Methicillin Resistant Staphylococcus Aureus (MRSA) Nasal Carriage among Health Care Workers in Intensive Care Units

Jawad R. Al-Zaidi
Foundation of Technical Education, Shatrah Technical Institute, Iraq
E-mail:jawadalzaidi@gmail.com

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Abstract

To detect the nasal carriage rate of Methicillin Resistant *S. aureus* (MRSA) among healthcare workers (HCWs) in the Intensive Care Units by Chromogenic medium and PCR assay, Out of 63 nasal samples, 30 (47.6%) *S. aureus* isolates were recovered. Out of the 30 isolates, 17 were methicillin resistant. Nasal carriage rate of MRSA was found to be 56.7% among HCWs. Regarding the prevalence of MRSA in relation to the work type, sex and age group of HCWs, the highest rate (83.3%) was seen in the Utility Worker, (64.7%) in the male and (47%) in age group 20 to 29 years. All MRSA isolates which detected by Chromogenic method and confirmed by PCR assay showed the presence of *mecA* gene. Also this study demonstrated higher compatible 100% in sensitivity, specificity, PPV and NPV between the two methods. In other side all MRSA isolates from HCWs were multidrug resistant (MDR) and high resistant (100%) to Beta-lactam antibiotics: oxacillin, amoxicillin, methicillin ampicillin, and cefoxitin followed by other antibiotics: erythromycin, ciprofloxacin, tetracycline, gentamycin and clindamycin which showed resistance rates 58.8%, 47%, 35.3%, 23.5% and 17.6%, respectively, but they showed high susceptibility to vancomycin.

Keywords: MRSA, Nasal carriage, Chromogenic medium, Health care workers (HCWs), ICU.

الحمل الأنفي للمكورات العنقودية الذهبية المقاومة للمثيسيلين بين عمال العناية الصحية في وحدات العناية المركزة

الخلاصة

لغرض الكشف عن نسبة الحمل الأنفي لبكتريا المكورات العنقودية الذهبية المقاومة للمثيسيلين MRSA بين العاملين في الرعاية الصحية HCWs في وحدات العناية المركزة استخدمت طريقتين هما طريقة وسط الكشف اللوني Chromogenic Medium وتقنية تفاعل البلمرة المتسلسل PCR، حيث أخذت ٦٣ مسحة أنفية من ٦٣ عاملا. عزلت ٣٠ عزلة من بكتريا المكورات العنقودية وبنسبة ٤٧.٦% من المجموع الكلي لعدد المسحات بينما كانت نسبة الحمل الأنفي لبكتريا MRSA ٥٦.٧% والتي سجلت اعلي نسب لها بين عمال الخدمة والإعانة 83.3% وبين الذكور٦٤.٧% كذلك بين الفئة العمرية ٢٠ . 29) ٤٧%.

تم تأكيد تشخيص جميع عزلات MRSA المعزولة بطريقة وسط الكشف اللوني بواسطة تقنية PCR والتي أظهرت وجود جين MecA 533bp . أظهرت الدراسة توافق تام وبنسبة ١٠٠% في قيم Sensitivity و Specificity و PPV وNPV لكلا الطريقتين كما بينت الدراسة المقاومة المتعددة للمضادات الحيوية لجميع عزلات MRSA المعزولة والتي سجلت أيضا مقاومة عالية ١٠٠% لمضادات البيتا لكتام بينما كانت مقاومتها منخفضة جدا لمضاد الفانكومايسين ٥.٩% .

Introduction

Methicillin was first introduced in human medicine in the 1960s for the treatment of infections caused by penicillin's resistant *Staphylococcus aureus*, but within a few years, methicillin resistant *S. aureus* (MRSA) emerged [1].

Despite the recent improvements of medical services and antimicrobial therapy, MRSA is recognized as a major cause of nosocomial infections which result in significant morbidity and mortality rates especially in places where people are in close contact such as hospitals and where hygiene is compromised [2-4]. Methicillin resistance in staphylococcii mediated by the *mecA* gene, which encodes for the penicillin-binding protein 2A (PBP2A) resulting in reduced affinity for the beta-lactam antibiotics including the penicillinase-resistant penicillin, therefore MRSA has become a major hospital pathogen in human medicine [5, 6].

The health care workers HCWs (technician, medical assistants, medical students, nurses, nurse assistants, utility workers, etc.), who are in close contact with the patients are possible sources of such hospital-acquired infection, with many of thepathogenic organisms transmitted by hand carriage, MRSA is the most important human pathogen cause of hospitals nosocomial infections [7]. Culture remains a common MRSA surveillance method. In Europe, culture is the standard method for MRSA detection, and the selective chromogenic agar has generated improvements over routine culture media [8]. Chromogenic medium offer advantages in turnaround time TAT and sensitivity over conventional agar [9].

