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Nasal Carriage of *Staphylococcus aureus* among Healthcare Workers in Althawra Hospital, Taiz City, Republic of Yemen

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Abstract: This study was conducted from October 2009 to May 2010 to identify the prevalence of *S. aureus* in the anterior nares of healthcare workers (HCWs) at Althawra Hospital, Taiz City, Republic of Yemen. The prevalence of nasal carriage of methicillin resistance *S. aureus* (MRSA) was 55.7% and of methicillin sensitive *S. aureus* (MSSA) was 30%. There was no significant difference between the sexes ($p = 0.251$), age ($p = 0.499$), smoking habits ($p = 0.767$), inhale water before praying ($p = 0.101$) and qat chewing ($p = 0.765$) with regard to the nasal carriage of MRSA and MSSA. A significant difference was only found for occupation ($p = 0.001$) between the carriage of MSSA and MRSA. The majority (97.44%) of MRSA isolates were resistant to penicillin G (97.43%), followed by ampicillin (87.18%), amoxicillin (82.05%), cloxacillin (71.79%), kanamycin (17.95%) and tobramycin (15.38%). 5.12 and 2.56% of MRSA isolates were resistant to vancomycin and gentamycin respectively. Only vancomycin showed 100% efficacy for MSSA. Antimicrobial activity of four types of natural honey was observed at concentrations of 50, 75 and 100% for MRSA and MSSA strains. Gentamycin application in the nose of patients reduced the risk of *S. aureus* burn wound colonization.

Key words: *S. aureus*, Nasal carriage, Antibiotic susceptibility, Honey susceptibility, Nasal gentamycin.

INTRODUCTION

Staphylococcus aureus is both a human commensal and a frequent cause of clinically important infections (Lowy, 1998). The ecological niches of *S. aureus* strains are the anterior nares (Vinodhkumaradithyaa *et al.*, 2009). One of the important sources of staphylococci for nosocomial infection is nasal carriage among hospital personnel. Almost 25% of the health care workers are stable nasal carriers, and 30% to 50% of them also possess the bacteria on their hands. Occasionally, health care workers who carry *S. aureus* in their nares can cause outbreaks of surgical-site infections (Luzar *et al.*, 1990 and Cespedes *et al.*, 2002). Most of invasive *S. aureus* infections are assumed to arise from nasal carriage (Von Eiff *et al.*, 2001). Methicillin-resistant *Staphylococcus aureus* (MRSA) is now a problem within health care organization and in the community (Kluytmans, 1997). The incidence of community-acquired and hospital-acquired *S. aureus* infections has been rising with increasing emergence of drug-resistant strains called methicillin-resistant *S. aureus* (Steinberg *et al.*, 1996 and Herold *et al.*, 1998). Methicillin resistant is due to the presence of mec A genes coding for penicillin binding protein (PBP2A) with a low affinity for β-lactam antibiotics (Ito *et al.*, 2001). In Indian hospitals, MRSA is one of the common causes hospital-acquired infections and different hospitals have reported anywhere from 30-80% methicillin-resistant based on antibiotic sensitivity tests (Anupurba *et al.*, 2003). Resistance toward antibiotics is associated with an increase in disease severity, which increases period of hospitalization, high mortality and increasing treatment costs, including a need for use of alternative drugs (Isturiz and Carbon, 2000 and Ogeer-Gyles, 2006). Honey has historically been known to have antimicrobial activity (Al-Haj *et al.*, 2009). Honey is produced from many sources, and its antimicrobial activity varies greatly with origin and processing (Molan, 1992). Honey has potential in the contamination of wounds colonized by antibiotic resistant strains of bacteria and non-resistant strains (Al-Haj *et al.*, 2009). Part of *S. aureus* wound colonizations in patients is of endogenous origin, i.e. their wounds become colonized by the *S. aureus* strain already present in the patients' nose or throat at the time of admission (Luzar *et al.*, 1990). By eliminating nasal carriage, nasal mupirocin could prevent endogenous *S. aureus* wound colonization (Kooistra-Smid *et al.*, 2008). The aim of the present investigation was to evaluate the prevalence of nasal carriage of MRSA among HCWs in Althawra Hospital, Taiz, Yemen and to determine the susceptibility of MRSA to various antibiotics and natural honeys. Also, the effect of a short course of nasal gentamycin on the incidence of *S. aureus* burn wound colonization was studied.

