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Surveillance of community acquired Methicillin-resistant *Staphylococcus aureus* and possible risk factors for health care providers in Hospitals and cancer centers

SOHA M. HUSSIEN
Nuclear and Radiological Regulatory Authority
Faculty of Pharmacy, MSA University
RANA SABRY
RAMY HELMY
NADA ABBAS
Faculty of Pharmacy, MSA University

Abstract:

Methicillin resistant Staphylococcus aureus (MRSA) infection has been increasing in the last decade specially the community acquired MRSA (CA-MRSA) which is a MRSA infection that is acquired in collage, schools, and even prisons, while the hospital acquired MRSA (HA-MRSA) is the one that infects patients in hospitals and cancer centers due to the immunedeficiency of the patients. MRSA generally causes pathogenic infections to humans, which could be life threatening and could lead to death. To determine the rate of MRSA carriers among MSA University medical students that candidate to be a health care providers and to compare the number of carriers among athlete and non-athlete participants. nasal and axillary samples were collected from 60 samples that varied between athletes and non-athletes. Samples were cultured on Mannitol Salt Agar (MSA) for phenotypic identification, then on Deoxyribonuclease (DNase) for confirmation of positive Staphylococcus Aureus samples. Antibiotic resistance tests were carried out using the positive samples against Oxacillin antibiotic disks on Mueller-Hinton agar, to check for methicillin resistance; hence, confirming the presence of MRSA. Random positive samples were genetically identified by

PCR, which proved according to their PVL gene count to be community acquired Methicillin resistant Staphylococcus Aureus (CA-MRSA). Out of 30 athletes 7 were positive MRSA carriers (23.3%), and out of 30 non-athletes 3 were positive (10%). The higher carrying rate among athlete participant is to the point of this research, that constant contact with unsanitary playgrounds, gym equipment, and usual professional injuries, are an effective risk factor for MRSA infections. There is no published information about MRSA among university Students in Egypt. The present study is the first to investigation done on the presence of MRSA among university students between athletes and non-athletes in an Egyptian university.

Key words: MRSA; *Staphylococcus Aureus*; MSA University; Athletes; Antibiotic resistance

INTRODUCTION

Staphylococcus aureus is a pathogenic bacterium that causes a lot of infections in humans and animals as confirmed by MRSA Research center (2010), **Deresinski (2005)** reports that MRSA is the leading source of skin and soft tissue infections in patients presenting to emergency departments in hospitals; and a higher percentage in intensive care unit Patients. Plenty of people may have MRSA yet have no symptoms as they only work as carriers for community acquired Methicillin resistant *Staphylococcus aureus* (CA-MRSA). The top popular medical conditions that are related to MRSA infections are septic shock, pneumonia, bacteremia, endocarditis, and cellulitis. MRSA is a worldwide problem as it survives in various climates and various environments. Community-acquired MRSA has a higher prevalence in Canada, the US, and Australia **(Kock, *et al.*, 2010).**

Following the introduction of Methicillin as a new treatment for *Staphylococcus aureus* infections caused by the penicillin resistant strains, reports of Methicillin resistance stormed the medical field. Nowadays, methicillin resistant *Staphylococcus aureus* strains are commonly found around the globe in both hospitals and among the community. Methicillin-resistant *Staphylococcus aureus* (MRSA) is no longer only hospital acquired. MRSA also is defined as community

acquired if the MRSA-positive specimen was obtained outside hospital settings or within 2 days of hospital admission, and if it was from a person who had not been hospitalized within 2 years before the date of MRSA isolation **(Salmenlinna et al., 2002)**

CA-MRSA is stronger and more vigorous than Hospital acquired Methicillin resistant *Staphylococcus aureus* (HA-MRSA) due to the difference in virulence factors which is responsible for the perseverance and the extended infection transmission according to **Liu., et al (2011).**

MRSA among health care workers increases the risk of spreading the organism in hospital settings, Alongside infections due to cross-contamination between patients and health workers and high risk of colonization with MRSA strains among health care providers especially in hospitals and cancer centers as patients being susceptible to common infections due to diminished immune responses. CDC (Center for Diseases Control and Prevention) is engaged in several short- and long-term MRSA surveillance (infection tracking) projects that involve collaboration with health departments, individual hospitals, and academic medical centers, among others. Understanding the burden of MRSA infections—how much is occurring, where it is happening, and how it is being spread – is essential for developing effective prevention programs and measuring their impact. Also separate events globally involving the evolution of CA-MRSA threaten to spoil any success that might have in controlling HA-MRSA and the risk factors for the incidence of community-associated Methicillin-resistant *Staphylococcus aureus* acquired infection or colonization in hospitals and cancer center Patients.

