



## The Prevalence of methicillin resistant *Staphylococcus aureus* and methicillin resistant *Staphylococcus epidermidis* in AL-Sulaimania city

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### Abstract

One hundred thirty seven *Staphylococcus* spp. isolates were isolated from one hundred fifty clinical specimens which were collected from several hospitals at Al-Sulaimaniya city. Seventy two *Staphylococcus aureus* isolates, 28 *Staphylococcus epidermidis* isolates and 37 isolates related to other coagulase negative staphylococci (*S. chromogenes*, *S. lugdunensis*, *S. cohnii*, *S. saprophyticus*, *S. hominis*, and *S. haemolyticus*) constituted 3.60%, 2.20%, 2.90%, 2.90%, 6.60%, and 8.80%, respectively). Burn specimens represented the highest ( $P < 0.05$ ) reservoir for *S. aureus* and *S. epidermidis* isolates. Staphylococci developed variable susceptibility to 4 antibiotics (cefoxitin; 30 µg, oxacillin; 1 µg, methicillin; 5 µg, and cefotaxime; 30 µg). Nevertheless, the results revealed that 68, 93, and 92% of *S. aureus*, *S. epidermidis*, and other Coagulase negative staphylococci (CONS) isolates developed methicillin resistance based on cefoxitin diffusion disc test. In a conclusion, methicillin resistant *S. aureus* (MRSA) and coagulase negative staphylococcus sp. (MRCONS) is an emerging subject even in our community, which requires further attention and support.

**Key words:** MRSA, MRSE, MRCONS, *Staphylococcus aureus*, *Staphylococcus epidermidis*

### انتشار العنقوديات الذهبية و البشروية المقاومة للمثيسيلين في محافظة السليمانية

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### الخلاصة:

عزلت 137 عذلة من العنقوديات من 150 عيرة سريرية جمعت من عدة مستشفيات في محافظة السليمانية. شخصت منها اثنان و سبعون على انها عنقوديات ذهبية و ثمان و عشرون عذلة على انها عنقوديات بشروية و سبعة و ثلاثون عذلة على انها عنقوديات اخرى سالبة للكواكوليز (*S. chromogenes* و *S. lugdunensis* و *S. cohnii* و *S. saprophyticus* و *S. hominis* و *S. haemolyticus*) ما نسبته 3.60% و 2.20% و 2.90% و 2.90% و 6.60% و 8.80% على التتابع). عزلت العنقوديات من عينات الحروق بمعدل اعلى ( $P < 0.05$ ) من بقية العينات. اظهرت العنقوديات مقاومة متباينة لاربعة مضادات حيوية (cefoxitin; 30 µg و oxacillin; 1 µg و methicillin; 5 µg و cefotaxime; 30 µg). بينت النتائج ان 68 و 93 و 92% من العنقوديات الذهبية و العنقوديات البشروية و العنقوديات الاخرى السالبة للكواكوليز، على التتابع كانت مقاومة للمثيسيلين بناء على اختبار قرص السيفوكسيتين. خلاصة النتائج، قد تكون العنقوديات الذهبية و البشروية المقاومة للمثيسيلين موضوعا اخذ بالصعود حتى في مجتمعنا الامر الذي يتطلب الدعم والانتباه.

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## Introduction

The Staphylococci are a diverse group of bacteria that cause diseases ranging from minor skin infections to life-threatening bacteraemia. In spite of large-scale efforts to control their spread, they persist as a major cause of both hospital and community acquired infections worldwide. The two major opportunistic pathogens of this genus are *Staphylococcus aureus* and *Staphylococcus epidermidis* [1, 2]. According to Moroni *et al.* (2011) [3] staphylococci are belonging to the family Staphylococcaceae, order Bacillales, class Bacilli, phylum Firmicutes, the genus *Staphylococcus*. The widespread use of methicillin and other semisynthetic penicillins in the late 1960s led to the emergence of methicillin resistance *S. aureus* (MRSA) and *S. epidermidis* (MRSE), which continue to persist in both the healthcare and community environments. Currently, greater than 60% of *S. aureus* isolates are resistant to methicillin and some strains have developed resistance to more than 20 different antimicrobial agents [2,4]. Antimicrobial resistance is the reduction in the susceptibility of pathogenic microorganism to one or more of the chemotherapeutic agents administered in clinical medicine [5].