MATERIALS AND METHODS

Stetting and Design:

The study was performed at Althawra Hospital, Taiz City, Republic of Yemen. Althawra Hospital is a 450-bed, tertiary-care and teaching hospital with approximately 165000 admissions per year. This cross sectional

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study was carried out from November 2009 to April 2010 among healthcare workers (HCWs) from different wards at the hospital.

Data Collection:

Data collected included: sex, age, occupation (doctor, nurse and auxiliary nurse), antibiotic therapy during last three months, inhale water five times every day before praying, smoking habits, qat chewing (qat chewing is Yemeni habit where some persons chew the leaves of plant *Catha edulus* daily for about four hours) and history of underlying disease such as ischemic heart disease (IHD), chronic obstructive pulmonary disease (COPD) and diabetes mellitus (DM).

Microbiology Methods:

To collect nasal swabs the vestibula of both right and left nares were swabbed with a sterile swab (Kooistra-Smid *et al.*, 2008). Swabs were plated on Baired Parker (BP) agar medium {pancreatic digest of casein 10.0g, beef extract 5.0g, yeast extract 1.0g, glycine 12.0g, sodium pyruvate 10.0g, lithium chloride 5.0g, agar 20.0g per 950 ml, after sterilization cool to 45-50°C and aseptically add 50 ml of egg yolk tellurite emulsion (containing potassium tellurite consists of 30% egg yolk suspension with 0.15% potassium tellurite). All were processed on the day of sampling. The plates were incubated at 37°C for 48h. The black, shiny and convex colonies with clear zone were counted and randomly one colony was selected and grown on nutrient agar and subjected to gram stain, catalase test and coagulase test. The gram positive, catalase positive and coagulase positive isolates were considered as *S. aureus. S. aureus* isolates were kept in refrigerator for further studies.

Coagulase Test:

Tube coagulase test was performed by diluting the plasma in freshly prepared normal saline (1:6). Three to four pure colonies were emulsified in 1 ml of diluted plasma and the tubes were incubated at 37ºC. Readings were taken at 1, 2, 3 and 4 h and further incubated over night at room temperature if no clot formation was observed (Baird, 1996)

Catalase Test:

The catalase test was done by transferring a small portion of the culture with a clean rod onto a slide with 3% (v/v) hydrogen peroxide (H_2O_2) which is kept under cover of a Petri plate to avoid aerosols. If the bacteria produce catalase, they will split hydrogen peroxide and oxygen will be evolved. The evolution of gas causes bubbles to form and is indicative of a positive test.

Methicillin-Resistance Test:

Methicillin resistance test was checked for all isolates of *S. aureus* by the disc diffusion method of the National Clinical and Laboratory Standards Institute using 1µg oxacillin discs (Hi-Media, India) on Mueller-Hinton agar. Zone diameters were measured and recorded after a 24h incubation at 37ºC, the results were classified as sensitive($\geq$ 13 mm), intermediate (11-12 mm), or resistant ($\leq$ 10 mm) (Mainous III *et al.*, 2006)

Antibiotic Susceptibility Testing:

The antibiotics sensitivity of MRSA and MSSA strains was carried out using Kirby-Bauer's disc diffusion method (Ortez, 2005). The antibiotic discs used were cloxacillin (5µg/disc), tobramycin (10µg/disc), kanamycin (30µg/disc), pencillin-G (10 IU/disc), gentamycin (10µg/disc), vancomycin (30µg/disc), ampicillin (10µg/disc), and amoxicillin (30µg/disc). *S. aureus* isolates were inoculated in Mueller-Hinton broth and placed in incubator for 24 hours at 37ºC. When its turbidity equivalent to that of a 0.5 McFarland standard, Mueller-Hinton agar plates were inoculated with each broth culture, these plates were inverted and incubated at 37ºC for 24-48h. Zones of inhibition were measured as recommended by the Clinical Laboratory Standard (CLSI, 2005), formerly National Committee for Clinical Laboratory Standards.

