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Rate of Methicillin-Resistant Staphylococcus Aureus (MRSA) Nasal Carriage among Healthy Students in Faculty of Veterinary Medicine/ University of Kufa and the Importance of Annual Screening

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Abstract

Aims: The current study aim to use simple procedures (detection of important virulence factors and antibiotic sensitivity testing) for investigating the rate of nasal carriage of Methicillin Resistant Staphylococcus aureus among healthy undergraduate students and to check the direct close contact to animals as a risk factor for the current carriage state.

Study design: cross sectional study.

Place and duration of study: The study conducted between February 2018 and April 2018 at the Faculty of Veterinary Medicine /University of Kufa/ Iraq.

Methodology: This study was conducted among 215 undergraduate students attending different study stages at the faculty of Veterinary medicine/ University of Kufa. According to certain exclusion and inclusion criteria only 71 students were included in this study, nasal swabs were taken as a samples and were inoculated into mannitol salt agar medium for the isolation of Staphyloccocus aureus strains. Also, certain virulence factors were determined in addition to antibiotic resistance testing to estimate Methicillin Resistant Staphylococcus aureus rate.

Results: The results showed that among 71 students from different stages, only 15 students (21.12%) were carrier for Staphyloccocus aureus out of which 11(73.33%) were methicillin-resistant Staphylococcus aureus (MRSA).

All Methicillin Resistant Staphylococcus aureus strains were isolated from Male (100%) with no carriage state recorded among female group. Seven (63.64%) of the Methicillin Resistant Staphylococcus aureus strains were able to produce Alpha and Beta-haemolysin toxin production. In addition, strains isolated showed (100%) positivity for mecA-mediated resistance test and (100%) coagulase production.

Conclusion: The study concluded that the rate of nasal carriage of Methicillin Resistant Staphylococcus aureus among apparently healthy students was high (73.33%). In addition, it found that animal contact was not a primary risk factor for the carriage state among the faculty students and recommended that annual screening of veterinarians (faculty members and students) should be performed annually. Moreover, submitting of prevention measures should be introduced as a routine checking to avoid pathogen transmission among students and between students and animals during their study years.

Keywords: MRSA, Nasal carriage, Healthy students.

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1. Introduction

Staphylococcus aureus (*S. aureus*) is a gram-positive bacteria that may commonly inhabit human anterior nares. Methicillin resistant *Staphylococcus aureus* (MRSA) is a strain of *S. aureus* that is genetically different from other strains, MRSA is responsible for many severe infections in humans and animals [1]. MRSA developed multiple drug resistance to β-lactam antibiotics through horizontal gene transfer or natural selection. The resistance of MRSA to those antibiotics is mediated by *blaZ* gene which encodes for enzyme called β-lactamase. The synthesis of this extracellular enzyme occur due to the exposition of this bacteria to continuous and high level of β-lactam antibiotics; this enzyme functions via the hydrolysis of β-lactam ring, rendering the β-lactam inactive [2] . Strains unable to resist these antibiotics are classified as methicillin-susceptible *Staphylococcus aureus* (MSSA) [3]. Nasal colonization with *S. aureus* is a dynamic process, certain time of carriage state may followed by loss of carriage and that might be associated with many factors [4]. Carrier individuals are in continuous risk for developing infections. In healthy individuals with no underlying diseases, there are at least three *S. aureus* nasal carriage patterns: persistent carriage, intermittent carriage, and non-carriage. Nasal carriage patterns were further divided to give a better description into occasional and intermittent carriers. Therefore, a patient classified as a carrier could either be a persistent or an intermittent carrier. This distinction is important because persistent carriers have higher *S. aureus* loads and a higher risk of acquiring *S. aureus* infection [5].

Although, a multifactorial genesis underlies *S. aureus* nasal carriage, but still the frequent and recurrent exposure to this bacteria in the environment is the most important determinant probably more important than the genetic background of individuals [6] . MRSA strains isolated from those who frequently visit or work at the healthcare facilities, is called healthcare associated MRSA (HA-MRSA) and were more resistant to clindamycin and other non β-lactam antimicrobials. While the MRSA strains that are commonly isolated from healthy people living in the community are named as community-associated MRSA (CA-MRSA). The strains of CA-MRSA can spread to others who are in close contact or share the same household [7]. CA-MRSA and HA-MRSA strains cause distinct clinical syndromes and affect different patient populations. HA-MRSA has been frequently associated with bacteraemia, pneumonia, and other invasive infections in patients exposed or admitted to hospitals or other healthcare facilities and with those who suffer from underlying morbid illnesses [5]. In contrast, CA-MRSA usually causes Skin and Soft Tissue infections (SSTIs) in healthy individuals. In some severe cases the infection might include pyomyositis, necrotizing pneumonia, sepsis, necrotizing fasciitis and osteomyelitis [7]. However, CA-MRSA strains are still the most common cause of invasive *S. aureus* infections in patients without risk factors for health care exposure [8]. Low personal hygiene standards and close body contact were the main cause for transferring infections among these groups [9]. Recently, CA-MRSA infections has been common among children, low income populations and patients attending emergency departments [9,10].

