NASAL CARRIAGE OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS(MRSA) AMONG MEDICAL AND NON-MEDICAL STUDENTS OF SHEIKHAN-POLYTECHNIQUE COLLEGE AND TECHNICAL INSTITUTE, IRAQ

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ABSTRACT

Background and Aims In population, Staphylococcus aureus is present in the nasal vestibule of 35%-50% and one tenth of these are methicillin resistant S aureus (MRSA). This study aimed to investigates the prevalence of MRSA nasal carriage among students of Sheikhan-Polytechnique College (Public health and Medical laboratory technology Department) and Technical Institute (TI). Methods: During December 2018 to February 2019 data collection has been achieved. About 150 students are screened, 66 were males and 84 females with age ranged (18-24 years). A cross-sectional study was conducted and 150 nasal swabs were taken from students groups of several grades in various departments (Public Health, Medical laboratory technology and IT department). The samples were examined through standard microbiological methods. Antibiotic susceptibility tests for all isolates were checked for methicillin resistance using disk diffusion agar. Molecular characterization using PCR test targeting mecA gene was studied. Results: The overall prevalence of MRSA was 27(18%), (12 males and 15 females). High prevalence found among public health students 12/27(44.4%) followed by information technology 9/27(33.3%) and less 6/27(22.2) cases in laboratory technology students. Students of first grade followed by second grade of various departments recorded high rates than third and fourth grade (12, 9, 3 and 3, respectively). Moreover, MRSA was higher among those students that live within their family 19/27(70.3%) than those that live in hostels 8/27(29.6%) and negative in those that live with family relatives. Out of 28 MRSA isolate 14(51.8%) were positive for mecA gene and public health students accounted more carriage 8/14 (57.1%). Conclusions: This study highlighted that students of several grades of medical study are more carriers and should admit enough information concerning control measures to limit contaminate with this pathogen.

KEYWORDS: *MRSA*, *Methicillin Resistant Staphylococcus aureus, Nasal swab, mecA gene, Medical and non-students.*

INTRODUCTON

ethicillin Resistant Staphylococcus aureus (MRSA) is gram positive bacteria, normal flora of skin, throat, and nasal nares as a prevalent site (Mat et al., 2014; Furberg et al., 2016). It is potential pathogen exhibits wide spectrum of virulence. Their spreading in the environment might produce carrier states that classically considered а source to hospital-acquired MRSA (HA-MRSA) or community-acquired MRSA (CA-MRSA) (Vijay et al., 2018). Infections could be endogenously, infectious bacteria of own patient's body, or exogenously, from various external sources (Kluytmans et al., 2005). Sometimes carrier states could be at growing

risk for developing infections ranged from mild skin infections to severe systemic infections like endocarditis, bacteremia, sepsis, and osteomyelitis (Jenkins *et al.*, 2015: Zheng *et al.*, 2015).

When β -lactamase enzyme was identified, methicillin soon later (semi-synthetic penicillinase-resistant β -lactams) was synthesized to combat the penicillinaseproducing S. aureus strains. Unfortunately, MRSA were reported in very short period and always difficult to treat due to its widespread resistance to multiple antibiotics (Pantosti et al., 2007; Ristagno et al., 2018). By clarity, MRSA strains harbors the mecA gene that encodes the low-affinity penicillin-binding protein (PBP) known as PBP2a. The Clinical and Laboratory Standards Institute (CLSI) claimed that the equivalent in sensitivity and specificity of disk diffusion test based on oxacillin and cefoxitin resistance are in parallel of the detection of *mecA*-mediated resistance in *S. aureus* (CLSI, 2021). Some researcher's emphasized on superiority of PCR targeting of *mecA* gene over phenotypic susceptibility test for confirmation of MRSA strains (Pu *et al.*, 2014; Zakai, 2015)

Medical students during their gradual phases of study years inside hospitals are being exposed to patients, the hospital environments and fomites and at high risk for colonization (Eveillard et al., 2004: D'Souza et al., 2013). The main routs of MRSA transmission are through direct contact with hands, contaminated stethoscopes , contaminated body fluid (Jones et al., 1995), identification badges (Hogue et al., 2017), neckties (Asciak et al., 2018), and white coats (Sande and Basak, 2015). Medical students exert potential hazardous as a part of the spreading of MRSA in healthcare community (Rampal et al., 2020). Since 2008, no data show any prevalence on MRSA nasal colonization rates among medical and non-medical students in Shekhan Polytechnique College of health and information Technology Institute. This study aimed to assess and compare prevalence of MRSA carrier states in medical and non-medical educated students belong to Shekhan Polytechnique College of Health and Technical Institute in Duhok city, Iraq, in addition to that detection of mecA gene among positive (MRSA group).

