Bulletin of High Institute of Public Health
Vol. 43 No. 1 [2013]

Prevalence of Methicillin Resistant *Staphylococcus aureus* Nasal Carriage and Its Antibiogram in Healthcare Workers from South of Jordan

Hamed M. Alzoubi*, Amin A. Aqel*, Munir Abu-Helalah**

Abstract:
Background: Healthcare workers (HCWs) can act as asymptomatic carrier in transmitting Methicillin Resistant Staphylococcus aureus (MRSA). Availability of epidemiological and antibiotic susceptibility data is necessary to limit the spread of HCWs-associated MRSA infections, and to help physicians in choosing the appropriate empirical antibiotic for management of such infections**. Objective:** to assess nasal carriage and antibiogram of MRSA in healthcare workers from Southern Jordan**. Methods:** a total of 276 nasal swabs were randomly collected from the HCWs. MRSA was identified by culture, biochemical and molecular methods. Antibiotic susceptibility was determined by the disc diffusion method**. Results:** The HCWs-MRSA nasal carriage was 8.7%. There was significant difference for nasal carriage of MRSA by nurse occupation (*p* value = 0.007), education level of less than a university degree (*p* value = 0.039) and years of HCW experience (*p* value = 0.023). No significant difference by age, sex, antibiotic exposure or smoking. Antibiotic resistance to Trimethoprim-Sulfamethoxazole and Tetracycline was detected in 37.5% and 12.5% of all MRSA isolates respectively. No resistance to the other antibiotics used in this study and no multidrug resistance was encountered in all MRSA isolates. **Conclusion:** MRSA nasal carriage among HCWs in this study was 8.7% with no alarming antibiotic resistance pattern. Nurses, less educated and more experienced HCWs are at increased risk of MRSA nasal carriage. Therefore, we strongly recommend screening and decolonizing positive HCWs who can act as asymptomatic carriers in MRSA transmission cycle.
Keywords: Methicillin resistant Staphylococcus aureus, Healthcare workers, Antibiogram

NTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) have become one of the major nosocomial and community pathogens worldwide since they were first isolated in the UK in early 1960s.[1,2] MRSA poses a *mec* A gene that codes for a penicillin binding protein (PBP2a) with low affinity to beta-lactam antibiotics which renders these bacteria resistant to virtually all beta-lactam antibiotics.[3,4] Additionally,

*Ph.D medical microbiology, Medical Microbiology and Immunology Department, Faculty of Medicine, Mu'tah University, Alkarak, Jordan
**Ph.D preventive medicine, Department of Public Health, Faculty of Medicine, Mu'tah University, Alkarak, Jordan

MRSA may also show resistance to several other antibiotics making it necessary to use specific antibiotics such as glycopeptides and linezolid to treat MRSA infections.[5] However, resistance to glycopeptides such as vancomycin is emerging and this may lead to further reduction in therapeutic options available for MRSA, which is in particular another serious aspect of MRSA infections.[6]

Factors such as improper infection control practices, antibiotic pressure, previous hospitalization and host factors have been suggested to increase the risk of MRSA colonization and spread.[7, 8] Colonized healthcare personnel were previously suggested to act as a reservoir, vectors or victims within the transmission cycle of MRSA which may increase the spread of MRSA within the hospital and into community.[9,10] Taking in consideration the resistance pattern of the MRSA, ease of spread and the fatal outcomes of infections caused by MRSA,[9] it is necessary to identify the prevalence and antibiotic resistance pattern of MRSA in HCWs. Screening of asymptomatic HCWs has been recommended by some studies as an essential part of a multifactorial approach in order to protect the health personnel, patients and community, and to encourage the application of better infection prevention precautions.[9, 11]

The aim of this study is to assess the prevalence of MRSA among healthcare workers at Al-Karak Governmental Hospital in the south of Jordan and to study its antibiotic susceptibility pattern.

