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Staphylococcus aureus nasal colonization among health care workers at an Egyptian tertiary care hospital

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ABSTRACT

Background: *Staphylococcus aureus (S. aureus)* colonization in health care workers (HCWs) is a major factor of nosocomial infections. Methicillin resistant *S. aureus* (MRSA) is incriminated in hospital-acquired infection. **Objectives**: To evaluate the colonization rates of nasal *carriage of S. aureus* and MRSA among HCWs at Al-Ahrar Teaching Hospital. **Methods**: We recruited 163 HCWs in a cross section study. *Staphylococcus aureus* was identified phenotypically by coagulase, with DNase tests and genotypically by nuc gene detection. Antibiotics sensitivity was tested by disk diffusion. Polymerase chain reaction (PCR) for mecA was done to confirm MRSA identification. **Results:** Forty-eight *S. aureus* strain were isolated. The frequency of nasal colonization of *S. aureus* in HCWs was 29.44% (48/163), the frequency of MRSA isolates in HCWs was 27.6% (45/163), and frequency of MRSA isolates among *S.aureus* isolates was 93.8% (45/48). Prior infection (last 3 months) and living in crowded places increased significantly risk of MRSA nasal carriage by 29.3 and 79.59 folds respectively **Conclusion:** We demonstrated high rates of nasal carriage of *S. aureus* and MRSA among HCWs. Prior infection and living in crowded places were risk factors for methicillin resistance among isolated *S.aureus* strains.

Introduction

Staphylococcus aureus (S. aureus) colonizes the anterior nares. Nasal colonization of *S.aureus* results in increasing the risk of nosocomial infections [1].

Moreover, methicillin resistant *S. aureus* (MRSA) is one of the important bacteria involved in hospital acquired infection [2,3], where health care workers (HCWs) are considered as a critical source [4]. The MRSA problem is observed all over the world, although, this burden is high in developing countries [5]. The MRSA burden ranges from 16% to 55% in most of the African countries. In Egypt, MRSA prevalence is about 45% -52% [6]. This high burden of MRSA has a negative effect on the long hospitalization, treatment cost, and high mortality and morbidity among the critically ill patients [5].

Evaluating MRSA colonization frequency and its antimicrobial susceptibility pattern is mandatory for proper choice of the empirical therapy for *S. aureus* [7]. Therefore, our aims were to investigate the colonization rates of nasal carriage of *S. aureus* and MRSA in HCWs at Al Ahrar Hospital, an Egyptian tertiary care hospital.

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Subjects, Material and Methods

Study settings and design

We conducted a cross section study at Al Ahrar Teaching Hospital, a tertiary care hospital, Zagazig, Egypt. All laboratory techniques were done in Department of Medical Microbiology and Immunology, Zagazig University, Faculty of Medicine, Zagazig, Egypt. This study was done during the period from November 2018 to June 2019. The present study was done on the bases of the guidelines of Strengthening the Reporting for Observational Studies in Epidemiology (STROBE) [8]

Study subjects

This study included 163 HCWs at Al Ahrar Teaching Hospital. Health care workers were selected from the available HCWs' hospital list randomly. Inclusion criteria included all HCWs (doctors, nurses, pharmacist, housekeeping, technician, and security guards).

Ethical approval

Institutional Review Board (IRB) Committee of Zagazig Faculty of Medicine no Zu-IRB # 4422/1-4-2018 approved the current work. An informed written consent was obtained from all subjects. The work was done according to the revised declaration of Helsinki.

Collection of data

Data was collected by questionnaire. It included: age, sex, occupation, department, the working years, history of antibiotic therapy during last three months, taking antibiotic at last week, exposure to mupirocin at last year, admission to hospital at last year, smoking habits, participating in contact sports, using intravenous drugs, history of surgical operation, history of recurrent skin lesions, and history of chronic diseases.

Sample collection

Anterior nares of HCWs were swabbed by sterile disposable cotton swab after moistening it with sterile distilled water. The swabs were rubbed very well three times over the mucous membrane of nasal septum and ala nasi [9]. The swabs were transferred to the laboratory of Medical Microbiology and Immunology Department, Faculty of Medicine, Zagazig University within one hour to be processed.

Microbiological identification

The swabs were cultured on mannitol salt agar (Oxoid, UK) to be incubated for 24 hours at 37°C. Mannitol salt isolated colonies were sub-cultured on nutrient agar plates at 37°C for 24 hours. *Staphylococcus aureus* colonies were identified as Gram-positive cocci, with positive catalase, deoxyribonuclease (DNase) and coagulase test. All microbiological tests were done according to the standards techniques **[**10].

