DHR International Journal Of Biomedical and Life Sciences (DHR-IJBLS)
ISSN 2278-8301, Vol.5(3), 2014
Available online *http://www.doublehelixresearch.com/DHRIJBLS*

Original Article

Detection of methicillin resistant *Staphylococcus aureus* (MRSA) in the surgical wards by Chromogenic agar medium and PCR assay

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Abstract

In order to have an evaluation and comparison of the chromogenic HiCromeMeReSa medium, (FOX, OX and ME) DD test and PCR assay for detection of methicillin resistant *Staphylococcus aureus* (MRSA) in the surgical wards, 296 samples (from 232 patients and 64 hospital environment items) were collected and examined. 192 isolates (64.8%) were identified as *S. aureus* 150 (64.6%) from patients and 42(65.6%) from environment. Among the 192 *S. aureus* isolates, 126 (65.6%) were MRSA of which 100 (66.6%) and 26 (61.9%) from the patients and environment, respectively. Also the prevalence of MRSA among all the samples was 42.5% (126/296) and 22.3% (66/296) of MSSA. The sensitivity, specificity, positive predictive value PPV and negative predictive value NPV between direct culture on HiCrome medium and DD test were 94, 100, 100 and 95.6%, respectively in the patients screening samples. While in the environment were 84.6, 100, 100 and 90.4%, respectively. Patient's samples demonstrated higher sensitivity and NPV than environment samples but the same rate in specificity and PPV. In contrast, no observed differences between results from subculture on HiCrome medium and (OX, FOX and ME) DD test whichagreed in their sensitivity, specificity, PPV and NPV. All MRSA isolates were multidrug resistant (MDR) and they were highly resistant (100%) to Beta-lactam antibiotics which used in this study. The study also revealed that High prevalence of MRSA among the male (70%), and the Maximum MRSA strains were found among the age group 21-41 years (36%). Also the rate of MRSA nasal carriage among patients with previously chronic diseases was higher (23.2%) than that in non-previously chronic diseases (19.8%). High rate (26.7%) of MRSA nasal carriage was observed among patients with previous use of antibiotics against in non-previous use (16.3%). High sensitivity 100% and high PPV 100% of PCR technique were demonstrated .The detection of mecA gene by PCR assay in all MRSA isolates that were

detected by phenotypic tests (HiCromeMeReSa, DD tests for FOX, OX and ME) indicated that these tests were reliable and accurate tests for the detection of MRSA isolates.

Key words: Methicillin resistant Staphylococcus aureus (MRSA), surgical wards, Chromogenic HiCromeMeReSa medium, mecA gene, nasal swab.

Introduction

After the introduction of methicillin in 1959, methicillin-resistant *Staphylococcus aureus* (MRSA) has quickly and widely emerged as a major nosocomial burden worldwide[1]. MRSA has become a major problem worldwide.It is able to cause a wide variety of different diseases, ranging from superficial skin inflammation to severe invasive infections such as bacteremia which leads to endocarditis and osteomyelitis[**2**]**.**MRSA is typically resistant to classes of antimicrobials other than β-lactams, therefore, it is an example of antimicrobial resistant (AMR) organism. MRSA has been recognized by the World Health Organization (WHO) as one of the top three threats to human health[3]. The resistance of Methicillin in s. aureus is caused by the mecAgene which is embedded within a mobile staphylococcal cassette chromosome SCC element known as SCCmec. It encodes an altered penicillin-binding protein 2a (PBP2a) with a low affinity for beta-lactam antibiotics such as penicillin, methicillin, and cephalosporins. The mecA gene allows the synthesis of cell wall in MRSA isolates regardless of the presence of β-lactam antibiotics[4,5]. The anterior nares are the most common site for MRSA carriage; which increases a patient's risk for developing a health care-associated infection with this bacterium especially after the surgery and in peritoneal dialysis[6]. Preoperative screening for nasal carriage and subsequent treatment of carriers reduces the risk for the development of hospital-acquired-MRSA infections[7]. Consequently, the mean duration of hospital stay and the costs are reduced in treated carriers[8]. Active surveillance for MRSA involves direct or non direct culturing the nares of patients at the time of admission or during hospitalization. The goal of this surveillance is to limit the transmission of MRSA and prevent outbreaks in Intensive care units ICUs as well as in other patient settings. Chromogenic selective agar for fast detection of MRSA gave overall results for detecting MRSA from nasal specimens[9]. The aims of this study was detecting the methicillin-resistant *Staphylococcus aureus* MRSA by using conventional and molecular methods in patients and the environment of the surgical wards of Al-Hussein teaching hospital in Nasiriyah, Iraq using a chromogenic selective agar (HiCromeMeReSa agar, HiMedia, India) and Polymerase Chain Reaction PCR assay which focused on identification of the mecA gene.