MATERIALS and METHODS

2.1 Study design, population and location

This cross-sectional study was conducted from March 2012 till June 2012. Results of previous study, conducted in Jordan, showed a prevalence of 5.8% positive MRSA amongst healthcare staff.[12] For our population of 580 with a precision of 2% (95% Confidence Interval specified limits 3.8% - 7.8%), the sample size required for this study was 276.[13]

Therefore, a total of two hundred seventy six nasal swabs were randomly collected from HCWs at Al-Karak hospital, the tertiary teaching hospital in south of Jordan. All workers included in the study were fulltime employees. Written informed consent was obtained from each participant before nasal specimen collection. The study proposal was reviewed and approved by the Scientific and Ethics Committee of Mu'tah University in Jordan.

2.2 Data collection

Using a questionnaire that was filled by all participants, data collected included age, sex, smoking history, education level, occupation (nurse and others; doctor, pharmacist, technicians and service employees), place of work within the hospital, years of healthcare work experience and antibiotic exposure over the last 3 months.

2.3 Nares cultures, bacterial strains and antimicrobial susceptibility testing

Sampling for each participant was performed by rotating a sterile cotton swab in the vestibule of both anterior nares as previously described by Wen-Tsung *et al*.[14] Cotton swabs were plated immediately on mannitol salt agar (MSA) (MSA; BBL Microbiology Systems, Becton Dickinson, Company, Sparks, MD, U.S.A.) The collected samples were transported within 4 hours at a temperature between 4-8℃ to the Microbiology Laboratory at Faculty of Medicine, Mu'tah University. Plates were incubated at 35 ± 1℃ and examined for growth after 24-48 hours. Each distinctive morphotype of mannitol-fermenting colony was selected from an MSA plate, subcultured to a nutrient agar (BBL Microbiology Systems, Becton Dickinson, Company, Sparks, MD, U.S.A.), and incubated at 37^{o}C in a humidified incubator.[15] Colonies growing on nutrient agar were identified as *S. aureus* by their typical colony morphology, Gram's staining, anaerobic utilization of glucose and mannitol, catalase production and tube

coagulase test. Screening for methicillin resistance was done using 30 µg/ml cefoxitin disk in Mueller-Hinton agar supplemented with NaCl (4% w/v; 0.68 mol/L) according to Clinical and Laboratory Standard Institute (CLSI) guidelines.[16] Antibiotic sensitivity tests were performed using Kirby Bauer's disc diffusion method according to performance standards of CLSI.[16] *S. aureus* ATCC 25923 was used as control strain. The panel of antibiotics tested included those that are recommended by CLSI or are commonly used locally in empirical treatment of *S. aureus* infections. Susceptibility testing was done and results were presented for the following antibiotics: Trimethoprim-sulfamethoxazole, ciprofloxacin, tetracycline, gentamicin, linezolid, mupirocin, fusidic acid, rifampicin, teicoplanin and vancomycin. For MRSA a multi-drug resistant (MDR) isolate was defined as those resistance to methicillin and to 3 other different antibiotics. All MRSA isolates were frozen at –70C^{o} for additional testing of organism characteristics.

2.4 Detection of *MecA*, *VanA* and *MupA* genes:

PCR assays were used to detect the carriage of *MecA, VanA and MupA* encoding high-level resistance to methicillin, vancomycin and mupirocin respectively. We used a novel primers, MI (5' CTT ACC AGT TGA ATT '3) and MII (5' TGG AGC ACT ATC CGA '3) for *MupA* gene amplification, *mecA1* (5'AAA TCG ATG GTA AAG GTT GGC 3') and *mecA2* (5' AGT TCT GCA GTA CCG GAT TTG C 3') for *MecA* gene amplification, *Forward* (5' ATG AAT AGA ATA AAA GTT GC 3') and *Reverse* (5' TCA CCC CTT TAA CGC TAA TA 3') for *VanA* amplification. Purification of total DNA and the amplification conditions were done as described by Aqel *et al*.[17] and Thati *et al*.[18] Briefly; isolates were grown overnight at 37 ºC on blood agar. Three to five colonies were suspended in 100 ml of sterile distilled water containing 1 mg of lysostaphin (Sigma, UK). This suspension was incubated at 37 ºC for 30 min and then heated

to 95 ºC for 5 min, 5µl from the solution was used for the PCR and then stored at –20 ºC. PCR's were run in a Techne Thermo Cycler (Bibby Scientific Limited, UK), under the following conditions, initial denaturation 95 ºC for 5 min.; 94 ºC for 1 min., 50 ºC for 2 min., 72 ºC for 3 min. repeated 30 times and final extension 72 ºC for 5 min. The efficiency of the amplification reactions was evaluated by electrophoresis on 2% agarose gel prepared in 0.5X TBE buffer and stained with ethidium bromide.