Antimicrobial sensitivity testing

Antibiotic susceptibility was done by a standardized Kirby–Bauer disk diffusion method on Mueller Hinton Agar (Becton-Dickinson, Sparks, USA) **[**11]. The technique was done as per the recommendations of the Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines [12]. The used antibiotics included: Mupirocin 20µg (Mup), Mup (5µg), cefoxitin (30µg), penicillin (10 units), ciprofloxacin (5 µg), clindamycin (2 µg), and rifampin (5 µg). All of these discs are manufactured by BD BBL™sensi disc™, USA except Mup 20µg and Mup 5µg are manufactured by Oxoid, UK.

The inhibition zone diameters were interpreted according to the CLSI 2020 guidelines where mecA negative (*S. aureus* ATCC® 25923) and mecA positive (ATCC ® 43300) controls were used as reference strains [12].

Definition of MRSA

MRSA was defined as isolates with a zone diameter ≥ 22 mm against 30µg cefoxitin disc were considered susceptible to methicillin, while isolates with a zone diameter ≤ 21 mm against 30µg cefoxitin disc were considered methicillin resistant [12].

Molecular confirmation of *S. aureus* and MRSA isolates

Multiplex PCR assay was used to detect *S.aureus* gene (nuc), and methicillin resistant gene (mecA). DNA Extraction was done using Thermo Scientific Gene JET Genomic DNA Purification Kit, USA, according to manufacturer's instructions**.** We used Platinum™ SuperFi II PCR Master Mix (Invitrogen, Thermo Fisher Scientific, USA) for amplification which was done according to manufacturer's instructions. Primers used are shown in **table (1)** [13].

The amplification reaction was done in a 0.2mL, nuclease-free, thin-walled PCR tube on ice. The total 50 ul volume PCR mix was formed from 25 ul 2X Platinum™ SuperFi™ II PCR Master Mix, 5 µL template DNA, 0.2 µM of each forward and reverse primer, and autoclaved distilled water to the rest of volume. The reaction was placed in the pre-heated thermal cycler with the following amplification conditions: the initial denaturation was achieved by

one cycle of 98°C for 1 min. DNA amplification was achieved by 40cycles (each cycle was 98°C for 10 sec, 57°C for 30 sec and 72°C for 1min). Final extension consists of one cycle for 5 min at 72 °C. The amplified PCR products were visualized by 2 % agarose gel electrophoresis [14]. The band of Nuc gene was at 279 bp, the band of MecA gene was at 112bp **(Table 1)**.

Table 1. Primers used in PCR.

Primer	Sequence	Relevant product size
Nuc	F-GCGATTGATGGTGATACGGTT	279bp
	R-AGCCAAGCCTTGACGAACTAAAGC	
MecA	F-GTGAAGATATACCAAGTGATT	112bp
	R-ATCAGTATTTCACCTTGTCCG	

Statistical analysis and quality control

Quantitative data were expressed as mean and standard deviations (SD). Qualitative data were expressed as numbers and percentages. Odds Ratio (OR) at 95 % confidence intervals (CIs) was used to assess the relation between methicillin resistance of *S.aureus* and certain variables. Univariate logistic regression was used to determine the relation between isolation rate of MRSA and the possible associated risk factors. SPSS version 20.0 software (SPSS INC, Chicago, IL, USA) was used in the statistical analysis. *P*-value *<0.05* was considered statistically significant.

Strict quality control measures, as specified by National Health Laboratory of the Ministry of Health of Egypt, were implemented throughout the study period such as implementing a policy and procedures to cover the laboratory cycle including: receiving specimens orders with patient's age, sex; collecting, identifying and processing specimens; safe transportation of specimens; retention and disposal of specimens. Moreover, the organization implements a laboratory safety program based upon the biosafety level of the laboratory. The organization ensures licensed competent healthcare providers are available to operate laboratory services and report its results. It also implements and develops procedures to control risks to the staff and organization whenever operating a laboratory test [15]. *Staphylococcus aureus* reference strains were used to ensure the accuracy and quality of testing procedures.

Results

One hundred sixty-three HCWs were included in the study. The mean age was 32.1 ± 8.22 years. One hundred twenty-three (75.5 %) were male while 40 (24.5%) were female. Seventy (42.9%) HCWs were from rural areas. Forty-one (25.2%) were smokers and 24 (14.7%) were suffering from chronic diseases. Fifteen HCWs (9.2%) received antibiotics in the last week, and seven (4.3%) were admitted to the hospital in the last year. Fifty-one (31.3%) of HCWs were physicians while 77(47.2%) were nurses. Twenty-eight (17.2%) of HCWs were working in the intensive care unit and 25(15.3%) were working in the Pediatric Department. The categorization of HCWs according to the occupation and the departments were illustrated in **table (2)**.