Beta-lactam antibiotics produce a bactericidal effect by binding penicillin protein PBP to  $\beta$ -lactam inhibiting the membrane-bound enzymes responsible for catalyzing vital stages in the biosynthesis of the cell wall. In contrast to the PBPs in methicillin-susceptible strains, which have high affinity for most  $\beta$ -lactam antimicrobials, PBP2a has low affinity for binding  $\beta$ -lactams. In methicillin-resistance strains, the essential function of PBP is undertaken by PBP2a to maintain survival of the bacterium in the presence of antimicrobials [6].

Methicillin resistance in clinical staphylococcal isolates is mostly mediated through acquisition of *mecA* gene encoding a mutant penicillin-binding protein, designated 2' or 2a, by bacterial genome. That has decreased affinity for  $\beta$ -lactams and catalyses effective cell wall synthesis [6, 7].

The gene encoding methicillin resistance (*mecA*) is carried by the chromosome of MRSA and methicillin-resistance *S. epidermidis* (MRSE), the mechanism for which is the synthesis of an altered low-affinity PBP termed PBP2a. *mecA* is part on a large mobile genetic element termed staphylococcal cassette chromosome *mec* (SCC*mec*) [8-10], is the players in the signaling pathway for methicillin resistance are Expression of *mecA* gene regulated by transcriptional regulators *mecR1*, *mecR2* and *mecI* located immediately upstream from the *mecA* promoter in staphylococci [11].

The regulation of methicillin resistance resembles that of  $\beta$ -lactamase expression, *mecA* gene activation by  $\beta$ -lactams is postulated to be the result of cleavage of *mecI* protein by activated *mecR1* gene product [12]. MRSA carrying SCC*mec* type I spread across the world in the 1960s, SCC*mec* II in the 1970s, SCC*mec* III in the 1980s, and SCC*mec* type IV in the 1990s [9]. The present work aimed to investigate the prevalence of MRSA in Sulaimania governorate.

## Materials and Methods

### Specimen collection

About 150 clinical specimens were collected from patients attending Pediatric Teaching Hospital, plastic surgery Teaching Hospital, surgical Teaching Hospital at Sulaimania governorate. The specimens included Burn swab, wound swab, catheter swab, sputum, indwelling devices swab, midstream urine, and blood, for the period from October 2012 to February 2013. Thereafter, all specimens were streaked on mannitol salt agar and Blood Agar. All plates were incubated aerobically for 24 h at 37°C. After the identity of the cultures was confirmed according to Bergey's manual including Gram's staining, catalase and coagulase test (slide and tube methods) [13]. MASTASTAPH-Latex kit, API Staph and VITEC 2 auto-system were employed for confirming the identification. All isolates were stored at -40°C in freezer vials containing 15% glycerol for further analysis [14].

### Antibiotic susceptibility test

Antibiotic susceptibility was investigated toward cefoxitin (30  $\mu$ g), oxacillin (1 $\mu$ g), methicillin (5 $\mu$ g), and cefotaxime (30  $\mu$ g) by modified Kirby-Bauer method [15]. Inocula were prepared by taking 3-5 colonies from fresh culture (that showed similar morphology) by loop and suspended in 4-5 ml of normal saline to obtain culture with  $1.5 \times 10^8$  CFU/ml by adjusting to turbidity standard of McFarland 0.5 that prepared.

A sterile cotton swab was dipped into the inoculums and then swabbed evenly across the surface of Muller-Hinton agar plate, after that within 15 minutes of inoculation, the antibiotic-containing disc (four to five discs for each plate) were applied to the agar with a sterile forceps pressed firmly to

ensure contact with the agar and then plate inverted and incubated at 37°C for 18-24 hours [14]. Inhibition zones were expressed in millimeters by an aid of a metric ruler [16].

