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Prevalence of methicillin resistance *Staphylococcus aureus* (MRSA) and methicillin sensitivity *Staphylococcus aureus* (MSSA) among hospitalized Iraqi patients.

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Abstract

Background: *Staphylococcus aureus* is an important nosocomial pathogen worldwide, with two major classes; Methicillin resistant *S. aureus* (MRSA) and Methicillin sensitive *S. aureus* (MSSA).
Aim: To compare the distribution frequency and antimicrobial sensitivity of MRSA and MSSA *S. aureus* isolates in different clinical specimen from hospitalized Iraqi patients.
Materials and methods: *S. aureus* isolates from clinical specimens were investigated in 203 hospitalizes patients with wide range of ages during the period from February to May 2017. API and Vitek were used for identification and a panel of antibiotics was used to define the antimicrobial sensitivity of the isolates.
Results: The highest *S. aureus* isolates were from burn swab (35%), followed by urine specimen and blood samples with (30 and 26% respectively). MSSA isolates represents (57.5%) of the total and the rest was MRSA isolates (42.5%). MRSA isolates was higher in burns and wound specimens (485 and 13% respectively) whereas the MSSA isolates were higher in blood, urine and ear specimens (29%, 38% and 3.5% respectively). MRSA were multidrug resistance to 7 antibiotics in comparison to MSSA (only two antibiotics).
Conclusion: MSSA isolate are more common than MRSA in clinical specimens with variable proportions in different clinical specimens. Multidrug resistance was more evident among the MRSA than MSSA.

Keywords: MRSA, MSSA, Antimicrobial test.

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Introduction

Staphylococcus aureus is pathogenic bacteria involved in serious infections which leading to a significant mortality and morbidity in children and adults [1,2]. *Staphylococcus aureus* is classified into two classes known as Methicllin Resistant *S. aureus* (MRSA) and Methicillin Sensitive *S. aureus* (MSSA), and both associated with the nosocomial infections [3,4]. They cause a wide spectrum of diseases (especially in patients with compromised immune systems) as skin infections, urinary tract infections, respiratory tract infection, burn and wounds infections, intravenous catheters, and others [5,6]. Such pathogenicity is augmented by the existence of colonization factor and numerous other virulence factors in these bacteria [7-9].

Both MRSA and MSSA had acquired many genes that are responsible for resistance to methicillin and other beta-lactam antibiotics [10]. Such feature turns the bacteria into an important nosocomial pathogen worldwide resistant to many common antibiotics and difficult to be treated [11-13].

Subjects, Materials and Methods

Subjects

203 patients with wide range of ages attending Al-Kindy Teaching Hospital during the period from February to May, 2017 were included in the study.

Isolation and identification

S. aureus were isolated by primary culture on mannitol salt agar plates (Oxoid) that were incubated for 24 hrs at 37°C and examined for the growth of bacteria and fermentation of mannitol by means of production of yellow colonies [14]. Identification was based on API and Vitek system.

Biochemical test

Catalase, Coagulase, DNase, gelatin liquefaction, mannitol fermentation, nitrate reduction, clumping factor, oxidase, protease, urease and β-Hemolysis were used in the biochemical tests.

Detection of MRSA

In a 0.5 equivalent McFarland standard, bacterial isolate suspension was made and lawn culture done on Muller-Hinton agar (MHA) plate. Cefoxitin disc (30 µg) [15] was placed on the culture surface and the plates were incubated at 37°C for 18 h. Growth inhibition zone diameters was measured (≤ 9 mm was reported as resistant whilst ≥ 14 mm was considered as sensitive).

Antibiotic susceptibility test

S. aureus isolates were tested for their susceptibility to antimicrobial agents by Kirby-Bauer method on MHA (Hi-media) [16]. Plates were incubated at 37°C for 18 h. Following the incubation, the diameter of inhibition zone was measured according to the criteria recommended by Clinical and Laboratory Standards Institute [17]. The tested antibiotics in this study were as follows: Cefoxitin (FOX: 30 µg), Ciprofloxacin (CIP: 5 µg), Imipenem (IMP: 10 µg), Penicillin (P: 10 IU), Ceftriaxone (CRO: 30 µg), Erythromycin (E: 15µg), Gentamycin (GN: 10 µg), Methicillin (10µg) and tetracycline (TE: 30µg).

