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Prevalence of nasal carriage of methicillin-resistant *Staphylococcus aureus* among healthcare workers in Tarhuna and Mesllata hospitals

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Abstract

Background: *Staphylococcus aureus* is among the most common causes of hospital-acquired infections. Methicillin-resistant *Staphylococcus aureus* considered a hospital pathogen associated with hospital admission and antibiotic use.

Objectives: The present study was conducted to determine the rate of *S. aureus* and MRSA among health care workers (HCWs) in Mesllata and Tarhuna hospitals. Nasopharyngeal swabs were taken to determine the rate of MRSA, estimate the frequencies distribution of MRSA carrier among each group of HCWs and characterize the antibiotic resistance profile.

Methods: samples collected were 210. HCWs included were doctors, nurses, technicians and pharmacists. Samples identified by standard methods of culturing, staining and biochemical testing. Antimicrobial susceptibility testing were done by Kirby-Bauer method and confirmed by BD phoenix IM system ®. The findings were statistically analyzed by *chi-square* test.

Results: Nasal carriages of *S. aureus* were (15.2%) and among them (12.5%) isolates were MRSA. *S. aureus* rate was (81.25%) among direct contact HCWs with patients along with MRSA (75%). *S. aureus* rate was high among nurses followed by doctors. The highest rate of *S. aureus* carriers by ward showed that 62.5% respondents of high risk infection wards; p=0.01. Moreover, 31.3% respondents of intermediate risk of infection wards and 6.3% respondents of low risk of infection wards. In relation to working period of participants hours/day (p=0.01) and working years (p=0.02) were significantly affect the occurrence of MRSA respectively. Moreover following of hygiene protocols significantly (p<0.05) affect MRSA occurrence. All isolates of *S .aureus* were susceptible to ciprofloxacin, oxacillin, cefoxitin, vancomycin and clindamycin and were susceptible to rifampin and mupirocin. MRSA isolates were 100% resistant to cefoxitin and ampicillin and sensitive to fusidic acid, tetracyclin, linezolid and moxifloxacin.

Conclusion: The rate of MRSA was low 1.9% as both hospitals followed continuous awareness lectures. This highlights the fact that educating HCWs continuously eliminates MRSA carriage.

Key words: *MRSA, Staphylococcus aureus, antibiotic resistance, health care workers*

Introduction

The human body is continuously inhabited by different types of microorganisms as a normal flora. The most common medical group causes of infections are *Staphylococcus aureus* (*S. aureus*), *Staphylococcus epidermidis* (*S. epidermidis*) and *Staphylococcus saprophyticus* (*S. saprophyticus*) they can be very difficult to treat especially those contracted in hospitals because of the remarkable ability of bacteria to become resistant to antimicrobial agents and frequently causing nosocomial

infections. Nosocomial infections can be defined as an infection in hospitalized patients or in health care workers. Previous studies have shown that spreading rates from patient to health care workers (HCWs) and HCWs to community were 23.7% and 4.6% respectively (1). The strains have been responsible for many nosocomial infections colonized strains often act as reservoirs for the spread of this organism within hospitals in many parts of the world (2 & 3). The most common way of transmission of MRSA is contact transmission; direct contact occurs when microorganisms are transferred directly from one person to another (4). *S. aureus* was reported to be the main cause of a variety of infections and diseases by infecting tissues typically creating abscesses and localized skin infections, hair follicles or deep localized infections, *S. aureus* causes acute and chronic infection of bone marrow, acute endocarditis, septicemia, necrotizing pneumonia and nosocomial infections. *S. aureus* is one of the most common causes of hospital-acquired infections related to wounds surgical or bacteremia associated with catheters (5 & 6). Among Libyan hospitals; Belgasim *et al*., 2010, screened HCWs in the intensive care unit and operating theaters in African Oncology institute in Sabrata city, Libya found that MRSA carriage rate was 11.6% of studied individuals. These results showed that doctors had higher carriage rate compared with nurses. The prevalence of MRSA was 20% and 7.14% respectively (7). In Alkhums hospital medical staff, Libya; Krima *et al*., 2014 stated that the prevalence of MRSA positive was in nasal swabs at 14.7% of tested individuals (8). Screening of MRSA in and outside of Benghazi-hospitals reported that the prevalence of MRSA nasal carriage was 21.4% among HCWs with some differences between hospitals also prevalence of MRSA nasal carriage was 9.6% among community hospital-acquired infections (9). Furthermore Ahmed *et al*., 2010 screened 569 subjects from 4 hospitals in Tripoli-Libya and found that 19% of them carrying MRSA (10).

