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## Prevalence and antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* isolated in Makkah hospitals

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### ABSTRACT

This study aimed to report the frequency of MRSA among *S. aureus* isolates from patients in Makkah hospitals in Saudi Arabia and described the antimicrobial resistance profile of the MRSA isolates. 206 non-duplicated *S. aureus* clinical isolates were identified in the five main tertiary care hospitals in Makkah with standard microbiological methods. MRSA were identified by Oxoid penicillin binding protein (PBP2') latex agglutination test, and confirmed by the oxacillin-salt agar-screening test. The antimicrobial susceptibility of all MRSA isolates were assessed using a VITEK 2 Compact 15 identification system. MRSA was 55.3% and most frequently isolated from the intensive care unit 37%. Of the patients infected with MRSA, the majority were men 60%, and 25% were over 60 years old. Of the MRSA strains isolated, 41% were from skin and wound swabs. The resistance rates among MRSA isolates for penicillin G, oxacillin, and vancomycin were 100%, 100%, and 0.9%, respectively. Our results indicated that the prevalence of MRSA in Makkah hospitals was generally high. vancomycin, teicoplanin, and the oxazolidinone linezolid were considered the drugs of choice for the treatment of MRSA infections.

### INTRODUCTION

The first methicillin-resistant *Staphylococcus aureus* (MRSA) isolates were detected in medical centres in the early 1960s (Durand *et al.*, 2006). MRSA is a major challenge to hospitals because of the emergence and spread of isolates with decreased susceptibilities to several classes of antibiotics classes including methicillin and the other members of the  $\beta$ -lactam family (Gomes *et al.*, 2006). Reports of *S. aureus* infection are increasing worldwide. The prevalence of methicillin-resistance among *S. aureus* isolates in intensive care units (ICU) in the United States is 60% (NNIS, 2004), and resistance is increasingly noted in Europe and Asia (Sader *et al.*, 2006).

MRSA developed by multiple insertions of a staphylococcal cassette chromosome (SCCmec) into successful methicillin-susceptible *S. aureus* (MSSA) lineages (Aires and De LH, 2004). The organism's resistance to antibiotics is due to the acquisition of the methicillin resistance gene *mecA*, which codes for the low-affinity penicillin-binding protein PBP2A, a transpeptidase (Jarvis *et al.*, 2007). Supported by the transglycolase domain of the native PBP2 of *S. aureus*, PBP2A maintains the biosynthesis of the cell wall, which is otherwise blocked in the presence of beta-lactam antibiotics (Grundmann *et al.*, 2006). For patients with an MRSA infection, treatment is then limited to a narrow range of potentially toxic antibiotics such as vancomycin and teicoplanin. Patients with MRSA infections have higher mortality, longer hospital stays, and higher healthcare costs than patients with MSSA infections.

MRSA are often sub-categorized as hospital-associated MRSA (HA-MRSA) or community-associated MRSA (CA-MRSA). MRSA, primarily a nosocomial pathogen, is a major cause of infection and colonization in hospitalized patients (Madani 2001). Recently, MRSA has also been implicated in the propagation of community-acquired infections in individuals with a recognized predisposing risk factor, such as recent contact with a health care facility, nursing home residence, or parenteral substance abuse (Madani 2001). As MRSA spreads, the line between CA-MRSA and HA-MRSA is becoming increasingly indistinct. Healthcare-associated MRSA no longer exclusively resides in health care facilities. To provide a platform for the initiation of epidemiological studies of staphylococcal infections in the Makkah area, this study reports the frequency of MRSA among *S. aureus* isolates from patients in Makkah hospitals and describes the antimicrobial resistance profile of the MRSA isolates.

## MATERIAL AND METHODS

In the five main tertiary care hospitals in Makkah [Al-Noor Specialist Hospital (ASH), Hera General Hospital (HGH), King Abdulaziz Hospital (KAH), Maternity Hospital (MH) and King Faisal Hospital (KFH)], 206 non-duplicated *S. aureus* clinical isolates were identified during September 2011. Patient demographic data were collected from the medical and laboratory records.