Statistical analysis

Data were statistically analyzed using Statistical Analysis System-version 9.1 (SAS) basing on Two-Way Analysis of Variance (ANOVA). The significant level of p-value ($P<0.05$) was considered for indicating the significance point.

Results and Discussion

Table 1 indicates that S. aureus isolates were with a high frequency from burn swab (35%), followed by urine specimen and blood samples with (% and 26% respectively), but with a low frequency from both wound and ear swab (7% and 2% respectively). These results correspond to those of Obajuluwa et al. [18] as they reported that 24.6% of the bacterial isolates in wound infection were caused by S aureus. The same was noticed with Srinivasan et al. [19] as they found that the high frequency (33%). of S. aureus isolates was from wounds and burns. On the contrary, Japoni et al. [20] found that S. aureus isolates were reported higher from urine samples (30.6%) and lower from both blood samples and deep wound (14, 13.5% respectively) [20,21]. Japoni et al. [20] found that the highest frequency of S. aureus isolates were in wound and burn infections (54.17%), followed by eye infections and UTIs as (20.8, 16.7% respectively), then ear infection (8.3%). While the results of Nada et al. [21] and Pirko et al. [22] study showed that the highest bacterial isolates were from UTIs infection (42%), whilst results of Sherwal et al. [23] showed that the highest rate of S. aureus isolates were from eyes infections (20.8%) (Figure 1).

Table 1. Number and percent of S. aureus and other bacteria isolated from clinical specimen.

Clinical specimen	Positive isolates				Negative isolates		Total		*P value
	S aureus		Other bacteria						
	No.	%	No.	%	No.	%	No.	%	
Burn	19	35	16	16	10	20.5	45	22	S
Urine	16	30	26	26	17	35	59	29	NS
Blood	14	26	19	19	4	8	37	18	S
Wound	4	7	29	29	13	26.5	46	23	HS
Ear swab	1	2	10	10	5	10	16	8	HS
Total	54	27	100	49	49	24	203	100	NS

*S= Significant, NS= Non-Significant, HS= Highly Significant

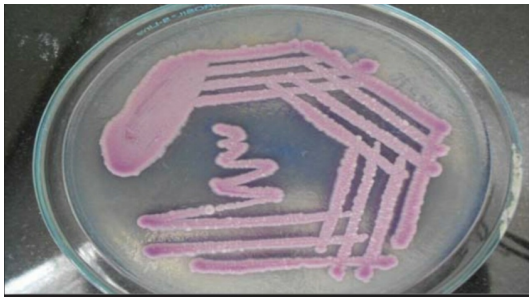

Figure 1. *MRSA on chromogen agar.*

According to results of API Staph, this system can be used for the diagnosis and identification of *S. aureus* isolates (Figure 2). The API Staph system is a rapid and simple method which used for identification of S aureus, also for differentiation between *Staphylococcus, Kocuria* and *Micrococcus.*