Antibacterial Activity of Natural Honey:

Susceptibility of 60 nasal *S. aureus* isolates (MSSA and MRSA) to bees honey was carried out on Mueller-Hinton agar by the agar disc diffusion method. Four types of bees honey, Crest's I honey and Crest's II honey of the plant source, *Ziziphus spina-christi* in two hilly regions in Yemen, Tohama and Sharaab region respectively, Acacia's honey of the plant source *Acacia asak* and pasture honey of mixed pasture source. A suspension of the *S. aureus* strains (0.1 ml of 10^9 cells ml^{-1}) was spread on the solid media plates by sterilized glass spreader. Honey was dissolved in sterile water to prepare solution of 25, 50 and 75% (v/v) honey immediately before use. Honey discs were prepared by impregnation of honey concentration (25, 50, 75 and 100%) in 6 mm diameter filter paper discs and placed on the inoculated plates. The plates were incubated at 37°C for 24h. The diameters of the inhibition zones were measured in mm. Three replicate plates were used at each concentration of honey.

Effect of Nasal Gentamycin on S. aureus Burn Wound Colonization:

Seven patients having nasal carriage of *S. aureus* and with burn wounds. The patients received nasal gentamycin after sampling nose and burn wounds. Gentamycin ointment (Medica) was applied three times daily for 7 days. After the start of gentamycin course, cultures of anterior nares and burn wounds for *S. aureus* were carried out after 3 and 7 days.

Statistical Analysis:

For elucidating interspecific variations, the variables were statistically analyzed by Chi-square test using the spss package release 9.0 (spss Inc. Hewlett, PC. USA). A logistic regression model was built to identify risk factors. $P < 0.05$ was considered significant.

Results:

In this study, 70 of health-care workers (HCWs) at Althawra Hospital, Taiz city, Republic of Yemen, were screened for nasal carriage of *S. aureus*. Of the 70 HCWs, 60(85.7%) of participants were nasal carriers of *S. aureus*. 60 *S. aureus* strains were isolated on Baird-Parker (BP) agar. All isolates were gram positive, catalase positive, coagulase positive and formed black colonies surrounded by clear zone on BP agar. Of the 60 nasal carriers of *S. aureus*, 39 (65%) carried MRSA and 21 (35%) carried MSSA (55.7% and 30% of all HCWs, respectively).

Table (1) shows the nasal carriage of MRSA and MSSA among HCWs in relation to potential factors. There is no significant difference between nasal carriage of MRSA and MSSA with regard to sexes ($p = 0.251$), age ($p = 0.499$), inhale water before praying ($p = 0.101$), smoking habits ($p = 0.767$) and qat chewing ($p = 0.765$). Some risk factors of HCWs were studied in relation to nasal carriage of MSSA and MRSA, such as ischemic heart disease (IHD), chronic obstructive pulmonary disease (COPD), antibiotic use through the last three months and diabetes mellitus (DM).There is no significant difference between these and nasal carriage of MRSA and MSSA. The only significant independent factor for nasal carriage of MRSA and MSSA was occupation ($p = 0.001$).

Table 1: Multivariate analysis of potential factors for MRSA and MSSA among HCWs at Althawra Hospital.