Standard microbiological methods were used to isolate and identify *S. aureus* clinical isolates. These methods included assessment of colony morphology on blood agar and mannitol agar, Gram staining, and catalase and coagulase tests. All collected strains were stored at -86°C in brain–heart infusion broth containing 15% glycerol until antibiotic susceptibility to be done. *S. aureus* clinical isolates were detected as MRSA (oxacillin-resistant) using the Oxoid penicillin binding protein (PBP2') latex agglutination test, and the findings were confirmed by the oxacillin-salt agar screening test, according to NCCLS guidelines (NCCLS 2004). The antimicrobial susceptibility of all MRSA isolates was assessed using a VITEK 2 Compact 15 identification system (bioMérieux, Durham, North Carolina, USA). The VITEK2 card panel contained doubling dilutions of the antimicrobial agents (Table 1). All clinical and microbiological outcomes were analysed using the IBM Statistical Package for Social Sciences Statistics 21.0 software (SPSS, Inc., Chicago, IL, USA). The significance of the differences in resistance rates was determined by means of the  $\chi^2$  test and Fisher exact test for independence using the GraphPad InStat 3.0 software (GraphPad, Inc., San Diego, California, USA).

A statistically significant difference was defined as a p value of <0.05 and a 95% confidence interval.

Table 1: Antimicrobial agents in the VITEK2 card panel (ATS-P580) and their concentrations

Antibiotic	Concentration ( $\mu\text{g/ml}$ )
Benzylpenicillin	0.125, 0.25, 1
Oxacillin	0.5, 1, 2
Gentamicin	8, 16, 64
Tobramycin	16, 32, 64
Levofloxacin	0.25, 2, 8
Moxifloxacin	0.25, 2, 8
Erythromycin	0.25, 0.5, 2
Clindamycin	0.5, 1, 2
Linezolid	0.5, 1, 2
Teicoplanin	1, 4, 8, 16
Vancomycin	1, 4, 8, 16
Tetracycline	0.5, 1, 2
Tigecycline	0.25, 0.5, 1
Fosfomycin	8, 32
Nitrofurantoin	16, 32, 64
Fusidic acid	0.5, 1, 4
Mupirocin	1
Rifampicin	0.25, 0.5, 2
Trimethoprim/Sulfamethoxazole	8/152, 16/304, 32/608

## RESULTS

Of the 206 of *S. aureus* pathogenic isolates, 114 (55.3%) were MRSA. MRSA was most frequently isolated from the intensive care unit (ICU) (37%), followed by the outpatient department (OPD) (19%), surgical ward (16%), and paediatrics ward (9%) (Table 2). The majority of patients with MRSA infection were men (60%). Of the infected patients with this strain, 25% were over 60 years old, 15% were 41–50 years old, and 14% children that were less than a year old (Table 2). Among the clinical specimens, 41% of the MRSA

strains were isolated from skin and wound swabs (41%), 26% from sputum and tracheal aspirates, and 19% from blood (Table 2). MRSA infections were distributed across 16 different nationalities. The majority of infected patients were Saudi (78.2%), followed by Pakistani (5.8%) and Yemeni (2.9%) (Table 2). The resistance rates among MRSA isolates for penicillin G, oxacillin, and vancomycin were 100%, 100%, and 0.9%, respectively. Resistance to linezolid, teicoplanin, tigecycline, and mupirocin was not detected (Table 3).

Table 2: Prevalence of MRSA in Makkah hospitals

Age	<1	1–10	11–20	21–30	31–40	41–50	51–60	>60
	14%	9%	10%	7%	11%	15%	9%	25%
Nationality	Saudi	Pakistani	Yemeni	Malaysian	Egyptian	Burmese	Indonesian	Others
	78.2%	5.8%	2.9%	2.0%	1.9%	1.9%	1.5%	5.8
Specimen	Skin & wound swabs	Sputum & tracheal	Blood	ENT & Eye	Urine	Genital swabs	Body fluid	Other
	41%	26%	19%	9%	3%	1%	1%	0
Wards	ICU	OPD	Surgical ward	Paediatrics	Ops/GYN	Burn units	ENT	Other
	37%	19%	16%	9%	10%	2%	7%	0
Sex	Men				Women			
	60%				40%			

