



## Antimicrobial Susceptibility Pattern of *Staphylococcus aureus* Strains Isolated from Hospitalized Patients in Tehran, Iran

Rasoul Soltani<sup>a</sup>, Hossein Khalili<sup>a,\*</sup>, Mehrnaz Rasoolinejad<sup>b</sup>, Alireza Abdollahi<sup>c</sup>,  
Kheirollah Gholami<sup>a</sup>

<sup>a</sup>Department of Clinical Pharmacy, Faculty of Pharmacy, Tehran University of Medical Sciences, Tehran, Iran

<sup>b</sup>Department of Infectious diseases, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

<sup>c</sup>Department of Pathology, Faculty of Medicine, Tehran University of Medical Sciences, Tehran, Iran

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### Abstract

*Staphylococcus aureus* is a major bacterial pathogen that causes different community- and hospital-acquired infections. Over time, strains of *S. aureus* have become resistant to different antibiotics including penicillinase-resistant penicillins. Having data on the local antimicrobial susceptibility pattern of this pathogen is necessary for selection of appropriate antibiotics for empirical treatment of infections due to it. To determine the antimicrobial susceptibility pattern of *Staphylococcus aureus* strains isolated from hospitalized patients in Tehran, Iran, In a prospective cross-sectional study performed at Imam Khomeini Hospital, samples were collected from hospitalized patients and were cultured. All positive cultures which yielded *S. aureus* underwent antimicrobial susceptibility testing using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar. The results were interpreted after 24 hours of incubation at 37 °C. A total of 160 clinical isolates of *S. aureus* were collected. Most isolates were obtained from blood (29%). The overall susceptibility of isolated *S. aureus* strains to antimicrobial agents was 100% for vancomycin, 49.4% for amikacin, 43.8% for gentamicin, 36.8% for co-trimoxazole and tetracycline, 36.3% for cefazolin, 30.6% for cephalexin, 24.4% for oxacillin, 23.8% for erythromycin, and 3.1% for penicillin. Other than vancomycin, none of the tested antibiotics are appropriate for empirical treatment of serious *S. aureus* infections in our area.

**Keywords:** Antimicrobials; Resistance; *Staphylococcus aureus*; Susceptibility.

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### 1. Introduction

*Staphylococcus aureus* is a major bacterial pathogen that can cause infection in a variety of body organs and tissues including skin and soft tissue. It may also cause infective endocarditis, bacteremia, pneumonia,

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\*Corresponding Author: Prof. Hossein Khalili, Department of Clinical Pharmacy, School of pharmacy, Tehran University of Medical Sciences, Tehran, IRAN, Postal Code:1417614411.  
Tel: (+98)912-2979329; Fax: (+98)21-66461178  
E-mail: khalilih@tums.ac.ir

osteomyelitis, infective arthritis, and urinary tract infection.

Virtually all *S. aureus* strains were susceptible to penicillin G until 1994, when the first reports of penicillin-resistant *S. aureus* were reported and today virtually all strains of *S. aureus* are resistant to natural penicillins [1]. Methicillin and other penicillinase-resistant penicillins were developed to treat infections caused by penicillin-resistant *S. aureus* and met with initial success; however, over time, strains of methicillin-resistant *S. aureus* (MRSA) began to appear and spread [1]. Infections caused by MRSA have been associated with high morbidity and mortality. MRSA is currently recognized as a major problem in hospitals throughout the world. In 2004, a report from the Surveillance and Control of Pathogens of Epidemiologic Importance (SCOPE) project, which monitors significant bloodstream infections in hospitalized patients in the United States, showed that methicillin resistance was present in 44% of bloodstream *S. aureus* isolates from intensive care unit (ICU) [2].

Although MRSA was initially observed in hospital settings, it is now clear that MRSA may be acquired in the community as well. The exact prevalence of community-acquired MRSA (CA-MRSA) has been difficult to determine, however, it appears to be increasing and CA-MRSA is now recognized as a growing problem worldwide [3].

There is no authentic data on the antimicrobial susceptibility pattern of *S. aureus* strains from Iran; this can complicate the selection of appropriate antibiotics for empirical treatment of infections due to this pathogen.