2.5 Statistical analysis

The statistical analysis was conducted using STATA10. For categorical variables, Chi Square test was used. For continuous variables that were normally distributed, T test was used to search for significant difference between the groups.

RESULTS:

Our results showed that a total of 24 healthcare workers (HCWs) were MRSA positive which represents 8.7% of the total 276 HCWs who were screened in this study. The positive and negative HCWs with nasal MRSA carriage according to different variables and statistical significance of each variable are shown in table 1. The only significant independent variables for nasal carriage of MRSA versus non carriage were nurse occupation compared to other occupations ($p = 0.007$), education level of less than university degree ($p = 0.039$) and mean number of years of experience ($P= 0.023$). There was no significant difference by the gender ($p = 0.94$), age ($p = 0.25$), antibiotic exposure ($p = 0.41$) and smoking ($p = 0.81$) with nasal carriage of MRSA amongst HCWs.

The job title and place of work of all MRSA positive HCWs are shown in table 2. Of all MRSA carriers, it was noticed that the highest MRSA carriage rate was among nurses and service employees with a total of 58% (14/24) and 33% (8/24) respectively. In terms of place of work, the highest MRSA carriage rate was within the intensive care unit with a total of 17% (4/24).

In figure 1, of all MRSA samples isolated

from HCWs, 100% (24/24), 37.5% (9/24) and 12.5% (3/24) were resistant to cefoxitin, trimethoprim-sulfamethoxazole and tetracycline respectively. No multidrug resistant MRSA was identified. However, there was only one MRSA isolate (4.1%) that showed double resistance to both trimethoprim-sulfamethoxazole and tetracycline. This sample was from a nurse in the sterilization unit. No resistance was encountered for glycopeptides (vancomycin or teicoplanin), linezolid, mupirocin, rifampicin, ciprofloxacin, gentamicin or fusidic acid among all MRSA isolates. For fusidic acid, the required diameter for the used 10µg disc susceptibility and resistance were >22mm and < 22mm, respectively according to the European Committee on Antimicrobial Susceptibility Testing.[19] *MecA* gene was detected in all MRSA isolates. No PCR product with the *VanA* and *MupA* primers was found with plasmid and genomic DNA extracted from all MRSA isolates.

Table 1: Potential variables affecting nasal MRSA carriage amongst HCWs

Variable	Nasal MRSA Carriage		P value
	Positive	Negative	
	no	no	
Gender			
Male	10	107	0.94
Female	14	145	
Age (Year)			
<25	1	30	0.25
25-50	23	222	
Occupation			
Nurse	14	78	0.007
Others[1]	10	174	
Education			
School / College (2 years degree)	7/12 (19)	73/72 (145)	0.039
University (≥ 4 years degree)	5	107	
Mean work experience in years*	12.7	8.97	0.023
Antibiotic exposure over the last 3 months			
Yes	10	84	0.41
No	14	168	
Smoking			
Yes	4	47	0.81
No	20	205	

[1] **Others:** Other professions including doctor, pharmacist, technicians and service employees
Significant P value < 0.05

Table 2: Distribution of MRSA positive HCWs by their Job title and place of work

Job title	no	Place of work	no
Nurse	**14**		
		ICU*	2
		Outpatient clinics	2
		Physiotherapy unit	2
		Theatre	1
		NICU**	1
		Sterilization unit	1
		Emergency department	1
		Endoscopy	1
		Pediatric ward	1
		Infection control unit	1
		Radiology	1
Others	**10**		
Service	8		
		Refectory	2
		ICU cleaners	2
		General Wards cleaners	2
		Driver	1
		Manager	1
Physician	1	Radiology	1
Writer	1	Filing	1
Total	**24**	**Total**	**24**

