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Prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) among Medical Staff in Three Syrian Provinces: Damascus, Daraa and Al-Swayda

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Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA) species represent a great challenge, in terms of their rising prevalence worldwide. In their capacity as health care professionals, hospitals' medical staff are at a greater risk of acquiring MRSA; being in contact with patients receiving antibiotics constantly for a variety of clinical conditions. Yet, perhaps due to limitations in research funds and a lack of incentives dedicated for survey purposes, the prevalence of MRSA among health care professionals in the Syrian community has gone undocumented so far. Hence, in this study, susceptibility testing was performed on *S. aureus* invasive isolates collected from 202 volunteers of the medical staff of major hospitals in three provinces in Syria: Damascus (the capitol), Daraa and Al-Swayda. The isolates were screened as recommended by the National Clinical and Laboratory Standards Institute (CLSI). All tests were carried out within the Microbiology laboratory at the Arab International University, Damascus, Syria. Appallingly, at the end of the survey, methicillin-resistant *Staphylococcus aureus* was found in the nasal cavities of 9.4% of the medical staff in the main hospitals in southern Syria. Vancomycin resistance was also detected in nearly 2% of the collected samples.

Key words: Methicillin resistant *Staphylococcus aureus* · Medical staff · Susceptibility testing · Vancomycin resistant *Staphylococcus aureus*

INTRODUCTION

Resistance developed against the antibiotics used in the treatment of *Staphylococcus aureus* related diseases represents a critical problem in the medical field worldwide. Long years of research and clinical trials aimed at the discovery and the development of new antibiotics have frequently been disvalued by the emergence of resistant species.

Staphylococcus aureus has long been recognized as an important pathogen responsible for different illness and conditions in humans (1) [1, 2]. It has been shown that people who carry *S.aureus* as part of their nasal flora may be at greater risk of acquiring infections with this pathogen. The nose is the main ecological niche where *S. aureus* resides in human beings. Yet, the determinants of a carrier state are still but vaguely understood [3]. Staphylococcal infections occur more frequently in hospitalized patients and they tend to have severe consequences [4]. Infections caused by *S. aureus* clinically range from minor skin infections to severe life threatening infections [5, 6]. The incidence rate is 20 to 50 cases/100,000 population per year and the mortality rate of *S. aureus* bacteremia has held up at roughly 10-30% despite the availability of several effective antibiotics [7].

The antibiotic Methicillin was introduced in 1959 and was employed in the treatment of *S. aureus* infections; however, in 1961, shortly after methicillin had started to be used, isolates of *S. aureus* were reported to have acquired resistance against it, which triggered much research on what was conveniently referred to as methicillin-resistant *Staphylococcus aureus* (MRSA) [8]. For the most part, the resistance was attributable to the production of β-lactamase by virtue of a relevant

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plasmid or chromosomal gene. However, some penicillin-resistant staphylococci also tend to be resistant to the newer β-lactamase-resistant semi-synthetic penicillins, such as methicillin, oxacillin and nafcillin. Thus, the resistance was primarily attributed to the presence of an unusual penicillin binding protein located in the cell walls of the resistant strains [9, 10].

The incidence of community-acquired and hospital-acquired *S. aureus* infections has been rising with the emergence of the drug-resistant MRSA [5]. MRSA is considered an established pathogen in most health care facilities. Initially, MRSA was limited to hospitals. Nowadays, MRSA isolates are being increasingly recovered from nursing homes and from the community [11]. The emergence of MRSA, usually multi-drug-resistant, strains renders the treatment of staphylococcal infections more challenging [12]. Being in close contact with the patients, health care workers (physicians, nurses, nurse assistants, medical students, utility workers, etc.) may be a possible source of hospital-acquired MRSA infections, since it is possible to transmit many of the pathogenic strains by physical hand contact with a carrier [13].