Methods

Study design

This 19 months prospective study was conducted from March 2019 to October 2020 at Mesllata and Tarhuna hospitals. Nasopharyngeal samples were collected from direct contact workers with patients including doctors, nurses and emergency and other indirect contact workers including technicians in (laboratory, radiology and physiotherapy), anesthesia departments, Pharmacists and other staff. Hospital's departments in this study were divided into high, intermediate and low risk. Participants who had active infections or any illnesses and who have taken antibiotic prior 21-days were excluded. Written informed consents were obtained from participants prior to taking nasal swabs. Participants were asked to fill out a questionnaire.

Microbiologic methods

Processing of the clinical samples

Sterilized pre-moistened swabs were used to collect samples from both anterior nares of HCWs who were involved in the study after they filled out the questioner form that were prepared to get a good data about involved HCWs. The swabs placed gently and allowed to remain in each nose for 2 to 3 seconds. The Swabs were put immediately after collection in tubes containing nutrient broth with 7% sodium chloride and labeled with name and code of tested person. Tubes were incubated at 37˚C for 24 hours. A loopful of nutrient broth was streaked onto Mannitol salt agar (MSA) and blood agar (BA) plates for isolation of *Staphylococcus* spp. All isolated *Staphylococcus* spp were identified using standard procedures and tested for Methicillin-resistance according to the method of the National Committee for Clinical Laboratory Standards (11).

Culture and Identification of Microorganisms

Swab specimens were inoculated on MSA for Mannitol fermentation test, BA for hemolysis detection and Trypticase soy broth (TSB) for recovery of MRSA. All plates incubated at 35°C - 37°C for 18-72 hours. The presumptive *S. aureus* isolates were confirmed by coagulase test. MRSA were identified by the cefoxitin disk-diffusion method according to the recommendations of Clinical and Laboratory Standard Institutes (11). In all investigations *S. aureus* (ATCC 25923) was used as a positive control organism.

Susceptibility testing

Susceptibility tests were performed by the disc-diffusion method of Bauer-Kirby using Mueller-Hinton agar (12) as described by the Clinical Laboratory Standard Institute (11) to 10 antibiotics. Namely; cefoxitin; chloramphenicol; penicillin-G; oxacillin; tetracyclin; augmentin; trimethoprim/sulfameth-oxazole; fusidic acid; erythromycin and ciprofloxacin. Cefoxitin

was used as an indicator of methicillin susceptibility disks and an inhibition zone diameter of ≤ 14 mm was reported as methicillin resistant, 15-17 mm as intermediate and ≥ 18 mm was considered as methicillin sensitive.

Automated method of antibiotic susceptibility testing system (BD Phoenix™)

Identification of *S. aureus* and MRSA isolates and susceptibility tests towards 20 antibiotics were performed by BD phoenix IM system quick reference guide (automated method) in the microbiology unit of Tripoli central hospital. This test applied to all positive results samples by manual method for confirmation of results.

Results

Prevalence of MRSA

The results of HCWs' questionnaires in both hospitals in this study shown in (Table1) there were 210 participants from both hospitals (47.6%) from Mesallata hospital and (52.4%) from Tarhuna hospital.

Morphological characteristics of isolates

Total 78 isolates (37.1%) of nasal samples were gram positive cocci and (34.8%) were positive Mannitol sugar fermenters.

Biochemical identification of tested isolates

The biochemical tests of 78 gram positive nasal swab specimens showed that (36.2%) were catalase positive and (15.2 %) coagulase positive and DNAse positive respectively.