### Statistical analysis

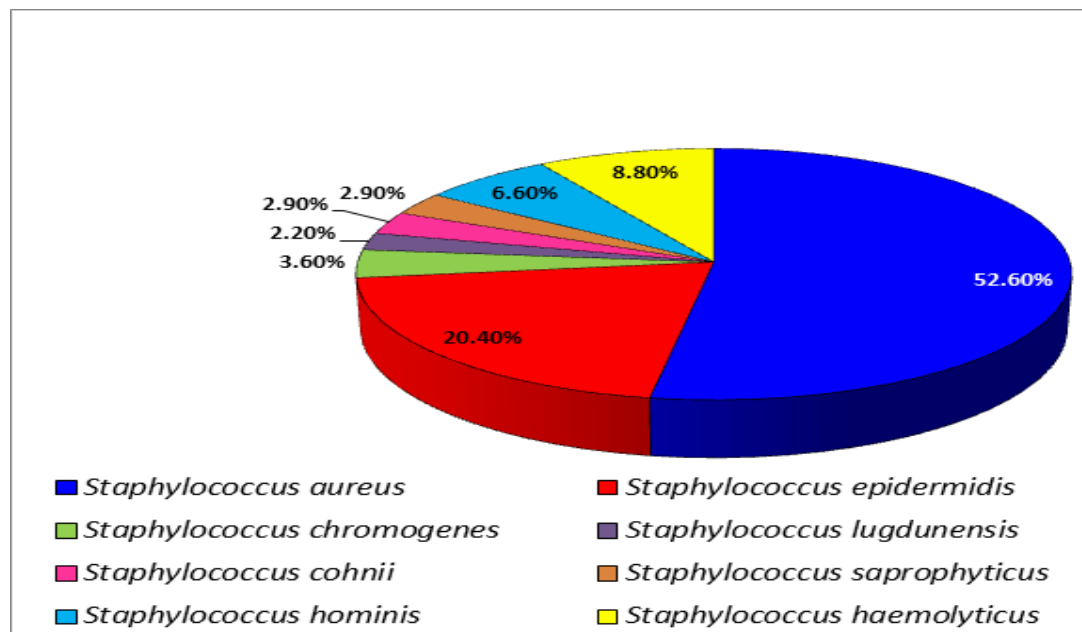
ANOVA test was adopted to compare the findings of present work.  $P < 0.05$  was considered significant.

### Results and Discussion

Only 137 isolates (91%) had the ability to grow on the Mannitol salt agar which considered selective and differential media for genus *Staphylococcus* [17]. The colonies appeared round, smooth, raised, mucoid and glistening. Consequently, the isolates belong to the genus *Staphylococcus*. Microscopic examination illustrated that all these isolates were gram positive cocci. What's more, all these isolates gave negative results to oxidase, positive results to catalase and resisted bacitracin (0.04 U). Moreover, 72 isolates were able to ferment mannitol while 65 isolates did not.

The present study findings revealed that all mannitol fermenters were coagulase positive, and DNase positive; consequently, they were considered as *S. aureus*; whereas the 65 non-mannitol fermenters were DNase negative, gamma haemolytic, sensitive to the Novobiocin (5 µg/disc) and free coagulase negative comprised *S. epidermidis* (n= 28), *S. chromogenes* (n= 5), *S. lugdunensis* (n= 3), *S. cohnii* (n= 4), *S. saprophyticus* (n= 4), *S. hominis* (n= 9), *S. haemolyticus* (n= 12). Nevertheless, *S. lugdunensis* developed positive result for the clumping factor, figure-1. MASTASTAPH-Latex kit, API Staph and VITEC 2 auto-system confirmed the results of identification.

Prevalence of *S. aureus* and coagulase negative staphylococci in accordance to the specimen type revealed that burn specimens achieved the highest number of isolates reached 36. However, the sputum specimen covered the lowest number of isolates reached 5 as it is summarized in table-1. Furthermore, the present work demonstrated that *S. aureus* and *S. epidermidis* were isolated with higher percentage from burn specimens than other specimens. Interestingly, the CONS other than *S. epidermidis* outnumbered *S. aureus* and *S. epidermidis* in sputum and indwelling devices specimens.



**Figure 1-** Isolation percentages of staphylococci (n= 137) isolated in the present study.

Various studies dealt with the isolation of staphylococci from clinical specimens. Babakir-Mina *et al.* (2012) [18] stated that *S. aureus* accounted for 22% of all patients in Sulaimania burn hospital, and constituted 36% from burn specimens.

The *S. epidermidis* in study of Eftekhar and Mirmohamadi (2009) [19] were mostly from blood (40%) followed by urine (14%), surgical wounds (14%), intravascular catheters (8%), exudates (8%) and other unknown sources (16%). But in a study conducted by Diamond-Hernandez *et al.* (2010) [20], 73.77% of CONS and 79.5% of *S. aureus* isolates were detected. Also in a study carried out by Gad *et al.* (2009) [21], out of 292 isolates of urine and catheter, 53 (18.2%) staphylococcal strains