Carriage of *Staphylococcus aureus* plays a key role in the epidemiology and pathogenesis of *S. aureus*-related infections,due to both the diversity and the severity of the infections caused by this organism whereas it is predicted that more than 25% of the healthy individuals carry *S. aureus* on their skin or mucous. [14, 15]. Several studies have indicated that the nasal carriage of *S. aureus* strains varies anywhere between 16.8% and 90% worldwide (- [16, 17]. Not only does the distribution of MRSA show considerable differences on the global scale, but it has also shown remarkable differences between geographically approximate regions [4, 18]. Unfortunately, the Mediterranean region appears to be a hyperendemic geographic area for MRSA. However, epidemiology data regarding the non-European countries in the southern and the eastern parts of the Mediterranean pool are sparse.

Compared to surveys done in the European side of the Mediterranean, in addition to being few in number, studies done in its southern region employed different methodologies, a fact which has rendered it difficult of a process to produce valid comparisons [4, 18, 19]. The aim of this study was to assess the prevalence of and the factors associated with, nasal carriage of *S. aureus* and its antibiotic sensitivity patterns among the medical staff in three different provinces in Syria. To the best of our knowledge, no previous data on the prevalence of MRSA in Syria exists. Hence, this study is to be considered the first to report the predominance of MRSA in three major Syrian provinces.

MATERIALS AND METHODS

Setting and Design Samples were collected from the staff members of the main hospitals and health centers in the period from March 2011 to November 2012, in the three major provinces in the southern part of Syria, Damascus (the capital), Daraa and Al-Swayda (Figure 1).

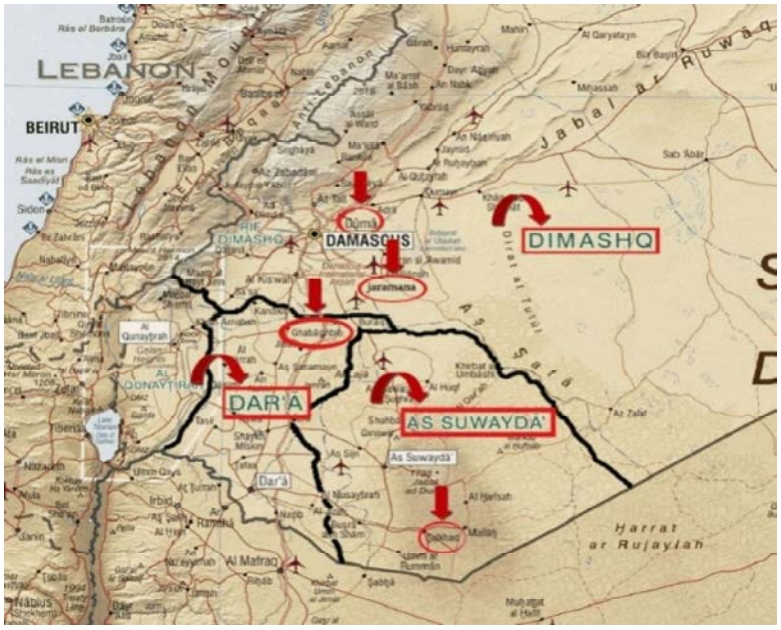

Fig. 1:

The following demographical data was collected from each of the subjects beforehand: age, gender, level of education, occupation (nurse, doctor, manager, cleaning staff or medical student), ward, history of antibiotic therapy during the past three months, smoking habits, nasal abnormalities (sinusitis, allergic rhinitis, nasal septal deviation) and history of previous surgeries.

Collection of Samples: Swabs were obtained from 202 staff members using sterile, dry cotton wool swab sticks, after the edges of the nostril cavity were cleaned with 70% ethanol. The swabs were carefully inserted into each nostril so that the tip was at the level of the nasal osteum (2.5cm from the edge of the nares), where they were gently rolled 5 times. Then, they were immediately placed in stuart transport medium (CONDA, Madrid, Spain)and were transported to the Microbiology laboratory at the Arab International University for analysis.

