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Nasal Carriage of Methicillin Resistant *Staphylococcus aureus* among ICU Patients in Al Quwayiyah General Hospital, Ryadh, KSA

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A B S T R A C T

The treatment of methicillin resistant *Staphylococcus aureus* (MRSA) infection is challenging because of their high resistance to different antibiotics. The objectives of this study were to detect the prevelance of *S. aureus* and MRSA carriage in ICU patients in Al Quwayiyah General Hospital and to evaluate the performance of MRSA-Select agar media for rapid screening of MRSA Furthermore, to describe the antibiotic susceptibility patterns of the *S. aureus* and MRSA isolates. During the period from January to July 2018, a total of 278 patients admitted to the ICUs were screened for MRSA. Culture of specimens was done on, MRSA-Select agar plate, Oxacillin Screen Agar™ BD (Mueller-Hinton oxacillin agar screen plate containing4%Nacl and oxacillin 6mg/ml) and blood agar. Suspected colonies of *S. aureus* were identified as by Gram stain, catalase, DNase, mannitol fermentation, and coagulase positivity or by Vitek2 ID-GP card. All strains were tested with Oxacillin disc diffusion method, Cefoxitin disc diffusion method, Latex agglutination test for detection of PBP2a and Susceptibility testing of oxacillin-susceptible *mecA*-positive isolates by the Vitek 2. The study showed that 56 (20.14%) isolates were MRSA, 45(16.18%) were MSSA, 164(58.99%) were CONS, 4 (1.44%) were *Strept viridians*, 2(0.71%) were gram negative bacteria, 3 (1.07%) were *Corynebacterium* spp., while 3 (1.07%) showed no growth. Antimicrobial susceptibility results for the MSSA and MRSA isolates showed that all MRSA isolates were totally resistant to β Ampicillin/sulbactam, Benzyle penicillin, Cefuroxime and Tetracycline while MSSA isolates susceptibilities to same antibiotics were 75%, 60%, 98.7% and 75% respectively. Susceptibility of MRSA to Quinupristin/dalfopristin, clindamycin, trimethoprim sulfamethoxazol, erythromycin, streptomycin were 98%, 72.4%, 65.6%, 55.4% and 63.8% respectively, while MSSA isolates showed more susceptibilities to same antibiotics which were 100%, 98.7%, 80.8%, 82.9 and 98.7% respectively. Both MRSA and MSSA isolates were 100% susceptible to levofloxacin, linzolide, Tigecycline, vancomycin. the sensitivity and specificity of diagnostic methods for MRSA by Oxacillin disc diffusion were 91.4% and 99.2% respectively, Cefoxitin disc diffusion were 98.5% and 99.2%, MRSA -select agar were 99.2% and 98.4% respectively, Oxacillin Screen Agar were 97.1% and 100% respectively and Latex agglutination were 100% and 99.2%. Risks and infection episodes due to *Staph. aureus* were significantly different between MRSA carriers versus MSSA carriers and Colonization MRSA versus Other microorganisms. Total ICU acquired *Staph. aureus* infection were occurred in 7.14% MRSA carriers, 3.05% MSSA carriers and 1.03% Other micro organisms colonization. Conclusions: The prevalence of MRSA is high and rapidly increasing at Al Quwayiyah General Hospital, as it is worldwide. Control measures to prevent the spread of MRSA in hospital should continue with reinforcement of hygienic precautions and development of policies to restrict the use of antibiotics.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen that causes severe morbidity and mortality worldwide (De San *et al.*, 2007). It has emerged as one of the commonest causes of hospital acquired infection and continues to remain an important factor contributing to failure of management (Mehta *et al.*, 1998).

MRSA shows a higher prevalence in ICUs than in general wards (Jin, 2006; Safdar and Maki, 2002). Rapid and accurate screening of MRSA carriers among high-risk patients is essential for MRSA control. Many screening protocols introduced for MRSA detection such as direct agar media including blood agar, mannitol-salt agar with oxacillin, and chromogenic agar or molecular methods (Brown *et al.*, 2005).

