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Molecular Study of Methicillin Resistant *Staphylococcus aureus* Isolated from Different Hospitals in Najaf-Iraq

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Abstract
Aim of Study: *The aim is to do molecular characterization of Staphylococcus aureus isolated from hospital staff and environment, in order to determine the gene(s) that is (are) responsible for antibiotics resistance especially mecA and SCCmec.*
Methodology: *This study that aimed to detect methicillin resistant Staphylococcus aureus in isolates from Al-Sader Teaching Hospital and al-Hakeem General Hospital in Annajaf during the period from November 2015 to April 2016. From a total of 250 clinical samples that were collected to isolate Staphylococcus aureus from the hospital staff (nurses, doctors, workers, medical student ---etc.), and also from different parts of hospital environments. A total of 50 Staphylococcus aureus isolate were detected and confirmed by different morphological and biochemical confirmatory test.*
Results: *These 50 isolates then studied by VITEC2 and also by cefoxitin disc susceptibility test .It was able to detect only 41 isolates that had cefoxitin resistance which represented 82% of the total Staphylococcus isolates which are regarded as Methicillin Resistant Staphylococcus aureus phenotypically. Polymerase chain reaction test was done to find out the genes that are responsible for methicillin resistance in these isolates, using three types of primers one was for Sa442 gene which was designed to confirm that the isolates were Staphylococcus aureus and two other genes to confirm that the isolates were methicillin resistant which were mecA and SCCmec with the different subtypes of the last gene, and it was found that from the 41 isolates which were phenotypically confirimed to be methicillin resistant only 32 were found to harbor these genes which represented only 78% of the phenotypically confirmed MRSA; It was found also that the subtype SCCmec type IV was the most dominant gene among SCCmec types in these isolates as it was detected in 23 out of 32 isolates (71.9%), followed by SCCmec type III Which was found in 5(15.6%) of the isolates.*
Conclusions: *Polymerase chain reaction is the golden slandered for identification of methicillin resistant Staphylococcus aureus MRSA. The SCC mec type IV was the most dominant among SCCmec genotypes of MRSA strains that were isolated from hospitals in the present study.*
Recommandation: *Vancomycin resistant staphylococcus aureas (VRSA) should be taken in consideration when we are working with hospital cross infection. Further studies on large scale should be performed in order to study MRSA in the whole country. Antibiotic prescribing policy should be put under observation and guidenece inside and outside hospitals.*
Keywords: *staphylococcus aureus, cefoxitin, SCCmec, mecA.*

INTRODUCTION

Staphylococcus aureus is an important pathogen in causing many diseases like pulmonary infections. Septicemia, cutaneous diseases, bones and heart, in addition to other diseases caused by it's toxins as food poisoning; and shocks[1]. *Staphylococcus aureus* is also a leading cause of nosocomial infections. This bacteria acquires resistance to many antibacterial agents that cause it's therapy difficult. Especially methicillin-resistant *Staphylococcus aureus* (MRSA) which become a troublesome bacteria for huge number of infections, the hospital acquire one is the most important [2].

Methicillin-susceptible *Staphylococcus aureus* (MSSA) acquires this phenomenon by gaining some additional gene elements inserted into it's genome. This element is called staphylococcal chromosomes casset *SCCmec*, which is composed of the, *mecA*. [3]. Certain situations are blamed in the acquisition of MRSA in hospitals like the antibiotics wide use, and many procedures and ecological factors. In man, colonization of *Staphylococcus aureus* is appeared in the nasal passages. Nasal passage acquisition of *Staphylococcus aureus* in hospital personnel gives a risk of infection in patients admitted to hospitals. and elimination of this risk factor is found to reduce in the incidence of *Staphylococcus aureus* infections [4].

MATERIALS AND METHODS

The study had been done in accordance with recommendation and ethical guidelines of the College of Medicine, Kufa University.

The present study was done without involving any biological materials or genetically modified organisms.

A total of 250 clinical samples were taken from both the hospital staff (50 samples) and the hospitals environments (Al-Hakeem and Al-Sader) (200samples).

The staff samples were taken from the anterior nares of persons who were working in different departments of the hospitals. The hospital environmental samples were taken from: hospital air current (80 samples), hospital surfaces(like door handles, floor, beds, 35 samples), hospital instruments like medical equipments, surgical theater lamp(lights), stethoscopes(85 samples.)