Subjects and Methods:

Isolation of bacteria

The samples were taken from patients and the environment items of the surgical wards during a Period from May to November 2012. Two hundred and ninety six samples (from 232 patients and 64 hospital environment items) were collected for detection of MRSA. Informed consent was obtained from patients who stayed in the hospital wards for at least 72 hours. Nasal samples were taken from each patient. A performa including gender, age, health status and relevant data were also collected from each patient. Sterile cotton swabs (dipped in normal saline 0.9) were used for nasal swabbing of the anterior nares of the patients. Swab was inserted simultaneously inside the anterior nares, first into one nostril and then the other nostril and rubbed very well by rotating 5 times over the inner wall of the nasal septum and immediately processed for culture and isolation. For the environment, surfaces of frequently handled items (beds, sinks, door handles, surgical trays, and table surfaces) were swabbed. Each nasal and environmental swab was cultured directly on HiCromeMeReSa Agar plate and subsequently on Mannitol Salt agar plate within one hour after collection by spreading as per the conventional technique. All effort was made to ensure equivalent specimen distributions between the two plates by each swab. The HiCrome culture plates were incubated at 35°C for 24 h, and the colonies showing bluish green colored growth on this medium were considered as MRSA positive, whereas the mannitol salt culture incubated at 37°C for 24 to 48 h and the predominant colonies per sample showing mannitol fermentation were selected and ***S. aureus*** was identified to species level by various bacteriological tests, though colony characteristics, microscopically cells morphology, gram stain and necessary biochemical tests[10,11]. The biochemical properties were determined using traditional biochemical tests were included catalase test, oxidase test, coagulase test, carbohydrate fermentation test and other properties by using API staph. System (bioMerieux, France). For Each *S. aureus* isolate, a bacterial suspension adjusted to 0.5 McFarland was used. Subsequently, a swab was dipped in the suspension, streaked onto the HiCromeMeReSa agar plate (for confirmation as MRSA) and incubated at 35°C for 24 h. All cultures showing bluish green colored growth on this HiCromeMeReSa agar were considered as MRSA positive, and subsequently**,** confirmed by PCR assay.

Detection of MRSA by chromogenic medium

HiCromeMeReSa Agar Base M1674 (Hi-Media, India) was used for detection of the MRSA among the clinical isolates of *S. aureus*. The medium was prepared by suspending 41.65 g of the medium into 500 ml of the distilled water and boiling. The medium was cooled to around 45 to 50°C and MeReSa selective supplement (FD229) reconstituted with 5 ml sterile distilled water into each methicillin vials having 2.0

mg of methicillin as per the direction of the supplier (HiMedia-India), was added and mixed very well. Soon after, the medium was poured into Petri plates and cooled then checked for sterility by keeping at 37°C overnight. In this study the detection of MRSA was determined by direct culture of each swab on HiCrome medium and by subculture of the identified *S. aureus* isolate from mannitol salt agar onto the HiCromeMeReSa agar. Plates were incubated at 35°C for 24 h after which, all cultures showing bluish green colored growth were taken as MRSA positive strains, while all others are recorded as MSSA strains (HiMedia Labs. Products, India).