The introduction of molecular methods (e.g., PCR) for the rapid detection of MRSA in nasal specimens has improved the reporting time of MRSA. However, not all laboratories can justify the added expense of molecular methods. An alternative, cost-effective approach is the use of chromogenic media for screening nasal specimens for MRSA carriage. Chromogenic media are selective and differential for the qualitative detection of MRSA from nasal surveillance specimens. Each contains specific chromogenic substrates, antimicrobials, and selective agents for the suppression of Gram-negative organisms, yeasts, and other Gram-positive cocci. Identification of MRSA from the primary culture is based on the cleavage of chromogenic substrate by a specific enzymatic activity of *S. aureus*, leading to coloration of colonies (Farley *et al.*, 2008).

The present study was done to detect the prevelance of of *S. aureus* and MRSA carriage in ICU patients in Al Quwayiyah General hospital and to evaluate the performance of MRSA-Select agar media for rapid screening of MRSA Furthermore, to describe the antibiotic susceptibility patterns of the *S. aureus* and MRSA isolates.

Materials and Methods

Study design

A cross sectional study and prospective cohort study was performed from January to July 2018 in Al Quwayiyah General hospital involving a total of 278 subjects inpatients were screened for MRSA after obtaining an informed consent. 278 Nasal cultures were performed form all patients admitted to the ICUs. MRSA surveillance cultures were done as part of routine infection control policy in the hospital.

Clinical details including duration of hospital stay, diagnosis, antibiotic intake and presence of other medical illnesses were recorded for the patients.

Definition of carriage states

The screening procedure for *S. aureus* nasal carriage, described by (Nouwen *et al.*, 2004), was carried out by performing a nasal swab culture obtained from every admitted patient. Those who had positive culture of *S. aureus* were defined as *S. aureus* carriers, whereas all of the others were defined as *S. aureus* non carriers.

An individual was classed as a methicillin-susceptible *S.aureus* (MSSA) carrier if the grown *S.aureus* strain was susceptible to oxacillin. If the grown strain was MRSA, the corresponding patient was defined as a MRSA carrier.

Both *S. aureus* non carriers and MSSA carriers were assigned as MRSA non carriers.

Collection of specimens

Nasal swabs were collected from the anterior nares ICU patients using a cotton swab in Amies transport medium. The swab was inserted into each nostril in turn to a depth of approximately one cm and rotated 4–5 times both clockwise and counterclockwise. The swabs were immediately transported to the microbiology laboratory for further processing. A total of 278 nasal specimens were collected from patients admitted to ICU.

Culture of specimens on

MRSA-select agar plate

Nasal swab specimens were streaked directly on MRSA-Select agar plate (Bio-Rad, France), incubated at 35°C, and then examined at 24 hr; is a new chromogenic medium for the identification of MRSA. For each strain, a bacterial suspension adjusted to 0.5 McFarland was used. Subsequently, a swab streaked onto a MRSA-Select agar plate. The growth of any pink colony was considered to be positive, indicating MRSA (Diederen *et al.,* 2005).

Oxacillin Screen Agar™BD (Mueller-Hinton oxacillin agar screen plate containing4%Nacl and oxacillin 6mg/ml (BD Companey, KSA).

Blood agar

The subcultures were examined at 24 hr. Suspected colonies of *S. aureus* were identified as by Gram stain, catalase, DNase, mannitol fermentation, and coagulase positivity or by Vitek2 ID-GP card (bioMérieux, Marcy l' Etoile, France.)

Oxacillin disc diffusion method

All strains were tested with 1 mg oxacillin discs (Hi-Media) on Mueller–Hinton agar plates. For each strain, a bacterial suspension adjusted to 0.5 McFarland was used. The zone of inhibition was determined after 24 h incubation at 35 ^{o}C. Zone size was interpreted according to (Clinical and laboratory standards institute (CLSI), 2012) criteria: susceptible, 13 mm; intermediate, 11–12 mm; and resistant 10 mm.