The present study was conducted to determine the susceptibility pattern of *S. aureus* strains isolated from hospitalized patients in a teaching hospital, Tehran, Iran.

## 2. Materials and methods

This is a prospective cross-sectional study

**Table 1.** Frequency of isolated *S. aureus* strains from different collected biologic samples.

Sample	Frequency (%)
Blood	46 (28.7)
Wound secretions	33 (20.6)
Urine	23 (14.4)
Respiratory secretions	15 (9.4)
Sputum	11 (6.9)
Abscess	10 (6.2)
Intra-articular fluid	9 (5.6)
Bone	6 (3.7)
Others	7 (4.4)

performed at Imam Hospital, a general teaching hospital affiliated to Tehran University of Medical Sciences during Nov. 2007 to Nov. 2008. Samples were collected from patients hospitalized in different medical wards including ICU. All *S. aureus* isolates were collected from patients with at least 48 h of stay in the hospital, and with evidence of infection, were included in the study. Samples included blood, urine, sputum, wound secretions, intra-articular fluid, bone, abscess, respiratory tract secretions, and other valuable clinical specimens (Table 1).

All isolates were cultured on blood and chocolate agar as growth medium and incubated at 37 °C for 18 to 24 h. All positive cultures which yielded *S. aureus* (identified by different tests including Gram Stain, catalase and coagulase tests, and culture of isolates on mannitol salt and DNase agar media), underwent antimicrobial susceptibility testing using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, according to Clinical and Laboratory Standards Institute (CLSI) recommendations [4]. The results were interpreted after 24 h of incubation at 37 °C, as sensitive, intermediately sensitive, and resistant according to the zone diameter around each antibiotic disk. The antibiotic disks were from Padten Teb Co. (Tehran, Iran), and included penicillin, cephalexin, cefazolin, oxacillin, gentamicin, amikacin, co-trimoxazole (TMP/SMX), erythromycin, tetracycline, ciprofloxacin, and vancomycin. *S. aureus* strain 25923 was used as the

**Table 2.** Antimicrobial susceptibility of isolated *S. aureus* strains from different samples to studied antibiotics.

Sample	Antibiotic	Sensitive	Intermediate	Resistant
		n (%)	n (%)	n (%)
Blood	Penicillin	0 (0.0)	0 (0.0)	46 (100)
	Oxacillin	3 (6.50)	4 (8.7)	39 (84.8)
	Cephalexin	8 (17.4)	8 (17.4)	30 (65.2)
	Cefazolin	7 (15.2)	3 (6.5)	36 (78.3)
	Co-trimoxazole	9 (19.6)	0 (0.0)	37 (80.4)
	Ciprofloxacin	10 (21.7)	6 (13.0)	30 (65.2)
	Tetracycline	8 (17.4)	9 (19.6)	29 (63.0)
	Erythromycin	4 (8.7)	6 (13.0)	36 (78.3)
	Gentamicin	11 (23.9)	2 (4.3)	33 (71.7)
	Amikacin	13 (28.3)	4 (8.7)	29 (63.0)
	Vancomycin	46 (100)	0 (0.0)	0 (0.0)
Wound	Penicillin	0 (0.0)	2 (6.1)	31 (93.9)
	Oxacillin	9 (27.3)	8 (24.2)	16 (48.5)
	Cephalexin	7 (21.2)	14 (42.4)	12 (36.4)
	Cefazolin	13 (39.4)	4 (12.1)	16 (48.5)
	Co-trimoxazole	13 (39.4)	2 (6.1)	18 (54.5)
	Ciprofloxacin	13 (39.4)	6 (18.2)	14 (42.4)
	Tetracycline	14 (42.4)	12 (36.4)	7 (21.2)
	Erythromycin	10 (30.3)	3 (9.1)	20 (60.6)
	Gentamicin	15 (45.4)	6 (18.2)	12 (36.4)
	Amikacin	15 (45.4)	3 (9.1)	15 (45.4)
	Vancomycin	33 (100)	0 (0.0)	0 (0.0)
Urine	Penicillin	1 (4.3)	0 (0.0)	22 (95.6)
	Oxacillin	6 (26.1)	3 (13.0)	14 (60.9)
	Cephalexin	9 (39.1)	8 (34.8)	6 (26.1)
	Cefazolin	10 (43.5)	0 (0.0)	13 (56.5)
	Co-trimoxazole	11 (47.8)	2 (8.7)	10 (43.5)
	Ciprofloxacin	14 (60.9)	4 (17.4)	5 (21.7)
	Tetracycline	8 (34.8)	9 (39.1)	6 (26.1)
	Erythromycin	6 (26.1)	5 (21.7)	12 (52.2)
	Gentamicin	12 (52.2)	2 (8.7)	9 (39.1)
	Amikacin	11 (47.8)	4 (17.4)	8 (34.8)
	Vancomycin	23 (100)	0 (0.0)	0 (0.0)