Variable	Carrier status		*p*- value	Logistic regression	
	MSSA, n(%)	MRSA, n(%)		OR (95%Cl)	*p*- value
Gender			0.251		
Male	14 (66.7)	20 (51.3)			
Female	7 (33.3)	19 (48.7)			
Age (years)	34.14±11.51	36.87±13.81			
Stratified age			0.499		
≤ 29	5(23.8)	10(25.6)			
30 – 49	14(66.7)	21(53.8)			
≥ 50	2(9.5)	8(20.5)			
Occupation			0.001	4.423(o.543-2.886)	0.0014
Doctors	10(47.6)	3(7.6)			
Nurse	8(38.1)	19(48.7)			
Auxiliary nurse	3(14.3)	17(43.6)			
Antibiotic use			0.399		
Absent	11(52.4)	16(44.o)			
Present	10(47.6)	23(58.9)			
Inhale water			0.101		
Absent	10(47.6)	27(69.2)			
Present	11(52.4)	12(30.8)			
Smoking habit			0.767		
Absent	16(76.2)	31(79.5)			
Present	5(23.8)	8(20.5)			
Qat chewing			0.765		
Absent	10(47.6)	17(43.6)			
Present	11(52.4)	22(65.4)			
IHD			0.950		
Absent	20(95.2)	37(94.9)			
Present	1(4.7)	2(5.1)			
COPD			NA		
Absent	21(100)	39(100)			
Present	0.00	0.00			
.DM			0.950		
Absent	20(95.2)	37(94.9)			
Present	1(4.7)	2(5.1)			

MSSA, methicillin-sensitive *Staphylococcus aurues*; MRSA, methicillin-resistant *Staphylococcus aurues*; IHD, ischemic heart disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus.

The percentage of resistance to various antibiotics for MRSA and MSSA strains from Althawra Hospital personnel was shawed in Table (2). None of the 39 MRSA strains and the 21 MSSA strains were susceptible to all antibiotics tested. Gentamycin was found to be effective antimicrobials with efficacy 97.4% for MRSA isolates and 95.23% for MSSA isolates. Vancomycin, tobramycin and kanamycin showed 94.87, 84.61 and 82.05% sensitivity respectively for MRSA and 100, 80.95, and 76.19 efficacy respectively for MSSA. Cloxacillin, amoxycillin, ampicillin and penicillin G demonstrated only 28.2, 17.95, 12.82 and 2.56 efficacy respectively for MRSA and 66.67, 76.19, 61.9 and 19.05% sensitivity respectively for MSSA.

Strains of 39 MRSA and 21 MSSA were tested against four types of bees honey in order to evaluate the antimicrobial potential of honey (Figs. 1&2). The concentration 25% of the four honeys proved to be totally ineffective. The minimum inhibitory concentration (MIC) values started at concentration of 50% with 17(43.58%) strains of MRSA and 14(66.67%) strains of MSSA for Crest's honey II followed by 15(38.46%) strains of MRSA and 14(66.67%) strains of MSSA for Acacia's honey, 14(35.90%) strains of MRSA and 14(66.67%) strains of MSSA for pasture honey and 13(33.33%) strains of MRSA and 14(66.67%) strains of MSSA for Crest's honey I. There is no remarkable difference in the effectiveness of concentration 50% and concentrations 75 and 100% of the four types of honey against MRSA and MSSA. The MIC value (50%) for four types of honey with MRSA and MSSA strains in this study indicate there is not much difference in the sensitivity.

Table 2: Antibiotic resistance of *S. aureus* (MSSA and MRSA) from AlthawraHospital personnel.

Antibiotic	MRSA		MSSA		χ^2	*p*- value
	N = 39	%	N = 21	%		
Gentamycin	1	2.56	1	4.76	0.20	0.65
Tobramycin	6	15.38	4	19.05	0.13	0.72
Vancomycin	2	5.12	0	0	1.11	0.29
Kanamycin	7	17.95	5	23.81	0.29	0.59
Cloxacillin	28	71.79	7	33.33	8.31	0.004
Amoxycillin	32	82.05	5	23.81	19.59	<0.0001
Ampicillin	34	87.18	8	38.10	15.66	<0.0001
Penicillin G	38	97.44	17	80.95	4.86	0.03

Seven patients with burns were undergone a short course of nasal gentamycin (Table 3). These patients were nasal carriers of *S. aureus*. On admission of nasal gentamycin, six of 7(85.7%) patients were positive for *S. aureus* burn wound colonization. During the gentamycin treatment, four of 7 (57.14%) patients were negative for *S. aureus* burn wound colonization and one of 7(85.7%) patients was negative for nasal carriage of *S. aureus* after 3 days of the start of gentamycin course. After seven days of gentamycin admission, five of 7(71.42%) patients were negative for *S. aureus* burn wound colonization and two of 7(28.57%) patients were negative for nasal carriage of *S. aureus*.

