Carriage of *Staphylococcus aureus* among Hajj pilgrims

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ABSTRACT

Objective: To evaluate the prevalence of methicillin resistant *Staphylococcus aureus (*MRSA) carriage among a cohort of pilgrims during 2004 Hajj season.

Methods: Pilgrims attending the 2004 Hajj season were recruited and screened for carriage of MRSA. Standard microbiological techniques were used to screen for the presence of MRSA.

Results: Out of 411 individuals screened, 85 (20.6%) were positive for *Staphylococcus aureus (S. aureus)* of which only 6 (1.46 %) were MRSA. Four individuals (4.6%) had the *S. aureus* organism in both nasal and axillary swabs, while 7 individuals (8%) had the organism in their axillae only. The other 74 individuals (87.1%) had the organism in their nares only. The 6 MRSA isolates were positive for the mecA gene by polymerase chain reaction method. None of the pilgrims examined had any risk factors for community-acquired methicillin resistant *S. aureus* (CAMRSA). Overall, the prevalence of MRSA in the population of pilgrims examined was found to be low (1.46%) in comparison with most community based studies.

Conclusion: A low rate of MRSA carriage was noticed among the screened cohort. Physicians treating patients suspected of *S. aureus* infection during the Hajj pilgrimage should bear in mind the possibility of community acquired - MRSA and should obtain appropriate samples for bacterial cultures and susceptibility testing so that antimicrobial agents could be introduced when necessary at a later stage.

Saudi Med J 2006; Vol. 27 (9): 1367-1372

Methicillin-resistant *Staphylococcus aureus* (MRSA) is now recognized as a serious and common cause of nosocomial and community acquired infections worldwide.[1-4] Infected persons may be simply colonized or develop infections ranging from wound, skin and soft tissue infections to severe infections such as bacteremia, septicemia, and necrotizing pneumonia. The virulence of community-acquired MRSA (CAMRSA) depends on their possession of 5 genes Panton-Valentine Leukocidin (PVL) which mediate the cytolytic toxin and seb, sec, seh and sek genes (*Staphylococcal* enterotoxins), which mediate enterotoxin production.[5] The CAMRSA isolates frequently share some genetic characteristics, including the SCCmec type IV and lukS-lukF genes.[6]

The epidemiology of MRSA in the Kingdom of Saudi Arabia (KSA) has varied from very low to high rates in different areas of the Kingdom. Madani et al[7] in 1998 reported that 222 (33%) of 673 *Staphylococcus*

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Received 14th February 2006. Accepted for publication in final form 11th June 2006.

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aureus (S. aureus) isolates collected from 2 tertiary health care centers in Jeddah were MRSA. In Abha, in the south, it was reported that MRSA was isolated from 5.1% of non-hospital patients and 18.3% of hospital patients, while 61% of *S. aureus* isolates from infected patients were MRSA.[8] However, it was not indicated if any of these subjects had CAMRSA disease or had risk factors for CAMRSA. Some reports have suggested that serious MRSA infections can be acquired in the community in rural as well as urban locations and that CAMRSA might have replaced the community acquired methicillin sensitive *Staphylococcus aureus* (MSSA) as the dominant strain in some communities.[9,10] An increase in the number of patients colonized or infected by MRSA who were transferred from the medical facilities in Makkah City to Jeddah hospitals prompted this study. Makkah city receives approximately 2 million pilgrims yearly from all over the world, who come to perform the Hajj religious rituals. It is uncertain at present, the contribution of the pilgrims to the epidemiology of MRSA in Makkah area.

The purpose of this study was to determine the rate of MRSA colonization among Hajj pilgrims in Makkah, Saudi Arabia during the 2004 Hajj pilgrimage, identify demographic and clinical variables associated with CAMRSA and their possible relationship to CAMRSA epidemiology in KSA.