Athletes have one of the biggest risk factors for getting Methicillin resistant *Staphylococcus aureus* (MRSA) infection; especially skin infection due to lack of hygiene, and sharing personal materials. Transmission of MRSA infection between athletes in the same team or even during practice, and competitive games like wrestling and rugby football results in MRSA infection outbreak, sharing towels, clothes, shaving tools, and soaps increase the rate of having MRSA outbreak. Also due to another outbreak that happened in August 2003 in a collage football team it was found that fields could cause MRSA infections because mot fields are made of grass, sand,

and asphalt **(Collins, and O'connell, 2012).** Also athletes that play sports that could easily cause skin break, and wounds are more susceptible for getting MRSA skin infection, since the human skin already carries colonies of MRSA on it that does not cause any harm until it come across open wound, or broken skin . Prevalence was made in Virginia Polytechnic Institute and State University on university athletes; it was found that thirty five percent (35%) of the participants were carriers for Methicillin resistant *Staphylococcus aureus* (MRSA), and seventy six percent (76%) of the wrestling participant were carrier for Methicillin resistant *Staphylococcus aureus* **(Lee, and Neild, 2007)** .Athletes have poor hygiene, and poor medical knowledge most athletes ignore the presence of boils, and rashes they will dismiss it and will think it is self-treated disease, however it could be serious infection specially athletes are at risk of having many infections because their life style. First Methicillin resistant *Staphylococcus aureus* (MRSA) infection case in high school wrestling team was reported in 1993 the student played from 1993 to 1994, because of this student an outbreak took place and seven out of thirty two (7/32) were diagnosed as MRSA infected patients, due to this outbreak the school did visual examination on each athlete before matches **(Deleo, et al., 2010)** .

According to CDC one in three humans is a carrier for MRSA, which approximately means that 33% are carriers. MRSA could be colonizes on skin, in nasal cavity, gastrointestinal tract, and throat. There are three types of MRSA carrier first there is intermittent carrier, second there is persistent carrier, and third there is occasionally carrier (**VandenBergh et al., 1999**). Axillary is one of the most suitable places for bacteria generally to be colonized in; due to the sweat, the fat cells, and it is considered to be closed due to the kin folds, so it could contain a lot of dirt, and in athletes it is a better media for MRSA because athletes train much more than normal persons so they sweat more, they get contact with fields full of dirt that could increase their risk factor of being a carrier of MRSA skin infection, they are susceptible to injuries, and as it has been said previously the lack of hygiene. On the other hand nasal site is also suitable for bacterial colonies, nasal site is wet, dark, and could have lots of dirt, and dust gets stuck in the nasal hair as a filtration for air before it enter the lungs, however nasal site in athletes could be the

same as normal personals same percentage for both athletes and non-athletes.

This study is focused on the medical students as they are candidate to be a health care provider and contact with patients in educational hospitals or due to their educational pathway and in athletes too due to their life style and also they have a high risk factor of getting MRSA infection, athletes have high contact with each other, with gym machines, and with fields. Also they sometime share their personal stuff like towels, and soap. There is increased in the risk factors for the incidence of CA-MRSA infection or colonization in hospitals and cancer center Patients. Also to investigate the percentage of MRSA carriers in athlete and non-athlete persons by determine the presence of MRSA among athlete and non-athlete participants in MSA university, Investigate the microbial content and count of the collected samples, determine the presence or absence of genes using PCR and improve the hygiene and awareness between MSA students which are susceptible to be health care providers and the risk factors for community-associated Methicillin-resistant *Staphylococcus Aureus.*

MATERIALS AND METHODS

Sample collection

Forty six participants were included in this study, all are MSA university medical students however age range between eighteen to twenty five years old (18-25yrs). Twenty three students are athletic students and twenty three students are non-athletic students; all students have signed a consent form for taking nasal samples and axillary samples from them. In the athletic students category only seven students agreed on giving both nasal and axillary swabs, while in the non-athletic category only ten students agreed on giving both nasal and axillary swabs which gives sixty samples in total, forty three nasal swabs and seventeen axillary swabs.