From the 50 hospital staff samples 11 *Staphylococcus aureus* isolates could be detected, while from the 35 hospital surface samples, 8 *Staphylococcus aureus* isolates were detected, and from the 80 air current samples of hospital 17 *Staphylococcus aureus* isolates were detected, while there were 14 *Staphylococcus aureus* isolates detected from the 85 hospital instrument samples .

All isolates then were confirmed by biochemical and enzymatic confirmative tests and finally by VITEC2 tests then subjected for PCR study.

RESULT AND DISCUSSION

Phenotypic Characterization of *Staphylococcus aureus*

Table (1) Number of *Staphylococcus aureus* isolates according to their source in the hospitals.

Type of sample	Number of samples	Number and percentage of *staphylococcus aureus*
Hospital staff nasal swabs	50	11(22%)
Hospital Air Current	80	17(21%)
Hospital surfaces	35	8(22%)
Hospital instruments	85	14(16%)
Total	250	50(20%)

From Table (1) we can see the distribution of *staphylococcus aureus* isolates according to their source in the hospital and we can find that the hospital air current and the hospital instrument represented the highest source of *Staphylococcus aureus* isolation followed by hospital- staff nasal swabs and the test source of isolation was the hospital surface.

Detection of methicillin resistant *Staphylococcus aureus.*

Cefoxitin (fox) 30 mg discs were used for detection of methicillin resistance *staphylococcus aureus* MRSA (Table2), because cefoxitin is regarded as a potent inducer for the regulatory system covering the mec A gene [(5)].

Table (2) Methicillin resistant *Staphylococcus aureus* according to their clinical source by cefoxitin susceptibility test.

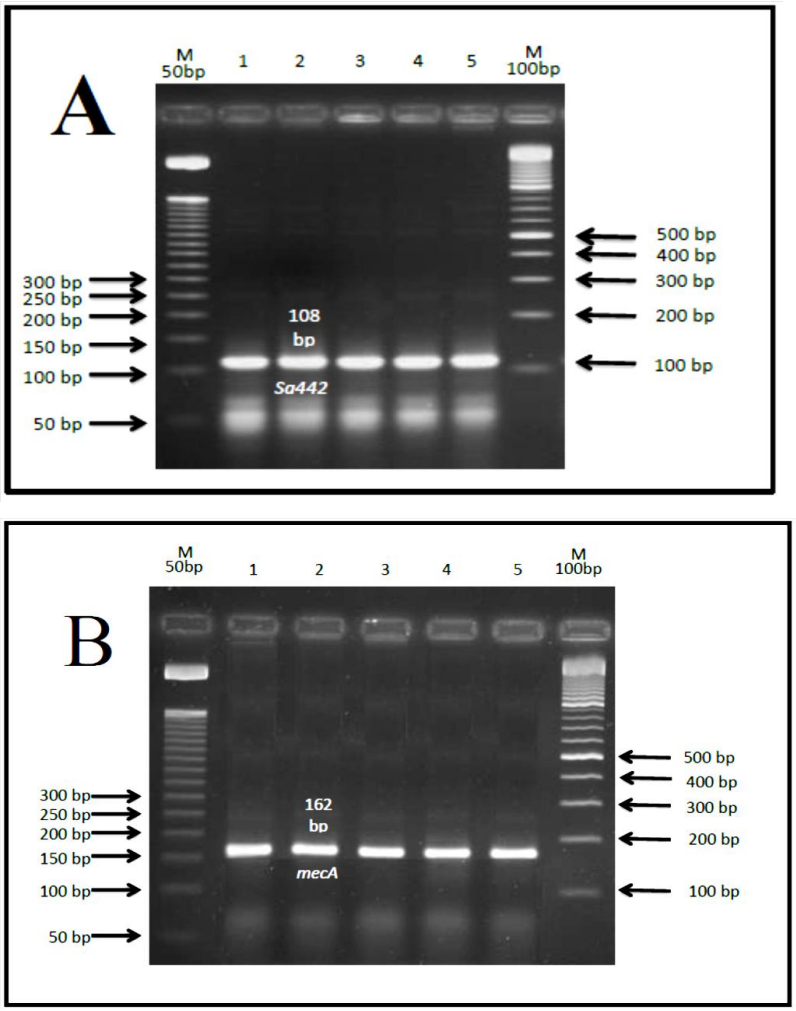
Clinical isolate	Number	Number of MARSA	Percent
Hospital staff nasal swabs	11	8	72.727%
Hospital air current	17	13	76.47%
Hospital surfaces	8	8	100%
Hospital instruments	14	12	85.71%
Total	50	41	82%

Susceptibility to cefoxitin 30 mg disc was determined on Muller-Hinton agar at 37°C using low inoculum. The cefoxitin 30 mg disc showed 100% specificity for detection of MRSA. Interpretive criteria were:- zone diameter <27 mm =MRSA [(5).]