MATERIALS AND METHODS

A. . Design and setting of study:

A cross sectional study design was conducted in Sheikhan-Polytechnique College of Health and Technical Institute. This affiliations located in Shekhan district, Duhok governorate, Iraq. Data collection was performed from December 2018 to February 2019

B. Ethical Approval: All students that agreed to participate in the study form were signed and informed consent was taking. The study was approved by scientific committee of Sheikhan-Polytechnique College of Health.

C. Study population and sampling

All students in grades (1-4) belong Sheikhan-Polytechnique College of Health of both its departments (Public health and Medical Laboratory technology) and students in grade (1-2) of Information Technology department (who were not exposed in the hospitals)at Technical Institute were included in this study. Then, questionnaire regarding demographic data and health history of students was filled by the students. The questionnaire consisted of variables like year of study, age, gender, department and class, student had ever tested for MRSA, relation to student's inhabitant and any recent antibiotics consumption. A Table (1) shows ages, genders, departments and classes of students participated in this study.

D. *Inclusion and Exclusion Criteria*: The nose swab (anterior nares) samples were taken from enrolled students not had clinical symptoms (pharyngitis, cryptic tonsillitis, sinusitis, otitis media, and other upper respiratory illnesses) and those students not taken antibiotics for 3 days ago and past 6 months were hospitalized.

Age	number	percent
18	18	12%
19	30	20%
20	24	16%
21	25	16.7%
22	23	15.3%
23+	30	20%
	Gender	
Male	66	44%
Female	84	56%
	Department of stu	dents
Public health	60	40%
Medical laboratory technology	38	25.3%
Information technology	52	34.7%
	Stage of stude	nts
1	68	45.3%
2	48	32%
3	20	13.3%
4	14	9.3%

Table (1):- Shows Ages ,Genders, Departments and Stages of participated students

E. Nasal Swabs: A moisten swab with sterile normal saline was used for collection of samples from both anterior nares of each student. Swabs carefully inserted into each nostril so that the tip is entirely at the nasal osteum level and gently rolled 5 times, then inoculated into Amies transport media. Samples were then processed within 1-3 hours for culture, and identification through microbiological methods.

F. Bacterial Identification: For this purpose, several media and tests used for the isolation, identification and testing the susceptibility of the isolates for common used antibiotics. The media were: blood (with 5-7% defibrinized blood), DNase, nutrient, mannitol salt and Mueller-Hinton agar. Coagulase and catalase tests were used for the identification. The media prepared according to manufacturer's instructions and sterilized by autoclaving at 121°C for 20 min. The plates were incubated at 37°C for 18-24 hr. Identification of the isolates done by using standard procedures as follow:

Morphological characteristics and gram stain to be performed. To check the growth pattern blood agar (with 5-7% defibrinized blood), mannitol salt agar were used. For biochemical characteristics, DNA hydrolysis, sugar fermentation, coagulase, catalase, oxidase test and novobiocin disc (5µg) will perform (Koneman et al, 1992).

E. Screen for methicillin resistance staphylococcus strains (MRSA); The methicillin susceptibility test made with Kirby-Bauer disk diffusion method according to CLSI (2021) criteria as described, briefly; a bacterium suspension in 0.5 McFarland turbidity standards were prepared from each S. aureus strain. The plantation made to Mueller-Hinton agar from these suspensions and the oxacillin disk or methicillin (1µg)(Oxoid) place to the culture plates and the plates incubated for 24 hrs at 37°C in the aerobic medium. The inhibition zones will interpret in accordance with standards of (CLSI. 2021).

F. Molecular characterization

All isolates of *S aureus* involved in this study were subjected to PCR assay. Genomic DNA extraction was performed according to (Bettin et al., 2012). Briefly, around 10 colonies of S. aureus cultivated on Blood agar plate were suspended into 1 mL of Tris (0.5 M, pH 8.0) then centrifuged at 13,000 rpm for 5 minutes. Supernatants were discarded, and pellets were re-suspended Tris into ethylenediaminetetraacetic acid (TE) buffer solution Tris, (10)mM mM 1 Ethylenediaminetetraacetic acid, pH 8.0) and boiled at 100°C for 30 minutes. Then the samples were incubated at 37°C for 20 minutes and at 65°C for 10 minutes then centrifuged at 13,000 rpm for 15 minutes. Supernatant that containing genomic DNA was collected and kept at -20°C for extended works.