Adherence to hand hygiene protocols among all participants

Hand hygiene protocols were always applied by only (9.3%) of HCWs who were nasal carriers of *S. aureus* although (90.6%) well no or sometimes applied the protocols of hand hygiene. Among MRSA carriers HCWs (25%) always applied hygiene protocols routinely and (75%) sometimes applied the protocols. On the other hand *S. aureus* HCWs carriers wash hands frequently before and after contact with patients (to treat the patients) were (18.7%) answer yes however (81.2%) had not always or occasionally didn't wash their hands before and after contact with patients. All the results are shown in (Table 2).

Antibiotic susceptibility profile

Of the total *S. aureus* isolates (n = 32) were resistant to penicillin G and ampicillin and 40.6% towards Fusidic acid, whereas all isolate were susceptible to rifampin and mupirocin. Additionally, 96.8% of isolates were susceptible to daptomycin, teicoplanin, gentamycin, vancomycin, clindamycin and moxifloxacin respectively. The MRSA isolates (n=4) were resistant to cefoxitin and ampicillin, respectively. However all were susceptible to tetracyclin, linezolid, rifampin and moxifloxacin respectively (tables 3 and 4).

Discussion

MRSA is one of the leading MDR pathogens responsible for nosocomial infections globally. The main source of this organism is healthy carriers including health care workers. The spread of this organism requires an infected persons transmit infection to healthy persons by direct contact. Detection of colonized HCWs and assessing the associated risk factors of colonization is one of the most effective methods for preventing its transmission. This study aimed to determine the frequency of S. aureus and MRSA nasal colonization in different HCWs in two hospitals in west of Libya, in addition to determination of antimicrobial resistant profiles of the isolates by using both manual and automated identification and susceptibility testing system (BD Phoenix™). Staphylococcal infections occur frequently in hospitalized patients and have severe consequences despite antibiotic therapy (13). *S. aureus* isolates are generally susceptible to β-lactam antibiotics but extensive use of this class of drugs has led to increasing emergence of resistant strains (14). In the present study a total of 210 HCWs enrolled.

Table (1) Demographic characteristics associated with S. aureus and MRSA colonization

	Staph. carriers	Non-*Staph.* carriers	Total (%)	MRSA (%)	X^2	*p*-95% CI
Gender					2.45	0.118
Female	26 (81.2)	120 (67.4)	146 (69.5)	**2** (50)		
Male	6 (18.7)	58 (32.5)	64 (30.4)	**2** (50)		
Age (year)						

20-35	15 (46.8)	101(56.7)	116 (55.2)	**3** (75)		
36-51	16 (50)	71 (39.9)	87 (41.4)	**1** (25)	1.152	0.562
52-67	1 (3.1)	6 (3.3)	7 (3.3)	-		
Occupation						
Direct contact	26 (81.2)	127 (71.3)	153(72.8)	**3** (75)		
Indirect contact	6 (18.7)	51 (28.6)	57 (27.1)	**1** (25)	1.34	0.24
Hospital wards						
High risk	20 (62.5)	77 (43.2)	97 (46.1)	**3** (75)		
Intermediate risk	10 (31.25)	43 (24.1)	53 (25.2)	**1** (25)	**9.29**	**0.010**
Low risk	2 (6.25)	58 (32.6)	60 (28.5)	-		
Working hours						
4-10	6 (18.7)	71 (39.8)	77 (36.6)	**2** (50)	**9.29**	**0.010**
11-17	7 (21.8)	50 (28)	57 (27.1)	-		
18-24	19 (59.3)	57 (32)	76 (36.1)	**2** (50)		
Working years						
1-10	20(62.5)	142(79.7)	162(77.1)	**4** (100)		
11-20	8 (25)	31 (17.4)	39 (18.5)	-	**7.83**	**0.020**
21-or more	4 (12.5)	5 (27.7)	9 (4.2)	-		

All participants signed a written consent and completed a questionnaire of demographic and professional information. The mean ages range between 20 to 60 years. Overall 32(15.2%) subjects had *S. aureus* colonization. Prevalence rate of *S. aureus* was 6 (18.75%) males and 26 (81.25%) females. Many studies indicated the same results as females mostly have higher prevalence rate of *S. aureus* colonization (1, 3, 13, 15 and 16). Four (12.5%) isolates were MRSA (50% isolated from each gender). Moreover according to contact with patients' participants the results shown that 3 MRSA isolates were from direct contact with patients HCWs. In addition to 26 (81.25%) *S. aureus* isolates were from direct contact with patients HCWs. Among HCWs around the globe, the nasal carriage rates of *S. aureus* have been reported as 14% in Nigeria, 27.5% in Turkey, 31.1% in Iran, 33.4% in France and 39.3% in Spain (17).

