagi *et al*., 2009). Different concentration of honey (v v^{-1}) were diluted in Muller-Hinton medium to give final concentrations of 5, 10, 15, 20, 30 and 40% which were used for MRSA and MSSA. The inhibition effect of honey was studied in vitro by streaking isolates on honey containing Muller Hinton medium. Different concentration of honey plates were incubated for 24 hours at 37 ℃. After overnight incubation, the plates were observed for inhibition of growth. The plate of Muller-Hinton/honey agar medium with the minimum honey concentration that completely inhibited the growth of isolates was taken as Minimum Inhibitory Concentration (MIC) for that isolate. The MIC found to be optimal, as being completely inhibitory to all the isolates tested, was taken as MIC of honey used for all the isolates.

RESULTS AND DISCUSSION

A total of 120 clinical specimens of pus samples from different body sites were collected. Out of 120 samples 63 isolates were of *Staphylococcus aureus*. This includes 40 isolates of methicillin resistance *S. aureus* & 23 isolates of methicillin sensitive *S. aureus*. Antibiotics susceptibility testing was also carried out by using other commonly used antibiotics which include Vancomycin, Cefotaxime, Erythromycin, Ciprofloxacin, Nalidixic acid, Tetracyclin, Amphicillin, Kanamycin, Levofloxacin, Clindamycin, Penicillin G, Chloramphenicol, Pipracillin and Piperacillin/Tazobactam.

The study was design to determine the antibiotic profile of MRSA isolates from clinical samples. According to the antibiotic sensitivity pattern, the prevalence of MRSA was found to be 63.4% the investigated *S. aureus* isolates which is comparable than the study of Dala *et al.* (2014). According to the gender, the prevalence of MRSA differed significantly between both male and female and also according to age groups (fig 1 & 2). People between age group 15-30 seemed to be *S. aureus* carriers more than those younger than 15 years. The antibiotic resistance patterns of MRSA isolates were found to be highly variable with methicillin showing highest resistivity followed by ciprofloxacin (61.90%), followed by vancomycin (54.14%). The lowest resistivity is shown by Ampicillin (28.57%). (Table 1)

Susceptibility of *S. aureus* to Honey

The antibacterial activity of honey clearly shows that it has the potential to be used as antibacterial agent to prevent and control infection with *S. aureus* (Table 2). The MIC of honey was 20% v/v in case of 18% MRSA isolates, however, the remaining MRSA shows MIC of honey as 15% v/v. The MIC of honey for MSSA was found to be 15% v/v in case of all the MSSA tested. Nagi *et al*., (2009) reported 25% v/v honey concentration as the MIC for MRSA and 30% v/v honey concentration as the MIC for MSSA. However, higher MIC of honey at about 30% v/v concentration was reported in the previous study (Chauhan and Desai, 2012). Variation in the MIC of honey might be due to the differences in the species of bees, which in turn results in difference

in the production and type of honey (Mulu *et al*., 2004) and the differences in the test methods and test organisms.

Honey mainly consists of sugars and water, but also contains several vitamins, especially B complex and vitamin C, together with a lot of minerals. Honey has been used for its healing, nutritional and therapeutic properties since ancient times. It possesses anti-bacterial, anti-inflammatory and anti-oxidant properties that may be beneficial for combating multi-drug resistant bacteria as well as for preventing chronic inflammatory processes, such as atherosclerosis and diabetes mellitus.

CONCLUSION

The present study shows a high level prevalence of MRSA against widely used antimicrobial agents. In present investigation, the resistant rate to different antibiotics among MRSA strains was higher than those sensitive to methicillin and this phenomenon was reported elsewhere. In the inhibitory action of honey shows that MIC of honey in the range of 15-20% (v/v) concentration. Thus, honey can be used as an antibacterial agent for treating MRSA infection.

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Table 1: Antibiotic profile of *S. aureus* isolates

Antibiotics	Resistance %	Intermediate %	Sensitive %
Methicillin (5 µg)	63.49%	30.15%	6.34%
Vancomycin (30μg)	54.14%	33.33%	9.52%
Cefotaxime (30 μg)	31.74%	53.96%	14.28%
Erythromycin (15 μg)	31.74%	28.57%	34.50%
Ciprofloxacin (5 μg)	61.90%	3.17%	34.92%
Nalidixic acid (30 μg)	74.60%	19.04%	6.34%
Tetracyclin (30 μg)	50.79%	28.57%	36.50%
Ampicillin (10 μg)	28.57%	9.52%	61.90%
Kanamycin (30 μg)	33.33%	42.85%	23.80%
Levofloxacin (5 μg)	30.15%	39.68%	30.15%
Clindamycin (2 μg)	41.26%	15.87%	42.85%
Penicillin G (10 μg)	53.96%	9.52%	36.50%
Chloramphenicol (30 μg)	39.68%	14.28%	46.03%
Pipracillin (100 μg)	33.33%	19.04%	47.61%
Piperacillin/Tazobactam (100 μg)	47.61%	33.33%	19.04%