Isolation and Identification of MRSA: In the laboratory, the nasal swabs were pooled in Mueller-Hinton Broth (OXOID, Hampshire, UK) and incubated for 24hr hours at 37°C. Selective enrichment was performed in mannitol salt agar (CONDA, Madrid, Spain), which contained 7.5% NaCl and inhibit the growth of any bacteria other than *S. aureus*. After 24hrof incubation at 37°C, the presence of *S. aureus* was recognized due to its mannitol-fermenting ability which yielded an acid that reacted with an indicator dye to produce a yellow color in the culture plate; and it was confirmed by latex agglutination.

Assessing the Sensitivity to Methicillin and Other Antibiotics: The samples were tested for the presence of MRSAand the *S. aureus* isolates were screened using the disk diffusion method of the National Clinical and Laboratory Standards Institute (CLSI). The antibiotics used in the study were recommended by CLSI for the treatment of *S. aureus* infections. Determination of sensitivity of MRSA was conducted using cefoxitin method. Cultured in liquid broth, *S. aureus* was grown on Mueller-Hinton agar (OXOID, Hampshire, UK) and 1-µg disks of cefoxitin, cefuroxime, clindamycin, cloxacillin, Azithromycin, cefaclor, vancomycin, Penicillin G and Augmentin wereplaced onthe inoculated plates. Diameters of zone of inhibition were measured and recorded after a 24-hour incubation period at 37°C.

Statistical Analysis: Data was presented as frequencies and percentages. Data obtained from the experiments were expressed as the mean ± standard error (± SEM). Statistical difference between the different groups were evaluated by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test using IBM SPSS software (Version 20). Differences were considered significant at $p < 0.05$, $p < 0.01$ and $p < 0.001$.

RESULTS

Carriage rate of *S. aureus*: Swab samples were obtained from 202 staff members. All the samples were analyzed for the presence of *S. aureus*. Out of 202 clinical isolates, 48% samples showed presence of*S. aureus* whereas, 52% samples were not carriers of the pathogen (Fig. 2).

Table 1 and Figure 2 depict the characteristics of the screened health care workers. Among the carriers of *S. Aureus*, it was revealed that 44% werephysicians, 52.3% were nurses, 60.0% werecleaning staff and 26.6% were managers. while (45.3%) of trained student were carried. The carriage rate (prevalence) of *S. aureus* in males was found to be 33% while in females it was about 46.4%.