Methods. All Hajj pilgrims visiting the National Guard Health Affairs health facility in Mina, KSA for medical reasons were invited to participate in the study after verbal consent. The Mina Health care facility includes a triage area, male and female outpatient clinics including dental and ophthalmology and a 21-bed inpatient area designed for minor and moderate trauma. All critically ill patients are transferred to a nearby tertiary care facility. A prepared questionnaire was completed with respect to each participant, which included demographic data, risk factors and type of specimens collected: nasal, axilla, groins and open wounds if present. Patients on antibiotic therapy as well as those who have consumed antibiotics 10 days previous to the study were excluded. For obtaining nasal swabs a sterile pre-moistened swab with sterile water was inserted into both nares and rotated 3 times. A 2nd pre-moistened swab with sterile water was used to swab both axilla, a 3rd was done for both groins and a 4th for wounds if present. All specimens were kept in the Stuart's transport medium at room temperature and transported to the Laboratory at King Khalid National Guard Hospital in Jeddah, within 12 hours of collection. In the Laboratory, the swabs were plated on 5% Sheep blood agar and mannitol salt agar with Methicillin susceptibility disc (5 ug) on the surface of the agar and incubated at 37°C in ambient air for 48 hours. After incubation, mannitol fermenting colonies (yellow) resembling *S. aureus* were subcultured on to Mueller Hinton agar with cefoxitin disk (30 ug) and other gram positive susceptibility panel. *Staphylococcus* latex test was performed on all *Staphylococci* isolated. The MRSA was confirmed by typical gram stain morphology, catalase test, Staph aurex test, and DNase tests and resistance to methicillin and cefoxitin. The *S. aureus* with cefoxitin zones of ≤19 mm is reported as oxacillin resistant (National Committee for Clinical Laboratory Standards for antibiotic susceptibility was followed). Further, confirmations of the isolates were carried out using the Microscan (Microscan Walkaway 40 SI. Dade Behring Inc. 1584 Enterprise Blvd., West Sacramento, CA 95691,USA.).

Molecular Biological technique. After boiling the *S. aureus* strains in phosphate-buffered saline for 20 minutes, bacterial DNA was obtained from the supernatant after centrifugation. All the DNA extracts were tested by a molecular biological assay using a commercial In-vitro Diagnostic Kit (Roche Diagnostics, GmbH, Roche Applied Science, Sandhofer Strasse 110, D-68305 Mannheim, Germany). This kit detects the mecA gene using the Light Cycler system and is designated for known *S. aureus* isolates. All assays were performed according to manufacturer's instructions. The Roche kit contains positive and negative internal controls for reliable interpretation of the results. Known MSSA isolates and mecA positive MRSA isolates were used as negative and positive controls.

Results. Out of 411 individuals screened, complete demographic information was available on 361 pilgrims, **Figure 1**. Out of 411 individuals screened 85 (20.7%) were positive for *S. aureus*.Four individuals (4.7%) had the organism in both nasal and axillary swabs while 7 individuals (8.2%) had the organism in their axilla only. The other 74 subjects (87.1%) had the organism in their nares only. *Staphylococcus aureus* was not isolated from any site samples in 326 pilgrims. Out of the 85 subjects with *S. aureus* colonization, only 6 (7.1%) had MRSA in their nares. The overall prevalence of MRSA among the pilgrims was 1.46% (6/411). The 6 isolates were positive for the mecA gene by polymerase chain reaction (PCR) and have been preserved at -80°C for testing for *Staphylococcal* Casette Chromosome type I - IV and the pri genes encoding PVL lukS-PV and lukF-PV genes to determine if they are typical CAMRSA of the same lineage or hospital related MRSA.