Detection of mecA; Genomic DNA was used as DNA templates for application of PCR assay targeting the presence or absence of mecA gene. To detect the existence of mecA gene; forward primer (GTGGAATTGGCCAATACAGGAAC) and reverse primer (GTTAGTTGAATATCTTTGCCATC) was used to amplify a product of 502 bp DNA fragment of the mecA gene. Program of PCR amplification consisted of: initial denaturation at 95°C for 4 minutes, followed by 35 cycles of denaturation at 95°C for one minute, annealing at 54°C for one minute, and extension at 72°C for one minute, and a final extension at 72°C for 5 minutes. The amplified product was run on 2% agarose gel electrophoresis and then visualized (Zakai, 2015).

G. Statistical analysis

Data were analyzed by interning data into Statistical Package for Social Sciences (SPSS, version 13) program for Microsoft Word version 2010.

RESULTS

Generally, out of 150 nasal swabs collected from enrolled students of Sheikhan-Polytechnique College of Health and internet technology department only 27(18%) were MRSA as shown in Table 2.

Regarding gender-related MRSA distribution, female students 15 (55.5%) was higher than male students 12(44.5%) and no statistically significant correlation found between gender (p=0.46) in males and (p=0.57) in females. Moreover, numbers of MRSA among students of various departments showed that students of public health department recorded higher numbers of MRSA carrier (12 over 60 student examined) followed by information technology (9 over 52 student examined), while lowest number of MRSA carrier found among medical laboratory technology that was (6 over 38 student examined) as in in Table (3).

Table(2): -Frequency of MRSA among gender of students Examined

MRSA nasal swab test	gender o	f students	Total
	male	female	
Positive	12	15	27
Negative	54	69	123
Total	66	84	150

MRSA nasal swab test	de	epartment of studer	nts	Total
	Public health No. (%)	Medical laboratory technology No. (%)	Information Technology No. (%)	
Positive	12 (44.4)	6 (22.3)	9(33.3)	27
Negative	48 (39)	32(26)	43(35)	123
Total	60	38	52	150

Table (3):- Frequency of MRSA among students of various departments

In other words, numbers of MRSA among medical students were higher than those students of nonmedical educated study that unexposed to health fields as in Table (3 and 4)

 Table(4): -Frequency of MRSA among medical and non-medical students

MRSA nasal swab	Medical Students	Non-Medical Students	Total
	Public health&	Information technology	
	medical laboratory technology No. (%)	No. (%)	
positive	18 (66.7)	9(33.3)	27
negative	80 (65)	43(35)	123
Total	98(63)	52(37)	150

Table (5) shows numbers of MRSA among students of various stages and classes, generally; students of first stage recorded higher number of MRSA (12) followed by second stage (9), while both third and fourth stage recorded (3) of each of them. Furthermore, numbers of students that previously tested for MRSA in nasal swab, it appeared that 13 student already tested for MRSA, 3 of them still considered positive carrier state as in Table (6).

Prevalence of MRSA carrier states in relation to various inhabitants of students, higher number of MRSA were registered among those students that live with their families, followed by those lived with friends in student's hostel and no MRSA was found among those students that stayed at family relatives as 19, 8 and 0, respectively (Table 7).

Tabl	e (5):- Freque				
MRSA nasal swab test	stage of college				Total
	1	2	3	4	
positive	12(44.4%)	9(33.3%)	3(11.1)	3(11.1%)	27
negative	56(45.5%)	39(31.8%)	17(13.8%)	11(8.9%)	123
Total	68	48	20	14	150

Ever tested for MRSA	number	Percent
Yes	13	8.7%
No	137	91.3%
Total	150	100%

Table (7):- Frequency of MRSA in relation to student's inhabitant

MRSA nasal swab test	Inhabitant			
	with family No. (%)	in hostel with friends No. (%)	with relative family No. (%)	
positive	19 (70)	8(30)	0	27
negative	79 (64)	37 (30)	7(6)	123
Total	98 (65)	45(30)	7(5)	150

Based on the PCR assay targeting *mecA* gene, 14 of 25 (51.8%) isolates were MRSA carrying the *mecA* gene and high rate as 8/14 (57.1%) were bearing among public health students (Table 8).