Forty-eight isolates were phenotypically and genotypically (nuc gene) identified as *S. aureus*. The isolation frequency of nasal *S. aureus* among HCWs was 29.44% (48/163). Among them, 45 isolates were considered MRSA using the cefoxitin disc resistance and the mecA gene. Accordingly, the frequency of nasal carriage MRSA among HCWs was 27.6% (45/163), and the frequency of methicillin resistance among isolated *S.aureus* was 93.8% (45/48).

Regarding gender, *S. aureus* carriage rate was highest among females HCWs (79.2%) Regarding the occupation, nurses showed the highest rates of nasal *S.aureus* carriage (52.1%). The rate among physicians was 14.6%, house-keeping workers was 22.9%, technicians and security guards were 4.2% for each, and pharmacists was 2.1%. Regarding the department, the highest percentage of *S.aureus* carriage was in surgery department and pediatric department 18.8% and 16.7% respectively **(Table 2)**.

There was no statistically significant relation between colonization frequency of MRSA or MSSA isolates and either age, gender, years of working,

occupation or department of the studied HCWs. Being less than 30 years age, female, nurses, working in intensive care unit, surgery and outpatient clinic were non-significant risk factors for MRSA colonization **(Table 3)**. On univariate analysis of certain risk factors for emerging MRSA, prior infection (last 3 months) and living in crowded places increased significantly risk of MRSA nasal carriage by 29.3 and 79.59 folds respectively **(Table 4)**.

For antibiotic susceptibility testing, about 96% of the isolated *S.aureus* were sensitive to MUP 5 ug, MUP 20 ug and rifampicin. Only one isolate was penicillin sensitive. About 10% and 15% were resistant to clindamycin and ciprofloxacin **(Table 5)**. Isolated MRSA strains showed higher sensitivity to mupirocin (95.6%), clindamycin (88.8%) and rifampicin (86.7%), while MSSA showed higher sensitivity to all used antibiotic discs except penicillin **(Table 6)**.

Table 2. Distribution of *S.aureus* isolates carriage on basis of age, gender, occupation, and department of HCWs.

Variable	**Total n=163**		***S. aureus isolates* n=48**	
Age (years)				
Mean ± SD	32.1 ± 8.22		31.64 ± 8.23	
Min-Max	18 – 57		18 – 57	
Gender, n (%)				
Female	40	(24.5)	38	(79.2)
Male	123	(75.5)	10	(20.8)
Occupation, n (%)				
Physicians	51	(31.3)	7	(14.6)
Pharmacists	4	(2.5)	1	(2.1)
Nurses	77	(47.2)	25	(52.1)
House keeping	21	(12.9)	11	(22.9)
Technicians	7	(4.3)	2	(4.2)
Security guards	3	(1.8)	2	(4.2)
Departments, n (%)				
Intensive care unit	28	(17.2)	7	(14.6)
Pediatric	25	(15.3)	8	(16.7)
Gastroenterology	13	(8)	5	(10.4)
Gynecology	20	(12.3)	4	(8.3)
Surgery	22	(13.5)	9	(18.8)
Laboratory	12	(7.4)	3	(6.25)
Outpatients clinics	21	(12.9)	6	(12.5)
Neurology	10	(6.1)	1	(2.1)
Nephrology	12	(7.4)	5	(10.4)

Table 3. Distribution of MRSA and MSSA carriage among the different HCWs.

Variable	MRSA[a] n=45		MSSA[b] n=3		*P-value*	OR[c] (95% CI[d])
	n	%	n	%		
Age, years						
<30	20	44.4	0	0	*0.306*	5.6(0.3 - 115.3)
30–40	1	33.3	2	66.7		
>40	10	22.2	1	33.3		
Sex						
Female	37	82.2	1	33.3	*0.106*	9.3(0.7-114.8)
Male	8	17.8	2	66.7		
Years of Working						
0–9	25	55.6	1	33.3	*0.182*	2.5 (0.2 - 29.6)
10–15	13	28.9	1	33.3		
16–29	7	15.6	1	33.3		
Occupation						
Doctors	6	13.3	1	33.3	*0.384*	0.31(0.02 – 3.94)
Pharmacists	1	2.2	0	0	*1*	0.24(0.01 - 6.93)
Nurses	25	55.6	1	33.3	*0.587*	2.5 (0.2 - 29.6)
House keeping	10	22.2	1	33.3	*0.551*	0.6 (0.05 – 6.97)
Technicians	1	2.2	0	0	*1*	0.24(0.01 - 6.93)
Security guards	2	4.4	0	0	*1*	0.4 (0.02- 10.13)
Department						
Intensive care unit	7	15.6	0	0	*1*	1.4 (0.06 – 29.2)
Paediatric	7	15.6	1	33.3	*0.429*	0.37 (0.03– 4.64)
Gastroentorology	5	11.1	1	33.3	*0.336*	0.25 (0.02-3.28)
Gynaecology	3	6.7	0	0	*1*	0.58(0.02-13.55)
Surgery	9	20	0	0	*1*	1.8 (0.08-38.39)
Laboratory	2	4.4	1	33.3	*0.18*	0.09(0.06 - 1.51)
Outpatients clinic	6	13.3	0	0	*1*	1.15(0.05-24.99)
Neurology	1	2.2	0	0	*1*	0.24(0.01 – 6.93)
Nephrology	5	11.1	0	0	*1*	0.95(0.04-20.98)