Some studies well documented the Transmission of MRSA between veterinarians, farmers, animal owners and mentioned that being close or dealing with animals might considered as a risk factor for MRSA carriage in humans and the likelihood of carriage correlates with the intensity of animal exposure [11,12,13]. The current study aims to use simple procedures to investigate the rate of MRSA among healthy undergraduate students and to check the direct close contact to animals as a risk factor for MRSA carriage. Such studies may increase awareness of the faculty community (teachers and students) to the importance of taking precautions to prevent spread of MRSA and its subsequently infections.

2. MATERIAL AND METHODS

i. Study area.

The study was conducted between February 2018 and April 2018 at the Faculty of Veterinary Medicine/ University of Kufa/ Iraq.

ii. Sample collection

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Out of 215 undergraduate students only (71) students involved to participate in the current study. The participated students were attending different study stages (first, second, third, fourth and fifth stage). Students how did not involve in the current study (144 students) either refused to volunteer or they were excluded from the study according to the exclusion criteria mentioned below.

The participants were 17(23.9%) female and 54(26.1%) male. All participants were asked to complete a questionnaire asking for the age, gender, previous nasal and upper respiratory tract infections, smoking and underlying and/or chronic diseases. In addition, the students were informed to declare if they have pets at home or not and all of them answered with no. Students included in the current study were screened at the Microbiology Department for being carriers for MRSA through collecting nasal samples from the anterior nares using a single sterile swab. Samples were taken by students themselves under the researcher supervision and samples were immediately processed. Based on the faculty curriculum and according to the degree of animal contacts and duration of exposure to pathogens, the students were classified into three groups as shown in table 1.

Table 1. Classification of Students into Groups according to Study Stage

Name	Student stage	Degree of contact with Animals and exposure to pathogens
1st Group	First stage	Low Degree
2nd Group	Second & third stages	Medium Degree
3rd Group	Fourth & fifth stages	High Degree

Sample analysis:

Samples were cultured directly on Mannitol salt agar (HIMEDIA, India) for selectively growing of *Staphylococcus* family. Plates were cultured aerobically for 24hours at 37 ^{o}C, appearance of golden yellow Mannitol fermenting colonies were considered as a positive indicator for *S. aureus* growth.

Biochemical analysis:

Different tests were used to identify *S. aureus* including cell morphology examination using Gram stain (Staphylococci appear as gram positive grape like clusters). Catalase test was used for further differentiating *Staphylococci* from other gram positive cocci bacteria.

Production of virulence factors:

Production of virulence factors was tested using the following tests:

- **Double zone hemolysis test:**
-

Using Sheep Blood Agar (SBA), *S. aureus* strains were plated and cultured in a candle jar for 24h at 37 ^{o}C, the results interpretation was as in (Table 2).

- **Coagulase production tests:**
-

Coagulase Test was carried out to differentiate potentially pathogenic *Staphylococcus* species from non-pathogens. Two types of tests were used, bound (slide) and free Coagulase production test (tube test). Tests were performed according to Tortora, *et al*.[14].

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Table 2. Double Zone Haemolysis Test Reading

Type of Toxin	**Appearance on SBA**
Beta-haemolysin toxin production	Appearance of narrow zone of complete haemolysis immediately around the colony
Alpha-Hemolysin toxin production	a wider zone of partial or incomplete haemolysis.

Antibiotic susceptibility test:

Antibiotic sensitivity testing was performed by Kirby–Bauer disc diffusion method for the following antibiotics: Ciprofloxacin, Erythromycin, Penicillin, Ceftriaxone, Cefotaxime, Cefoxitin, Norfloxacin and vancomycin (Toxoid) . Discs were manipulated on a Mueller–Hinton agar plate previously inoculated with 0.5 McFarland bacterial suspension. Following overnight incubation at 37°C, zones were measured and compared to the standard zones obtained from the Clinical and Laboratory Standards Institute (CLSI). Test for methicillin resistance was performed by Kirby–Bauer disc diffusion method 30-µg Cefoxitin disk test to detect *mecA*-mediated resistance in *S. aureus.* Cefoxitin disk was manipulated on a Mueller–Hinton agar (HiMedia Labs, Mumbai) plate previously inoculated with bacterial suspension equivalent to 0.5 McFarland standard, plates were incubated at 35°C for 24 hours. Results were interpreted according to the criteria of the CLSI [15].