Table (2) Adherence to hand hygiene protocols among all health care workers in both hospitals.

	***Staph.* Carriers (%)**	**Non- *Staph.* Carriers (%)**	**Total (%)**	**MRSA (%)**	***p*-** **X^2 95%CL**
Apply PR- HG*					
Always	3 (9.3)	46 (25.8)	49 (23.3)	**1** (25)	
Some time	29 (90.6)	132 (74.1)	161(76.6)	**3** (75)	4.11 **0.043**
HW B & A PT-contact*					
Yes	6 (18.7)	61 (34.2)	67 (31.9)	**4** (100)	
No	26 (81.2)	117(65.7)	143(68.1)	-	3.00 0.083

Hand washing time/day						
5-10 times	28 (87.5)	119(56.6)	147 (70)	**2** (50)	5.50	**0.019**
More than 10 times	4 (12.5)	59 (28.1)	63 (30)	**2** (50)		

Apply PR-HG*= Apply protocol of hand hygiene, **HW B & A PT-contact***= hand washing before

Table (3) Antibiotic susceptible profile of *S. aureus* and MRSA isolated from the HCWs of both hospitals done by Kirby-Bauer Disk Diffusion Susceptibility Test Protocol

I =

Antibiotics	**Isolates of both hospitals** Total *S. aureus* n=32 (%) / Total MRSA n=4 (%)					
	SA			**MRSA**		
	(S) %	(I) %	R %	(S) %	(I) %	R%
Cefoxitin	**24 (75)**	4 (12.5)	4 (12.5)	-	-	**4(100)**
Oxacillin	15(46.8)	7 (21.8)	10(31.2)	1(25)	3(75)	-
Chloramphenicol	**22(68.7)**	5 (15.6)	5 (15.6)	2(50)	2(50)	-
Tetracycline	16(50)	12(37.5)	4 (12.5)	1(25)	3(75)	-
Ciprofloxacin	20(62.5)	12(37.5)	0 (0)	**3(75)**	1(25)	-
Fusidic acid	17(53.1)	2 (6.2)	13(40.6)	**4(100)**	-	-
Erythromycin	19(59.3)	9 (28.1)	4 (12.5)	2(50)	2(50)	-
Amoxicillin+ Clavulanic acid	**22(68.7)**	3 (9.3)	7 (21.8)	2(50)	1(25)	1(25)
Trim-sulfa. (SXT)	20(62.5)	7 (21.8)	5 (15.6)	2(50)	1(25)	1(25)
Penicillin-G	3 (9.3)	1 (3.1)	**28(87.5)**	2(50)	-	2(50)

Resistant, **(S)** = *Sensitive, and* **(I)** = *Intermediate.*

Table (4) Antibiotic susceptible profile of *S. aureus* and MRSA isolated from the HCWs of both hospitals done by BD Phoenix™ system

Antibiotics	Isolates of both hospitals Total *S. aureus* n=32 / Total MRSA n=4					
	Pattern of susceptibility					
	(SA)			**(MRSA)**		
	(S) %	(I) %	R %	(S) %	(I) %	R %
Cefoxitin	26 (81.2)	-	6 (18.7)	-	-	**4 (100)**
Oxacillin	29 (90.6)	-	3 (9.3)	2 (50)	-	2 (50)
Ampicillin	-	-	**32(100)**	-	-	**4 (100)**
Daptomycin	31 (96.8)	-	1 (3.1)	3 (75)	-	1 (25)
Teicoplanin	31 (96.8)	-	1 (3.1)	3 (75)	-	1 (25)
Gentamycin	31 (96.8)	-	1 (3.1)	3 (75)	-	1 (25)
Tetracycline	28 (87.5)	1 (3.1)	4 (12.5)	**4 (100)**	-	-
Ciprofloxacin	30 (93.7)	1 (3.1)	1 (3.1)	3 (75)	1(25)	-
Vancomycin	31 (96.8)	-	1 (3.1)	3 (75)	-	1 (25)
Clindamycin	31 (96.8)	-	1 (3.1)	3 (75)	-	1 (25)
Linezolid	28 (87.5)	3(9.3)	1 (3.1)	**4 (100)**	-	-
Rifampin	**32 (100)**	-	-	**4 (100)**	-	-