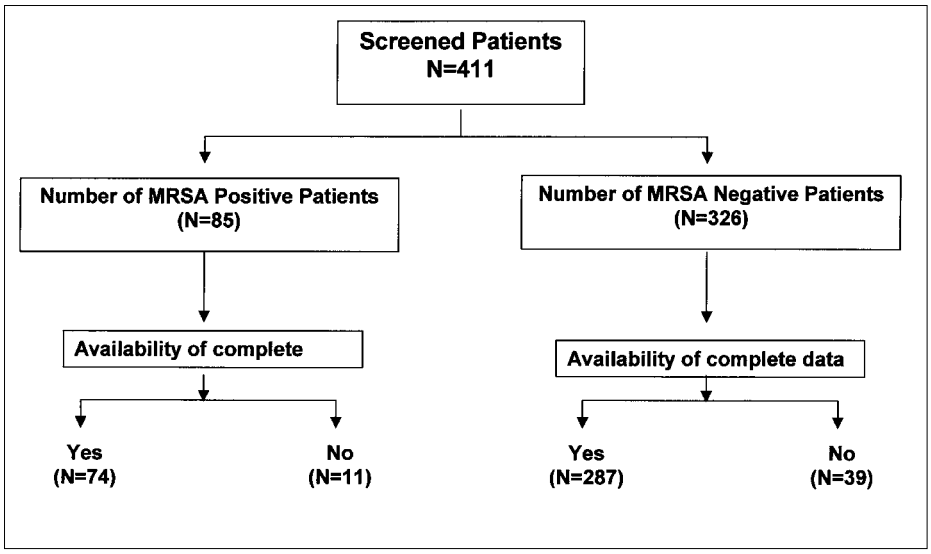

Figure 1 - Number of Hajj pilgrims screened and availability of complete data.

Among the pilgrims found positive with *S. aureus*, there were 71 (85.5%) males and 12 (14.5%) females, **Table 1**. The ages of the subjects ranged from 1 to over 50 years, but the majority was between 20 and 50 years. Forty-six (54.1%) of those colonized by *S. aureus* were aged between 20 and 40 years. Out of the 411 subjects screened, 191 were Saudis, 66 were Egyptians and the rest were Asians and Middle Easterners. Three hundred and twenty (77.9%) of the pilgrims were residing in Saudi Arabia before going on the pilgrimage, **Table 1**. Three (3.5%) of the 85 subjects harboring *S. aureus*, were hospitalized 3 months before going on the Hajj pilgrimage, while none of them worked in a hospital. Seven (8.2%) had taken antibiotics prior to going on the pilgrimage and 15 had some underlying medical conditions listed in **Table 2**. Throughout the period of the study, we did not encounter any patient with disease or clinical syndromes caused by CAMRSA. The 21 inpatients involved in our study were victims of heat stroke and minor trauma.

All the *S. aureus* strains gave positive DNase, catalase and coagulase tests. Both the screening with methicillin 5 ug disc and cefoxitin 30 ug disc gave identical results in identifying MRSA. All the identified MRSA strains possessed the mecA gene by PCR (**Table 3**).

Discussion. Methicillin resistant *S. aureus* is no longer acquired in hospitals only, but there are increasing reports of its acquisition from the community (CAMRSA). Methicillin resistant *Staphylococcus aureus* 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.[11]

Methicillin resistant *Staphylococcus aureus* infection has emerged in normal patients who do not possess the recognized risk factors for MRSA infections.[12,13] However, the clinical implications of this are not yet clear especially in the Kingdom where there have been very few studies on the CAMRSA infections or colonization. In the USA, where surveillance studies have been carried out on CAMRSA, infections have increased significantly between 1990 and 2000.[12-14] The CAMRSA have been reported to be genetically distinct from the strains prevalent in healthcare facilities and can cause infections in young persons with no risk factors. It has been documented that CAMRSA typically possessed different exotoxin gene profiles (such as, PVL genes), which is a cytolytic toxin, compared with health care-associated isolates, as well as an enterotoxin (*Staphylococcal* enterotoxin (sec).[5,15] None of the subjects in the study had any skin lesions or disease syndromes suggestive of CAMRSA at the time of culture.

The only study in the Kingdom on CAMRSA was carried out in Al-Khobar in the Eastern province, the number of patients with CAMRSA disease increased from a single patient in 1998 to 15 in the year 2000 and the percentage of CAMRSA/ total number of MRSA increased from 5-33%.[16] Up till recently, MRSA infections have been acquired primarily

Table 1 - Demographic data of Hajj surveillance 1425H (2004).