organism for quality control of antibiotic disks.

### 3. Results

A total of 160 clinical isolates of *S. aureus* were collected, of which, 32 isolates were from ICU patients. As shown in Table 1, most of the isolates were obtained from blood (28.7%), wound secretions (20.6%), and urine (14.4%). Table 2 shows the susceptibility of isolated *S. aureus* strains from blood, wound and urine samples to studied antibiotics.

Antimicrobial susceptibility pattern of

isolated *S. aureus* strains to studied antibiotics is shown in Table 3. The overall susceptibility of isolated *S. aureus* strains to antimicrobial agents was 100% for vancomycin, 49.4% for amikacin, 43.8% for gentamicin, 36.8% for co-trimoxazole and tetracycline, 36.3% for cefazolin, 30.6% for cephalexin, 24.4% for oxacillin, 23.8% for erythromycin, and 3.1% for penicillin.

According to these results, vancomycin, amikacin, and gentamicin were the most effective agents against isolated *S. aureus* strains, while penicillin, oxacillin, and

**Table 3.** Antimicrobial susceptibility of isolated *S. aureus* strains to studied antibiotics.

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Penicillin	5 (3.1)	3 (1.9)	152 (95.0)
Oxacillin	39 (24.4)	22 (15.0)	99 (60.6)
Cephalexin	49 (30.6)	43 (26.9)	68 (42.5)
Cefazolin	58 (36.3)	16 (10.0)	86 (53.7)
Co-trimoxazole	56 (35.0)	6 (3.8)	98 (61.2)
Ciprofloxacin	59 (36.8)	34 (21.3)	67 (41.9)
Tetracycline	56 (35.0)	43 (26.9)	61 (38.1)
Erythromycin	38 (23.8)	26 (16.3)	96 (59.9)
Gentamicin	70 (43.8)	18 (11.3)	72 (44.9)
Amikacin	79 (49.4)	18 (11.3)	63 (39.3)
Vancomycin	160 (100)	0 (0.0)	0 (0.0)

erythromycin were the least effective antimicrobial agents.

Antimicrobial susceptibility of *S. aureus* isolates from ICU patients is shown in Table 4. Vancomycin (100%), amikacin (71.88%), gentamicin, and cefazolin (46.87%) were the most effective agents against these isolates.

Comparison of resistance pattern of *S. aureus* strains to antimicrobial agents in different studies is shown in Table 5.

#### 4. Discussion

*S. aureus* is known as an important bacterial pathogen that can cause community- and hospital-acquired infections with high morbidity and mortality rate in spite of the use of antibiotics. In the current study, *in vitro* susceptibility pattern of this gram-positive pathogen was assessed using clinical specimens isolated from hospitalized patients. In this study, most *S. aureus* strains (29%) were isolated from blood samples. This is consistent with the study of Mamishi et al. that reported *S. aureus* as the second most frequent isolated pathogen from blood cultures of hospitalized patients at Children's Medical Center (CMC) of Tehran, Iran [5]. Also, some other reports from the United States and Europe have shown *S. aureus* as a frequent blood stream pathogen [6, 7].

Table 5 shows reported susceptibility pattern of *S. aureus* strains to antibiotics in some other studies compared to the present

document. As expected, resistance to penicillin was high (96%) in the present study that is consistent with many reported results of other studies in different countries [8-11].