Table 2: MIC (v/v) of honey on MRSA and MSSA isolates

Concentration of honey on Muller Hinton Agar	% of MRSA inhibited	% of MSSA inhibited
5%	0% (all isolates have growth)	0% (all isolates have growth)
10%	0% (all isolates have growth)	0% (all isolates have growth)
15%	18% isolate shows growth	No growth
20%	No growth	No growth
30%	No growth	No growth
40%	No growth	No growth

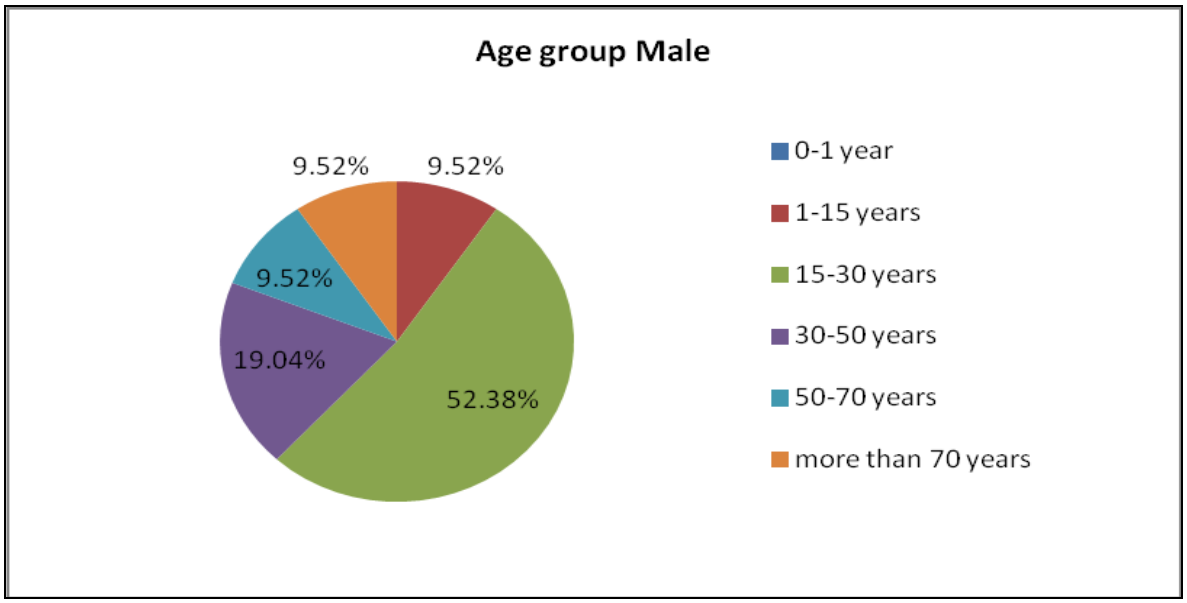

Figure 1: Age wise distribution of MRSA in Male

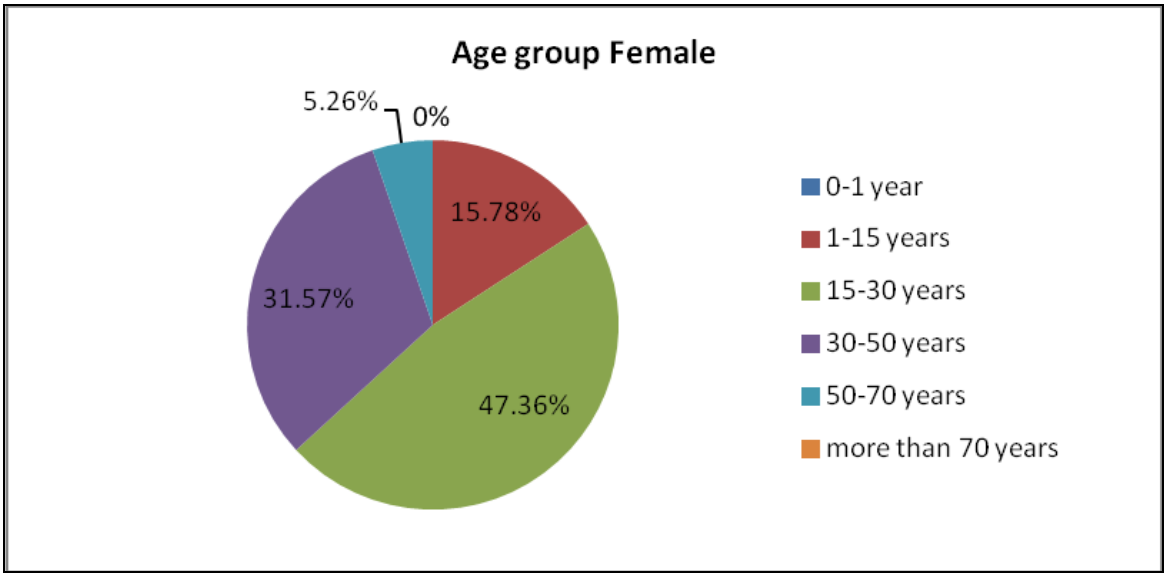

Figure 2: Age wise distribution of MRSA in Female

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