

# Surveillance of Antibacterial Resistance in *Staphylococcus aureus* Isolated in Kuwaiti Hospitals

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## Key Words

Methicillin-resistant *Staphylococcus aureus* · Fusidic acid resistance · Reduced susceptibility to vancomycin

## Abstract

**Objective:** To investigate the prevalence of antibiotic resistance among *Staphylococcus aureus* isolated in Kuwaiti hospitals. **Materials and Methods:** *S. aureus* were isolated and identified following standard microbiological methods. Antibacterial susceptibility test was performed by disk diffusion and the measurement of minimum inhibitory concentration with E-test strips. **Results:** A total of 1,846 *S. aureus* isolates were analyzed from 13 hospitals between 1 March and 30 October 2005. They were isolated from 1,765 (95.6%) inpatients and 81 (4.4%) outpatients. Methicillin resistance was detected in 588 (32.0%) of the isolates. The methicillin-resistant *S. aureus* (MRSA) consisted of 461 (78%) multiresistant and 127 (22%) nonmultiresistant isolates. The nonmultiresistant MRSA consisted of epidemic MRSA-15 and community-associated MRSA. The community-associated MRSA was detected in all hospitals with MRSA, indicating its establishment in Kuwaiti hospitals. The proportion of isolates resistant to gentamicin, kanamycin, erythromycin, tetracycline, ciprofloxacin, fusidic acid and trimethoprim was higher among MRSA than methicillin-susceptible *S. aureus*

(MSSA) isolates. Twenty-four and 22% of MRSA and MSSA isolates, respectively, expressed reduced susceptibility to vancomycin (minimum inhibitory concentration = 3–4 mg/l). **Conclusion:** The study revealed the presence of methicillin resistance in 32% of *S. aureus* isolated in Kuwaiti hospitals and revealed an increase in the number of MRSA and MSSA with reduced susceptibility to vancomycin.

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## Introduction

*Staphylococcus aureus* remains one of the most frequently isolated pathogens in hospitals and community settings. Methicillin-resistant *S. aureus* (MRSA) is an important cause of nosocomial and community-acquired infections worldwide. The global epidemiology of MRSA infections is changing significantly. MRSA was initially isolated in healthcare facilities such as hospitals, nursing homes and long-term care facilities [1, 2] and was often resistant also to antibacterial agents such as the aminoglycosides, macrolides, tetracyclines, fluoroquinolones, chloramphenicol, heavy metals and antiseptics, making antibiotic resistance a global public health problem with significant economic and social costs [1, 3, 4]. In recent years, MRSA has also emerged in the community, iso-

lated from patients without risk factors traditionally associated with hospital-acquired MRSA, such as recent hospitalization, transfer from another hospital, prior antimicrobial use, invasive procedures and underlying diseases [5, 6]. These new MRSA clones have been described as community-acquired or community-originated MRSA (CA-MRSA) [5–9]. The CA-MRSA differ from the health-care-associated MRSA (HA-MRSA) strains phenotypically and genotypically. The CA-MRSA tend to be susceptible to a variety of non- $\beta$ -lactam antibiotics, whereas HA-MRSA are typically resistant to multiple antibiotics [6–9]. In addition, CA-MRSA strains carry a novel methicillin resistance gene cassette element [staphylococcal cassette chromosome *mec* (SCC*mec*) type IV or V], whereas the HA-MRSA carry SCC*mec* types I, II and III [6]. The CA-MRSA is now being isolated in hospitals in many countries [6–9].

MRSA isolated from patients in Kuwaiti hospitals have been studied previously for their resistance to antibacterial agents [10–12]. However, data on the prevalence of MRSA as a proportion of *S. aureus* isolated in these hospitals is limited [13]. In response to this information gap, the present study was conducted to ascertain the prevalence of MRSA among *S. aureus* isolated in Kuwaiti hospitals.