***ICU: Intensive care unit, NICU** ** Neonatal Intensive Care Unit**

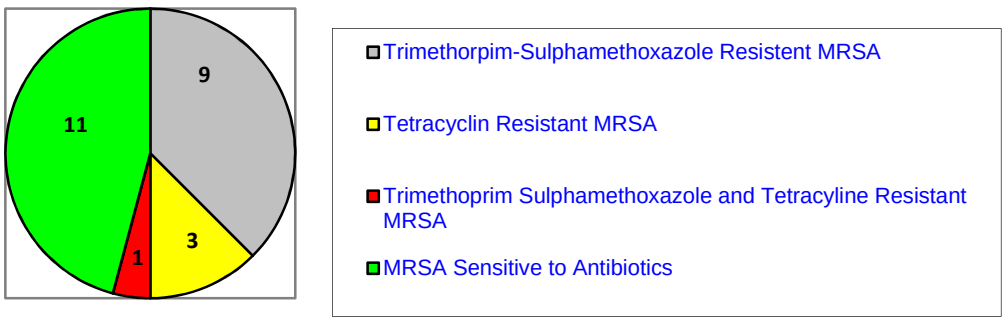

Figure 1: Antibiogram of MRSA Isolates (n=24)

DISCUSSION

HCWs are on the interface between community and patients. This might put them at increasing risk of being carriers or victims of cross transm ission of MRSA.[9] Therefore, periodical and pre-employment screening of HCWs has been previously recommended.[11, 20, 21] Screening of HCWs for nasal MRSA carriage in the current study, which was carried out in the south of Jordan at Al-Karak governmental hospital, has shown a carriage rate of 8.7%. In a previous study carried out by Na'was and Fakhory in north of Jordan in 1991, the MRSA nasal carriage amongst HCWs was 5.8%.[12] The higher rate found in the current study could be due to factors such as difference in the study population and their exposure to the organism, geographical area and sampling technique. In a study by Abed El-Jalil *etal*,[22] the author screened nursing students who were having training sessions in the hospital and considered as the hospital-exposed study group. The nasal carriage rate in this group was 3.5%. However, nursing students would not likely had the same long hospital exposure as in the case of proficient HCWs of the current study who work regularly in a hospital based environment. The average worldwide MRSA carriage in health care workers was found to be 4.6% in 127 studies with an average of 6.1% in the Middle East region where our study was carried out.[9] Higher rate in our study might be explained by factors previously mentioned such as difference in study population, geographical areas, years of experience for HCWs and implication of infection prevention practices. However, 8.7% MRSA carriage rate among HCW found in the current study is still within the worldwide range of 1.6-15.5% found in different studies in the literature.[9]

Nasal carriage amongst HCWs in this study was significantly higher in workers

with high number of years of hospital work experience. This would have been expected as the exposure to hospital environment is a known risk factor to MRSA carriage.[23, 24] Additionally, nurse occupation in the current study was significantly associated with higher rate for MRSA nasal carriage than other occupations which was similar to the results found by Na'was and Fakhory.[12] This might be explained by the fact that nurses usually spend longer time with patients in comparison to other occupations as required by the nature of their job. On the other hand, education level of less than a university degree was also significantly associated MRSA nasal carriage which could be due to lack of proper awareness of MRSA and application of infection prevention measures such as hand washing. Therefore, it is important to increase the awareness of MRSA methods of transmissions and emphasize on proper infection prevention education for all hospital personnel and in particular the nurses and HCWs with long experience.

Based on the location of work within the hospital, the highest rate of MRSA nasal carriage was 17% in HCWs of the six-bedded Intensive Care Unit in the hospital. Such a trend could be related to the fact that patients in the ICU are usually having multiple co-morbidities with higher likelihood of being MRSA carriers [25] and as a consequence, transmission to HCWs was higher than in other hospital places. However, larger sample of HCWs from the ICU is required to prove the significance of this trend and that was difficult to carry out in this study because of the small six-bedded ICU in the hospital and the low number of HCWs based in the ICU. Nevertheless, strict infection prevention precautions must be followed strictly in hospital in general and in the ICU unit in particular. Our results, also, showed that 42% of all MRSA positive HCWs have

received antibiotics in the last 3 months with 60% of them were given a broad spectrum antibiotic. Antibiotic exposure is expected to increase the risk of MRSA carriage.[23] However, antibiotic exposure was not statistically significant in terms of increasing the risk of MRSA nasal carriage in our study. This might be related to low sample size of those exposed to antibiotics when compared to non exposed sample. Still, it is important to have proper antibiotic guidelines that must be followed strictly within the hospital. No significant risk was associated with age, sex and smoking.

