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RESEARCH ARTICLE

Nasal Carriage of Methicillin Resistant Staphylococcus Aureus among Medical Personnel of Salah Al-Din Hospitals using Classical Method and PCR Technique

Nihad Abdul-Hussain Jafar[1*], Adnan F. Al-Azaawie[2], Osama Hashim Muhammed[3]

1. *Department of Microbiology, College of Veterinary Medicine, Tikrit University, Iraq.*
2. *Department of Biology, College of Science, Tikrit University, Iraq.*
3. *Samarra General Hospital, Salah Al-Din, Iraq.*

***Corresponding Author: Nihad Abdul-Hussain Jafar**

Abstract

Objectives: To screen medical personnel of four hospitals at Salah Al-Din governorate for MRSA, and to compare between the classical method and PCR method for detection of *Staphylococcus aureus* and MRSA. Materials and Methods: A total of 279 nasal swabs were taken from medical personnel of four hospitals in Salah al-Din (Salah Al-Din General Hospital, Tikrit Teaching Hospital, Samarra General Hospital and Baiji General Hospital). *Staphylococcus aureus* isolates diagnosed by both classical methods based on morphology, coagulase test, DNase test and mannitol salt agar fermentation, and by PCR by detection of *fem*A gene .The isolates diagnosed as MRSA using classical method by its sensitivity to Cefoxitin in disc diffusion method and by detection of *mec*A gene with PCR. Results: The results showed that out of the 279 swabs taken from the medical staff of four hospitals in Salah al-Din, 88 were isolated as *S. aureus* (31.5%) using the classical method used in the hospitals of the governorate under study, and 58 (65.9 %) of them were methicillin resistant *S. aureus* using the Cefoxitin antibiotic method. However, the PCR results showed that only 57 isolates (20.4%) were diagnosed as *S. aureus* and that the number of isolates of *S. aureus* that possessed the *mec*A gene was only 24 isolates (42.1%) from the total isolates. Conclusion: The classical methods used in most Iraqi hospitals are not accurate in diagnosing *S. aureus* isolates and MRSA isolates, since 31 (35.2 %) isolates of the 88 isolates were misdiagnosed as *S. aureus* by the classical method. Furthermore, 34 (58.6 %) isolates of *S. aureus* were misdiagnosed as MRSA by the classical disc diffusion method.

Keywords: *MSRA, Nasal carriage, Meca.*

Introduction

Staphylococcus aureus (*S. aureus*) is a widespread commensal bacterium and pathogen. Approximately 50% to 60% of individuals are intermittently or permanently colonized with *S. aureus* and, thus, there is relatively high potential for infections [1, 2]. Although these bacteria are often part of the normal flora, they have the potential to produce a variety of infections ranging from relatively minor skin infections to life-threatening systemic diseases [3].

The focus on these bacteria has been demonstrated by the emergence of resistant strains to many antibiotics, which has become the leading cause of nosocomial infections worldwide and at alarming high rates [4]. Antibiotic resistances by this pathogen are due to the presence of genes responsible from that resistance, which are carried on the chromosome or plasmids, and the possibility of transmission of these genes between strains of the same species. The antibiotic Penicillin was used as the first antibiotic to treat the infections caused by the *S. aureus* bacteria at the beginning of 1940s and was considered a successful treatment.

Penicillin resistance was initially within a small number of hospital patients, but the large increase in use of penicillin lead to

resistance spread [5]. Because of this resistance, another antibiotic was needed to treat the infection. Methicillin was chosen as a second treatment weapon and other semi-manufactured agents such as oxacillin and nafacillin were used [6]. As a result of the indiscriminate use of these antibiotics, the methicillin resistant *S. aureus* (MRSA) strains have emerged as the leading cause of hospital infections [7, 11].

The emergence of resistance to natural and semi-processed penicillins such as methicillin and oxacillin made it very difficult to treat infections caused by *S. aureus* and posed a strong challenge to health care workers. Due to the seriousness of these strains and their importance and the lack of information on their prevalence rates in the hospitals of Salah al-Din Governorate, the present study aimed at comparing the traditional method used in Salah Al-Din hospitals and the PCR method in diagnosing *S. aureus* and MRSA isolates among medical staff.

Materials and Methods

Sample Collection and Culture

A total of 279 nasal swabs were taken from medical personnel of four hospitals in Salah al-Din (Salah Al-Din General Hospital, Tikrit Teaching Hospital, Samarra General Hospital and Baiji General Hospital). The swab were taken from both nostrils [13] and cultured on mannitol salt agar which is a selective and differential medium for *S. aureus* [14]. The diagnosis of S. aureus isolates by classical method based on morphology, coagulase test, DNase test and mannitol salt agar fermentation.