Table (3) Antibiotic susceptibility test for MRSA isolates.

Antibiotic	Resistance (R)	Intermediate (F)	Sensitive(S)	Total
Penicillin	41(100%)	0(0%)	0(0%)	41
Oxacellin	40(97.5%)	1(2.4%)	0(0%)	41
Erythromycin	38(92.6%)	2(4.8%)	1(2.4%)	41
Clindamycin	12(92.2%)	5(12.1%)	24(58.5%)	41
Kanamycin	8(0.19%)	3(0.07)	30(73.1%)	41
Vancomycin	1(2.4%)	0(0%)	40(97.5%)	41
Azithromycin	35(85.3%)	4(0.09%)	7(0.17%)	41
Chloramphenical	10(24.3%)	4(0.09%)	27(65.8%)	41
Tetracyclin	9(21.9%)	5(21.1%)	27(65.8%)	41
Cefoxitin	41(100%)	0(0%)	0(0%)	41

From table (3) It was confirmed that all MRSA isolates were found to be resistant to cefoxitin (100%) oxacillin (97.5%), penicillin G(100%), but also expressed high degrees of resistance to macrolids (85.3%) , clindamycin (92.2%), tetracyclin (21.2%) .



Figure(1): Conventional PCR results showing genotypic identification of MRSA isolates. A: MRSA with *Staphylococcus aureus* genotypic confirmation (*Sa442* gene); B: MRSA with methicillin resistance detection (*mecA* gene). Lanes 1-5: MRSA isolates demonstrating *Sa442* gene (A); Lanes 1-5: MRSA isolates demonstrating *mecA* gene (B); A and B: Lanes M 50bp: 50bp DNA steps ladder; Lanes M 100bp: 100bp DNA ladder; PCR products analyzed on 3% agarose gel at 80 V for 1 hours.

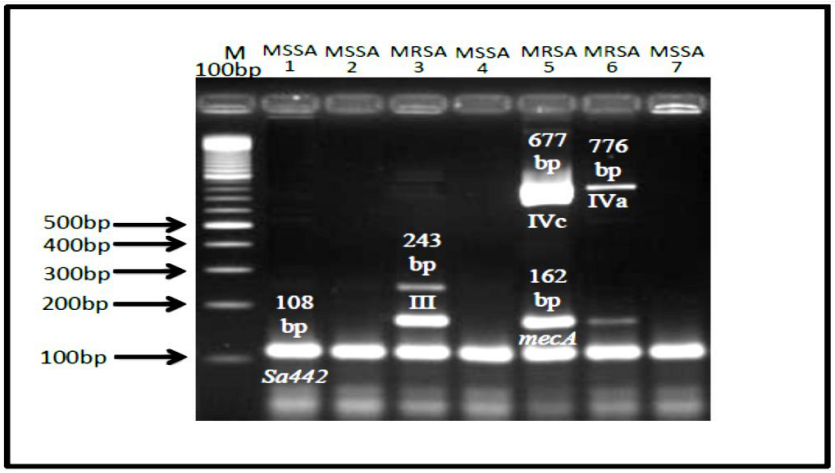


Figure (2): Multiplex PCR results showing simultaneous genotypic discrimination of MRSA from MSSA isolates. Lanes 3,5,6: MRSA isolates showing a certain *SCCmec* type and detected *mecA* gene.; Lanes 1,2,4,7: MSSA isolates showing only *Sa442* gene. Lanes M 100bp: 100bp DNA ladder; PCR products analyzed on 3% agarose gel at 70 V for 1.30 hours.

Table (4). Characteristics and distribution of *SCCmec* types and subtypes in MRSA isolates.

SCCmec types	HA or CA-MRSA	Length size (bp)	MRSA Isolates N=32 No (%)
SCCmec type I	Not detect	613	-
SCCmec type II	HA-MRSA	287	-
SCCmec type III	HA-MRSA	243	5 (15.6)
SCCmec type IV	CA-MRSA	-	23 (71.9)
SCCmec IV subtypes			
SCCmec type IVa	CA-MRSA	776	19 (59.4)
SCCmec type IVb	Not detect	1000	-
SCCmec type IVc	CA-MRSA	677	2 (6.3)
SCCmec type IVd	Not detect	1242	-
SCCmec type IVh	CA-MRSA	663	2 (6.3)
SCCmec type V	CA-MRSA	325	3 (9.4)
Multibands *SCCmec* type			
Multibands *SCCmec* type II and V	Unidentified	287+325	1 (3.1)
Total	32		

From tabel (4) the types SCCmec I,II,III,IV,V were type I is not detect and suptype IV is(Iva,IVb,IVc,IVd,IVh) were IVb and IVd not detect.