Resistance pattern in some isolates was observed only for trimethoprim-sulfamethoxazole and tetracycline. No multidrug resistant isolates were found. A total of 37.5% and 12.5% of HCWs samples were resistant to trimethoprim-sulfamethoxazole and tetracycline respectively. Resistance to trimethoprim-sulfamethoxazole is within the range found in previous studies.[22, 24, 26] On the other hand, our finding tetracycline resistance has never been detected in previous studies on clinical isolates in Jordan,[22, 26] but is still lower than resistance range values found in other studies in different countries.[27,28] No resistance was encountered for the remaining antibiotics in particular, glycopeptides, Mupirocin and Linezolid which could be due to many reasons but mainly the strict control on prescribing these antibiotics when available.

Finally, periodical screening of HCWs in hospital settings is strongly recommended. Where limited resources can be a hindrance in applying such a policy; screening HCWs can be restricted to specific hospital areas such as ICU, surgical and orthopedic wards. Screening of HCWs should be more cost effective than treating MRSA epidemic infections or before MRSA become endemic in healthcare setting.[9] Proper policies should also be applied for HCWs with positive MRSA as previously suggested,[9, 29] and

should include education of HCWs on infection precaution and hand hygiene together with either immediate decolonization, decolonization if no spontaneous clearance after six months or mobilizing HCWs to less critical areas.

Acknowledgment:

We would like to acknowledge the scientific research committee at Mu'tah university/Jordan for financial support of this work. We would also like to acknowledge Ahmad Khazar Zayed Makableh, 4th year medical student for his help in part of the laboratory work of the current study.

REFERENCES

1. Jevons M. Celbenin-resistant *staphylococci*. BMJ. 1961;i:124-5.
2. Report of a combined working party of the Hospital Infection Society and British Society for Antimicrobial Chemotherapy: Guidelines for the control of epidemic methicillin-resistant *Staphylococcus* aureus. J Hosp Infect. 1986;7:193–201.
3. Monecke S, Coombs G, Shore A, Coleman D, Akpaka P. A field guide to pandemic, epidemic and sporadic clones of methicillin-resistant *Staphylococcus* aureus. PLoS One. 2011;6(4):e17936.
4. Pinho M, de Lencastre H, Tomasz A. An acquired and a native penicillin-binding protein cooperate in building the cell wall of drug-resistant *staphylococci*. Proc Natl Acad Sci. 2001;98:10886–91.
5. Chambers HF. Methicillin resistance in *staphylococci*: molecular and biochemical basis and clinical implications. Clin Microbiol Rev. 1997;10:781–91.
6. Gosbell I. Epidemiology, clinical features and management of infections due to community methicillinresistant *staphylococcus* aureus (cMRSA). Intern Med J. 2005;35(S2):S120-S35.
7. Herwaldt L. Control of methicillin-resistant *Staphylococcus* aureus in the hospital setting Am J Med. 1999;106:11s-8s.
8. Monnet D. Methicillin resistant *Staphylococcus* aureus and its relationship to antimicrobial use:possible implications for control. Infect Control Hosp Epidemiol. 1998;19:552-9.
9. Albrich WC, Harbarth S. Health-care workers: source, vector, or victim of MRSA? Lancet Infect Dis. 2008;8:289-301.
10. Sax H, Allegranzi B, Uçkay I, Larson E, Boyce J, Pittet D. "My five moments for hand hygiene": a user-centred design approach to understand, train, monitor and report hand hygiene. J Hosp Infect. 2007;67: 9–21.
11. Stein M, Navon-Venezia S, Chmelnitsky I, Kohelet D, Schwartz O, Agmon O. An outbreak of new, nonmultidrug-resistant, methicillin-resistant *Staphylococcus* aureus strain (sccmec type iiia variant-1) in the neonatal intensive care unit transmitted by a staff member. Pediatr Infect Dis J. 2006;25:557–9.
12. Na'was T, Fakhoury J. Nasal carriage of methicillin-resistant *Staphylococcus* aureus by hospital staff in north Jordan. J Hosp Infect. 1991;17:223-9.
13. Daniel W. Biostatistics: A foundation for Analysis in the Health Sciences. 7th ed. New York: John Wiley & Sons; 1999. P. 156.