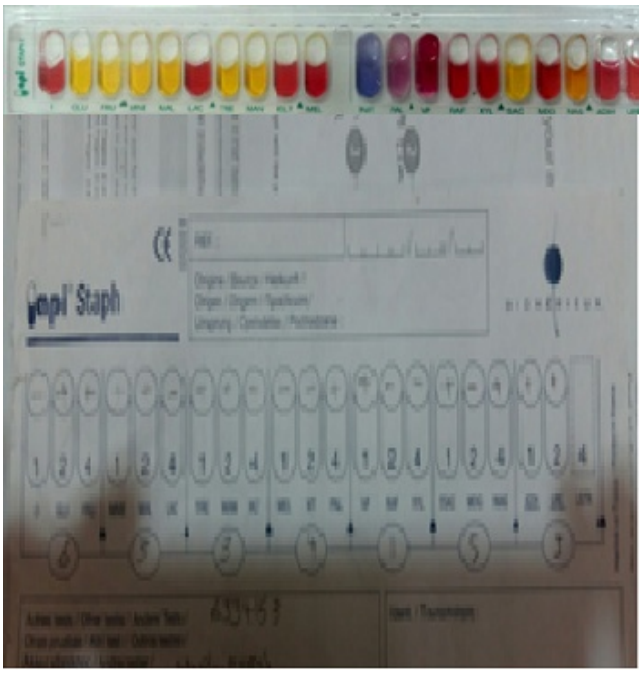

Figure 2. *Results of API staph system of S. aureus (code number 6334 153).*

Table 2 demonstrated the frequency of MRSA and MSSA isolates from different clinical specimens. The percentage of total MSSA isolates was (57.5%) compared to MRSA (42.5%). MRSA isolates were higher than MRSA in burns (48% vs. 26%) and wounds specimens (13% vs. 3.5%), whereas MSSA isolates where higher than MRSA in urine (38% vs. 17%), blood (29% vs. 22%) and ear specimens (1% vs. 0%). Current results were fully incompatible with the results of Peck et al. [24] and Al-Alem [25] who showed that only (48.6%) of isolates respectively MSSA. The same was true with Al-Hasani [26] and Al-Geobory [27] who found a higher frequency of the MRSA isolates among clinical specimens (83.7% and 90.9% respectively). These differences may due to the different collection site of isolates, the sources of clinical specimens, genetic background and variations in geographic area [28].

Concerning the high frequency of isolates in burns, it could be due to the skin damage as skin represent first line (protective barrier) of defense in immune system and when damaged by burn, the inner layers of skin would exposed to air, increasing the contact with the opportunity pathogenic bacteria as S. aureus and causing infections [22,29,30].

Table 2. *Distribution of MRSA and MSSA in clinical specimens.*

Clinical specimen	MRSA		MSSA		Total		*P value
	No.	%	No.	%	No.	%	
Burn	11	48	8	26	19	35	S
Blood	5	22	9	29	14	26	NS
Urine	4	17	12	38	16	30	HS
Wound	3	13	1	3.5	4	7	HS
Ear swab	0	0	1	3.5	1	2	HS
Total	23	42.5	31	57.5	54	100	S

*S= Significant, NS= Non-Significant, HS= Highly Significant

Biochemical tests include positive results as (100%) for DNase, gelatin liquefaction, mannitol fermentation, nitrate reduction, clumping factor, protease, and positive results as (90%) for β-hemolysis, whilst negative results as (100%) for oxidase and (77%) for coagulase (Table 3).

Table 3. *Biochemical test of MRSA and MSSA.*

Biochemical test	Results (%)
Catalase	+(100)
Co-agulase	-(77)
DNase	+(100)
Gelatin liquefaction	+(100)
Mannitol fermentation	+(100)
Nitrate reduction	+(100)
Clumping factor	+(100)
Oxidase	-(100)
Protease	+(100)
Urease	+(100)
ß-Hemolysis	+(90)

As in Table 4, MRSA isolates had exhibited a 100% resistance to both methicillin and penicillin, followed by 89%, 86%, and 85% to ceftriaxon, cefoxitin and tetracycline respectively. For MSSA isolates, they exhibited resistance to both methicillin and penicillin (75% and 70% respectively) and 100% sensitivity to ciprofloxacin, gentamycin and imipenem. These results indicate that MRSA isolates were multidrug resistance (7 antibiotics) compare to MSSA isolates which were resistance to only two antibiotics. Results of Al-Geobory [27],

Al-Saadi et al. [31], Tektook et al. [12] and Tektook et al. [32] studies showed similar results.

Table 4. Resistance of MRSA and MSSA towards antibiotic panel.

Antibiotics	MRSA			MSSA		
	*R (%)	I (%)	S (%)	R (%)	I (%)	S (%)
Cefoxitin	86	0	14	10	0	90
Ceftriaxon	89	0	11	0	26	74
Ciprofloxacin	70	25	5	0	0	100
Erythromycin	84	0	16	10	0	90
Gentamycin	79	0	21	0	0	100
Imipenem	11	15	74	0	0	100
Methicillin	100	0	0	75	0	25
Penicillin	100	0	0	70	0	30
Tetracycline	85	0	15	10	0	90
No of antibiotics resistance	7	–	–	2	–	–

*R= Resistance, I= Intermediate, S= Sensitive.

Conclusion

The highest *S. aureus* isolates was obtained from burn swab followed by urine specimen and blood samples. The MSSA isolates were more frequent than MRSA. All MRSA isolates have high resistance to both methicillin and penicillin compare to MSSA isolates, so MRSA were multidrug resistance compare to MSSA.

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