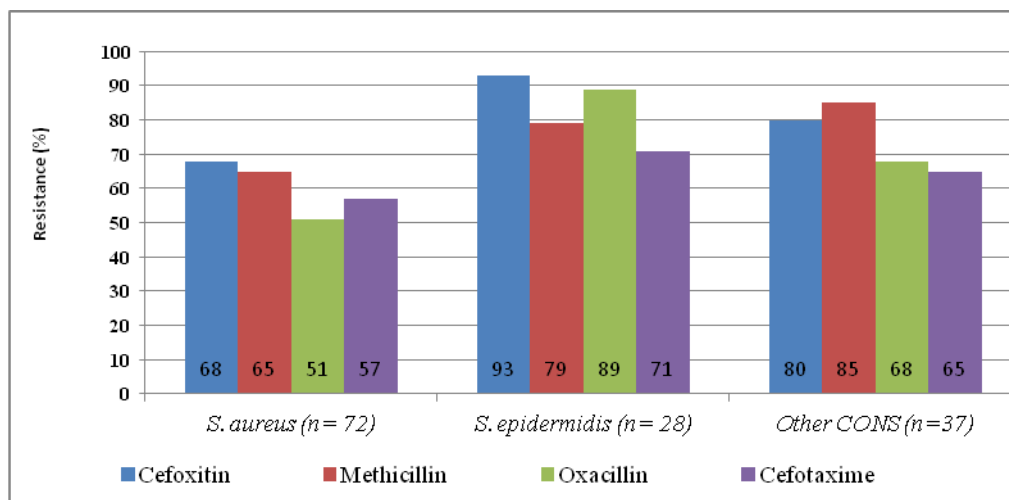
were identified (*S. aureus* represented 6.2% and *S. epidermidis* represented 12%). *S. aureus* in a study done by Vaez *et al.* (2011)[22] nearly 30.8%, 26.5%, 22.7%, 10.8 and 9.2% of them were isolated from urine, wound, blood, sputum, and other specimens such as abscess, respectively.

**Table 1-** Prevalence of staphylococci according to the source of specimen

Specimen type	<i>S. aureus</i> (%) n= 72	<i>S. epidermidis</i> (%) n= 28	Other CONS (%) n= 37
Burn	24 (33%)	8 (29%)	4 (11%)
Blood	15 (21%)	6 (21%)	9 (24%)
Catheter	7 (10%)	7 (25%)	3 (8%)
Indwelling Device	N.A	3 (11%)	5 (14%)
Sputum	N.A	1 (4%)	4 (11%)
Midstream urine	18 (25%)	2 (7%)	6 (16%)
Wound	8 (11%)	1 (4%)	6 (16%)
Total	72 (53%)	28 (20%)	37 (27%)

CONS= Coagulase negative staphylococci. P< 0.05

To investigate the distribution of methicillin resistance staphylococci among the patients, the antibiotic sensitivity test was applied to all 137 isolates that proved to belong to the genus *Staphylococcus*, the test was performed by cefoxitin (30µg/disc) disc diffusion method. Variable resistance to this antibiotic was observed among *Staphylococcus* sp. The results revealed that 68% (49/72), 93% (26/28), and 92% (34/37) of *S. aureus*, *S. epidermidis*, and other CONS isolates developed methicillin resistance respectively figure-2.



**Figure 2-** Antibiotic resistance of staphylococci. CONS= coagulase negative staphylococci.

Figure-2, also depicts that 65, 51, and 57% of *S. aureus* isolates were resistant to methicillin, oxacillin, and cefotaxime, respectively. In regard to *S. epidermidis*, 79, 89, and 71% of isolates were resistant to methicillin, oxacillin, and cefotaxime, respectively. Whereas CONS other than *S. epidermidis* showed resistance towards methicillin, oxacillin, and cefotaxime in 85, 68, and 65% of isolates, respectively.

Locally, depending on study of Al-Dahbi and Al-Mathkhury (2013) [23], the incidence of MRSA among *S. aureus* was 94.3%. Babakir-Mina *et al.* (2012) [18] found that among *S. aureus* positive cases, 88% were MRSA. Al-Hasani (2005) [24] indicated that 41/49 (83.7%) were MRSA and 30/37(81%) were found to be MRCONS. Abd-Elateef [25] reported that MRSE covered 84%.

From an international stand, our data are in the same range as Argentina and Mexico in Latin America [26]. Mean Prevalence of MRSA in Iran is moderately higher than Australia and lower than the United States [28, 27]. However, reports have shown that MRSA rates are declining in United States [29, 30]. Prevalence of MRSA in Europe is heterogeneous with average lower than other continents but Portugal seems to have a similar rate of MRSA to our country [31].

In a study accomplished in Korea, Song *et al.* (2001) [32] reported that the incidence of MRSA was 98% within a burn center. This is markedly higher than those reported from other countries.