[a]MRSA: methicillin resistant s. aureus; [b] MSSA: methicillin sensitive *s. aureus* [c]OR: odds ratio, [d]CI: Confidence interval

Table 4. Risk factors of MRSA infection.

Risk factor	Number of MRSA[a] isolates	OR[b]	95%CI[c]	*P*-value
Prior exposure				
Prior MRSA colonization (6months)	4	10.7	0.7 - 205.3	*0.115*
Prior Infection (3 months ago)	10	29.3	1.66 – 516.26	*0.021**
Prior Admission (1 year)	3	8.15	0.41 – 162.4	*0.169*
Prior Antibiotics (1 week)	5	13.44	0.72 – 250.46	*0.082*
Prior Intra Venous Drugs (1 week)	1	3.34	0.13 – 84.04	*0.464*
Skin integrity				
Skin lesion	5	13.44	0.72 – 250.46	*0.082*
Pressure ulcer	1	3.34	0.13 – 84.04	*0.464*
Comorbidities				
Renal failure	2	5.69	0.27 – 121.79	*0.266*
Diabetes	3	8.15	0.41 – 162.4	*0.169*
Heart failure	1	3.34	0.13 – 84.04	*0.464*
Chronic respiratory disease	1	3.34	0.13 – 84.04	*0.464*
Living in crowded places	20	79.59	4.62 – 1370.2	*0.003**
Sharing in contact sports	3	8.15	0.41 – 162.4	*0.169*

[a] MRSA: methicillin resistant *S.aureus*; [b] OR: odds ratio; [c] CI :Confidence interval

Table 5. Antibiotic susceptibility of all *S.aureus* isolates (n=48).

Antibiotic concentration (µg/ml)	Sensitive		Intermediate		Resistant	
	N	%	n	%	n	%
Cefoxitin 30	3	6.3	-	-	45	93.7
Mupirocin 20	46	95.8	-	-	2	4.2
Mupirocin 5	46	95.8	-	-	2	4.2
Clindamycin 2	43	89.6	-	-	5	10.4
Rifampicin 5	46	95.8	-	-	2	4.2
Ciprofloxacin5	38	79.2	3	6.3	7	14.5
Penicillin10 u	1	2.1	-	-	47	97.9

Table 6. Antibiotic susceptibility pattern of MRSA and MSSA isolated from HCWs.

Antibiotic concentration (µg/ml)	MRSA[a] (n=45)				MSSA[b] (n=3)			
	Sensitive		Resistant		Sensitive		Resistant	
	n	%	n	%	n	%	n	%
Cefoxitin 30	0	0	45	100	3	100	0	0
Clindamycin 2	40	88.8	5	11.1	3	100	0	0
Rifampicin 5	37	82.2	8	17.8	3	100	0	0
Ciprofloxacin 5	39	86.7	6	13.3	3	100	0	0
Penicillin10 u	1	2.2	44	97.8	0	0	3	100
Mupirocin 20	43	95.6	2	4.4	3	100	0	0
Mupirocin 5	43	95.6	2	4.4	3	100	0	0

[Ba]MRSA: methicillin resistant *s. aureus*; [b]MSSA: methicillin sensitive *s.aureus.*

Discussion

In the current work, the isolation frequency of *S.aureus* was 29.4% that was comparable to previous studies in other developing countries [14, 16-19], and developed countries [20-23]. On the contrary, our colonization frequency of nasal *S. aureus* was more than that found in other Arabic countries [24,25]. This discrepancy in colonization rates of *S. aureus* could be attributed to local infection control policies, different sampling techniques, and microbiological procedures [26].