Detection of MRSA by Disc Diffusion method

According to the CLSI guidelines the phenotypic methicillin susceptibilities for the confirmed *S. aureus* isolates were determined by using the standard methicillin, cefoxitin and oxacillin diffusion techniques[12,13]. A colony suspension of each isolate was prepared to the density of a 0.5 McFarland standard and plated on Mueller-Hinton agar. 5 µg methicillin disc ME, 1 µg oxacillin disc OX and 30 µg cefoxitin disc FOX were added onto the plate. The inhibition zone diameters were measured manually after 24 h of incubation at 37°C.

Antibiotic susceptibility testing

The susceptibilities of the isolates to 13 antibiotics (methicillin 5µg, cefoxitin 30µg, oxacillin 1µg, ampicillin 10µg, amoxicillin 10µg, meropenem10µg , imipenem 10µg, ciprofloxacin 15µg, erythromycin 5µg, vancomycin 30µg, tetracycline 5µg, gentamycin 10µg and clindamycin 2 µg) were determined on Mueller-Hinton agar, by the Kirby Bauer disk diffusion method (MacFaddin, 2000). Results were interpreted on the basis of the guidelines by the Clinical and Laboratory Standards' institute (CLSI, 2011, 2012).

Genomic DNA extraction

The Genomic DNA of 38 selected MRSA isolates (12,13,17,20, 21,22, 25,30, 34,36,37,42,43,49,52,57,60,63,68,76,77,81,83,85,89,90,91,92, 95,97,98,100,102,113,116, 117,123 and126, which showed high resistance for methicillin, cefoxitin and oxacillin) was extracted by DNA extraction kit of Geneaid, Korea for the detection of mecA gene by PCR. TheDNA extract was confirmed by gel electrophoresis. The concentration (Nanogram /µl) of the template DNA of MRSA isolates was confirmed by nanodrop apparatus (Korea) and kept in -20˚C until use.

PCR amplification of mecA gene

According to the manufacturer's instructions (Bioneer, korea), the PCR amplification mixture has been prepared. The forward primer 5' AAAATCGATGGTAAAGG TTGGC3' and reverse primer 5' AGTTCTGCAGTACCGGATTTTGC 3' were used to amplify a 533-bp DNA fragment of mecA gene. The final

volume of reaction mixture was 20µl containing the following:- 5 µl (5-50ng/µl)from template DNA from selected MRSA isolates which prepared as described above was added to AccuPower PCR PreMix tube, subsequently 1 µl from each forward and reverse primer was added. Then the final volume 20µl was completed with deionized sterile distilled water ddH_2O. The lyophilized blue pellet was dissolved by vortexing and briefly spins down. Centrifuged for 5 seconds and the PCR of samples were performed. PCR was carried out in thermocycler apparatus (Cleaver scientific LtD, USA) with reaction cycles, that is, initial denaturation at 94°C for 3 min; 30 cycles of 94°C/ 1 min, 60°C/1 min, 72°C/1 min and a final extension of 72°C/5 min. PCR products were visualized by running on 1% agarose gel which prepared as described above containing 0.5 µl /ml ethidium bromide (Sigma-Aldrich, UK) in TBE buffer by Gel-Electrophoresis apparatus (Cleaver,UK)and images of bands were taken using a digital camera.

Statistical analysis

The Microsoft Excel data analysis tool was used to compare if there is a difference between HiCromeMeReSa, PCR assay and DD test result. Also, analysis was performed by displaying the data graphically. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the MRSA HiCromeMeReSa test (direct and subculture) were calculated by comparing the results of these culture protocols with the OX, FOX and ME DD Test, and with PCR assay.