In the current study, 62% of isolated *S. aureus* strains were resistant to oxacillin. This could be the representative of hospital-acquired MRSA rate in Tehran, Iran. The prevalence of MRSA varies among different countries and different areas of a country. In a study performed by Alborzi *et al.* in Shiraz, Iran, 33% of all *S. aureus* isolates were reported as MRSA [9]. It appears that MRSA has emerged as an important endemic pathogen in our hospitals. Fridkin *et al.* reported a median increase of 2.4% in the prevalence of oxacillin-resistant *S. aureus* in U.S. hospitals from 1996 to 1999 [12]. According to the reports, prevalence of MRSA is increasing in Europe. In Austria 21.6%, Belgium 25.1%, Spain 30.3%, and France 33.6% of isolated *S. aureus* strains are methicillin resistant [13]. In a survey performed in Pakistan, 61.29% of isolated *S. aureus* strains were resistant to oxacillin [10]. In the study of Ikeagwu *et al.* in Nigeria, 87% of resistance to cloxacillin was recorded among isolated *S. aureus* strains [14].

Resistance to cefazolin and cephalexin (both as first-generation cephalosporins) among the isolates were 55% and 41%, respectively. Among the strains isolated from urine samples, resistance to cefazolin was

**Table 4.** Antimicrobial susceptibility of isolated *S. aureus* strains from ICU patients to studied antibiotics.

Antibiotic	Sensitive n (%)	Intermediate n (%)	Resistant n (%)
Penicillin	2 (6.25)	1 (3.12)	29 (90.62)
Oxacillin	12 (37.5)	2 (6.25)	18 (56.25)
Cephalexin	12 (37.5)	5 (15.62)	15 (46.87)
Cefazolin	15 (46.87)	2 (6.25)	15 (46.87)
Co-trimoxazole	14 (43.75)	0 (0.0)	18 (56.25)
Ciprofloxacin	10 (31.25)	8 (25.0)	14 (43.75)
Tetracycline	13 (40.63)	4 (12.5)	15 (46.87)
Erythromycin	14 (43.75)	4 (12.5)	14 (43.75)
Gentamicin	15 (46.87)	4 (12.5)	13 (40.63)
Amikacin	23 (71.88)	1 (3.12)	8 (25.0)
Vancomycin	32 (100)	0 (0.0)	0 (0.0)

56.5%, while in the study of Haghi-Ashteiiani *et al.* conducted at 2003 in Children's Medical Center of Tehran, Iran, only 20% of *S. aureus* strains isolated from urinary tract of children with urinary tract infection (UTI), were resistant to cefazolin [8]. The difference in evaluated population (children vs adult) and different patterns of antibiotic use in these groups may be responsible for these significantly different resistance rates.

It is noteworthy that in the current study, about 10% and 15% of MRSA isolates were sensitive to cefazolin and cephalexin, respectively. These show that cross-resistance between penicillinase-resistant penicillins (e.g., oxacillin) and first-generation cephalosporins is not absolute.

In the present study, the overall resistance rate to co-trimoxazole was 61%. Most of co-trimoxazole-resistant strains were isolated from blood samples with resistance rate of 80.4%. This is higher than resistance rate of 52% that was reported by Mamishi *et al.* [5]. Based on these data, co-trimoxazole resistance among *S. aureus* strains is high in Iran. Co-trimoxazole is an inexpensive and available antibiotic in Iran and because of its broad spectrum of activity, it is prescribed for different infections. Martin *et al.* showed that resistance to co-trimoxazole increased from 0% to 48% in *S. aureus* isolates obtained from HIV-infected patients during a 16-year period at San Francisco General Hospital

[15]. The authors explained this increase of resistance by extensive use of this drug as prophylaxis against *Pneumocystis carinii* pneumonia. In contrast to these reports, a study in Israel showed an increase in the susceptibility to co-trimoxazole among MRSA isolates from 31% in 1988 to 92% in 1997 [16]. The authors attributed this increased sensitivity to significantly reduced usage of this drug in their institution.

In the present study, there was significant consistency in the susceptibility pattern of isolated *S. aureus* strains to oxacillin and co-trimoxazole. Similar results were reported in the study of Alborzi *et al.* [9].