Inclusion and Exclusion criteria:

All apparently healthy students from different stages were included in the current study.
Students who excluded from the study were with, Previous upper respiratory tract problems, Long time hospital admission, Long term antibiotic use and chronic underlying diseases.

Statistical analysis: The statistics for the current cross sectional study was described by frequencies and proportions.

3. RESULTS and DISCUSSION

MRSA is a pandemic antimicrobial-resistant pathogen. With the increasing rate of Nasal colonization with this pathogen, the risk for development of clinical infection and chance of high mortality and morbidity rate among carriers is increasing [16,17]. Many studies investigated different risk factors leading to nasal carriage of MRSA among healthy individuals [18,19]. Others mentioned that, nasal carriage considered as an important source for spreading infection among susceptible patients and the students are one of the main sources for spreading such pathogen [20].
In the current study we tried to investigate the rate of MRSA nasal carriage among the faculty students as well as predicting whether animal contact was a risk factor for the carriage state. In our study we tried to exclude any participant with a chance of being infected with MRSA from a source other than animals contact specifically contact with animals.
Out of 215 students only 71 were participated in the current study, the participation was voluntary and the student filled a questionnaire and signed an informed consent for participation. The laboratory work revealed that 15 student (21.12%) were carriers for *S. aureus*, eleven out of the fifteen (11/15= 73.33%) were carriers for MRSA strains (Table 3). Whereas the prevalence rate of MRSA among the healthy students was (11/71=15%).

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Table 3. Number of MRSA Positive Cases among *S. aureus* Carriers

Results	*S. aureus* in Male	*S. aureus* in Female	MRSA in male	MRSA in female	Total out of 71
Carriers	4(26.67%)	0 (0%)	11(73.33%)	0(0%)	15(21.12%)
Non-carriers	-	-	39(69.64%)	17(30.35%)	56 (78.87%)
Total					71(100%)

All MRSA strains were isolated from Male (100%) with no carriage among female group. 1st group students showed the highest rate of MRSA carriage (54.54%) followed by the 2nd group and the least percentage recorded by the 3rd group (18.18%) (Table3 and 4).

Table 4. Distribution of MRSA Positive Cases according to Study Stage

Study group	Number (%)	Male	Female	Smoker
1st group	6(54.54%)	6(54.5%)	0	1(100%)
2nd group	3(27.27%)	3(273%)	0	0
3rd group	2(18.18%)	2(18%)	0	0
Total	11(100%)	11(100%)	0	1(100%)

Our result was in agreement with Humphrey *et al*. [10] results which revealed that male gender is a significant risk factor for MRSA carriage.

All the participants in the current study were with no history of hospital admission or any type of underlying diseases so the carrier cases were considered as community-acquired methicillin-resistant *Staphylococcus aureus* (CA-MRSA). Recently, the emerging CA-MRSA is forming a huge concern that requires a considerable attention infection with such bacteria may lead to severe and difficult to treat infections in healthy individuals. Such infection would increase the need for Vancomycin hydrochloride therapy which may lead to increase chance of emerging of Vancomycin resistant *S. aureus* (VRSA) strains [21]. Among our study groups, smoking was a non-significant factor for developing MRSA nasal carriage, in which 90.9 % of our study MRSA carriers were non-smokers and these results disagree with the results of Abdella and colleagues who stated that smoking is a significant risk factor for MRSA nasal colonization [22].

The percentage of *S. aureus* carriers in our study agree with that published in other studies among Iranian, Egyptian, Ethiopia and Chinese peoples which were 22.7%, 22.9%, 28.8% and 21.6% respectively, while disagreed with the rate of MRSA carriage among *S. aureus* isolates of the same countries mentioned above as, 32.8%, 58.8%, 44.1% and 4.7% respectively [23].

The importance of MRSA increases with the number and type of virulence factors secreted by it. Numerous secreted virulence factors are produced by *S. aureus*, including a number of membrane damaging toxins capable of forming pores in the cytoplasmic membrane of host cells leading to cell lysis[16]. in our study we

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tried to use a simple methods for detection of MRSA strains and indicating the most important virulence factors that increases the pathogenicity of such strains. Hemolysin toxins production, coagulase production and gene for *mecA*-mediated resistance is an important factors contributing in MRSA virulence. The results showed that 63.64% of the isolated MRSA strains have both Alpha and Beta hemolycin toxins and 100% coagulase enzyme producing strains. Also all the isolated strains showed 100% positivity for surrogate *mecA*-mediated resistance (Table4 and 5).