Results

Detection of MRSA by Phenotypic assay

As per the conventional techniques of all nasal and environmental swabs which cultured on Mannitol Salt agar, the predominant colonies showed mannitol fermentation were selected as presumptive S*. aureus.* Eventually there were confirmed by using standard cultural and biochemical tests such as coagulase, catalase**,**API Staph tests and others. Out of 296 samples (232 nasal swabs and 64 environment swabs) 192 (64.8%) were identified as *S. aureus* of which 150 (64.6%) from patients and 42(65.6%) from environment. The detection of MRSA was determined by direct culture of each swab on the chromogenic agar (HiCromeMeReSa medium) and by subculture of the identified *S. aureus* isolates from mannitol salt agar onto the HiCromeMeReSa. All cultures showing bluish green colored growth were considered as MRSA positive isolates and the results compared with OX, FOX and ME DD test. Out of 192 S. aureus isolates 126 (65.6%) were MRSA of which 100 (66.6%)and 26(61.9%) from the patients and the environment respectively (Table:1). OX, FOX and ME DD test was used to detection of MRSA among *S. aureus* isolates that grew on Mannitol salt agar from the same swab which cultured on HiCromeMeReSa. Table 1 showed the detection rate of MRSA among patients and hospital environment by DD test were (100/150 and 26/42, respectively) and prevalence was 42.5 (126/296) of all samples

and the report time was 48h**.** In this study each isolate that could be identified as MRSA by DD test was confirmed by subculture on HiCromeMeReSa. Results showed similar detection rate of MRSA by subculture on HiCromeMeReSa compared with DD test in patients and environment isolates (100/150 and 26/42, respectively) and prevalence was 42.5 (126/296) of all samples. In contrast the direct culture on HiCrome medium showed lower detection rate of MRSA in patients and environment (94/150 and 22/42, respectively) and prevalence was 39.1 (116/296) compared with the other two methods.

Sensitivity and Specificity of phenotypic tests:

In the patients samples, sensitivity, specificity, PPV and NPV between direct culture on HiCrome agar medium and (OX, FOX and ME) DD test were 94%, 100%,100% and 95.6% respectively, whereas in the environment samples were 84.6%, 100%, 100% and 90.4% respectively. Results demonstrated higher sensitivity and NPV in patient's samples than in environment samples but the same rate in specificity and PPV. In contrast, no differences were observed between results from subculture on HiCrome Agar medium and (OX, FOX and ME) DD test, which were compatible in sensitivity, specificity, PPV and NPV (Tables1, 2).

Table 1. MRSA prevalence (%) by different methods

Isolate source n =296	Staph. aureus	ME, Ox, FOX DD test (48 hr)		Direct culture on HiCrome (24hr)		Subculture on HiCrome(48 hr)	
		MRSA	MSSA	MRSA	MSSA	MRSA	MSSA
Patients n = 232	150	100	50	94	56	100	50
Environment n = 64	42	26	16	22	20	26	16
Prevalence %	64.8 (192/296)	42.5 (126/296)	22.3 (66/296)	39.1 (116/296)	25.7 (76/296)	42.5 (126/296)	22.3 (66/296)

MRSA: Methicillin Resistant *Staphylococcus aureus* MSSA: Methicillin Sensitive *Staph. aureus*

OX: oxacillin FOX: cefoxitin ME: methicillin

Table 2. Sensitivity, specificity, PPV and NPV of direct culture and subculture on HiCromeMeReSa Agar and OX, FOX and ME DD test

Test	Subculture on HiCromeMeReSa Agar and Oxacillin, Cefoxitin and Methicillin D D test					
Direct culture on HiCromeMeReSa Agar	Patients isolates			Environment isolates		
	Positive	Negative	Total	Positive	Negative	Total
Positive	94	0	94	22	0	22
Negative	6	132	138	4	38	42
Total	100	132	232	26	38	64
sensitivity	94%			84.6%		
specificity	100%			100%		
PPV	100%			100%		
NPV	95.6%			90.4%		

PPV: positive predictive value, NPV: Negative predictive value

Antibiotics susceptibility tests

The antimicrobial susceptibility patterns (Kirby-Bauer method) of MRSA isolates against 13 different types of antibiotics are summarized in Figure1. The drug resistance patterns of MRSA isolated from clinical specimens was found to be highly variable. All the 126 MRSA isolates screened from nasal samples and environment items were resistant to oxacillin 1 µg, cefoxitin 30 µg, methicillin 5 µg, ampicillin 10µg, and amoxicillin 10µg (100%). In MRSA from patients, resistance to erythromycin 15 µg, gentamycin 5 µg, ciprofloxacin 5 µg and meropenem 10 µg was(66%, 44%, 42%, and 40% respectively), whereas resistance to tetracycline 30 µg, imipenem10 µg , clindamycin 2 µg and vancomycin 10 µg was relatively low (30%, 26%, 26%, and 12%, respectively). The resistance of environment MRSA to antibiotics; erythromycin, gentamycin, ciprofloxacin, meropenem, tetracycline, imipenem, clindamycin