Imipenem	27 (84.3)	1(3.1)	4 (12.5)	-	1(25)	3 (75)
Amoxicillin+ Clavulanic acid	28 (87.5)	-	4 (12.5)	2 (50)	-	2 (50)
Mupirocin	**32 (100)**	-	-	3 (75)	-	1 (25)
Nitrofurantoin	31 (96.8)	-	1 (3.1)	3 (75)	-	1 (25)
Moxifloxacin	31 (96.8)	1(3.1)	-	**4 (100)**	-	-
Trim-sulfa. SXT	30 (93.7)	-	2 (6.2)	2 (50)	1(25)	1 (25)
Penicillin-G	-	-	**32(100)**	2 (50)	-	2 (50)

I = *Resistant,* **(S)** = *Sensitive, and* **(I)** = *Intermediate.*

The prevalence of MRSA (12.5%) in our study is consistent with the frequency of HCWs MRSA nasal carriage in a previous study in Nigeria however less than other reported data as mentioned by Mahmoud and; Albadawy et al., 2015 (17). All *S. aureus* isolates were strongly coagulase positive. Our results are in agreement with previous study by Alturki, 2015 (16) who stated that 100% strains of *S. aureus* produce clumping factor and protein A in his study. Furthermore, Jeljaszewicz, 1983 (18) observed that 97% isolates were coagulase positive. According to Mossel, 1962 (19) Mannitol fermentation has been related to pathogenicity of *S. aureus*. Present study is in consistent with this investigation as 100% of isolates were Mannitol fermenting. *S. aureus* rate was high 73.1% among nurses followed by doctors 19.2% and emergency department workers 7.7%. This is reported previously by (20). HCWs in our study colonized with *S. aureus* in their interior nares asymptomatically. Furthermore low MRSA presence (12.5%) in our study amongst *S. aureus* isolates reported previously by some workers in percentages less than our study rate (3, 16, 21 &22). Many researchers in different Libyan hospitals showed results in agreement with our study result where MRSA prevalence rates were 37%, 11.6%, 21.4%, 14.7% and 19% respectively (7, 8,9,10 & 23). According to statistical analysis of 32 questionnaires in this study, the rates of *S. aureus* among direct contact HCWs (26) 81.25 % (19.2% doctors, 73% nurses, 7.7% emergency), and 18.75% of indirect contact HCWs were (33.3% pharmacy, 50% technicians, and 16.6% other staff). A similar previous study (3) showed that the prevalence rates of *S. aureus* were: 37.5% doctors which was the highest rate unlike our study and there was dissimilarity regarding prevalence rates in nurses (34.5%), technicians (25%) and (20 %) pharmacists. In our study the prevalence rates of nasal carriers of *S. aureus* 62.5% at high risk hospital wards and 31.2% at intermediate risk wards. The same rate shown for MRSA among HCWs were nurses (50%) and doctors (25%) representing high risk wards and technicians (25%) from intermediate risk wards and no MRSA carriers detected in low risk wards in both hospitals. These findings completely agreed with the same previous study (3). Concerning the length of working hours in hospital statistically it was found that there is a significant correlation with the prevalence of *S. aureus* and MRSA among HCWs because hours of working has direct effect on contact time with patients in this study. As per hours working from 18-24 hours has more percentage of *S. aureus* carrying in comparison with workers with less hours (4-10 and 11-17 hours) 18.7%, 21.8% and 59.3% respectively ($p<0.05$). These results were proven in another study by Alturki, 2015 (16).