However, there is limited data on MRSA prevalence in Iraqi Health Care Workers. Here, the present study was aimed to detection the nasal carriage rate of MRSA among healthcare workers (HCWs)in the Intensive Care Units of Alhabbobi teaching Hospital in Al-Nasiriyah city using a chromogenic selective agar which is a specific and selective method for detection of MRSA and by the molecular method Polymerase Chain Reaction (PCR) technique.

Materials and Methods

collection of Specimens

Sixty three nasal samples were taken from sixty three Health Care Workers (HCWs) of Intensive Care Units (ICU) in the Al-Habbobi teaching hospital in Al-Nasiriyah city. A Performa including sex, age, health status, work type, and other data were also recorded from each HCWs. Sterile cotton swabs (dipped in normal saline) were used for nasal swabbing of the anterior nares of each HCW. 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 and immediately processed for culture and isolation. Each nasal swab was cultured directly on HiCrome MeReSa Agar plate, HiMedia, India (Chromogenic method) and subsequently on Mannitol Salt agar plate within one hour after collection by spreading as per the conventional technique. 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 to48 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 other biochemical tests [10]. The biochemical properties were determined using traditional biochemical tests were included coagulase, catalase, oxidase and Carbohydrate fermentation tests and other properties were determined by API staph System (BioMerieux, France). All cultures showing bluish green colored growth on this HiCrome MeReSa agar were considered as MRSA positive, and subsequently, confirmed by PCR technique as golden standard test.

MRSA detection by chromogenic method

According to instructions of Hi-Media lab, the Chromogenic medium (HiCromeMeReSa Agar Base M1674) was used for detection of the MRSA among HCWs isolates. 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, 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 Chromogenic medium. Plates were incubated at 35°C for 24 h after which, all cultures showing bluish green colored growth were taken as MRSA positive isolates, and all others are recorded as MSSA isolates (HiMedia Labs. Products, India).

MRSA detection by PCR technique

Extraction of Genomic DNA

The Genomic DNA of 30 *S. aureus* isolates which confirmed by Chromogenic method was extracted by DNA extraction kit of Geneaid, Korea for the detection of mecA gene by PCR. The DNA 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

The PCR amplification mixture has been prepared according to the manufacturer's instructions (Bioneer, Korea). The forward primer 5' AAAATCGATGGTAAAGGTTGGC3' and reverse primer 5' AGTTCTGCAGTACCGGATTTTGC 3' were used to amplify a 533bp 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 HCWs 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 ddH2O. 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, UK) 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, USA) in TBE buffer by Gel-Electrophoresis apparatus (Cleaver, UK) and images of bands were taken using a digital camera (Sony, Japan).

Antibiotic susceptibility testing

The antibiotic susceptibility testing was performed using the Kirby-Bauer disc diffusion test (DDT) on Mueller-Hinton agar [10] according to Clinical and Laboratory Standards Institute (CLSI) guidelines [11, 12]. The following antibiotics discs (Hi-media Mumbai, India); methicillin 5µg, amoxicillin 10µg, cefoxitin 30µg, ampicillin 10µg, oxacillin 1µg, erythromycin 15µg, vancomycin 10µg, ciprofloxacin 5µg, gentamycin 5µg, clindamycin 2 µg and tetracycline 30µg) were used. The results of DDT were interpreted according to CLSI guidelines [12].

Statistical analysis

Frequencies were obtained and percentages were calculated for study variables of all methods which used in this study. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of the MRSA Chromogenic test were calculated by comparing the results of this culture protocol with the PCR technique.

Results

Nasal carriage of *S. aureus* and MRSA

A total of 30 *S. aureus* isolates were isolated from nares of 63 Health Care Workers in this study. The nasal carriage rate of *S. aureus* was 47.6%. The nasal carriage of *S. aureus* was 13 (43.3%) in females and 17 (56.7%) in males. Highest nasal carriage 13 (43.3%) and 6 (66.7) of *S. aureus* was recorded in the age group of 20-29 yrs and in the Utility Worker, respectively. Out of 30 *S. aureus* isolates 17 (56.7%) were MRSA detected by the two methods Chromogenic medium and PCR assay, of which 4 (44.5%), 8(57.1), 0(0%) and 5(83.3) from the Medical Assistant, Nurse, Technician and Utility Worker, respectively; while the nasal carriage rate of MRSA was 6(35.3) and 11(64.7) in female and male, respectively. Highest nasal carriage, 8 (47%) of MRSA was recorded in the age group of 20-29 yrs followed by 5 (29.4%), 3 (17.7%) and 1 (5.9%) in the age groups of 30-39 yrs, 40-49 yrs and<49 yrs, respectively (Table 1 and 2).