and vancomycin were (69.2%, 53.8%, 46.1%, 38.4%, 38.4%, 15.3%, 23%, and 7.6%, respectively). However, higher rate of MRSA isolates tested in this study were recorded sensitive to vancomycin. The results of this study demonstrated the high prevalence of MRSA among the male (70%) than the female, and the Maximum MRSA strains were found among the age group 21-41years (36%) and the minimum MRSA among age group 1-20 years(12%). Table 3 showed the rates of MRSA nasal carriage among previously chronic diseases was higher (23.2%) than that in non-previously chronic diseases (19.8%). Also this result demonstrated high rate (26.7%) of MRSA nasal carriage among patients with previous use of antibiotics against in non- previous use 16.3%. In contrast, these results represented there is no association between MRSA nasal carriage among patients and previous admission in the hospital or their previously admission for surgical operation.

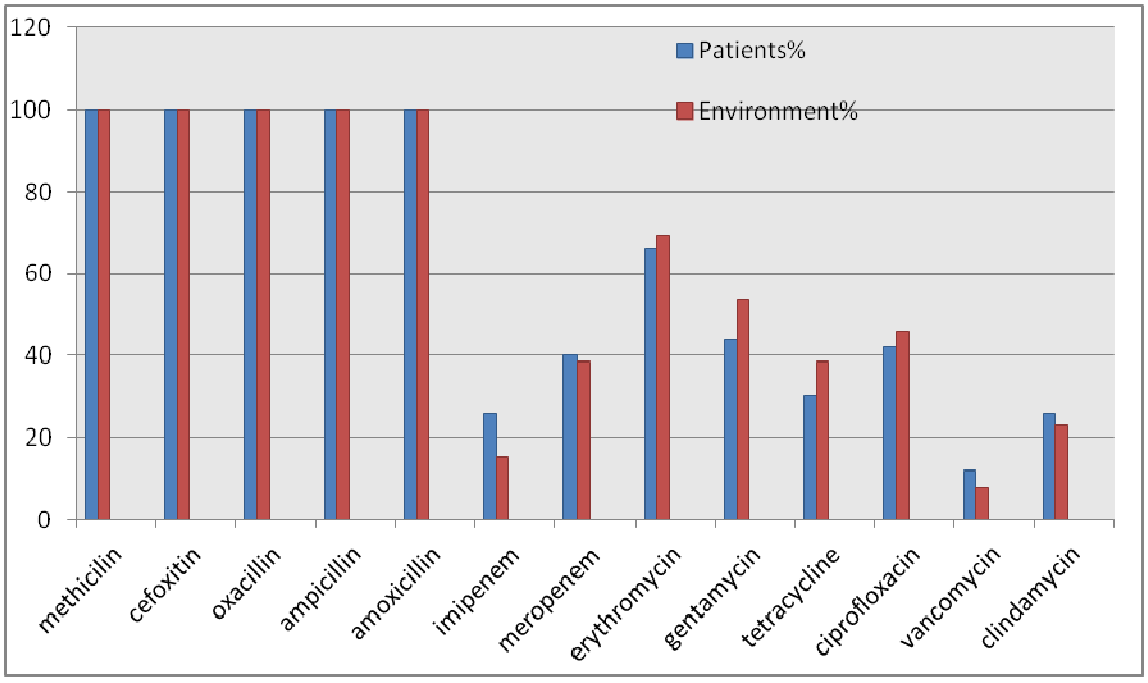

Figure 1. Antibiotics resistance patterns of MRSA

Confirmation of MRSA by genotypic assay

To confirm methicillin resistance in MRSA isolates on the molecular level, the presence of mecA gene in the 38 MRSA selected isolates which gave the highest level of methicillin, oxacillin and cefoxitin resistance were tested. A volume of 20µl of reaction mixture and reaction cycles program were set up as described above. Negative control (MSSA isolate) was used to confirm the absence of mecA gene. Results of this assay demonstrated that all MRSA isolates were selected for the detection of mecA gene showed the

presence of amplified bands of this gene(533-bp), while mecAwas never detected in case of the negative control (Figure 2).