Characteristics	Result of culture				Total number of pilgrims cultured		*P*-value
	Positive		Negative				
	N	(%)	N	(%)	N	(%)	
Gender							
Male	71	(85.5)	292	(92.7)	363	(91.2)	0.041
Female	12	(14.5)	23	(7.3)	35	(8.8)	
Age group (in years)							
0-19	9	(11.7)	19	(6.2)	28	(7.3)	
20-29	23	(29.9)	70	(23)	93	(24.3)	
30-39	23	(29.9)	98	(32.1)	121	(31.7)	
40-49*	10	(13)	72	(23.6)	82	(21.5)	0.04
50+	12	(15.6)	46	(15.1)	58	(15.2)	
Nationality							
Saudi*	48	(59.3)	143	(46.1)	191	(48.8)	0.03
Egyptian	14	(17.3)	52	(16.8)	66	(16.9)	
Pakistani	5	(6.2)	13	(4.2)	18	(4.6)	
Yemeni	3	(3.7)	9	(2.9)	12	(3.1)	
Sudanees*	2	(2.5)	32	(10.4)	34	(8.7)	0.01
Indian	2	(2.5)	6	(1.9)	8	(2)	
Chadi	2	(2.5)	1	(0.3)	3	(0.8)	
Others	5	(6.2)	54	(17.5)	59	(15.1)	
Country of residence							
Saudia	70	(88.6)	250	(83.1)	320	(84.2)	
Egypt	3	(3.8)	12	(4)	15	(3.9)	
Others	6	(7.6)	39	(13)	45	(11.9)	
Source							
Inpatient*	21	(24.7)	52	(16)	73	(17.8)	0.03
Outpatient	64	(75.3)	272	(84)	336	(82.2)	
Hospitalization within 3 months							
Yes	3	(3.5)	3	(0.9)	6	(1.5)	
No	82	(96.5)	322	(99.1)	404	(98.5)	

*P-value <0.05

in the healthcare setting. There are several reports indicating that serious MRSA infections can be acquired in the community in rural as well as in urban locations.[5,6,10,12]

Our study suggests that MRSA is not circulating to a great extent in our pilgrim population, as the prevalence in our study population is extremely low (1.46%; 6/411). It is possible that since a large number of pilgrims receive prophylactic ciprofloxacin before leaving home or at the point of entry into the Kingdom, colonization with MRSA was limited in our study population. Carriage of MRSA has been identified in only a small proportion of the pilgrims (1.46%). There is a strong possibility that due to the crowded living conditions and hygiene difficulties during the Hajj pilgrimage, transmission of MRSA from colonized individuals to other pilgrims may occur. However, with transmissibility, the carriage can accelerate and spread to the community when the colonized pilgrims return to their homes and local communities, and probably leading to the increase of CAMRSA infections or outbreaks in the Kingdom in the future. This fear is supported by the reports of several outbreaks of CAMRSA in the last 8 years occurring in situations where human live in close proximity.[5,17]

The CAMRSA infections in the absence of identified risk factors have been reported by a number of studies.[18] Furthermore, the clinical syndromes associated with MRSA in children without identified risk factors were similar to those associated with community-acquired MSSA.[18]

The rate of prevalence of CAMRSA in people without risk factors who live in a community is on the rise, at least according to reports from the USA.[15,17,19] Thus, confirming the transition of MRSA

Table 2 - The 85 *Staphyloccus aureus* postive cases distributed according to risk factors during Hajj 1425 (2004).