14. Wen-Tsung L, Wei-Jen L, Min-Hua T, Sheng-Ru W, Chih-Chien W. Dissemination of Methicillin-resistant *Staphylococcus* aureus among Healthy Children in Northern Taiwan. J Med Sci. 2010;30(2): 47-53.
15. 1Pathak A, Marothi Y, Iyer R, Singh B, Sharma M, Eriksson B, et al. Nasal carriage and antimicrobial susceptibility of *Staphylococcus* aureus in healthy preschool children in Ujjain, India. BMC pediatr. 2010;10 (100):1-8.
16. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. CLSI Document 2007;27(1):100-17.
17. Aqel A, Ibrahim A, Shehabi A. Rare occurrence of Mupirocin resistance among clinical *Staphylococcus* isolates in Jordan. Acta Microbiol Immunol Hung. 2012;59(2):239-47.
18. Thati V, Shivannavar C, Gaddad S. Vancomycin resistance among methicillin resistant *Staphylococcus* aureus isolates from intensive care units of tertiary care hospitals in Hyderabad. Indian J Med Res. 2011;134(5):704-8.
19. The European Committee on Antimicrobial Susceptibility Testing (EUCAST):Breakpoint tables for interpretations of MICs and zone diameters.Version 1.1, April 2010.
20. Jones L, Baggott J. Control and elimination of an MRSA outbreak. Med J Aust. 1982;1:480.
21. Boyce J, Cookson B, Christiansen K. Meticillin-resistant *Staphylococcus* aureus. Lancet Infect Dis. 2005;5:653-63.
22. Abed El- Jalil H, Jallad M, Thwaini AJ. Nasal carriage of methicillin resistant *Staphylococcus* aureus in individuals exposed and not exposed to hospital environments. European Journal of Scientific Research. 2008;22(4):570-4.
23. Hidron A, Kourbatova E, Sue Halvosa J, Terrell B, McDougal L, Tenover F, et al. Risk factors for colonization with methicillin-resistant *Staphylococcus* aureus (MRSA) in patients admitted to an urban hospital: emergence of community-associated MRSA nasal carriage. Clin Infect Dis. 2005;41:159–66.
24. Azeez-Akande O. Global trend of methicillin-resistant *Staphlococcus* aureus and emerging challenges for control. African Journal of Clinical and Experimental Microbiology. 2010;11(3):150-8.
25. Huang S, Rifas-Shiman S, Warren D. Improving methicillin-resistant *Staphylococcus* aureus surveillance and reporting in intensive care units. Lancet Infect Dis. 2007;195:330-8.
26. Alzoubi K, Hayajneh W, Ayoub A, Al-Safi S, Azzam S, Mhaidat N. Prevalence of methicillin-resistant *Staphylococcus* aureus (MRSA) at a tertiary hospital in North Jordan. Jordan Journal of Pharmaceutical Sciences. 2010;3(1):37-43.
27. Askarian M, Zeinalzadeh A, Japoni A, Alborzi A, Memish Z. Prevalence of nasal carriage of methicillin-resistant *Staphylococcus* aureus and its antibiotic susceptibility pattern in healthcare workers at Namazi Hospital, Shiraz, Iran. Int J Infect Dis. 2009;13:e241-e7.
28. Akhtar N. *Staphylococcal* nasal carriage of health care workers. J Coll Physicians Surg Pakistan. 2010;20(7):439-43.
29. Eveillard M, Martin Y, Hidri N, Boussougant Y, Joly-Guillou M. Carriage of methicillin-resistant *Staphylococcus* aureus among hospital employees: prevalence, duration, and transmission to households. Infect Control Hosp Epidemiol. 2004; 25:114-20.