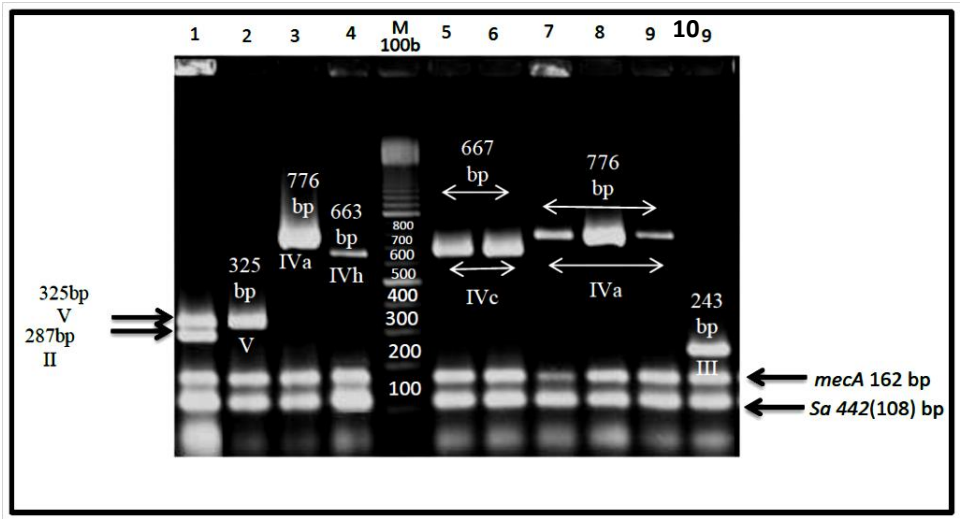


Figure (3): Multiplex PCR results identifying types and IV subtypes of *SCCmec* element with simultaneous detection of *Sa442* and *mecA* genes of MRSA isolates. Type III: Lanes 10; Type IVa: lanes 7-9 and 2; Type IVc: lanes 5-6; Type IVh: lane 4, Type V: lane 2; Multiband *SCCmec* type II and V; Lane M100bp: 100bp DNA ladder; PCR products analyzed on 3% agarose gel at 70 V for 1.30 hours.

Discussion

Evaluation of antibiotic susceptibility test: MRSA isolated is Known to have a remarkable genetic versatility, which allows for the adaptation to the present of antibiotics, such that many isolated can be multiresistant to several classes of antibiotics [6].

In the present study one aim is to find out the resistant profile of the MRSA isolates.

From table (3) It was confirmed that all MRSA isolates were found to be resistant to cefoxitin (100%) oxacillin (97.5%), penicillin G(100%), but also expressed high degrees of resistance to macrolids (85.3%) , clindamycin (92.2%), tetracyclin (21.2%) .

As a result of erythromycin and Azithromycin widespread use for treatment of *Staphylococcal* infections in Najaf province and in whole of Iraq , the level of resistance in MRSA isolates was high in this study.

Vancomycin is almost universally accepted as the drug choice for treatment of MRSA infection [7].According to this, all *Staphylococcus aureus* isolates with Vancomycin resistance should be reported to public health and infection control committee immediately.

The first clinical Vancomycin resistant *Staphylococcus aureuas* was detected from a dialysis patient in USA in 2002[8].

The possible emergence of VRSA may be due to a building of selective pressure of vancomycin [9].

There are about three reports of emergence of VRSA from Iraq [10,11]. and one report in Najaf

Genotypic characterization of Methicillin resistant *Staphylococcus aureus.*

In the current study, *Sa442* gene detection was done in all MRSA isolates for confirmation of *Staphylococcus aureus* genotype, which is concerned as unique to *Staphylococcus aureus* [12]. All biochemically detected MRSA isolates were also evaluated for the presence *mecA* gene (determinant of methicillin resistant) in order to certify the MRSA identification [13&14].