## Materials and Methods

### *S. aureus* Isolates

The surveillance was conducted between 1 March and 30 September 2005 with the participation of 13 hospital-based microbiology laboratories. The hospitals consisted of 2 university-affiliated hospitals and 2 general hospitals with bed capacities >500 and smaller general hospitals with <500 beds. The participating microbiology laboratories were requested to submit nonduplicate consecutive *S. aureus* isolates from each patient to the MRSA reference laboratory during the surveillance period. Each participating laboratory provided isolate-level information such as specimen collection date and source (e.g. blood, wound). They also provided some patient details, such as hospital number, location, age, sex and preliminary susceptibility pattern. The isolates were identified by cultural characteristics, growth on mannitol salt agar, Gram's stain and positive tube coagulase test. Isolates were preserved at  $-80^{\circ}\text{C}$  in 20% (v/v) glycerol and subcultured in brain heart infusion broth and brain heart infusion agar when required.

### Antibiotic Susceptibility Testing

Susceptibility testing was performed by the disk diffusion method on Müller-Hinton agar according to the Clinical and Laboratory Standards Institute guidelines [14]. The Clinical and Laboratory Standards Institute susceptibility breakpoints were used for interpretation of inhibition zone diameters except for

mupirocin and fusidic acid, which were interpreted based on their minimum inhibitory concentration (MIC) values as described previously [11]. Susceptibility testing for methicillin was performed on Müller-Hinton agar plates with disks containing 5  $\mu\text{g}$  of methicillin and incubated at  $30^{\circ}\text{C}$  for 24 h. Methicillin resistance was confirmed by detecting PBP2a in culture supernatants using a rapid latex agglutination kit (Denka-Seiken, Japan) according to the manufacturer's instruction. MIC of mupirocin, vancomycin and teicoplanin were determined with E-test strips (AB Biodisk, Solna, Sweden) according to the manufacturer's instructions for the detection of resistance. *S. aureus* strain ATCC25923 was used for quality control of susceptibility testing.  $\beta$ -Lactamase production was tested with nitrocefin (Oxoid, Basingstoke, UK) according to the manufacturer's instructions. *S. aureus* strain ATCC29213 was used as a positive control. The differentiation between epidemic MRSA-15 (EMRSA-15) and CA-MRSA was based on susceptibility patterns, urease production and SCC*mec* typing as reported previously [11]. Multiresistance for MRSA was defined as resistance to >3 classes of antibiotics among aminoglycosides, macrolides, tetracyclines, quinolones, rifampicin, chloramphenicol, mupirocin, glycopeptides, trimethoprim and fusidic acid. Nonmultiresistance was defined as resistance to not more than 3 classes of these agents.

## Results

During the 7-month surveillance period, a total of 1,846 *S. aureus* isolates were obtained from different clinical specimens in 13 hospitals. Although the source of *S. aureus* was not provided for 39.6% of the samples, the major sources of *S. aureus* were skin/soft tissues (34.8%), nasal swabs (7.5%) and miscellaneous sources which included catheter tips, high vaginal swabs, axilla, eye and semen. A total of 1,765 (95.6%) of the 1,846 *S. aureus* isolates were from inpatients and only 81 (4.4%) were from outpatients.

The distribution of *S. aureus* isolates by hospital is presented in table 1. The data show that 588 isolates constituting 32.0% of the *S. aureus* isolates were MRSA. A high prevalence of MRSA was observed in Mubarak Al-Kabeer Hospital, which is a university teaching hospital, Ibn Sina Hospital (the only burn centre in the country), Al-Razi Hospital (an orthopaedic hospital) and the general hospital at Al-Jahra. No MRSA was isolated at the Infectious Disease and Chest hospitals. A total of 578 (98.0%) MRSA isolates were from inpatients and 10 (2%) were from outpatients. Similarly, 1,187 (94%) of the 1,258 methicillin-susceptible *S. aureus* (MSSA) were from inpatients and 71 (6.0%) were from outpatients.