Table 5. Results for production of hemolycin toxins and some virulence factors

Coagulase test	Slide coagulation	Tube coagulation	*Double zone hemolysis	**Single zone hemolysis	Gamma hemolysis	*mecA*-mediated resistance
Positive	10(90.9%)	11(100%)	7 (63.64%)	3(27.27%)	1(9%)	11
Negative	1(9.1%)	0	4(36.36%)	8	10	0
Total	11(100%)	11(100%)	11(100%)	11(100%)	11(100%)	11

* =Beta And Alpha Hemolysin Toxin Production.
** = Beta-Hemolysin toxin production only.

The mecA gene encode for low affinity penicillin-binding protein PBP2a (or PBP2) and make it resistant to most beta lactam antibiotics [24]. While the alpha-hemolysin (Hla), is an important cytolytic, pore-forming toxin implicated in the pathogenesis of S.aureus. Hla has cytolytic activity toward a variety of host cell types, including human keratinocytes, epithelial cells and lymphocytes. Other types of hemolysin is Beta-hemolysin (Hlb) is a magnesium-dependent sphingomyelinase C that induces lysis of sheep erythrocytes and human monocytes [16]. All those toxins make carriers in a high risk for developing SSTIs and other infections.

Previous studies mentioned that MRSA in veterinary clinical practice is a professional hazard and hazard is dependent on Intensity of animal contact [25,26]. Our results showed that animal contact is not a significant risk factor as well as the period of contact, in which students of 3rd group whose considered as having a long term and strong contact with animals showed no carriage state. MRSA strains are usually resistant to several groups of broad-spectrum antibiotics that are used on a large scale in the hospital. The isolated MRSA strains in our study were 100% resistant to penicillin and Cefoxitin and were 100% sensitive to Vancomycin and ciprofloxacin (Table 6).

The results of antibiotic sensitivity testing (AST) showed the presence of more than one option for treating MRSA carriers, and this is a good news. However, choosing of suitable treatment is critical and should be based on antibiotic sensitivity results because Vancomycin till now considered as the last choice for treatment of MRSA infections and development of Vancomycin-resistant MRSA strains in different regions of the world is a major cause for concern. Therefore, prevention of staphylococcal infections and reduction of the spread and emergence of MRSA are essential [17].

Using of simple laboratory procedures for estimating the rate of MRSA carriers among student in medicine faculties encourages the start of using such procedures as a routine work for the annual checking of students and faculty members. Checking and treating MRSA carriers according to the AST results may help in controlling spread of MRSA in community.

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Table 6. Antibiotic Sensitivity Test Results

Case number / Type of antibiotic	1	2	3	4	5	6	7	8	9	10	11	Percentage of Resistance
Ciprofloxacin	S	S	S	S	S	S	S	S	S	S	S	0%
Erythromycin	R	S	R	S	S	I	R	R	R	S	R	55%
Vancomycin	S	S	S	S	S	S	S	S	S	S	S	0%
Penicillin	R	R	R	R	R	R	R	R	R	R	R	100%
Ceftriaxone	R	R	R	S	S	S	S	R	S	S	R	45.5%
Cefotaxime	R	R	R	S	S	S	S	R	S	S	R	45.5%
Cefoxitin	R	R	R	R	R	R	R	R	R	R	R	100%
Norfloxacin	S	S	S	S	S	S	S	S	S	S	S	0%

S= Sensitive, I= Intermediate sensitivity, R=Resistant.

CONCLUSION

The study concluded that the rate of nasal carriage of MRSA among apparently healthy students was (73.33%) and this is a high rate and a matter of concern. In addition, the study found that, Animal contact was not a primary risk factor for the carriage state among the faculty students. The study recommend that annual screening of veterinarians (faculty members and students) in addition to submitting prevention measures should be introduced as a routine checking to avoid pathogen transmission among students and between students and animals during their study years.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

AUTHORS'CONTRIBUTIONS

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This work was carried out in collaboration between all authors and all authors read and approved the final manuscript.

CONSENT

All students were given a written consent informing them that their participation was voluntary and that all data collected would remain unknown and confidential the informed Consent was signed by each student.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. In addition, the ethical permission for this study was obtained from the ethics committee of the Faculty of Veterinary Medicine at the University of Kufa.

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