ENT: ear, nose, and throat, ICU: intensive care unit, OPD: outpatient department, Ops/GYN: obstetrics and gynecology

Table 3: Antibiotic sensitivity pattern of the MRSA isolates (n = 114)

	Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
1	Benzylpenicillin	0	0	100
2	Oxacillin	0	0	100
3	Gentamicin	43	0	57
4	Tobramycin	36.8	0	63.2
5	Levofloxacin	48.2	29.8	21.9
6	Moxifloxacin	79.8	0	20.2
7	Erythromycin	40.4	0.9	58.8
8	Clindamycin	43.9	0	56.1
9	Linezolid	100	0	0
10	Teicoplanin	100	0	0
11	Vancomycin	97.4	1.8	0.9
12	Tetracycline	60.5	0	39.5
13	Tigecycline	100	0	0
14	Fosfomicin	93.9	0	6.1
15	Nitrofurantoin	98.2	0	1.8
16	Fusidic acid	15.8	57.9	26.3
17	Mupirocin	100	0	0
18	Rifampicin	91.3	2.6	6.1
19	Trimethoprim/Sulfamethoxazole	82.5	0	17.5

## DISCUSSION

Between 1990 and 1996, the National Nosocomial Surveillance System (USA) identified *S. aureus* as the most common etiological agent of nosocomial infection, principally causing nosocomial pneumonia and surgical wound infections (Rubin *et al.*, 1999). Reports of MRSA infections are increasing worldwide. In Saudi Arabia, many institutions have reported an increase in the incidence of MRSA in recent years; other institutions have reported a variation in incidence. The present study found that 55.3% of *S. aureus* isolates were MRSA, whereas earlier studies conducted in the Jeddah hospitals showed a lower prevalence, with only minor variation between 6.5% and 8.9% at King Khalid National Guard Hospital (Zaman and Dibb, 1994; Al-Anazi 2009).

We studied five main tertiary care hospitals in Makkah. MRSA was most frequently isolated from the ICU (39%), followed by the outpatient department (19%) and surgical ward (16%). In Korea, MRSA from tertiary hospitals accounted for 60–70% of all *S. aureus* isolates (Chong and Lee, 2000), and MRSA was more prevalent in an ICU setting than in non-ICU settings (Sista *et al.*, 2004).

This is because antibiotic usage is greatest in the ICU settings. Tyagi *et al.* (2008) found a high occurrence of MRSA in surgical wound infections, especially in neurosurgical and orthopaedics patients, a result of overcrowding, high workload, and understaffing of wards.

We found that the majority of patients with MRSA were men (60%). This is in agreement with the finding of Khanal and Jha (Khanal and Jha 2010), who explained that men might be more likely to contract community-acquired infections because of their involvement in outdoor activities. Lack of knowledge, comparative over-use of antibiotics without prescription, and failure to complete the prescribed treatment course among the men might have led to more men having MRSA than women. In this study, 25.7% of patients with MRSA infections were over 60 years old, 15% were 41–50 years old, and 14.1% were children less than 1 year old. This is in agreement with the findings of Madani, who reported that 36% of MRSA infections at King Fahad Hospital (KFH) in Jeddah occurred among patients over 60 years old [17]. In our study, 27.6% of MRSA infections occurred in the 31–60 age group. Madani *et al.* (2001) reported

higher numbers at King Abdulaziz University Hospital (KAUH) in Jeddah and KFH in Jeddah (33% and 46%, respectively). Of the MRSA strains isolated in this study, 41% were from wound and skin swabs, 26% from sputum and tracheal aspirates, and 19% from blood samples (19%). The high rate of MRSA infection in wounds might be due to the increased chance of MRSA infection in deep-seated lesions (Shittu *et al.*, 2011). Alanazi (2009) reported that 28% of MRSA isolates were cultured from the skin and tissues. The epidemiology of infections caused by MRSA is rapidly changing. In the past 10 years, MRSA infections have emerged in the community. The MRSA clones in many countries have mostly been associated with skin and soft tissue infections (Deresinski 2005; Chambers 2005). The majority of patients with MRSA were Saudi (78.2%), followed by Pakistani (5.8%) and Yemeni (2.9%) (Table 2). Similarly, Madani *et al.* (2001) found that number of Saudi patients with MRSA was more than two times the number of non-Saudi patients. This is explained by the fact that most patients admitted to the hospitals during the study periods were Saudi men.