High resistance of isolated *S. aureus* strains to ciprofloxacin, erythromycin, and tetracycline recorded in the current study (42%, 60%, and 38%, respectively), is consistent with other studies in Iran [9, 17] and other countries [11, 14, 18, 19]. According to these results, it seems that these three antibiotics are not appropriate choices for empirical therapy of *S. aureus* infections unless *in vitro* susceptibility test confirms the sensitivity of the pathogen to them.

In our study, amikacin and gentamicin were the second most effective agents against isolated *S. aureus* strains with the susceptibility rates of 49.4% and 43.8%, respectively. Also, with the susceptibility rate of 71.88%, amikacin showed good efficacy against isolates from ICU patients. Similarly,

**Table 5.** Comparison of resistance pattern of *S. aureus* strains to antimicrobial agents in different studies.

Antimicrobial agent	Resistance (%)			
	present study (Iran, 2008)	Ekrami & Kalantar <sup>27</sup> (Iran, 2004)	Farzana & Hameed <sup>10</sup> (Pakistan, 2006)	Ikeagwu et al. <sup>14</sup> (Nigeria, 2006)
Penicillin	95.0	-	92.0**	81.0**
Oxacillin	60.6	58.0	61.3	89.0
Cephalexin	42.5	74.0	60.0***	-
Cefazolin	53.7	73.3*	-	-
Co-trimoxazole	61.2	89.0	-	94.0
Ciprofloxacin	41.9	73.3	59.3	35.0****
Tetracycline	38.1	-	-	84.0
Erythromycin	59.9	-	-	-
Gentamicin	44.9	73.3	58.0	-
Amikacin	39.3	-	-	-
Vancomycin	0.0	0.0	0.0	-

\*For cephalothin, \*\*For ampicillin, \*\*\*For cephadrine, \*\*\*\*For ofloxacin

in a study in Pakistan (2006), gentamicin with the susceptibility rate of 42% was the second most effective antibiotic (after vancomycin) against isolated *S. aureus* strains [10]. However, the role of aminoglycosides as monotherapy in the treatment of infections due to Gram-positive organisms has not been well defined. Currently, these antibiotics are used as combination therapy with other effective antibiotics for synergistic effects and for prevention of resistance in the treatment of gram-positive related infections.

Finally, as stated earlier, our study showed vancomycin as the most effective agent against isolated *S. aureus* strains with the susceptibility rate of 100%. Infections caused by *S. aureus* with reduced vancomycin susceptibility (MIC $\geq$ 4 mcg/ml) including vancomycin-intermediate *S. aureus* (VISA; MIC $\geq$ 8 mcg/ml) and vancomycin-resistant *S. aureus* (VRSA; MIC $\geq$ 32 mcg/ml) are a new clinical and public health dilemma [20]. The first case of VISA was reported in Japan in 1996 [21]; but the first case of *S. aureus* truly resistant to vancomycin (VRSA) was reported from the USA in 2002 [22, 23]. Until 2006, seven cases of VRSA infection were reported from the USA [24].

In Iran vancomycin resistance rates of 11%, 21%, and 42.5% have been reported in different studies that have evaluated pediatric population [5, 8, 25]. However, VRSA had not

ever been reported from Iranian adult patients until recently that Emameini *et al.* reported the first isolate of MRSA for which the MIC of vancomycin was 512 mcg/ml [26]; this VRSA was isolated from a post-heart surgery wound specimen of a patient at a teaching hospital in Tehran. This is an alarming report that warns about the emergence of VRSA in Iran.

## 5. Conclusion

According to this study, other than vancomycin, none of tested antibiotics are appropriate for empirical treatment of serious *S. aureus* infections in our area. Also, these data shows that antimicrobial resistance is increasing among *S. aureus* strains in our country. This increase along with the emergence of VRSA highlights the value of prudent prescribing of antibiotics (including vancomycin) and avoiding their irrational use. It is necessary to establish an antimicrobial susceptibility surveillance system and to improve current infection control programs in our hospitals to prevent the spread of resistant microorganisms including MRSA and VRSA.

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