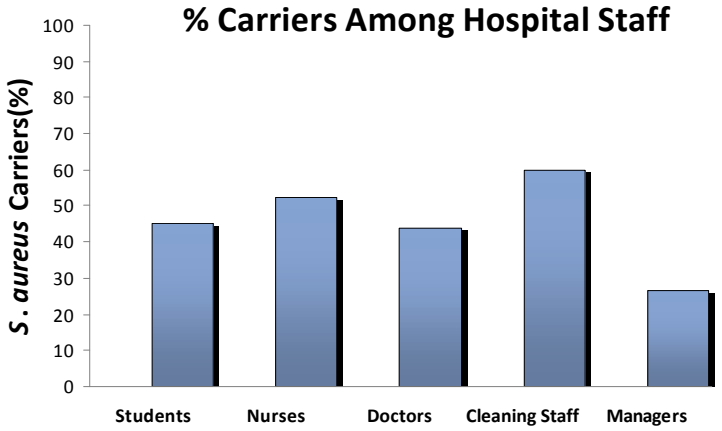

Fig. 2: Carriers percentages according to workers groups

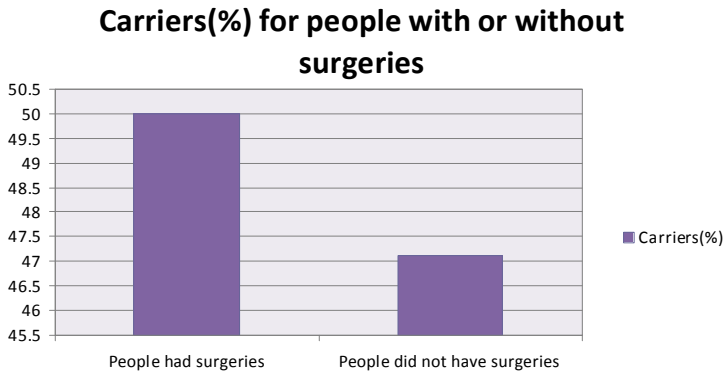

Fig. 3: Carriers(%) for people with or without surgeries

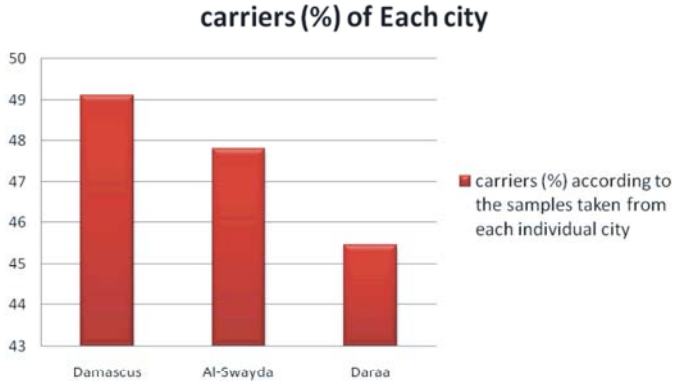

Fig. 4: Carriers (%) of each city

Table 1: Characteristics of the screened health care workers in hospitals

Variables		N=202 \carriers n (%)
Profession	managers	15\4 (26.6)
Hospital acquired	Nurses	65 \34 (52.3)
	Physician	27 \12 (44)
	Cleaning staff	25\ 15 (60.0)
	Training students	70\ 32 (45.3)
Gender	Male	64 \ 33 (51.6)
	Female	138 \ 64 (46.4)
Surgeries	With surgery	32 \ 16 (50)
	Without surgery	170 \81 (47.6)
Smoking	Smokers	23 \15 (65.2)
	Non-smokers	179 \82 (45.8)
Syrian provinces	Damascus	112 \ 55 (49.1)
	Al-Swayda	46 \22 (47.8)
	Daraa	44 \ 20 (45.4)

This study showed that there was a significant difference between sex for carriage rate of *S. aureus*. Among all the answered volunteer about their previous surgeries we have found out that (50%) of people who had surgeries were carried to *S. aureus* while (47.80%) were not (Table1) and (Figure3).

The prevalence of *S. aureus* according to the area is (49.11%) in Damascus which is the capital of Syria and (47.80%) in Al-Swayda and (45.45%) in Daraa (Table1) and (Fig.4).

Frequency of Occurrence of *Staphylococcus aureus*: Fig. 6 shows the frequency of occurrence of *Staphylococcus aureus* isolated from medical staff In three major areas. It showed that Methicillin susceptible *S. aureus* (MSSA) [32.2%] was most predominant over Methicillin resistant *S. aureus* (MRSA) which was 15.8% (Table 3). It showed that there was a significant difference between carriage rate of MRSA and MSSA

Antibiotic Sensitivity Pattern: Table 2 and Fig. 8 shows the antibiotics sensitivity and resistanceprofile of the *S. aureus* isolates among carriers. It showed that the percentage sensitivity(32.2%) and (15.8%) is percentage resistance. The in-vitro antibiotic sensitivity pattern of isolates of methicillin susceptible *Saureus* (MSSA) is shown in Table (2)., **VA :***Vancomycin*, (4.1%***P -G :****Penicillin G (32.2%)* **AZM** *:Azithromycin (29.9%),* **CX** *Cloxacillin (100%),* **CEC:** *Cefaclor*