Table 1 Frequency of *S. aureus* and MRSA in health care workers

Isolates	Subject	*S. aureus*		MRSA	
		NO.	%	NO.	%
Medical Assist.	22	9	40.9	4	44.5
Nurse	28	14	50	8	57.1
Technician	4	1	25	-	-
Utility Worker	9	6	66.7	5	83.3
Total	63	30	47.6	17	56.7

Table 2 Demographic variables among health care workers with *S. aureus* and MRSA nasal carriage

Variable	Total n=63	*S. aureus* (n=30)		MRSA n=17	
		NO.	%	NO.	%
Sex					
Female	34	13	43.3	6	35.3
Male	29	17	56.7	11	64.7
Age/year					
20-29	32	13	43.3	8	47
30-39	19	10	33.3	5	29.4
40-49	8	4	13.4	3	17.7
>49	4	3	10	1	5.9

Sensitivity, specificity, PPV and NPV between direct culture on Chromogenic medium and PCR assay were calculated. Results demonstrated higher compatible 100% in sensitivity, specificity, PPV and NPV between the two methods (Table 3) .

Negative control (MSSA isolate) was used to confirm the absence of mecA gene by PCR technique. Results of this assay demonstrated that all MRSA isolates which detected by Chromogenic method showed the presence of amplified bands of mecA gene (533bp), while mecA was not detected in negative control (Figure 1).

The resistance pattern of MRSA to various antibiotics was shown in Table 4. All the 17 MRSA strains screened from nasal samples of HCWs were resistant to oxacillin, amoxicillin, methicillin ampicillin, and cefoxitin (100%). Whereas resistance to erythromycin, ciprofloxacin, tetracycline, gentamycin, clindamycin, and vancomycin were 10 (58.8%), 8 (47%), 6 (35.3%), 4 (23.5%), 3 (17.6%), and 1 (5.9%) respectively.

Table 3 Sensitivity, Specificity, PPV and NPV of Chromogenic medium and PCR

Test / Chromogenic medium	PCR technique (mecA gene)		
	Positive	Negative	Total
Positive	17	0	17
Negative	0	13	13
Total	17	13	30
sensitivity	100%		
specificity	100%		
PPV	100%		
NPV	100%		

PPV: positive predictive value, NPV: Negative predictive value

Sensitivity =(true $^{+ve}$/ true $^{+ve}$ +false $^{-ve}$) *100%

Specificity =(true $^{-ve}$/ true $^{-ve}$ +false $^{+ve}$) *100%

PPV =true $^{+ve}$/ true $^{+ve}$ +false $^{+ve}$) *100%

NPV =true $^{-ve}$/ true $^{-ve}$ +false $^{-ve}$) *100%

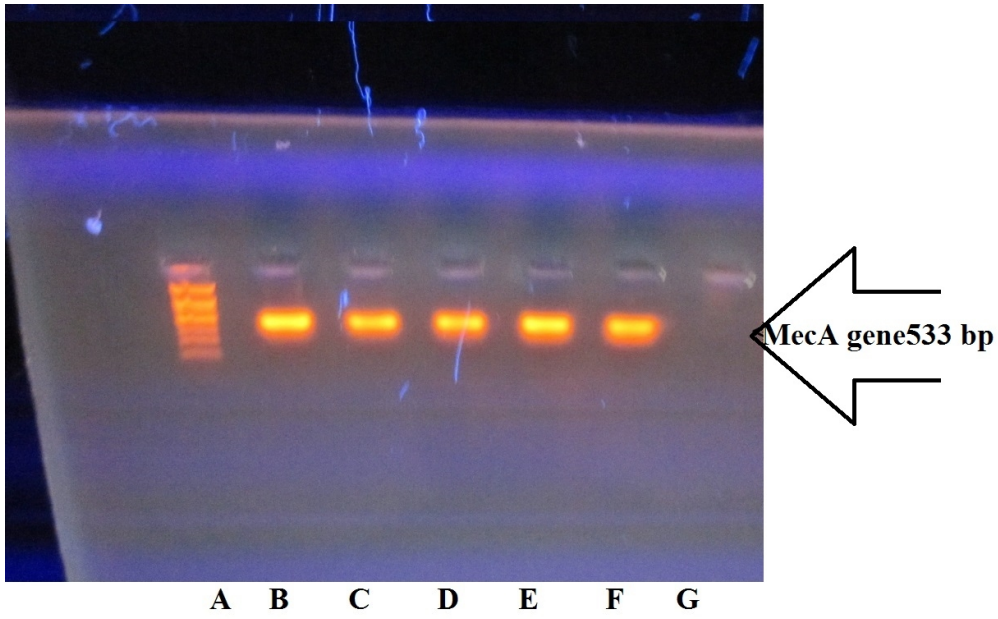

Figure 1 Agarose gel electrophoresis of mecA gene (533bp) amplification by PCR products of MRSA isolates. Lane A: DNA ladder (sizes Marker of bands are 100, 200, 300,400, 500, 1000, 2000 bp), Lane B: MRSA isolate number 1 ,Lane C: MRSA isolate number 2, Lane D: MRSA isolate number3, Lane E: MRSA isolate number4, Lane F: MRSA isolate number 5, Lane G: Negative control (MSSA).