The study showed that the resistance rates among MRSA isolates were 100%, 100%, and 0.9% for penicillin G, oxacillin, and vancomycin, respectively. Thus,  $\beta$ -lactam antibiotics such as penicillin are ineffective against *S. aureus*. Most clinical MRSA isolates were susceptible to vancomycin, whereas sensitivity to other drugs was poor. This indicates that vancomycin resistance may also spread. Therefore, among carriers, patients, and health care workers, it is important to continue to isolate and identify MRSA so that the effectiveness of newer glycopeptides such as teicoplanin can be monitored regularly. Statistically, our data showed an extremely significant correlation between the resistance to oxacillin and the

resistance to gentamicin and erythromycin ( $p > 0.0001$ ). This study also supports a relationship between oxacillin and aminoglycoside resistance in MRSA (Kim *et al.*, 2004; Schmitz *et al.*, 1999). Historically, gentamicin and erythromycin have had wide clinical application; they are inexpensive and available from diverse sources. In Makkah, they are sold with or without prescription. Misuse and overuse of these antibiotics may have contributed to increasing antibiotics resistance in Saudi Arabia. Therefore, to prevent treatment failures in the absence of data on antibiotic susceptibility, public awareness of the ineffectiveness of gentamicin and erythromycin against MRSA infections is urgently needed. No resistance to linezolid, teicoplanin, tigecycline, and mupirocin was detected in the MRSA isolates assessed in this study. The glycopeptides vancomycin and teicoplanin and the oxazolidinone linezolid have been considered the drugs of choice for the treatment of MRSA infections (Zarakolu *et al.*, 2009). Gram-positive cocci are rarely resistant to linezolid. A recent study examined the linezolid susceptibility of 1930 MRSA isolates collected from different regions of the United States; 99.9% were susceptible to linezolid (Jones *et al.*, 2008). Tigecycline represents an exciting new class of glycylcycline antimicrobial agents for the treatment of multidrug-resistant gram-positive bacteria (Squires and Postier, 2006). During this study, the authors have not declared any conflicts of interest. The results of our study indicate that the overall prevalence of MRSA in Makkah hospitals is high, especially in ICU units, and that vancomycin, teicoplanin, and linezolid are the drugs of choice for the treatment of MRSA infections. Strategies to control the spread of MRSA should include early detection of MRSA and surveillance.

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## ARABIC SUMMARY

انتشار العنقوديات الذهبية المقاومة للميثيسيلين ومدى حساسيتها للمضادات الحيوية بمستشفيات مكة المكرمة

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هدفت الدراسة الى معرفة مدى انتشار العنقوديات الذهبية المقاومة للميثيسيلين في مستشفيات مكة المكرمة ومدى حساسيتها للمضادات الحيوية. تم التعرف على 206 عينة من العنقوديات الذهبية بعد عزلها من خمس مستشفيات بمكة المكرمة بالطريقة القياسية المعروفة وتحديد تلك التي تقاوم الميثيسيلين بطريقة تراس اللاتكس (اوكسويد) وتأكيدها بطريقة التزريع على آجار الأوكسيللن الملحي ثم عمل إختبارات الحساسية لها بجهاز الفايتيك 2 فكانت نسبة العنقوديات المقاومة للميثيسيلين كانت 55.3% وأن أكثرها كان في العناية المركزة 37% ونسبتها في الرجال كانت 60% بينما 25% منها كان من ذوي الأعمار فوق الستين عاما وكانت نسبة عينات الجلد والجروح قد بلغت 41%. نسبة المقاومة للبنيسيلين والأوكساسيلين والفاנקومايسين كانت 100%، 100%، 0.9% على التوالي وختاما أعتبر أن إنتشار العنقوديات المقاومة للميثيسيلين في مكة عاليا كما أن الفانكوميسين والتكلوبلانين واللينوزوليد هي المضادات الحيوية الأكثر فعالية للقضاء على العنقودية الذهبية في مكة المكرمة.