The results achieved on the basis of working years showed that the incidence rates of both *S. aureus* were higher in working years 1-10 years (62.5%) in comparison to workers 11-20 (25%) and 21 years or more (12.5) respectively which was statistically significant ($p<0.05$). For MRSA all the isolates were from workers from 1-10 years (100%). This could be explained on basis that first, and second group of those may have more direct patient contact with no good idea to applied protocols of hand hygiene and usage of healthcare tools due to less experience. In Akhtar, 2010 study the carriage rates depend on HCWs' years of working were high in 26-30 years (40%), 6-10 years (20.51%) and least carriage rate 16 - 20 (13.04%) which is different from our results (22).

In this investigation the prevalence of *S. aureus* among HCWs depending on place of working (High, Intermediate, and Low risk departments) was 62.5% respondents of high risk ward, 31.25% respondents of intermediate risk and 6.25% respondents of low risk wards. Our results revealed that the prevalence of *S. aureus* among HCWs was high in surgery unit (high risk ward). Higher than the result obtained by Rongpharpi's *et al*., 2013 Alturki, 2015; Akhtar, 2010; Rahbar *et al*., 2006; and Pan *et al*., 2006 with rates of 26.32%, 26 %, 35%, 10.84% and 31% respectively (13, 16, 22, 24 & 25) . Additionally, the

prevalence rate in delivery unit (intermediate risk ward) was 50% and it was more than other studies namely, Rongpharpi's *et al*., 2013 (30.30%); Akhtar's, 2010 (34.9%) and Alturki, 2015 (34.62%), (13, 22 & 16). Our findings showed that only 50 % from 6.25% low risk respondents were carriage of *S. aureus* among ICU ward's HCWs. In emergency and medicine wards the rates were 20% and 45%. In addition high rate of HCWs who were nasal carriages of *S. aureus* (9.3%) respondents was well known and always applied the protocols of hand hygiene and (90.6%) were only applied the protocols some times which affecting their MRSA carriage significantly (p<0.05). All MRSA carriers (100%) in this study did not washing their hands before and after contact with patients. In addition washing hands daily (time of washing) with perfect way as written in the protocol of hand hygiene (5-10 seconds) was followed by 87.5% of *S. aureus* carriers and 12.5% wash their hands >10 seconds each time (p<0.05) these results also agreed with a previous study 89.93%, 10.07% respectively (16). The high rate of nasal MRSA carriage among healthcare workers found in this study indicates the need for adjusted infection control measures to prevent MRSA transmission in our healthcare settings. The optimal use of antibiotics in chemotherapy depends on reliable *in vitro* susceptibility testing of the infecting pathogenic organisms. The present study revealed that *S. aureus* isolates (no. 32) were all (100%) resistant to ampicillin and penicillin G and 100% susceptible towards cefoxitin, rifampin and mupirocin by both manual results and automated method (BD phoenix) ® system. This susceptibility profile agreed with the study done by Onwubiko and Sadiq, 2011 which was reported that 92.9 % of their isolates were resistant to penicillin-G. Another studies by Alturki, 2015 and Rongpharpi *et al.*, 2013 reported that 83.25% and 90% isolates respectively were resistant to penicillin. Our findings showed that 12.50%, 12.50% and 15.6% of *S. aureus* isolates were resistance to tetracycline, erythromycin and chloramphenicol respectively. Another study done by Onwubiko and Sadiq, 2011 showed higher resistance towards both antibiotics 86.8 %, 38.1 % respectively (26). The sensitivity pattern is good as the resistance towards used antibiotics is not high and 50% of the isolates were sensitive to tested agents. The same results achieved by automated method with different antibiotics wherever most isolates were up to 75% sensitive to tested antibiotics. MRSA isolates were 100% resistant to cefoxitin and ampicillin and 100% sensitive to fusidic acid, tetracyclin, linezolid and moxifloxacin. Resistance towards cefoxitin is a good indication for our results accuracy as cefoxitin is a potent inducer of the **mecA** regulatory system and it is being recommended for detection of methicillin resistance in MRSA. Cefoxitin resistance is a surrogate marker for the detection of MRSA.

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