Ethical Consideration

Ethical approval was obtained from faculty of pharmacy's ethics committee, MSA University Written informed consent was obtained from each participant.

All the participants are healthy with the condition of not being hospitalized in the last three months, did not use anti-biotic or anti-microbial in the last three months, and that they do not have any infectious diseases. Included sports that participated in this research were football, volleyball, gymnastics, basketball, swimming, and mixed material arts (table 1).

	Football	Volleyball	Gymnasium	Basketball	Swimming	MMA
Nasal	**8**	**6**	**5**	**2**	**1**	**1**
Axillary	**3**	**2**	**1**	**0**	**1**	**0**

Table (1) showing number of participants in each sport

Within two months swabs were collected from athletes and non-athletes students to be cultured immediately and if not possible to culture immediately swabs were to be put in the university refrigerators for a period not more than twenty four hours (24hrs). Sterile swabs were used for the sample collection, all safety measurement were followed, all the participants signed a consent form before the collection, all samples were collected in privet place for the participants' convenience, all risks and side effects were told to participants before the sample collection took place. Risks and side effects included irritation in the nasal area, teary eyes, sneezing, feeling ticklish, and the feeling of needing to scratch the area.

After the collection of samples the sterile swabs were preserved in its sterile tube and named according to the agreed naming protocol; the sample name contain three parts first part is a capital alphabetical litter (A, B), (A) is for athletic participants while (B) is for non-athletic participants. The second part is for the sample number it consist of three digit numbers (001, 002,…etc.). And finally the third and last part is a symbol either (α) or (ß), alpha (α) symbol is for nasal swabs while beta (ß) symbol is for the axillary swabs. So for example an athletic, nasal sample will be (A001α) while non-athletic, axillary sample will be (B001ß). After the collection and the sample naming sample form is to be filled, to ensure that all the conditions are being followed and to increase the chance of prevailing community acquired MRSA.

MRSA isolation

Swabs of the anterior nares and axillary swabs were cultured on Mannitol salt agar (MSA) using sterile loops and incubated for seventy two hours (72 h) in thirty eight Celsius (38°C). Yellow colonies were streaked on DNAse agar for forty eight to seventy two hours (48-72 h) in thirty eight Celsius (38°C) for confirmation. Positive samples were streaked on Mueller-Hinton agar and Oxacillin disc were placed on the middle of the petri dish for resistance test to confirm the colonies are MRSA or not, Mueller-Hinton agar is incubated for (48-72 h) in thirty eight Celsius (38°C) for confirmation.

PCR Genotyping

DNA extraction

Random positive samples were used for the genotyping identification three to five (3-5) colonies were taken from Mannitol salt agar (MSA) and boiled for ten minutes (10 min) after being suspended in 100µl of water to extract the bacterial DNA. After that the solution was centrifuged at 1000 rpm for 5 minutes to extract the supernatant, the supernatant needs to be suspended by shaking or vortex.

Primers were used for this identification of the samples; primers used were for SCC*mec gene* IVa, and IVb detection (table 2), these primers were chosen according to Zhang et al.

Oligonucleotide sequence (5′-3′)	Amplicon size (bp)	Specificity
GCCTTATTCGAAGAAACCG	776	*SCCmec* IVa
TCTGGAATTACTTCAGCTGC	493	*SCCmec* IVb

Table (2) showing the primers used in this research.

After extraction PCR was performed by taking five microliter (5µl) of the DNA added to the master mix which contain 1.25 unit of Taq DNA polymerase, 20 mM $(NH_4)_2SO_4$, 75mM HCL, 1.5mM $MgCl_2$, 0.01% (v/v) Tween 20, 100 pmol of the chosen primers mentioned in (table), and deoxynucleotide triphosphates "dATP, dCTP, dGTP and dTTP". PCR expected sizes of the products for the two used primers are 776bp, and 493bp. For an initial step the mixtures were incubated at temperature 95 °C for 5 minutes, then 32 cycles of alternating temperature are followed; 94 °C for one minute, 57°C for one minute, 70 °C for one minute and as a final step 72 °C for 10 minutes.