Sensitivity and PPV of Genotypic test

Table 4 represented the Sensitivity and positive predictive value PPV in (HiCromeMeReSa Agar and OX, FOX, ME DD test) and PCR technique.

Table3: MRSA nasalcarriage and medical history variables amongpatients.

Variable	Total No. n=232	MRSA n=100	
		No.	%
Previous admission to the hospital Yes No Don't know	29 203 ---	16 84 ---	6.8 40.5 ---
Previous use of antibiotic Yes No Don't know	97 135 ---	62 38 ---	26.7 16.3 ---
Previous infection with MRSA Yes No Don't know	--- 11 221	--- 3 97	--- 1.3 41.8
Previous admission for surgical operation Yes No Don't know	18 214 ---	7 93 ---	3 40 ---

Previous chronic disease	**73**	**54**	**23.2**
diagnosis	**159**	***46***	**19.8**
Yes	**---**	**---**	**---**
No			
Don't know			

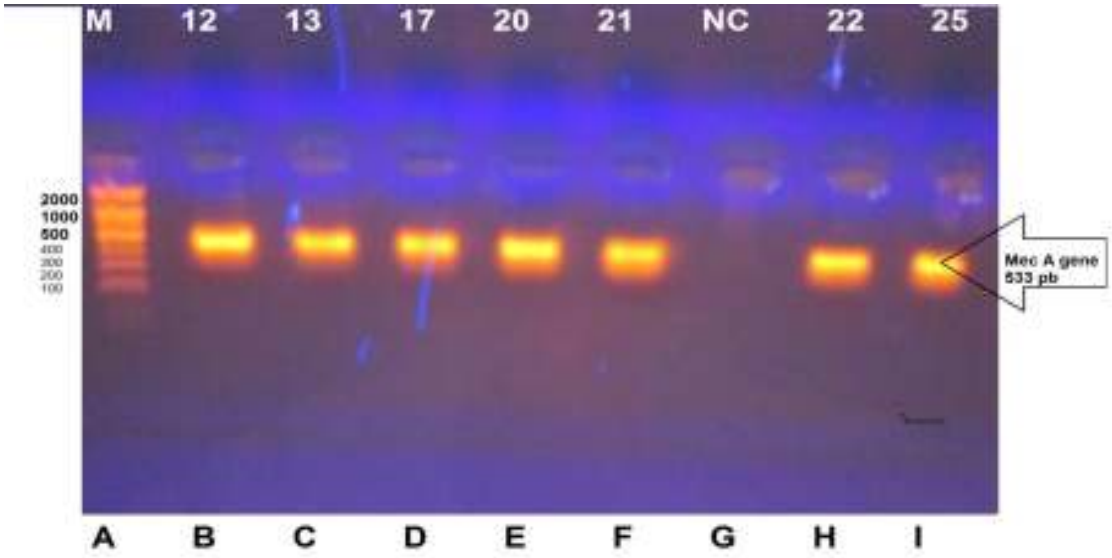

Figure 2. Agarose gel electrophoresis of mecA gene amplification by PCR products of MRSA isolates. Lane A: DNA ladder (sizes Marker of bands are 100, 200, 300,400, 500, 1000, 2000 bp), Lane B: MRSA isolate number 12 ,Lane C: MRSA isolate number 13, Lane D: MRSA isolate number17, Lane E: MRSA isolate number20, Lane F: MRSA isolate number 21, Lane G: Negative control, Lane H: MRSA isolate number 22, Lane I: MRSA isolate number25.