Cefoxitin disc diffusion method

All strains were tested with 30 mg cefoxitin discs (Hi-Media) on Mueller–Hinton agar plates. For each strain, a bacterial suspension adjusted to 0.5 McFarland was used.

The zone of inhibition was determined after 16–18 h incubation at 35 ^{0}C. Zone size was interpreted according to (CLSI, 2012) criteria: susceptible, 22 mm; resistant, 21 mm.

Latex agglutination test for detection of PBP2a

A latex agglutination MRSA screen test (Slidex MRSA Detection; bioMérieux) was carried out for all strains according to the manufacturer's instructions (Hussain *et al.,* 2000; Van leeuwen *et al.,* 1999).

Susceptibility testing of *mecA*-positive isolates

Susceptibility testing for 14 antimicrobial agents was done by the Vitek 2 AST-GP586 card and disc diffusion method, and the MICs of oxacillin and cefoxitin was determined by the Etest method according to (CLSI, 2012)

Quality control testing

Quality control testing was performed on each plate of chrome agar and oxacillin screening agar by inoculating ATCC 29213 as a negative control and ATCC 33, 592 as a positive control.

Statistical analysis

The results were tabulated and analyzed using version 20 of the Statistical Package for the Social Sciences (SPSS). Frequencies cross tabulation and appropriate statistical tests as Chi-square test and fisher exact test were performed. A *P*-value of less than 0.05 was considered significant.

Results and Discussion

Table 1 showed that total screened patients upon admission were 278.The patients were aged between 23 and 86 years at entry, mean age for MRSA carriers was 60.97±14.48 years while mean age for MRSA non carriers was 60.09 ± 11.82 years MRSA carriers. Male patients were 127, out of them 22(17.32%) were MRSA carriers while 105(82.68) were MRSA non carriers. 151 female, out of them 34 (22.51) were MRSA carriers while 117 (77.48) were MRSA non carriers. 202/278 (72.66%) patients were receiving antibiotics, out of them 51/56 (62.17%) were MRSA carriers while 151/222(68.01%) were MRSA non carriers. Also 73/278 (26.3%) patients were diabetic, out of them 20 (27.39%) were MRSA carriers while 53(72.61%) were MRSA non carriers.

MRSA colonization status

The isolated bacteria in the nasal samples were demonstrated in table 2, which showed that 56 (20.14%) isolates were MRSA, 45(16.18**%**) were MSSA, 164(58.99**%**) were CONS, 4 (1.44**%**) were Strept viridians, 2(0.71**%**) were gram negative bacteria, 3 (1.07**%**) were *Corynebacterium*, while 3 (1.07**%**) showed no growth.

Antimicrobial susceptibility results for the MSSA and MRSA isolates are shown in table 3. All MRSA isolates were totally resistant to β Ampicillin/sulbactam, Benzyle penicillin, Cefuroxime and Tetracycline while MSSA isolates susceptibilities to same antibiotics were 75%, 60%, 98.7% and 75% respectively. Susceptibility of MRSA to Quinupristin/ dalfopristin, clindamycin, trimethoprim-sulfamethoxazol, erythromycin, streptomycin were 98%, 72.4%, 65.6%, 55.4% and 63.8% respectively, while MSSA isolates showed more susceptibilities to same antibiotics which were 100%, 98.7%, 80.8%, 82.9% and 98.7% respectively. Both MRSA and MSSA isolates were 100% susceptible to levofloxacin, linzolide, Tigecycline, vancomycin.

Table 4 showed that the sensitivity and specificity of diagnostic methods for MRSA by Oxacillin disc diffusion were 91.4% and 99.2% respectively, Cefoxitin disc diffusion were 98.5% and 99.2%, MRSA -select agar were 99.2% and 98.4% respectively, Oxacillin Screen Agar were 97.1% and 100% respectively and Latex agglutination were 100% and 99.2%.