### Antibacterial Susceptibility Patterns

The antibacterial susceptibilities of both MRSA and MSSA are shown in table 2. Except for penicillin resis-

**Table 1.** Distribution of *S. aureus* isolates from Kuwaiti hospitals

S/N	Hospitals	Isolates	MRSA	MSSA
1	Amiri	372	55 (15)	317 (85)
2	Al-Sabah	304	80 (26)	224 (74)
3	Adan	263	56 (21)	207 (79)
4	Ibn Sina	234	106 (45)	128 (55)
5	Mubarak	201	97 (48)	104 (52)
6	Jahra	175	87 (50)	88 (50)
7	IDH	11	nil	11 (100)
8	Farwaniya	77	19 (25)	58 (75)
9	KOC	41	5 (12)	36 (88)
10	Armed Forces	16	3 (19)	13 (81)
11	IDH	11	nil	11 (100)
12	Maternity	6	1 (17)	5 (83)
13	Chest	3	nil	3 (100)
Total		1,846	588 (32)	1,258 (68)

Figures in parentheses are percentages. IDH = Infectious Disease Hospitals; KOC = Kuwait Oil Company.

tance, the proportion of isolates resistant to gentamicin, kanamycin, amikacin, ciprofloxacin, erythromycin, tetracycline, trimethoprim, fusidic acid and mupirocin was higher among MRSA than MSSA isolates. Most of the isolates were susceptible to chloramphenicol, rifampicin and mupirocin, and all were susceptible to linezolid.

#### *Reduced Susceptibility to Vancomycin and Teicoplanin*

The distribution of MIC values for vancomycin and teicoplanin showed that 75.9% of MRSA and 77.6% of MSSA had a vancomycin MIC of  $\leq 2$  mg/l and 75 and 82% of MRSA and MSSA had a teicoplanin MIC of  $\leq 2$  mg/l. None of the isolates expressed intermediate resistance to vancomycin or teicoplanin (MIC = 8–16 mg/l). However, 24.1 and 24.6% of the MRSA isolates expressed reduced susceptibility to vancomycin and teicoplanin (MIC = 3–4 mg/l), respectively. Similarly, 22.2 and 18.0% of the MSSA isolates expressed reduced susceptibility to vancomycin and teicoplanin (MIC = 3–4 mg/l), respectively.

#### *Nonmultiresistant MRSA*

The MRSA isolates were either multiresistant (mMRSA, i.e. resistant to  $\geq 3$  classes of non- $\beta$ -lactam antibiotics) or nonmultiresistant (nmMRSA, i.e. resistant to 1 or 2 classes of non- $\beta$ -lactam antibiotics). The proportion of mMRSA and nmMRSA isolates is presented in table 3.

**Table 2.** Prevalence of antibiotic resistance in *S. aureus* isolates

Antibacterial agents	Number resistant	
	MRSA n = 588	MSSA n = 1,258
Penicillin G	582 (98.9)	994 (79.0)
Gentamicin	453 (77.0)	26 (2.0)
Kanamycin	529 (89.9)	89 (7.0)
Amikacin	476 (80.9)	26 (2.0)
Erythromycin	494 (84.0)	101 (8.0)
Chloramphenicol	12 (2.0)	13 (1.0)
Tetracycline	488 (83.0)	227 (18.0)
Trimethoprim	59 (10.0)	26 (2.0)
Ciprofloxacin	500 (85.0)	38 (3.0)
Rifampicin	6 (1.0)	2 (0.1)
Fusidic acid	494 (84.0)	63 (5.0)
Mupirocin (low level)	394 (67.0)	10 (0.8)
Mupirocin (high level)	12 (2.0)	2 (0.1)

Figures in parentheses are percentages. Mupirocin: low level = MIC 8–256 mg/l; high level = MIC  $\geq 512$  mg/l.

**Table 3.** Distribution of MRSA isolates in Kuwaiti hospitals

Hospitals	MRSA	mMRSA	EMRSA-15	CA-MRSA
Ibn Sina	106	91 (86)	1 (1)	14 (13)
Mubarak	97	83 (86)	6 (6)	8 (8)
Jahra	87	70 (81)	1 (1)	16 (18)
Al-Sabah	80	55 (69)	6 (8)	19 (24)
Al-Razi	79	71 (90)	1 (1)	7 (9)
Adan	56	43 (77)	3 (5)	10 (18)
Amiri	55	32 (58)	5 (9)	18 (33)
Farwaniya	19	15 (79)	–	4 (21)
KOC	5	1 (20)	1 (20)	3 (60)
Armed Forces	3	–	2 (67)	1 (33)
Maternity	1	–	–	1 (100)
Total	588	461 (78.0)	26 (5)	101 (17)

Figures in parentheses are percentages. KOC = Kuwait Oil Company.