Table4. Sensitivity and PPV of (HiCromeMeReSa and OX, FOX, ME DD test) and PCR

Test / **PCRtechnique (mecA gene)**	**HiCromeMeReSa Agar and (Oxacillin, Cefoxitin and Methicillin) D D test**		
	Positive	**Negative**	**Total**
Positive	**38**	**0**	**38**
Negative	**0**	**0**	**0**
Total	**38**	**0**	**38**
sensitivity	**100%**		

specificity	**_**
positive predictive value (PPV)	**100%**
Negative predictive value (NPV)	**_**

Discussion

HiCromeMeReSa agar, DD test and MRSA PCR assay were used in this study In order to have an evaluation and Comparison for detection of methicillin resistant *Staphylococcus aureus* (MRSA) among patients and environment of surgical wards. Results of this study demonstrated high prevalence of *s.aureus* in patients and in the hospital environment (64.8%). This result is in accordance with several studies which stated that *S. aureus* is usually the highest in hospitalized patients and the anterior nares which are the most common site of this bacterium. Therefore, it has become a major pathogen associated with both hospital and community acquired infections[14]. A total of 192 *S. aureus* isolates were identified, the prevalence of MRSA was 65.6% (126/192) among patients 66.6% (100/150) and hospital environment 61.9% (26/42) respectively**.**

In this studyMRSA prevalence was 42.5 (126/296) among all the samples for patients and environment which is less than that reported in other studies[15]**.** showed that MRSA prevalence in the surgical wards of Kampala was 100% and 46% among S*. aureus*, Perry et al, 78%, and Nasser et al, 77.9% from all *S. aureus* isolates in Indian hospitals[16, 17]. However, result of this study was more than that obtained by Al-Charrakh et al,2012, who found that 32.3% of clinical *S. aureus* isolates in Al-Dewaniya /Iraq were MRSA[18]. The result of present study showed higher rates compared to other countries studies in 2012, such as France at 14.5% [19,] Taiwan at 6.9% [20], Netherlands at 3.1%[8]. In Western Europe, the percentage of MRSA among *S. aureus* clinical isolates ranged between 5% and 54% [21] .Also Khadri&Alzohairy pointed out that MRSA isolates constituted 54.2% among all *S. aureus* isolates in India[22]. Whereas, in a recent study in Egypt, reported the detection of 53% of MRSA among the studied *S. aureus* clinical isolates[23]. For comparison, the direct culture on the chromogenic agar is the basic diagnostic tool to monitor the nosocomial spread of MRSA. MRSA-Select (HiCromeMeReSa Agar) used in this study was the most rapid (18-24 hr) for identification of MRSA from primary isolation plates obviating the need for additional biochemical tests and the most specific (100%) protocol for patients and environment screening. This result was similar to that reported by [15] and [9]. Although the Direct

culture on MRSA-Select protocol showed slightly low sensitivity in both patients and environment screening (94%, 84.6%, respectively), but the subculture of *S. aureus* isolates from manitol salt agar on HiCrome medium increased the detection rate of MRSA from 94/150 to 100/150 and from 22/42 to 26/42,respectively. This in turn increased the rate of sensitivity in both patients and environment samples, though the report time could be delayed as much as 48 hr. It is likely that the pre-enrichment step enhances the growth of MRSA with low-level resistance (Lee et al, 2008)[24].The specificity was higher in all MRSA select protocols (100%) as there was no detection of any false positive sample. The specificity reported in the present study is higher than that reported by James et al [25]. There was a little difference in sensitivity, specificity, PPV and NPV among the three methods for the isolation of MRSA from nasal swabs and environment items swabs (94, 84.6%; 100,100%; 100,100%; 95.6, 90.4% respectively) which agreed with results of Hoecke, et al [26]. The chromogenic agar gave the best overall results for detecting MRSA from nasal specimens [9]. However, selective chromogenic agar used in this study has generated improvements over routine culture media. Chromogenic agar offer advantages in turnaround time TAT, specificity and sensitivity over conventional agar[27, 28]. Figure3 demonstrated that all MRSA isolates were multidrug resistant (MDR) and high resistant (100%) to Beta-lactam antibiotics: oxacillin, cefoxitin, methicillin, ampicillin and amoxicillin followed byother antibiotics: erythromycin, gentamycin, ciprofloxacin, meropenem, tetracycline, and (imipenem, clindamycin) which showed resistance rates 66%, 44%, 42%, 40%, 30%, 26%, 26% respectively, but showed much lower resistant to vancomycin. High resistance to Beta-lactam antibiotics can be attributed to the hyper production of Beta–lactamases and the low affinity penicillin binding protein 2a encoded by mecA gene. Therefore none of the beta-lactam antibiotics generally used in the first-line empiric treatment for severe infections is effective in MRSA infections[29]. MRSA isolates are resistant to all the beta-lactam antibiotics, nevertheless, MRSA resistance of meropenem and imipenem was lower than other Beta-lactams used in present study in both patients and environment isolates. This can be attributed to the low exposure to these antibiotics because of the limited or little usage of these antibiotics as drug in the Iraqi hospitals. On other hand, MRSAshowed very low **resistance** (12%&7.6%) against vancomycin. This result is in accordance with several studies reported that vancomycin is a glycopeptides antimicrobial, have been the mainstay for therapy of severe MRSA infections and all MRSA isolates still remain susceptible to glycopeptides, and these agents are recommended as the first choice alternatives. Also for empiric treatment of suspected invasive infections in which MRSA is regarded as a potential causative agent[30, 31]. According to CLSI, 2011 Table 2C, the vancomycin 30 microgram disk test detects the isolates of staph aureus resistant to vancomycin. Such isolates will show no zone of