The results of present single target PCR for *Sa442* and *mecA* genes revealed the expected size of fragment band; 108bp (Fig.1.A) and 162bp (Fig.1.B) respectively. The *Sa442* PCR results showed that all the 41isolates were positive for *Sa442* gene with 100% concordant with Vitec2 results, giving rise to documentation that all isolates were *Staphylococcus aureus*. While *mecA* PCR results found 32 out of 41 (78%) of biochemically detected MRSA isolates were positive for *mecA* gene and certified as MRSA and the 9 remaining isolates (22%) were *mecA*-negative and identified as Methicillin Sensitive *Staphylococcus aureus* (MSSA). This confirmed the ability of *mecA* PCR detection in accurate differentiating MRSA from MSSA isolates [13&14]. found that 47.4% and 71% of clinical isolates from An Najaf hospitals were MRSA and MSSA respectively depending on *mecA* PCR detection.

Molecular typing of MRSA- *SCCmec* element

Among 41 isolates, 32 MRSA isolates were *mecA*-positive with certain type or subtype of *SCCmec* with exception of one was multiband. While the remaining 9 MSSA isolates were *mecA*-negative and positive for *Sa442* gene without any *SCCmec* typing Fig.(2). The *mecA* and *Sa442* genes results were 100% concordant with that obtained by conventional PCR results, demonstrating the simultaneous ability of the current M-PCR to discriminate MRSA from MSSA isolates. Therefore, M-PCR assay included the *mecA* target may play a scientific role in this differentiation of MRSA from MSSA[13]. target may play a scientific role in this differentiation of MRSA from MSSA[13].

The majority of MRSA isolates (71.9%) carried *SCCmec* type IV which is regarded as community-acquired MRSA (CA-MRSA) that showed highest *SCCmec* distribution compared to the commonly isolated hospital-acquired strains (HA-MRSA) *SCCmec* types III (15.6%), while CA-MRSA type V was (9.4%) and only one isolate (3.1%) was not-type able with multiple bands for types II and V, at the same time HA-MRSA *SCCmec* types I and II were not detectable (Fig 3).

Accordingly, the present results revealed that the most current isolates are CA-MRSA (type IV and V) with predominance of *SCCmec* type IV in consistent with that previously reported in Najaf province in a study done by Al-Hassnawi et al,[21] who found that most of his clinical isolates (100%) from Najaf hospital are *SCCmec* type IV. Moreover, another study conducted in Hila city demonstrated that 95.8% of clinical MRSA isolates were CA-MRSA *SCCmec* type IV [15] but another research detected this type was less frequently isolated (15.8%) in Baghdad hosptials[16]. The two later studies did not find types I-III of *SCCmec* element among their isolates in contrast to study done by Sabri et al (2013) who observed that 36.4% of his MRSA isolates were *SCCmec* type III. Their rate in India and Philippines found to be much lower than other East Asian countries [17&18]. In fact, the variation in the prevalence of *SCCmec* types among Iraqi studies may be related to differences in the way of collecting the samples, or to hospital or community population involved as well as the difference in power and ability of PCR assays used in detection of *SCCmec* types. However, the increased *SCCmec* type IV incidence was documented worldwide and become common among hospital MRSA isolates. Among nosocomial bloodstream MRSA gathered in Brazil, 95% of MRSA isolates were *SCCmec* type IV recorded very high frequency [19]. and Chen et al, [20]. reported that 40% of MRSA harbored *SCCmec* type IV in Twain. It is recently demonstrated that CA-MRSA has emerged within hospitals in many places of the world causing nosocomial infections.

According to the all current data obtained, the present study successfully identified the subtypes of *SCCmec* type IV which demonstrated that the IVa subtype was the commonest among them and detected the presence of *SCCmec* type IVh giving rise to the possibility of nosocomial EMRSA-15 emergence in our country. Regarding the fact that the most common MRSA strains in hospitals may become CA-MRSA strains in replacing of HA-MRSA strains[21]. the rapid and reliable molecular typing method of *SCCmec* element involving most known types and subtypes with simultaneous methicillin resistance and *Staphylococcus aureus* genotypic identification as single M-PCR assay done in the present study should be applied. In order to be implemented in the rapid and precise detection of MRSA stains that assisted in the successful treatment, epidemiological identification and suppression of CA-MRSA strains outbreaks to the hospital.

Conclusions

Polymerase chain reaction is the golden slandered for identification of methicillin resistant *Staphylococcus aureus* MRSA. The *SCC mec* type IV was the most dominant among *SCCmec* genotypes of MRSA strains that were isolated from hospitals in the present study.

Recommendations

Vancomycin resistant staphylococcus aureas (VRSA) should be taken in consideration when we are working with hospital cross infection.

Further studies on large scale should be performed in order to study MRSA in the whole country.

Antibiotic prescribing policy should be put under observation and guidenece inside and outside hospitals.

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