Other Materials used

Bacterial Isolates, Culture media ,Samples, PCR kit, Autoclave, Petri dishes., Sterile swabs, Sterile loops and Incubator.

RESULTS

Sixty collected sample were included in this study .out of the forty six participants twenty three were non-athletic students and twenty three were athletic participants, first group the non-athletic group, and the second group the athletic group, thirty samples from each group. All participants did not have any infectious diseases, did not take any anti-biotic in the last three months or have been hospitalized.

Non-athletic group

The non-athletic group contained fifteen male's sample (15/30), fifteen female's sample (15/30), seven axillary samples (7/30), twenty three nasal sample (23/30), and the age of the participants various between twenty years old (20yrs) to twenty four years old (24yrs); one sample from age twenty years old (20yrs) 3.6% of samples, four sample from age twenty one years old (21yrs) 14.3% of samples, nine samples from age twenty two years old (22yrs) 28.6% of samples, eleven samples from age twenty three years old (23yrs) 35.7% of samples, and finally five samples from age twenty four years old (24yrs) 17.9% of samples representing in the following table and chart.

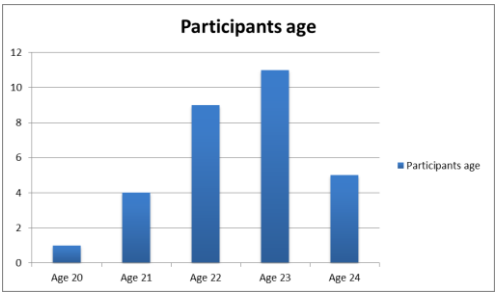

Chart (1), showing the different ages of participants

Three positive samples were obtained from the non-athletic group (3/30), all of the three samples are nasal samples (3/23), none of the six axillary samples are positive (0/7), and even though two out of the

three samples are collected from participants that agreed to give both nasal and axillary samples (2/3), however only nasal swabs were positive for MRSA (table 3), so 10% of the collected samples are positive (chart 2).

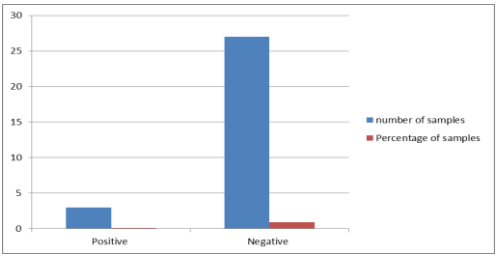

Chart (2), showing the number, and percentage of the positive and the negative samples.

Sample Number	Gender	Age	Any infectious Diseases	Athletes or non-athletes	Sample Result
B001 alpha	Female	23	No	non-athletes	Negative
B002 beta	Female	23	No	non-athletes	Negative
B004 alpha	Female	22	No	non-athletes	Positive
B005 alpha	Female	22	No	non-athletes	Negative
B008 alpha	Male	23	No	non-athletes	Positive
B009 alpha	Female	23	No	non-athletes	Negative
B010 alpha	Female	22	No	non-athletes	Negative
B011 alpha	Female	22	No	non-athletes	Negative
B012 Beta	Male	23	No	non-athletes	Negative
B013 alpha	Female	22	No	non-athletes	Negative
B014 alpha	Male	23	No	non-athletes	Negative
B015 beta	Male	22	No	non-athletes	Negative
B016 alpha	Male	22	No	non-athletes	Negative
B017 alpha	Male	21	No	non-athletes	Negative
B018 alpha	Male	23	No	non-athletes	Positive
B019 alpha	Female	22	No	non-athletes	Negative
B020 alpha	Female	21	No	non-athletes	Negative
B021 Beta	Male	21	No	non-athletes	Negative
B023 Alpha	Male	24	No	non-athletes	Negative
B024 Beta	Male	24	No	non-athletes	Negative
B025 alpha	Male	23	No	non-athletes	Negative
B026 Beta	Male	24	No	non-athletes	Negative
B003 alpha	Female	22	No	non-athletes	Negative
B006 alpha	Female	23	No	non-athletes	Negative
B007 Beta	Female	23	No	non-athletes	Negative
B022 alpha	Male	20	No	non-athletes	Negative
B027 alpha	Male	21	No	non-athletes	Negative
B028 alpha	Female	24	No	non-athletes	Negative
B029 alpha	Female	23	No	non-athletes	Negative
B030 alpha	Male	24	No	non-athletes	Negative

Table (3), showing information (gender, age, group, and results) of the non-athletic group.