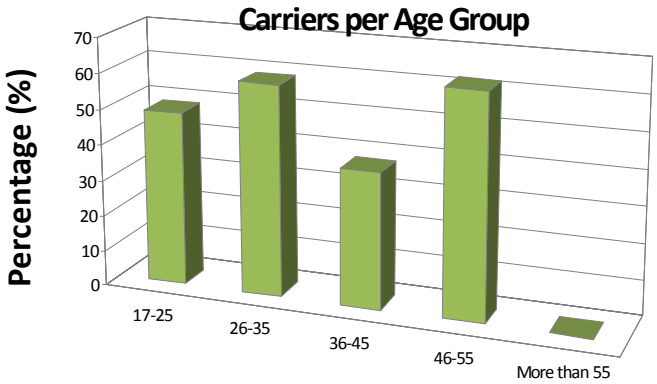

Fig. 5: The carriers according to age in the answered questionnaire. Three quarters of the Volunteers agreed to answer concerning their age.

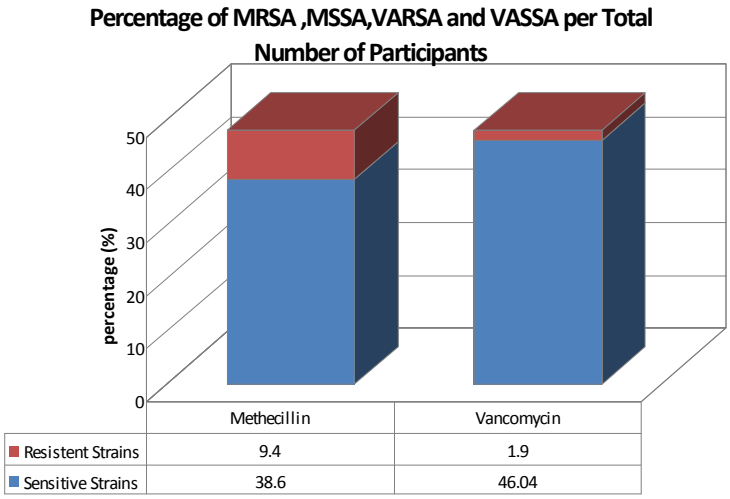

Fig. 6: The percentage of (MSSA), (MRSA),(VARSA) and (VASSA)

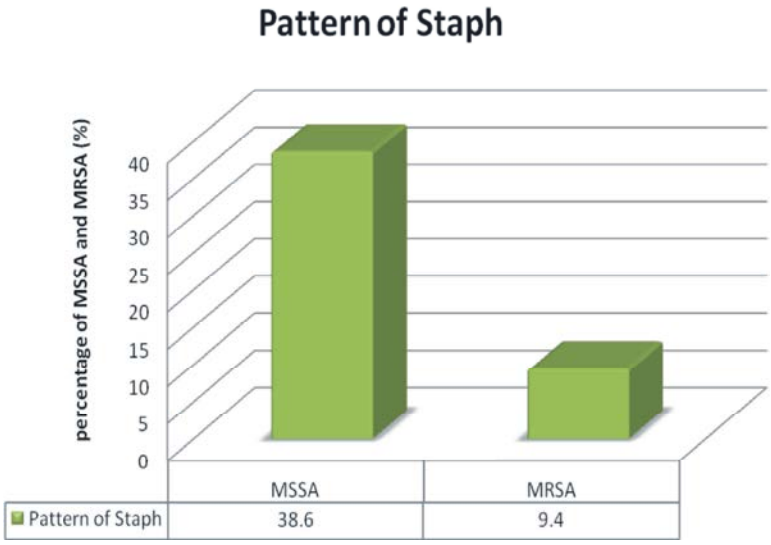

Fig. 7: Pattern of staff Penicillin G -Augmentin (PG - AMC)