The nmMRSA comprised 22% of the MRSA isolates and were isolated in 11 of the 13 hospitals. The nmMRSA were further classified as EMRSA-15 and CA-MRSA on the basis of their SCCmec types, phage types, urease and toxin production [15, 16]. Fifty-two (51.5%) of the CA-MRSA were isolated from skin/soft tissue samples.

## Discussion

The results of this study showed that MRSA constituted 32% of all *S. aureus* isolated in Kuwaiti hospitals. This indicated that the prevalence of MRSA among *S. aureus* isolates has remained stable in Kuwait since 1996, when it was reported to be 31% of *S. aureus* isolated in large and small hospitals [13]. MRSA constituted 35% of all *S. aureus* isolated in a Northern Indian hospital [15] and 47–55% of *S. aureus* in US hospitals [17]. In the Gulf countries, MRSA constituted 5% of *S. aureus* isolated in a tertiary hospital in the United Arab Emirates [18] but 38.9% of *S. aureus* in Saudi Arabian hospitals [16, 19] and 38.8% of *S. aureus* isolated in an Omani hospital [20]. Bell and Turnidge [21] reported an even higher prevalence of MRSA in other Asian and Western Pacific region countries. The differences in the prevalence of MRSA in different countries emphasize the importance of local surveillance in generating relevant local resistance data that can guide empiric therapy [9].

This study identified an increase in the proportion nmMRSA among MRSA in Kuwaiti hospitals. The 22% prevalence of nmMRSA observed in this study represents a substantial increase from a prevalence of 5% in Kuwait hospitals between 2000 and 2003 [11]. Furthermore, the nmMRSA were isolated in all of the 11 hospitals where MRSA was detected. Similarly, Asghar and Momenah [16] reported that 21.2% of MRSA in 4 Saudi Arabian hospitals were nmMRSA. The increase in the prevalence of nmMRSA in Kuwait and Saudi Arabia highlights the changing epidemiology of MRSA in these countries, which is consistent with current global trends [6–9, 17]. The nmMRSA consisted of EMRSA-15 and CA-MRSA. Although both the EMRSA-15 and CA-MRSA have the SCCmec type IV genotype, EMRSA-15 is an epidemic HA-MRSA strain and differs from the CA-MRSA in its lack of the urease enzyme, which results in a negative urease test and weak lysis with phage 75 of the international phage typing set [22]. In contrast, the CA-MRSA produces urease yielding a positive urease test and is lysed by different typing phages [11]. EMRSA-15 is a pandemic MRSA clone that was originally isolated in healthcare facilities in the UK [22] but has now spread internationally [11, 23]. The CA-MRSA is often associated with skin and soft tissue infections in children and young adults [6]. This is consistent with the results that 50% of the CA-MRSA in this study was associated with skin and soft tissue samples. The CA-MRSA was isolated in 2 hospitals in 2003 [11]. Its detection in 11 hospitals in this study indicates that it is now fully established in Kuwaiti hospitals.

This study showed that 24% of MRSA and 22% of MSSA expressed reduced susceptibility to vancomycin (MIC = 3–4 mg/l). This is significant because to date only MRSA isolates have been reported with reduced susceptibility to vancomycin [24–27]. *S. aureus* isolates with reduced vancomycin susceptibility is clinically significant because these isolates have been shown to express heterogeneous resistance to vancomycin that resulted in treatment failure [26, 27]. In heterogeneous resistance, a subpopulation of cells expresses resistance, whereas the majority of the cells appear susceptible. The expansion of *S. aureus* populations with reduced vancomycin susceptibility will further reduce the options available for treating *S. aureus* infections and put more financial burdens on the healthcare system.

## Conclusion

This study showed that 32% of *S. aureus* isolated in Kuwaiti hospitals during a 7-month period were MRSA. Although the prevalence of MRSA was stable compared to the data obtained in 1996 [14], there was a shift in the proportion of mMRSA and nmMRSA, with the nmMRSA showing a steady increase while the number of mMRSA isolates were decreasing. The results also revealed an increase in the number of MRSA and MSSA with reduced susceptibility to vancomycin and teicoplanin.

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