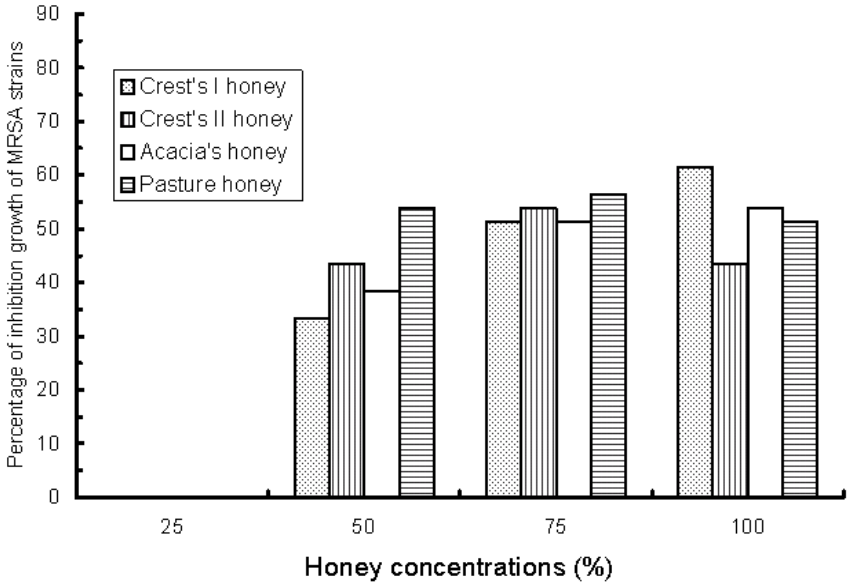

Fig. 1: Susceptibilty of nasal *S. aureus* isolates (MRSA) to four types of honey at different concentrations (V/V).

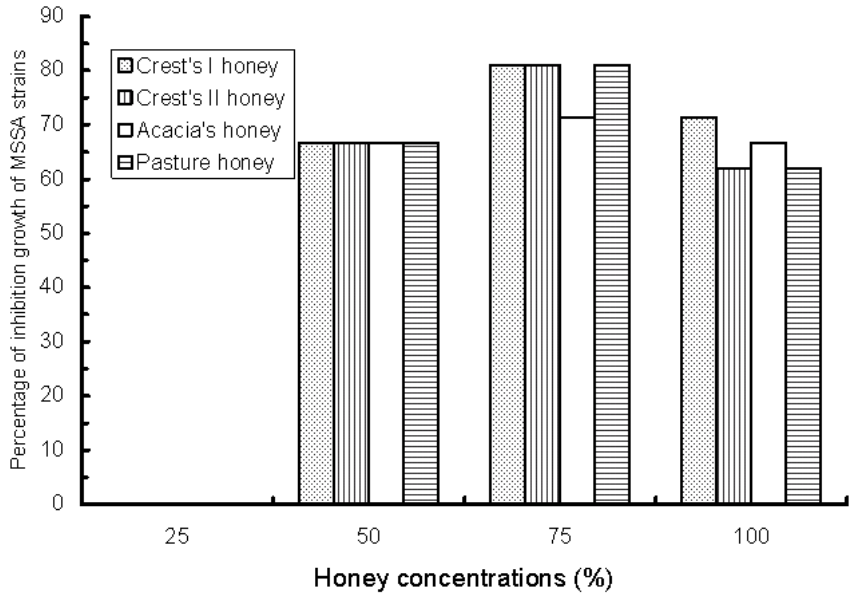

Fig. 2: Susceptibilty of nasal *S. aureus* isolates (MSSA) to four types of honey at different concentrations (V/V).