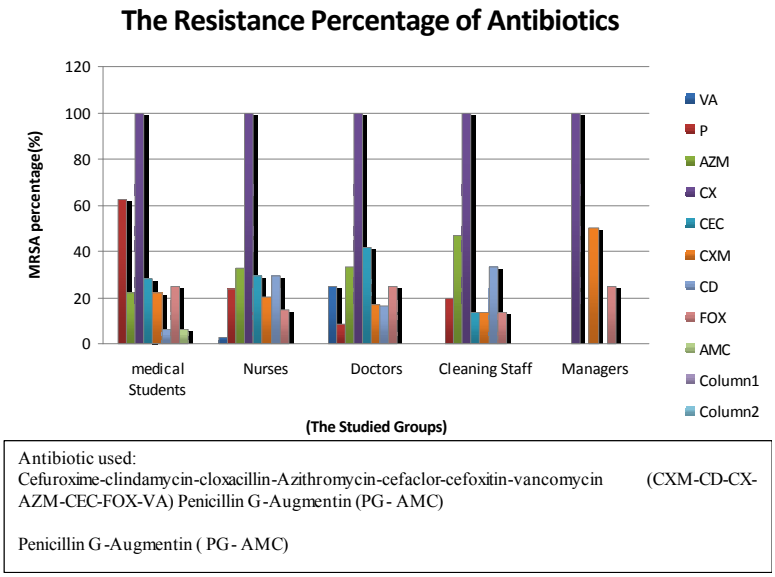

Fig. 8: The resistance percentages of antibiotics among the carriers

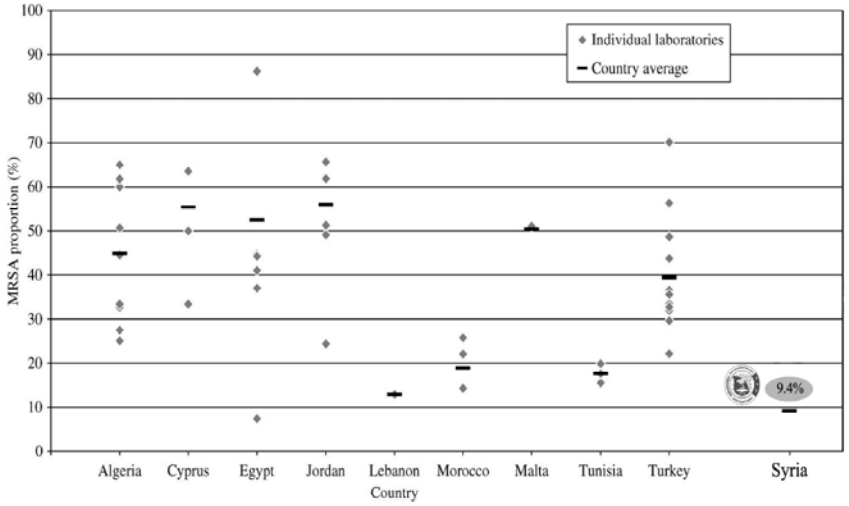

Fig. 9: MRSA Prevalence in Syria in Comparison to its Neighboring Countries (Modified from [20])

Table 2: Demographic profile

Age Groups	Numbers	Carriers(#)	Carriers (%)
17-25	76	37	48.7
26-35	44	26	59.1
36-45	21	8	38.1
46-55	8	5	62.5
More than 55	1	0	0

Table 3: Differences among different professionals

		Number of resistant samples								
Group	No. (Positive Carriers)	VA	P	AZM	CX	CEC	CXM	CD	FOX	AMC
Managers	4	0	--	0	4	0	2	0	1	--
Nurses	34	1	--	11	34	10	7	10	5	--
Doctors	12	3	--	4	12	5	2	2	3	--
Cleaning Staff	15	0	--	7	15	2	2	5	2	--
Students	32	0	32	7	32	9	7	2	8	2
All Groups	97	4	32	29	97	26	20	21	19	2