Our results are close to other studies in Iran and some countries. In three separated studies in Iran, which reported by Japoni *et al.* (2004) [33], Ekrami *et al.* (2007) [34] and Mehdinejad *et al.* (2008) [35], the average prevalence of methicillin resistance *Staphylococci* in burn patients was between 60%-80%. Also a report about rate of MRSA in nosocomial infections in Isfahan, Iran showed that 67.2% of isolates were MRSA [36]. Different epidemiological factors such as geographical, health system capability in running infection control program has role in variability of prevalence of MRSA. Most isolates of MRSA were observed in wound specimens (43%), this study nearly is in conformity to our study.

In a study achieved by Habibi *et al.* (2008) [37], the prevalence of MRSA was 55% among all studied strains. Nevertheless, it was highest among specimens from respiratory tract (66.2%) and was lowest among isolates associated with urinary tract infections (2.9%). The prevalence of methicillin resistance was 20.6% and 5.9% among wound and blood infection isolates respectively. Considerable differences were observed when the distributions of MRSA isolates in different wards were compared. Almost 21% of the *S. aureus* isolates from intensive care units (ICUs) and 11.3% of the isolates from operation wards were MRSA, whereas only 1.4% of the isolates from emergency rooms were MRSA. Cefoxitin is a cephamycin antibiotic and has been described as an inducer of methicillin resistance [38]. The performance of cefoxitin either as a disc or as a supplement in agar medium for the detection of MRSA has been confirmed extensively [39-42].

By the results of Ekrami *et al.* (2007) [34] from the multiplex PCR assay were correlated very well with those from conventional disc diffusion susceptibility tests. Sensitivity in cefoxitin disc diffusion test was slightly better than oxacillin disc. Also in study of Velasco *et al.* (2005) [42] cefoxitin was the appropriate disc for detecting MRSA isolates. The addition of cefoxitin is principally aimed at inducing the expression of methicillin resistance and inhibiting the growth of methicillin-susceptible *S. aureus* (MSSA) [38].

Phenotypic techniques as disc diffusion and microdilution methods are employed in routine laboratories for the detection of methicillin resistance. However, these methods are often not entirely reliable at detecting some strains that harbor the *mecA* gene [42]. Identification of the *mecA* gene remains the most reliable method of detecting MRSA isolates, however not all laboratories can include molecular biology techniques in their routine clinical practice, because detection of methicillin resistance by disc diffusion method in study of Ghazal *et al.* (2011) [43] show in *S. aureus* strains cefoxitin disc diffusion tests was 100% sensitive for MRSA detection. Alternatively, only (76.47%) isolates were resistance to oxacillin disc, and (88.24%) to methicillin and all strains were resistance to cefoxitin disc (100%).

The results of the present work revealed variable methicillin resistance in respect to the type of specimen. In cultures of *S. aureus* and *S. epidermidis* from the Burn specimens had the highest resistance to methicillin. Burn infection seems to be more resistant to most other antibiotics compared to other sites too. Sputum seemed to have the lowest methicillin resistance percentage in comparison to other specimens. Moreover, CONS other than *S. epidermidis* isolated in higher percentage from blood specimens table-2.

**Table 2-** Prevalence of methicillin resistance staphylococci according to type of specimen

Occupation	<i>S. aureus</i>	<i>S. epidermidis</i>	Other CONS
<b>Burn</b>	14 (29%)	8 (31%)	4 (12%)
<b>Blood culture</b>	13 (27%)	4 (15%)	9 (26%)
<b>Catheter</b>	7 (14%)	7 (27%)	3 (9%)
<b>Indwelling devices</b>	N.A	3 (12%)	4 (12%)
<b>Sputum</b>	N.A	1 (4%)	4 (12%)
<b>Midstream urine</b>	10 (20%)	2 (8%)	5 (15%)
<b>Wound</b>	5 (10%)	1 (4%)	5 (15%)
<b>Total</b>	49	26	34

CONS= coagulase negative staphylococci

**Conclusions :-**

*Staphylococcus aureus* (52.6%) outnumbered *Staphylococcus epidermidis* (20.40%) and other CONS (27%) isolates. Furthermore, *S. aureus* (33%) and *S. epidermidis* (29%) were frequently isolated from Burn specimens than other specimens; however, other CONS were highly recovered from blood (24%) specimens. Screening for Methicillin resistance by Cefoxitin D.D. test revealed that 68%, 93%, and 80% of *S. aureus*, *S. epidermidis*, and CONS isolates developed Methicillin resistance.

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