MRSA No.	de	Total		
		Medical laboratory	Information	
	Public health No.(%)	technology No. (%)	Technology No. (%)	
Positive mecA	8 (57.1)	4 (28.6)	2(14.3)	14
Negative mecA	4 (30.1)	2(15.4)	7 (54.5)	13

There is a statistically significant correlation between the prevalence of MRSA in first and second year study compared with third and fourth (p<0.05).Moreover, there is statistically significant correlation between the prevalence of MSSA in the IT students (not exposed to health works in hospital) compared with medical students (p<0.01). Furthermore, there was a statistically significant correlation between the prevalence of MSRA and both living at home and in hostels (p < 0.01).

DISCUSSION

The medical and health educated students will be handling dealing with many types of patients within and outside the hospitals. Healthy carrier state of *S aureus* MRSA among the

medical-students is a major problem. They can disseminate this pathogen to the patients or vice-versa. In addition it could be transmitting to their colleagues in college when they working in the same hospital notably when infection control measures are compromised. Therefore, limiting the spread of MRSA can be highlighted by stressing proper hand hygiene and educating patients, healthy student's carriers on MRSA colonization (Zakai, 2015; Rampal et al., 2020; Sharma et al., 2020). This study shows that 27/154 (18%) of students carried MRSA strains in their nares, providing evidence that these strains are swarming in Sheikhan-Polytechnique College of Health and IT institute. Although the rate of MRSA carriers among medical students department and (Public health Medical laboratory technology department) was higher than IT institute students (unexposed to health works) as 66.7 % and 33.3 %, respectively. The finding of this study was higher than two studies conducted in Iraq performed on medical students; one in Duhok city that carriage rates of MRSA was 50% (Yassin and Hassan, 2013) and other in Krabala city was 40 % (Mohammed et al., 2015). Data produced from this study showed that our obtaining results is less than a study carried out in Baghdad University/Pharmacy college students that was 90% MRSA colonization (Saeed et al., 2014). Furthermore, findings of current study was still higher when compared with to other studies in surrounding and international countries; in Turkey 4.4% of medical students were carried MRSA (Baliga et al., 2008), in Saudi Arabia 6.7 % (Zakai 2015), in India and Iran only one MRSA isolate was detected over 150 and 108 participated medical students (Vijay et al., 2016: Sharma et al., 2020), respectively, in Louisiana 3.1% (Bellows et al., 2013), in Colombia 14 % (Marín et al., 2015), in Nepal 4% (Ansari et al., 2016) and in Ethiopia 8.4% (Efa et al., 2019),. These data can be alarming due to apparently healthy medical students could also pose a risk for the frequent and direct transmission and contact with patients. Multiple causes could play role of the nasal carriage of MRSA in certain setting notably community host status, geographical area, environmental and bacterial virulence factors (Assafi et al., 2015; Rampal et al., 2020).

Interestingly, in this study, less prevalence of MRSA colonization 33.3% was observed among IT institute students (not exposed to clinical or health works) compared with medical students.

This could provide proof that common exposure to MRSA sources in hospitals may play an important role in successful gaining nasal colonization by MRSA. This finding was matching to several studies; Zakai (2015) in Saudai Arabia claimed no MRSA colonization was found in 32 control group students who were not exposed to clinical work. Kitti et al., (2011) announced that only (1%) of university non-medical students were colonized by either MRSA or MSSA in Thailand put forward that individuals who are not exposed to the pathogen are at low risk of nasal colonization. In a Hungarian study by Laub et al., (2011) on university non-medical students, MRSA colonization was very less as (0.7%). Assafi et al., (2015) in Iraq stated that (4.2 %) MRSA colonization was among healthy collegiate nonmedical students. Mentioned results is back to clear indication that medical students who are rottenly in contact with hospitals can acquire the MRSA pathogen during their hospital works hours inversely, students who are not exposed to clinical works are less contact with the pathogens and are at low risk of nasal colonization. Thus, it is necessary to identify MRSA carriers and apply educational sessions for medical students to limit the spread of this pathogen either in nasal carriage or through contaminated stethoscopes, identification badges, neckties, stethoscope, white coats and even identification badges.