inhibition around the disk (zone =6mm), but the staphylococci producing vancomycin zones of larger than or equal 7mm should not be reported as susceptible without performing a vancomycin MIC test . Regarding the prevalence of MRSA in relation to the age group, the lowest rate (12%) was seen in the age group 1-20 years, while the highest rate (36%) was seen in the age group 21-41 years, which probably reflects the fact that people at this age group are more involved in different life activity. The results demonstrated higher MRSA prevalence in males (70%) than in the females which are different than reported by Kang et al**.** who found that 68.4% in female and 52,6% in the age group 30-59 years[20]. The study showed the rates of MRSA nasal carriage among previously chronic diseases was higher (23.2%) than that in non-previously chronic diseases (19.8%).This result is in agreement with Johnson et al. study in the USA [32]. Also this result demonstrated high rate (26.7%) of MRSA nasal carriage among patients with previous use of antibiotics against in non- previous use 16.3%[33, 34]. In previous study of WHO pointed that widespread use of antibiotics both inside and outside of medicine is playing a significant role in the emergence of resistant bacteria[35]. The major problem of the emergence of resistant bacteria is due to misuse and overuse of antibiotics by doctors as well as patients[36]. It is worth noting that our study was the first in the Nasiriyah city which investigated and studied the prevalence of MRSA among patients, and the lacked medical culture for MRSA was observed when asked the patients about the previous infection with MRSA and the highest rate 221 from 232 patients were answered don't know**(**Table3**).** The confirmatory detection of mecA gene by PCR assay in all MRSA isolates that were detected by phenotypic tests (HiCromeMeReSa, DD test) indicated that these tests were reliable and accurate for the detection of MRSA isolates. Most molecular methods for identification of S. aureus and MRSA have been PCR based. PCR are now used extensively to confirm the presence of the genetic determinants of methicillin resistance (mecA gene) in S. aureus [37]. Results of PCR showed high sensitivity (100%) and high PPV (100%).These results were in correspondence with several recent studies that reported the commercial PCR sensitivity varied between 95 and 100%[38,37]. While specificity and NPV for PCR assay cannot be estimated because the true negative samples cannot be calculated when using 38 selective isolates of MRSA. PCR technique is a rapid and reliable method for identifying MRSA isolates and totally consistent with those obtained from conventional methods of identification[39, 40].Therefore, methicillin resistance in MRSA isolates of this study was confirmed by PCR amplification of a 533-bp fragment of mecA gene in38 isolates of MRSA.

Conclusion:All isolates positive for MRSA by conventional methods were defined positive by the chromogenic method, and confirmed here positive also by PCR technique. These results enhance the

confidence in that the chromogenic methodology is suitable for direct screening of MRSA colonization samples without any prior steps of isolation.

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