Risk factors	Result of cultrure				Total no. of pilgrims	
	Positive		Negative			
	N	(%)	N	(%)	N	(%)
Hospitalization within 3 months						
Yes	3	(3.5)	3	(0.9)	6	1.5
No	82	(96.5)	322	(99.1)	404	98.5
Working in a hospital						
Yes	0	(0)	2	(0.6)	2	0.5
No	85	(100)	323	(99.4)	408	99.5
More than 1 household member with any of the above risk factors						
Yes	4	(4.7)	14	(4.1)	18	4.2
No	81	(95.3)	311	(25.4)	392	40.1
Recent antibiotic use						
Yes	7	(8.2)	20	(6.3)	27	6.7
No	78	(91.8)	305	(93.7)	383	93.3
Underlying conditions	15	(17.6)	46	(13.2)	61	14.8
Hypertension	7	(8.2)	27	(8.3)	34	8.3
Asthma	5	(5.9)	12	(3.7)	17	4.1
Diabetes	5	(5.9)	15	(4.6)	20	4.9
Steroid usage	2	(2.4)	4	(1.2)	6	1.5
Renal failure	1	(1.2)	0	(0)	1	.2
Liver disease	1	(1.2)	3	(0.9)	4	1
Immunodeficiency	1	(1.2)	0	(0)	1	.2
Cardiac disease	0	(0)	5	(1.5)	5	1.2
Malignancy	0	(0)	0	(0)	0	0

Table 3 - The 85 *Staphylococcus* aureus positive cases distributed according to type of test.

Test	N	(%)
Catalase		
Positive	85	(100)
Negative	0	(0)
Coagulase test		
Positive	85	(100)
Negative	0	(0)
Deoxyribonuclease test		
Positive	85	(100)
Negative	0	(0)
MET5		
Sensitive	79	(92.9)
Resistant	6	(7.1)
FOX30		
Sensitive	79	(92.9)
Resistant	6	(7.1)
MecA gene		
Positive	6	(7.1)
Negative	79	(92.9)

MET 5 - Methicillin 5 ug ; FOX 30 - Cefoxitin 30 ug.

from a hospital pathogen to a community-associated emerging disease. This therefore, calls for vigilance and yearly monitoring during the Hajj pilgrimage for this potentially dangerous pathogen, and to prevent the acquisition of this pathogen as much as possible during Hajj pilgrimage. Molecular analysis of the 6 isolates of CAMRSA from the pilgrims will be desirable for epidemiological reasons. It will be interesting if they currently belong to any of the 5 SCCmec types known example, types I – V and to see whether they are related to those found in North America. For now, based on the findings of this small study, surveillance of pilgrims should be continued and every attempt must be made to screen a larger number of pilgrims for CAMRSA colonization or disease, since the Hajj pilgrimage offers an excellent milieu of overcrowding and close proximity of persons, which will foster the spread and acquisition of CAMRSA.

It is the policy of the Ministry of Health of the Kingdom that all intending pilgrims should be given 500 mg ciprofloxacin capsules for prophylaxis against meningococcal meningitis at the point of entry into the Kingdom or before leaving home if they are residents of the Kingdom. This policy needs to be reviewed in view of the present findings. The contribution of prophylactic ciprofloxacin to the sequential selection of CAMRSA among Hajj pilgrims is presently unknown and therefore, yearly screening of pilgrims will need to be undertaken, including in particularly those with skin and soft tissues infections, to determine the prevalence of CAMRSA among pilgrims.

Physicians treating patients suspected of *S. aureus* infection during the Hajj pilgrimage should bear in mind the possibility of CAMRSA and should obtain appropriate material for bacterial cultures and susceptibility testing so that appropriate antimicrobial agent could be instituted, when necessary at a later date.