Table 4: The resistance percentages of antibiotics among the carriers

Group	Number of Participants	Number of *S. aureus* Carriers	Percentag of *S. aureus* Carriers	Percentage of Resistance per *S. aureus* Positive Participants								
				VA-	P	AZM	CX	CEC	CXM	CD	FOX	AMC
Medical Students	70	32	45.3	0	62.5	21.9	100	28.1	21.9	6.25	25	6.25
Nurses	65	34	52.3	2.9	23.5	32.4	100	29.4	20.1	29.4	14.7	__
Doctors	27	12	44	25.0	8.3	33.3	100	41.6	16.7	16.6	25	__
Cleaning staff	25	15	60	0	20	46.6	100	13.3	13.3	33.3	13.3	__
Managers	15	4	26.6	0	__	0	100	0	50	0	25	__
All Groups	202	97	48.02	4.1	32.9	29.9	100	26.8	20.62	21.64	19.6	2

-The study revealed an alarming percentage of Vancomycin resistance among Syrian hospital staff members.

*(26.8%),,***CXM :***Cefuroxime (20.62%)***,CD :***Clindamycin (21.64%*, **FOX** *:Cefoxitin, (19.6%)***AMC** *:and Augmentin ((Amoxicillin + Clavulanic acid)) (2%).*

DISCUSSION

This study shows the distribution of MSSA and MRSA nasal carriage rate through HCWS (health care workers) according to gender, profession, hospital acquired and community acquired. Other studies which were carried out in Saudi Arabia and Kuwait didn`t discuss MSSA and MRSA nasal carriage rate in HCWS. Hence, there is no possibility for comparison.

In this study, 48% of total studied samples grew staph. Aureus. Among the screened health care workers,32.2% of them were MSSA carriers and 15.8% with MRSA.

A study in the gulf area conducted in 1999 in Abha, Saudi Arabia showed that the prevalence rate of S.aureus colonization in nasal swabs was 25.4%.

In this study of Saudi Arabia 20.8% showed MSSA isolation and 4.7% of MRSA. The prevalence rate of MSSA in Saudi Arabia is lower than to study finding in our study and the prevalence of MRSA was higher in our study. In an infectious disease hospital in Kuwait, the nasal carriage rate of MSSA in both physicians and nurses was 15.8% and none of them were MRSA carrierwhich is comparable to rate the rate found in the present study.

Results regarding antimicrobial susceptibility of MSSA isolates were cephalosporin second and third generation and macrolides.On the other hand, the result regarding MRSA sensitivity to clindamycin, vancomycin and cefaclor.

We have found through our research that there is a need to be wary of a resistance of antibiotics that used today in the Syrian market to treat Staphylococcus aureus because.

- The percentage of doctors that have a resistance to vancomycin antibiotic is high and that is very dangerous,
- While the percentage of patient that have a resistance to vancomycin antibiotic is low

So we are afraid of transfer the resistance from doctors to their patients and for that doctors will be obligate to use vancomycin to treat patients and that cause with time a resistance to vancomycin and we should think seriously for this reason to find a new generation if vancomycin is fall down.

To find a solution for this problem, we can think about:

- Adding a more accurate cleaning system inside hospitals.
- Counselling of antibiotics prescribed by doctors and pharmacist.
- Make an Infection Control Department that can be responsible for frequently testing or examination of presence of S.aureus between medical staff. So if there is any infection by *S. aureus* between medical staff, we can treat them by mupiricin
- We should think seriously to find a new generation of antibiotics if vancomycin is fall down.

CONCLUSION

To the best of our knowledge, this was the first report conducted in Syrian medical staff to examine the prevalence of methicillin resistant *Staphylococcus aureus*. Our findings indicate that there is a resistance to MRSA about 9.4%. Noteworthy this is the highest resistance rate of vancomycin among the Arab countries.

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