Table 5 showed that risks of and infection episodes due to *Staph. aureus* were significantly different between MRSA carriers versus MSSA carriers and Colonization MRSA versus Other microorganisms. Total ICU acquired *Staph. aureus* infection were occurred in 7.14% MRSA carriers, 3.05% MSSA carriers and 1.03% Other microorganisms colonization.

MRSA is primarily a nosocomial pathogen that emerged in the 1980s as a major cause of infection and colonization in hospitalized patients (Madani, 2002).

Our results showed that the prevelance of MRSA carriers were 20.14%. Also Panlilio AE, 1992 showed that in the last 20 years, the National Nosocomial Infection Surveillance data show that within all hospitals, there was an increase from 2% to 29% in the proportion

of methicillin resistance among *S aureus*, and an increase to 38% in those hospitals with more than 500 beds. A study done on MRSA by Zaman and Dibb (1994) in Jeddah over a period of three years (1990-1992) revealed that about 7.5% per annum of all isolated *S. aureus* were MRSA. While a retrospective study in the year of 2014 for the review of MRSA clinical experience in two tertiary care hospitals in Jeddah resulted in highest rates of MRSA in intensive care units (26.6%). Moreno *et al.*, 1995 also reported that Long term care facilities have become reservoirs of MRSA with mean monthly patient colonization rates as high as 23% with 5%–15% of colonized long term care facility residents subsequently develop MRSA infections.

In the present study the patients were aged between 23 and 86 years, mean age for MRSA carriers 60.97±14.48 years while mean age for non MRSA carriers was 60.09 ± 11.82 years MRSA carriers. Male patients were 127, out of them 22(17.32) were MRSA carriers while 105(82.68) were MRSA non carriers. 151 female, out of them 34 (22.51) were MRSA carriers while 117(77.48) were MRSA non carriers. 202/278 (72.66%) patients were receiving antibiotics, out of them 51/56 (62.17%) were MRSA carriers while 151/222(68.01%) were MRSA non carriers. Also 73/278 (26.3%) patients were diabetic, out of them 20 (27.39%) were MRSA carriers while 53(72.61%) were MRSA non carriers.

Soriano *et al.*, 2000 also reported that old age patients are at an increased risk of MRSA infection when colonization is present in the anterior nares. Patients in an ICU, especially a surgical ICU, have wounds, drains, and invasive monitoring devices that break the skin and increase the risk of developing infections. Additionally, impaired neutrophil function as a result of chronic liver disease, diabetes, or corticosteriod therapy may render these patients more susceptible to MRSA. Specific defects associated with granulocyte function, such as decreased chemotaxis and impaired phagocytosis associated burst activity have been documented with elderly in the present study and diabetes (Table 6).

Madani (2002) study also showed that about 80% of the patients were given either prophylactic or therapeutic antibiotics, 53% of them had multi-antibiotics at the same time while 72% of these used antibiotics were considered to be misused. Thus, the study concludes that more effective measures must be implemented for the high infection rate and the misuse of multiple antibiotics, and there must be a valid antibiotic use policies as well as an effective infection control committee in each hospital for monitoring these infections.

Table.1 Demographic data of nasal carriage MRSA in ICU patients

Characteristics	**MRSA carriers NO=56**		**non MRSA carriers NO=222**	
	NO	**%**	**NO**	**%**
Male=127	22/127	17.32%	105/127	82.68%
Female=151	34/151	22.51%	117/151	77.48
Age (SD) years	60.97±14.48		60.09 ± 11.82 years	
Length of stay (SD) days	7.47±7.94		5.11±3.45	
Use of antibiotics	51/56	91.07%	151/222	68.01%
Diabetes	20/56	35.71%	53/222	23.87%

Table.2 Showed *MRSA* colonization status, MRSA carriers were 56 (20.14%) while non MRSA carriers were 222(79.86%)

MRSA carriers		non MRSA carriers		TOTAL	
NO.	%	NO.	%	NO.	%
56	20.14%	222	79.86%	278	100%