Table 3: Effect of nasal gentamycin on prevention of *S. aureus* burn wound colonization.

Time of admission (days)	Patients with *S. aureus* nasal colonization n (%)	Patients with *S.aureus* burn wound colonization n (%)
0.0	7 (100)	6 (85.7)
0.3	6 (85.7)	3 (42.85)
7.0	5 (71.4)	2 (28.570)

Discussion:

The nasal carriage of *S. aureus* (MRSA and MSSA) among HCWs at Althawra hospital, Taiz city has not been previously determined. In the present study 60 (85.7%) of participants carried *S. aureus*. Out of the 60 carriers, 39 (65%) carried MRSA and 21 (35%) carried MSSA. Nasal carriage rate among hospital personnel and patient (60-70%) are much higher than those in community carriers (30-50%) (Lowy, 1998). The prevalence of nasal carriage of MRSA among HCWs in this study was higher than that found in the studies of Mainous III *et al.* (2006) where they recorded that the prevalence of MRSA among *S. aureus* isolates was 2.58% for an estimated population carriage MRSA of 0.84% or 2.2 million persons and lower than that reported by Naseer and Jayaraj (2010) where they found among 327 *S. aureus* strains, 255 MRSA (77.9%) was detected. The proportion of MRSA amongst *S. aureus* isolates was found to be 51.6% and these isolates were multidrug resistant (Vidhani *et al.* 2001). Akoua *et al.* (2004) conducted a similar study and reported the carriage rate of *S. aureus* 45.5%, out of these 38.7% strains were resistant to methicillin, whereas Alghaithy *et al.* (2000) reported 26.1% *S. aureus* carriage and out of which 18.3% were MRSA positive. High prevalences of MRSA reported earlier ranged from 34.8 to 70.6% (Preetha *et al.*, 2000 and Rahbar *et al.*, 2006). Nasal carriage of MRSA or MSSA varies in different geographical areas (Abudu *et al.*, 2001). The prevalence of carriage of methicillin resistance is high and increasing in hospital environments (Alghaithy *et al.*, 2000).

Only occupation (Doctors, nurses and auxiliary nurses) showed significant association with the nasal carriage of MRSA and MSSA and this my lead to cross-contamination of MRSA between personnel and patients. Other variables studied were not risk factors for nasal carriage of *S. aureus* strains. Munoz *et al.* (2008) found a significant rate of nasal carriage of *S. aureus* among patients undergoing major heart surgery (27%) and demonstrated that it is an independent risk factor for the develioment of surgical site infection and increased mortality in this population. Also they found that nasal carriage of *S. aureus* and diabetes mellitus were independent preoperative risk factors for surgical site infection. Askarian *et al.* (2009) reported univariate analysis suggests that only occupation is a risk factor for nasal carriage of MRSA among HCWs. Logistic regression showed that having a nuring occupation is independently associated with MRSA. Few demographic or clinical characteristics are related to either *S. aureus* carriage or, more specifically, MRSA carriage (Mainous III *et al.*, 2006).

Rate of resistance of MRSA against penicillin G and ampicillin was highest in this study. 97.77% and 87.18% resistance was observed against penicillin G and ampicillin. This agrees with a previous study (Farzana *et al.*, 2008) which found more than 80% resistance against penicillin and ampicillin. Penicillin resistant was found to be 92% (Naseer and Jayaraj, 2010). Amoxicillin resistance shown by MRSA and MSSA strains

isolated in this study was 82.05% for MRSA and 23.81% for MSSA. None of the MRSA isolates was found to be sensitive to amoxicillin (Vidhani *et al.*, 2001). Lowest rate of resistance was seen in gentamicin (2.56%), vancomycin (5.12%), tobramycin (15.38%) and kanamycin (17.95) for MRSA. Improper use of antibiotics creates problems such as the emergence of bacterial resistance to antibiotics (Chambers, 1997). Sierdzki *et al.* (1999) found that all the MRSA strains (PC-1, PC-2 and PC-3) examined showed heterogenous vancomycin-resistance phenotypes. They reported that the therapeutic failure of vancomycin for MRSA infections have aroused considerable concern regarding the emergence of MRSA strains for which there will be no effective therapy.