References

1. Fang H, Hedin G. Rapid Screening and identification of methicillin-resistant *Staphylococcus aureus* from clinical samples by selective-broth and real-time PCR Assay. ***J Clin Microbiol*** 2003; 41: 2894–2899.
2. Levi K, Bailey C, Bennett A, Marsh P, Cardy DL N, et al. Evaluation of isothermal signal amplification method for rapid detection of Methicillin-Resistant *Staphylococcus aureus* from patient screening swabs. ***J Clin Microbiol*** 2003; 41: 3187–3191.
3. Grisold AJ, Leitner E, Muhlbauer G, Marth E, Kessler HH. Detection of methicillin-resistant *Staphylococcus aureus* and simultaneous confirmation by automated nucleic acid extraction and real-time PCR. ***J Clin Microbiol*** 2002; 40: 2392–2397.
4. Buhairie HA, Abdelhadi MS. The epidemiology of methicillin-resistant *Staphylococcus aureus* at a Saudi University Hospital. ***Microb Drug Resist*** 2001; 7: 413–416.
5. Shukla SK. Community-associated methicillin-resistant *Staphylococcus aureus* and its emerging virulence. ***Clin Med Res*** 2005; 3: 57-60.
6. Ribeiro A, Dias C, Silva-Carvalho MC, Berquo L, Ferreira FA, Santos RN, et al. First report of infection with community –acquired methicillin-resistant *Staphylococcus aureus* in South America. ***J Clin Microbiol*** 2005; 43: 1985–1988.
7. Madani TA, Al-Abdullah NA, Al-Sanousi AA, Ghabrah TM, Afandi SZ, Bajunaid HA. Methicillin–resistant *Staphylococcus aureus* in two tertiary-care centers in Jeddah, Saudi Arabia. ***Infect Control Hosp Epidemiol*** 2001; 22: 211–216.
8. Alghaithy AA, Bilal NE, Gedebou M, Weily AH. Nasal carriage and antibiotic resistance of *Staphylococcus aureus* isolates from hospital and non-hospital personnel in Abha, Saudi Arabia. ***Trans R Soc Trop Med Hyg*** 2000; 94: 104-507.
9. Groom AV, Wolsey DH, Naimi TS, Smith K, Johnson S, Boxrud D, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in a rural American Indian community. ***JAMA*** 2001; 286: 1201-1205.
10. Charlebois ED, Perdreau-Remington F, Kreiswirth B, Bangsberg DR, Ciccarone D, Diep BA, et al. Origins of community strains of methicillin-resistant *Staphylococcus aureus*. ***Clin Infect Dis*** 2004; 39: 47-54.
11. Salmenlinna S. Lyytikainen O, Vuopio-Varkila J. Community-acquired methicillin-resistant *Staphylococcus aureus*, Finland. ***Emerg Infect Dis*** 2002; 8: 620-627.
12. Fridkin SK, Hageman JC, Morrison M, Sanza LT, Como-Sabetti K, Jergan JA, et al. Active bacterial core surveillance program of the emerging infections program network. Methicillin-resistant *Staphylococcus aureus* disease in three communities. ***N Engl J Med*** 2005; 352: 1436-1444.
13. Fergie JE, Purcell K. Community–acquired methicillin-resistant *Staphylococcus aureus* infections in South Texas. ***Pediatr Infect Dis J*** 2001; 20: 860-863.
14. Chambers HF. Community -Associated MRSA-resistance and virulence converge. ***N Engl J Med*** 2005; 352: 1485–1487.
15. Naimi TS, LeDell KH, Como-Sabetti K, Borchardt SM, Boxrud DJ, Etiene J, et al. Comparison of community- and health care-associated methicillin-resistant *Staphylococcus aureus* infection. ***JAMA*** 2003; 290: 2976–2984.
16. Bukharie HA, Abdelhadi MS, Saeed IA, Rubaish AM, Larbi EB. Emergence of methicillin-resistant *Staphylococcus aureus* as a community pathogen. ***Diagn Microbiol Infect Dis*** 2001; 40; 1-4.
17. Eguia JM, Chambers HF. Community –acquired methicillin-resistant *Staphylococcus aureus* : epidemiology and potential virulence factors. ***Curr Infect Dis Rep*** 2003; 5: 459-466.
18. Herold BC, Immergluck LC, Maranan MC, Lauderdale DS, Gaskin RE, Boyle-Vavra S, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. ***JAMA*** 1998; 279: 593–598.
19. Frazee BW, Lynn J, Charlebois ED, Lambert L, Lowery D, Perdreau-Remington F. High prevalence of methicillin-resistant *Staphylococcus aureus* in emergency department skin and soft tissue infections. ***Ann Emerg Med*** 2005; 45: 311–320.