Table.3 Microorganisms identified in nasal samples collected from ICU patients

Organism	No	%
MRSA	56	20.14%
MSSA	45	16.18%
CONS	164	58.99%
Strept viridians	4	1.44%
GNC	2	0.71%
Corynebacterium	3	1.07%
No growth	3	1.07%
Total	278	100%

Table.4 Antibiotic susceptibility pattern of *Staph. aureus* isolates

Antibiotics	MSSA	MRSA
Ampicillin/sulbactam	75%	-
Benzylepinicillin	60%	-
Cefuroxime	98.7%	-
clindamycin	98.7%	72.4%
Erythromycin	82.9%	55.4%
levofloxacin	100%	100%
linzolide	100%	100%
Quinupristin/dalfopristin	100%	98%
streptomicin high level	98.7%	63.8%
Teicoplanin	98.7%	93.7%
Tetracycline	75%	-
Tigecycline	100%	100%
Trimethoprim/sulpha	80.8%	65.6%
Vancomycin	100%	100%

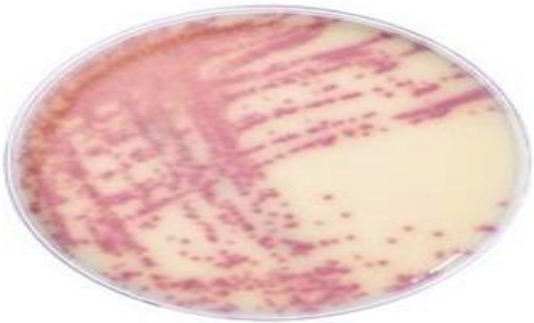
Table.5 Comparison of methods used to detect MRSA

METHOD	NO. OF FALSE NEGATIVE	NO. OF FALSE POSITIVE	SENSITIVITY	SPECIFICITY	NPN	PPV
Oxacillin disc diffusion	28	5	91.4%	99.2%	95.5%	98.4%
Cefoxitin disc diffusion	5	0	98.5%	100%	99.2%	100%
MRSA-select agar	9	5	97.1%	99.2%	98.4%	98.5%
Oxacillin Screen Agar	10	0	97.1%	100%	98.4%	100%
Latex agglutination	0	5	100%	99.2%	100%	98.5%

Table.6 Incidence of ICU-acquired *S. aureus* Infection, by the Nasal Colonization Status on Admission (per 1,000 ICU-days)

Type of infection	Colonization status at admission			P value	
	MRSA n=56	MSSA n= 45	Other micro organisms	Colonization MRSA versus MSSA	Colonization MRSA versus Other micro organisms
ICU acquired MRSA infection	4(7.14%)	1(2.22)	0.68	0.001	0.001
ICU acquired MSSA infection	0	1.35	1.71	-	-
Total ICU acquired *Staph. aureus* infection	4(7.14%)	4.40	1.03	0.05	0.001

Fig.1 Represent growth of MRSA strain MRSA *Select* as pink colonies



Various studies also have demonstrated that the risk factors for MRSA include the use of broad-spectrum antibiotics (Duran *et al.,* 2006). In the present study the isolated bacteria in the nasal samples were demonstrated in table 2, which showed that 56 (16.76) isolates were MRSA, 45(13.47) were MSSA, 221(66.16) were CONS, 4 (1.19) were *Strept viridians*, 2(0.6) were gram negative bacteria, 3 (0.89) were *Corynebacterium* while, 3 (0.89) showed no growth.

In our study all MRSA isolates were totally resistant to β Ampicillin/sulbactam, Benzyle penicillin, Cefuroxime and Tetracycline while MSSA isolates susceptibilities to same antibiotics were 75%, 60%, 98.7% and 75% respectively. Susceptibility of MRSA to Quinupristin/dalfopristin, clindamycin, trimethoprim-sulfamethoxazol, erythromycin, streptomycin were 98%, 72.4%, 65.6%, 55.4% and 63.8% respectively, while MSSA isolates showed more susceptibilities to same antibiotics which were 100%, 98.7%, 80.8%, 82.9 and 98.7% respectively. Both MRSA and MSSA isolates were 100% susceptible to levofloxacin, linzolide Tigecycline, vancomycin.