All MRSA isolates were resistant to ampicillin, followed by cephalexin (37.5%), ciprofloxacin (37.5%), tetracycline (37.5%), gentamycin (25.0%), erythromycin (0.0%) and vancomycin (0.0%) (Shakya *et al.*, 2010).

The antimicrobial property of honey is thought to be due to non-specific mechanisms (physicochemical and peroxidial properties); this value is more similar to disinfectants than to antibiotics. Thus can be expected to honey should be possess broad-spectrum antimicrobial potency and very low microbial resistant to it. Researches relating honey show that pure honey is bactericidal for many pathogenic organisms, including various gram-negative and gram-positive bacteria (Haffejee and Moosa, 1985; Ceyhan and Ugur, 2001 and Al-Jabri *et al.*, 2003). Strains 39 MRSA and 21 MSSA isolated in this research were tested against four bees honeys of known floral source, where there is no remarkable difference in the effectiveness of diluted (50 and 75% v/v) and undiluted honey against MRSA, while MSSA strains are more sensitive to diluted honey (75% V/V) than undiluted honey. In undiluted honey, the osmolarity and acidity undoubtedly limit bacterial growth. When many honeys are 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). This activity varies markedly from honey to honey (Molan, 1992). The antibacterial activity of these natural honeys was, therefore undoubtedly not attributable to sugar content alone. Variability in the composition of honey is expected (White 1979). The MIC value in this study was at concentration of 50% which was much higher than that reported by Al-Haj *et al.* (2009) and Cooper *et al.* (2002). The findings of Cooper *et al.* (1999) show that honey offers promise as an effective wound antiseptic, with broad spectrum antimicrobial. Antimicrobial activity of honey is thought to be due to physicochemical properties (high content of reducing sugars, high viscosity, high osmotic pressure, low pH, low water activity, low protein content and hydrogen peroxide (Hyslop *et al.*, 1995 and Molan and Cooper, 2000).

S. aureus nasopharyngeal colonization increased the risk of burn wound colonization, which supports the importance of the endogenous infection route (Kooistra-smid, *et al.*, 2008). The risk of *S. aureus* burn wound colonization in this research was reduced in the period during which a short course of nasal gentamycin was administered to all patients. Nasal carriage of *S. aureus* was eliminated in 81.5% of patients receiving mupirocin and 46.5% of patients receiving placebo (Konvalinka *et al.*, 2006). Nasal carriage of *S. aureus* significantly increases the rate of nosocomial surgical site infection (SSI) after major heart surgery (MHS) and is an independent risk factor for postoperative wound infections (Munoz, *et al.*, 2008). Data from our Burn Centre showed that 34% of the patients who developed burn wound colonization were colonized by the endogenous route and 66% by the exogenous route (Kooistra-Smid *et al.*, 2004). An alternative for the eradication of *S. aureus* has recently been proposed by Segers *et al.* (2006). The authors showed that perioperative decontamination of the nasopharynx and oropharynx with 0.12% chlorhexidine gluconate reduces the rate of lower respiratory tract infections and deep SSI after cardiac surgery. After decontamination of the nose with chlorhexidine gluconate, positive cultures for *S. aureus* were reduced by 57.5%.

In conclusion, my study confirms the high prevalence of *S. aureus* nasal carriage among HCWs in Althawra Hospital, Taiz City, Yemen. It also shows that the rate of MRSA carriage is high. Occupation is related to either MRSA and MSSA nasal carriage. The injudicious use of the antibiotics in Ymen, enhanced chances of emergence resistant strains. The findings of this study show an antimicrobial activity of natural honeys against the MRSA and MSSA strains at low concentrations. The results suggest that a short course of prophylactic intranasal gentamycin to all patients admitted to a Burn Centre may help to decrease the overall rate of *S. aureus* burn wound colonization.

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