Data of this study regarding MRSA carriage rates related to students studying years, more numbers of students in first year study 12/27(44.4%) followed by second vear carriers 9/27(33.4%) were than third 3/27(11.1%) and fourth students year 3/27(11.1%). Dissimilar to Assafi *et al.*, (2015) in Iraq reported highest nasal carriage 24.7% was in third year students. Also in study at Belgrade University, no MRSA carriers were detected in medical students of first and second year study, only 0.3 % in third and fourth year students (Cirkovic et al., 2013). In India, internship students were more carriage of MRSA than students of other phases (Vijay et al., 2016). Another study executed in China claimed first and second year medical students harbored less rate of MRSA 9.4% (Xiao et al., 2011). Study findings in Nepal reported low rate (4%) of MRSA among first year students (Ansari et al., 2016). The reasons behind of current results might be students of third and fourth year are more committed with frequent wearing gloves before coming in contact with patients , proper application of infection control rules and proper hand washing during long hour works inside hospital or health centers than first and second year students that spend less fixed hours clinical works.

Obtaining results of current study shows that main part of the student's participants of MRSA carriage (70 %) were belonged to family and 30 % were living with friends in hostel and no MRSA carriers detected among students living with relatives. In contrary to our data, prevalence of MRSA colonization was higher in rural resident's students 20% than urban (13 %) (Ansari et al., 2016). A number of studies emphasized on more common transmission and circulation of MRSA at home (Babb et al... 1983; Loh et al., 2000; Perry et al., 2001). The risks for transmission of MRSA within family members to another member depend partly on the length of time the individual carries MRSA and the number of bacteria that they carry and shed. Furthermore, the domestic animals may be colonized (Boost et al., 2008). Moreover, changing style of life manner such as usual visit of shopping malls, attending parties and using public vehicles could come peoples to be in close contact and chance of transmit this pathogens to family members(Ansari et al., 2016). Data of this study revealed that the 13/27(48.1%) MRSA carried-students have previously tested for nasal MRSA carriage, 3(23%) of them were still positive carriage. Colonized students are most often transiently colonized, but they may become persistent carriers if they have skin lesions that leading to prolonged MRSA transmission (Debby et al., 2008). Round about 20 % of individuals could represent as persistent carriers of MRSA and near to always carry one kind of strain (Armstrong, 1976). Methicillin resistant Staphylococcus aureus (MRSA) strains were once confined to hospitals however, in the last 20 years MRSA infections have emerged in the community in people with no prior exposure to hospitals. Strains causing such infections were novel and referred to as community-associated MRSA (CA-MRSA) (Kateete et al., 2019). In this study, those students (mainly third and fourth class) that their applications in the hospitals regularly are considered hospitalacquired MRSA, while other carriers were community-acquired MRSA.

Detection of *mecA* gene using PCR assay in current study indicated that out of 27 phenotypic

oxacilln resistance isolates 14(52%) were positive to mecA gene and was statistically significant. This is a confirmation identification of MRSA strain. Changing (mutation) of PBP in bacterial cell wall to other variant such as PBR2a may explain non detection of mecA gene among phenotypic oxacilln resistance isolates (SR et al., 2022). Zakai, (2015) in Saudi Arabia found 10 (6.8%) isolates of MRSA were contained mecA gene by PCR that included phenotypice oxacillin sensitive strains also. Similarly, Pu et al., (2014) out of 103 S. aureus isolates 49 (47.6%) harbored mecA gene and some isolates were susceptible to oxacillin in disk diffusion test. Recent study in Duhok city, Iraq (2019) identified (50.4%) MRSA isolates carrying *mecA* gene among healthy workers staff of general hospitals (Hussein et al., 2019).

The study concluded that relatively high carriers of MRSA were among students of Duhok Polytechnique University in Shekhan notably, public health and medical laboratory students of first and second year compared with less carriers in IT students that were not exposed to clinical and medical fields. The study highlighted on routine screening of MRSA in Shekhan College particularly among medical students who might pose a risk to patients, hospital personnel and college environments. Giving of educational sessions on frequent hand hygiene, patient's health safety, infection control managements, and awareness of severity of MRSA infections would be mandatory. The study emphasize on application of PCR assay targeting mecA gene for accurate identification of MRSA strain besides of phonotypic antibiotic susceptibly test.

CONFLICT OF INTERESTS

There are no conflicts of interest associated with this publication.

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