Priat *et al.,* 2011 also reported that Susceptibility test profiles revealed a higher levelof resistance to commonly prescribed antimicrobial agents among MRSA than MSSA. Also showed that isolates were sensitive to vancomycin, linezolid and dalfopristin/quinpristin. These results were comparable to studies carried out by Anupurba *et al.,* 2003 who showed that more than 80% of MRSA were found to be resistant to penicillin, cotrimoxazole, ciprofloxacin, gentamicin, tetracycline, 60.5% to erythromycin and 47.5% to netilmicin. However, no strains were resistant to vancomycin. Many MRSA strains (32.0%) were multi-drug resistant. Our study showed that the sensitivity and specificity of diagnostic methods for MRSA by Oxacillin disc diffusion were 91.4% and 99.2% respectively, Cefoxitin disc diffusion were 98.5% and 99.2%, MRSA -select agar were 99.2% and 98.4% respectively, Oxacillin Screen Agar were 97.1% and 100% respectively and Latex agglutination were 100% and 99.2%. Various workers have shown that the cefoxitin disc method has better sensitivity than the oxacillin disc method for MRSA detection **(**Velasco *et al.,* 2005; Boutiba-Ben Boubaker *et al.,* 2004; Skov *et al.,* 2003**)**. This higher sensitivity to cefoxitin can be explained by the increased expression of the mecA-encoded protein PBP2a, cefoxitin being an inducer of the mecA gene (Velasco *et al.,* 2005).

MRSA -select agar were 99.2% and 98.4% respectively, among the recently developed methods, CHRO Magar showed 97.1% sensitivity and 99.2% specificity. This sensitivity could be increased to 100% by increasing the incubation period of CHRO Magar from 24 to 48 h (Diederen *et al.,* 2005). However, the delay in obtaining the information will reduce the efficacy of this method. Oxacillin Screen Agar were 97.1% and 100% respectively and Latex agglutination were 100% and 99.2%. Agglutination was 100% and 99.2%. This study found that, for the detection of MRSA, the latex agglutination test had 100% sensitivity and 99.2% specificity. Many recent studies have reported the sensitivity of the latex agglutination test to be ¢97% (Cavassini *et al.,* 1999; Louie *et al.,* 2000; Udo *et al.,* 2000). Latex agglutination has the advantages of being rapid, giving results on the same day, and easy to perform with very good sensitivity. This method could detect even low levels of PBP2a that are usually missed in routine disc diffusion methods. The only disadvantage is the cost factor. A study by Rohrer *et al.,* 2001 proved that the sensitivity

of the latex agglutination test can be improved (93.5 to 100 %) by induction with cefoxitin using growth from the edge of the inhibition zone of cefoxitin to perform the test.

The present study showed that risks of and infection episodes due to *Staph. aureus* were significantly different between MRSA carriers versus MSSA carriers and Colonization MRSA versus Other microorganisms. Total ICU acquired *Staph. aureus* infection were occurred in 7.14% MRSA carriers, 3.05% MSSA carriers and 1.03% Other microorganisms colonization. Which is similar to previous studies of *S. aureus* infection incidence in ICUs (2% to 6%) (Archanda *et al.*, 2014; Boyce, 2001).

Poor hand hygiene compliance, non-judicious use of antibiotics and ineffective infection control measures may explain the relatively high nasal carriage of *S. aureus* and MRSA among ICU patients. Therefore, reducing antibiotic overuse, adherence to hand hygiene, screening and decolonization of carriers, education and training in antibiotic prescribing, environmental cleaning, routine environmental cultures, contact precautions, and active surveillance are recommended strategies for the prevention and control of MRSA transmission in our healthcare setting.

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