Our reported colonization frequency of MRSA in HCWs was 27.6 %. This result was comparable to a study done by **Rashad et al.** in the surgical intensive care unit of El-Demerdash Hospital where the colonization frequency of nasal MRSA in HCWs was 28.6% [27]. A previous study reported MRSA prevalence more than 50%, however it had recruited hospital outpatients **[**28]. On the opposite side, the reported rates in our study were more than the observed frequencies in the Middle East region [28, 29].

That high carriage rate of MRSA in Egypt could be explained by the improper antibiotics consumption, the main cause of emerging multidrug resistant isolates. Some studies documented a relation between consumption of beta-lactam and MRSA emergence. Poor hygiene, overcrowding, and nutritional status could be other contributing factors **[**30].

In contrast, other studies reported lower rates of MRSA nasal carriage as studies conducted in Nepal (3.4%) [26]; and Bangalore (8%) [31] and the West of Ireland (7.7%) [32].

In our study, we found that *S.aureus* isolates were higher in female gender (79.2%) and nursing occupation (52.1%). Our finding was comparable to other studies [23, 33, 34]. On the other hand, a study was done by **Chakolwa et al.** showed higher rates of *S.aureus* colonization among doctors (17.9%), to be followed by nurses (17.5%) [35]. **Rongpharpi et al.** illustrated that the colonization frequency of nasal *S.aureus* was high in male HCWs (54.28%) [36]. We reported that *S. aureus* colonization frequency was the highest in surgery department (18.8%). On the other hand, other study found that prevalence was the highest in the orthopedics ward, followed by the surgery and the gynecology wards [36].

In the current study, MRSA isolates were more among females (82.2%) and among nurses (55.6%) with higher MRSA isolates among HCWs working in the surgery department (20%). Also, similar results were reported **[**27,37]**.** In contrary, another study reported that MRSA colonization were the highest among doctors (50%) followed by nurses (25%), also MRSA colonization frequency in females was high (51.28%) [38]. This high rate of methicillin resistance among nurses could be due to the lack of infection control policies knowledge; particularly hand hygiene, and contact precautions.

In our study, univariate analysis of certain risk factors for developing MRSA, prior infection (in the previous 3 months) and living in crowded places increased significantly risk of nasal carriage of MRSA by 29.3 and 79.59 folds respectively. In contrast, Peters et al reported another two risk factors that significantly increased the risk of MRSA carriage which were chronic skin diseases, chronic wounds, decubitus and using indwelling devices [39]. **Schubert et al.** reported that methicillin resistance was not related to diabetes, hospital admission, chronic respiratory disorder, or antibiotics consumption. But, contact with MRSA patients, and previous history of MRSA colonization were possible factors associated with high frequency of MRSA colonization [40].

Our antibiotic susceptibility pattern of all isolated *S. aureus a*nd MRSA showed that about 96% of *S.aureus* were sensitive to mupirocin and rifampicin. About 97.9% *S.aureus* isolates were penicillin resistant and 93.7% *S.aureus* isolates were cefoxitin resistant. This result was comparable to **Bukhari et al.** results who reported that isolated *S.aureus* showed 100% resistant to penicillin and oxacillin , about 95.2% showed sensitivity to rifampicin and 63% showed sensitivity to clindamycin. In our study, 14.5% *S.aureus* isolates showed ciprofloxacin resistance, but **Bukhari et al.** reported ciprofloxacin resistance by 75.8% [41].

We reported that MRSA isolates were 45 and MSSA are 3 isolates. Isolated MRSA strains had high sensitivity to mupirocin (95.6%), clindamycin (88.8%) and rifampicin (86.7%), while MSSA shows high sensitivity (100%) to all used antibiotic discs except penicillin. Another study reported different results where MRSA isolates showed high sensitivity to linezolid (100%), rifampicin (94.1%) and mupirocin (70.6%), While MSSA showed high sensitivity (90%) to all used antibiotic discs except gentamycin [42].

Conclusion and recommendation

Our study reflects the burden of methicillin resistance among HCWs. We reported high rates of

nasal carriage of *S.aureus* and MRSA among HCWs, particularly nurses. Then, screening of MRSA carriage among HCWs, particularly nurses, should be done in all hospitals. Prior infection and living in crowded places were risk factors for methicillin resistance among isolated *S.aureus* strains.

Limitation

This work presented data that were collected from a hospital where there was extremely limited information about antimicrobial resistance pattern. The actual source of contamination among the colonized HCWs was not investigated. We did not test the sensitivity of the isolated strains to vancomycin and linezolid.

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Conflicts of interest

The authors declare no conflicts of interest.

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