Table 4 Antibiotics resistance patterns of MRSA isolates

Antibiotic	Disc potency (µg)	MRSA of HCWs = 17	
		Resist. No.	Resist. %
Oxacillin	1	17	100
Amoxicillin	10	17	100
Methicillin	5	17	100
Ampicillin	10	17	100
Cefoxitin	30	17	100
Erythromycin	15	10	58.8
Ciprofloxacin	5	8	47
Tetracycline	30	6	35.3
Gentamycin	5	4	23.5
Clindamycin	2	3	17.6
Vancomycin	10	1	5.9

Discussion

The screening of HCWs to detect MRSA colonization and subsequent isolation of carriers is a cost-effective infection control measure to reduce the risk of transmission [13, 14]. The chromogenic mixture incorporated in the medium is specifically

cleaved by *S. aureus* to give bluish green colored colonies, this method allows identification of MRSA from primary isolation plates within 24 h obviating the need for additional biochemical tests, therefor it is reduce the time and the cost of diagnosis and provide overall results that are equivalent to the results of the PCR method [15, 16].

Result of this study showed that the nasal courage rate of *S. aureus* and MRSA were 47.6% (30/63); 56.7% (17/30), respectively among all the samples for HCWs (Table 1). This result was similar or less than that reported in other studies in different areas of the world such as: Kateete et al. in 2011 which showed that *S. aureus* and MRSA prevalence among HCWs in the surgical wards of Kampala was 41.9% and 100% respectively [17], whereas 50% *S. aureus* and 16.7% MRSA among HCWs in Ramallah Governmental hospital in Palestine [18]. Also in India, Khadri & Alzohairy pointed out that MRSA isolates constituted 54.2% among all S. aureus isolates in hospital [19]. However, result of present study showed higher rates of this pathogen among HCWs compared to other hospitals in different countries, such as in the largest hospital in Iran, 31.1% [20], in Singapore General Hospital 20.2% of HCWs were found to be colonized with MRSA[21], In Turkey a study was conducted in Ankara University Hospital to determine the rate of nasal MRSA carriage among staff, showed 9.1% [22], also in Portugal hospitals the prevalence of MRSA nasal carriage among HCWs was 5.1% [23].

Regarding the prevalence of MRSA in relation to the work type, sex and age group, the highest rate (83.3%) was seen in the Utility Worker,(64.7%) in the male and (47%) in age group 20 to 29 years, which probably reflects the fact that the male HCWs at this age group were more activity in contact with patients (Table 2). These results were similar to that reported by Abu-Rabie Maaliin [18], but they were different from reported by Kang et al [24] who found that 68.4% in female and 52,6% in the age group 30 to 59 years. Moreover, the high prevalence of *S. aureus* and MRSA nasal carriage among HCWs may be due to the fact that patients and HCWs were more overcrowded in this hospital, and due to the fact that a significant number of HCWs acquired MRSA on their hands after removal of their gowns and gloves during patients' care [25].

Results of present study (Table3) demonstrated that all MRSA isolates (which detected by Chromogenic method and confirmed by PCR assay) were harboring *mecA* gene (Figure 1). Also these results demonstrated higher compatible 100% in sensitivity, specificity, PPV and NPV between the two methods which were agreed with results of Hoecke et al and Denys et al who found that the chromogenic medium gave the best overall results for detecting MRSA from nasal specimens [26, 27].

Results in Table 4 demonstrated that all MRSA isolates from HCWs were multidrug resistant (MDR) and high resistant (100%) to β-lactam antibiotics: oxacillin, amoxicillin, methicillin ampicillin, and cefoxitin followed by other antibiotics: erythromycin, ciprofloxacin, tetracycline, gentamycin and clindamycin which showed resistance rates 58.8%, 47%, 35.3%, 23.5% and17.6%, respectively, but showed much lower resistant to vancomycin5.9%. 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 [28]. This result was in accordance with study of Courvalin in 2006 who reported that vancomycin inhibits bacterial growth by binding to the C terminal end of late peptidoglycan precursors, preventing the effective formation of a bacterial cell wall, therefore, MRSA is sensitive to this type of antibiotics [29].

Conclusion

Based on the results of this study we conclude that Chromogenic medium for MRSA screening demonstrated a higher sensitivity and specificity for detection of MRSA nasal colonization isolates among

HCWs, and provided lower time (24h) in generating positive and negative results.

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