Athletic group results

Thirty samples were collected from twenty three participants after signing the consent form, out of the twenty three participants only one is a female athletic student (1/23), and only seven agreed on giving axillary samples. Ages of participants ranged between eighteen to twenty five years old (18yrs to 25yrs).

Three samples were collected from eighteen years old students (3/30) 10% of samples, one sample was collected from nineteen years old participant (1/30) 3.3% of samples, eleven sample were collected from twenty years old participants (11/30) 36.7% of samples, six samples were collected from participants of age twenty one (6/30) 20% of samples, three samples were collected from twenty two years old participants (3/30) 10% of samples, four samples were collected from twenty three years old participants (4/30) 13.3% of samples, one sample from twenty four years old participant (1/30) 3.3% of samples, and another one from twenty five years old participant (1/30) 3.3% of samples (chart 3).

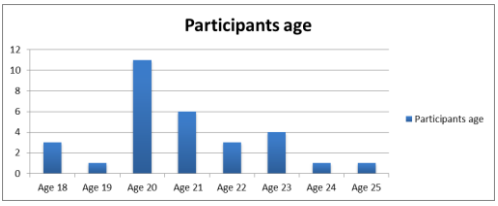

Chart (3), shows the different age of participants.

Seven positive samples were found and obtained from the athletic group (7/30), out of these seven one is axillary (1/7), while the other six is nasal samples (6/7). The positive axillary swab is from a participant that also has a positive nasal sample. So 26.7% is positive MRSA samples (chart 4)

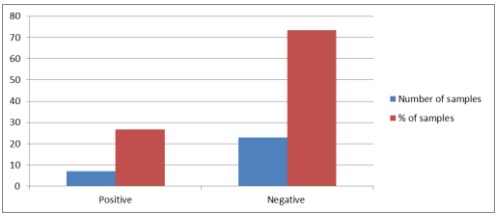

Chart (4), shows the number and percentage of positive and negative samples

Participants in the athletic group played deferent sports (table 1), out of this sports three positive samples were obtained from football sport (3/7), from gymnasium sport two samples were positive (2/7), one sample was positive from mixed material arts (1/7), and from basketball one sample was positive (1/7) (table 4). Time of sample collection was divided in to three categories before training, after training, and during training, the percentage of each one is different (chart 5).

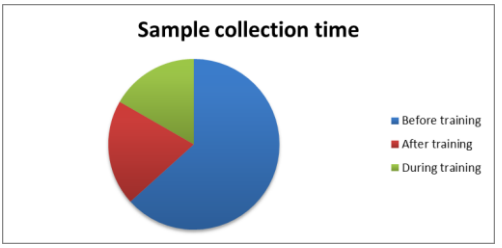

Chart (5), showing sample collection time

Sample Number	Gender	Age	Athletes or non-athletes	sport	Time of sample collection	Sample Result	Any infectious Diseases
A001 alpha	Male	23	athletes	Gym.	Before training	Negative	No
A006 alpha	Male	22	athletes	Gym	Before training	Negative	No
A023 alpha	Male	18	athletes	Gym	After training	Negative	No
A017 alpha	Male	24	athletes	Gym	After training	Negative	No
A019 alpha	Male	21	athletes	Gym	Before training	Positive	No
A024 beta	Male	21	athletes	Gym	Before training	Positive	No
A004 alpha	Male	20	athletes	Football	During training	Negative	No
A014 alpha	Male	20	athletes	Football	During training	Negative	No
A009 alpha	Male	19	athletes	Football	Before training	Positive	No
A015 alpha	Male	21	athletes	Football	During training	Negative	No
A005 alpha	Male	20	athletes	Football	After training	Negative	No
A020 alpha	Male	20	athletes	football	Before training	Negative	No
A025 beta	Male	20	athletes	football	Before training	Negative	No
A007 alpha	Male	22	athletes	Football	During training	Positive	No
A026 beta	Male	22	athletes	Football	During training	Negative	No
A003 alpha	Male	20	athletes	Football	Before training	Positive	No
A029 beta	Male	20	athletes	Football	Before training	Negative	No
A013 alpha	Male	21	athletes	MMA	After training	Positive	No
A010 alpha	Male	23	athletes	Basketball	Before training	Negative	No
A018 alpha	Male	23	athletes	volleyball	After training	Negative	No
A022 alpha	Male	21	athletes	Volleyball	Before training	Negative	No
A016 alpha	Male	25	athletes	volleyball	After training	Negative	No
A012 alpha	Male	21	athletes	volleyball	Before training	Negative	No
A021 alpha	Male	20	athletes	volleyball	Before training	Negative	No
A030 beta	Male	20	athletes	volleyball	Before training	Negative	No
A027 beta	Female	20	athletes	volleyball	Before training	Negative	No
A008 alpha	Female	20	athletes	volleyball	Before training	Negative	No
A002 alpha	Male	18	athletes	Swimming	Before training	Negative	No
A028 beta	Male	18	athletes	Swimming	Before training	Negative	No
A011 alpha	Male	23	athletes	basketball	Before training	Positive	No

Table (4), showing information (Gender, age, sport, time of collection, and results) of the athletic group.

SCCmec typing

Different samples that were chosen randomly and isolated harbored *SCCmec* IVa and IVb (table 1), according to Champion, A. CA-MRSA contain *SCCmec* IV and V, and the most common *SCCmec* IV genes are IVa, IVb, and IVd, IVc is not common. On the other hand HA-MRSA contains *SCCmec* I, II, and III. So the random samples have been proven to be positive for being community acquired MRSA (CA-MRSA).

DISCUSSION

The interior nares is known to be a *staphylococcus aureus* reservoir according to **Borchardt SM (2005)**, that is why the percentage of positive sample are much larger than the axillary samples, according to a study done by **Champion *et al.,* (2014**). 53% of the positive samples were collected from the interior nares while 47% were collected from both axillary and inguinal sites. **Nguyen *et al., (2005)*** did a group study on an American football team; this study is known to be the largest group study, a skin and soft tissue infection was reported in eleven (11) players on a collage team, after collection of samples it was found 26 out of 99 players were *staphylococcus aureus* in the interior nares and 8% of these players were positive for MRSA. Although some reported cases of infection was not caused by MRSA like **Kazakova *et al*., (2005)** that collected nasal samples from a professional football team and found that there is no MRSA carriers, also another case study was reported of a MRSA outbreak and nasal swabs were collected, however the outbreak was caused by MSSA rather than MRSA **Begier *et al., (2004)***

In previous studies **Cohen PR, (2008) and Kazakova SV *et al.(2005)*** sports with high contact with persons or equipment have high rate of causing athletes with MRSA infection than sports that has less contact. In our study football players and gymnasium athletes have higher rate of MRSA carrying than other sports like volleyball. Also since our study is to compare between athletes and non-athletes students the rate of MRSA carriers in athletes are higher than non-athletes students.

According to **Zong Z *et al.(2011)*** *SCCmec* IV and V are specified for the community acquired MRSA, while I, II, and III are

specified for Hospital acquired MRSA, that is why we chose *SCCmec* IV to confirm that what we isolated was community acquired MRSA not hospital acquired MRSA. Also we chose SCCmec IVa and IVb (table 2) because they are the most common, while IVc is not found in most of the time, according to **Champion *et al.(2014)*** IVc is rare to be found.

As for this research, the results confirmed to the past researches that used the same methodology to achieve a similar aim of work; as it showed that athletes are indeed a higher risk group for carrying and getting infected with MRSA and this confirmed the possible risk factors for health care workers in hospitals and cancer centers as all the participants were medical students in the university and are candidate to be a health care provider in different hospitals and contact with different patients especially immunodeficiency patients due to their educational pathway and due to their presence in the educational hospitals.

CONCLUSION AND RECOMMENDATION

This study has proven that athletes are more compromised to be MRSA carriers than non-athletic persons, and that the colonies could present in more than one site. Precaution should be taken under consideration to avoid infections to themselves and to others as they may contact with different patients especially immunodeficiency patients as heath care providers. Also any institute that has athletes work or study in it should teach them about infections, causes, symptoms and precautions.

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