Detection of MRSA by Classical Method

The susceptibility of all *S. aureus* isolates to the antibiotic Cefoxitin was tested at a concentration of 30 µg. This antibiotic was used as an alternative to methicillin and oxacillin in the identification and diagnosis of MRSA. Isolates with inhibition zones of 21 mm or less were considered to be resistant to methicillin, and isolates with a zone of inhibition more than 22 mm were considered to be sensitive to methicillin [15].

Diagnosis of *S. aureus* and MRSA by Polymerase Chain Reaction (PCR)

The present study was designed to investigate the *fem*A gene for the diagnosis of *S. aureus* [16] and *mec*A, the antibiotic resistance gene [17]. Using PCR technology, the prevalence of these genes recorded in the isolates of bacteria spread among the medical staff of Salah al-Din Hospitals. Total genomic DNA was extracted from 88 isolates using a method described by Onasanya *ET al* [18].A single colony was inoculated on 5 ml of brain heart infusion broth and incubated over night at 37ºC.

Then 1.5 ml of a saturated culture was harvested with centrifugation for 5 min. at 14,000 rpm, then phenol: chloroform method was performed for DNA extraction. DNA Quality was determined using 1% agarose gel electrophoresis and Nanodrop (Thermo scientific, Germany) was used for determination of DNA concentration.

A Duplex PCR method was used for the diagnosis of *S. aureus* and MRSA [19].A pair of primers, one of which was used to diagnose *S. aureus* (*fem*A) bacteria with 314 bp and the other with 533 bp for the detection of methicillin resistant gene (*mec*A). The primers were prepared in the form of a lyophilized powder by Bio basic company, the specification of the primers used is shown in Table (1).

Table 1: Primers used in the present study

No	Gene	Sequence	Size (bp)
1.	*mec*A	Forward 5'AAAATCGATGGTAAAGGTTGGC'3 Reverse 5'AGTTCTGCAGTACCGGATTTGC'3	533
2.	*fem*A	Forward 5'CATGATGGCGAGATTACAGGT'3 Reverse 5'GTCATCACGACCAGCGAAA'3	314

PCR reactions were performed in a total volume of 25 µL of the Go Tag Master Green kit from the American company Promega and according to the attached instructions. Each sample tube contains 12.5 µL of master mix, 2.5 µL of genomic DNA, 2 µL of each primer (four primers) and the reaction volume completed with (2 µL) DNase/RNase free water to 25 microliters. PCR reaction was accomplished using the program consisting of primary denaturation at 95°C for 5 min, 35 cycles were contained 50 seconds at 95°C, 55 seconds at 54°C and 50 seconds at 72°C, the reaction ended with an additional 5 minutes of extension at 72°C. After the reaction time, five microliters were withdrawn from each

tube placed in 2 % agarose gel electrophoresis and then electrolyzed by 5 V/cm for 90 minutes. The gel stained by immersing it in ethidium bromide for 45-60 minutes with stirring and imaged using the Gel Documentation System.

Results and Discussion

A total of 57 isolates of *S. aureus* were collected from (279) swabs from the medical staff of four hospitals in Salah al-Din governorate

Isolation and Diagnosis of *S. aureus*

The isolates diagnosed by two methods: The first method is the classical method which is used in the hospitals under study and based on the cultural and microscopic characteristics of the colonies and the biochemical tests. The second method is PCR technique. The results of the present study showed that of the 279 swabs taken from the medical staff of four hospitals in Salah al-Din, 88 were isolated as *S. aureus* (31.5%) of the total number of swabs (Fig. 1) using the classical method used in the hospitals of the governorate under study. The study also showed that from the 88 isolates of *S. aureus*, which were diagnosed by classical methods, 58 (65.9 %) of them were methicillin resistant *S. aureus* using the Cefoxitin antibiotic method, which is the method used in hospitals for the presence of *mec*A gene [15].

When the 88 isolates of *S. aureus*, which diagnosed by the classical method, were re-diagnosed using Polymerase Chain Reaction (PCR) method, the results showed that only 57 isolates were diagnosed as *S. aureus* (% 20.4) of the total number of swabs and that the number of isolates of *S. aureus* that possessed the *mec*A gene was only 24 isolates from the total 57 *S. aureus* isolates (42.1%) as shown in Figure (1). That is mean that 8.6% of medical staff carry MRSA.

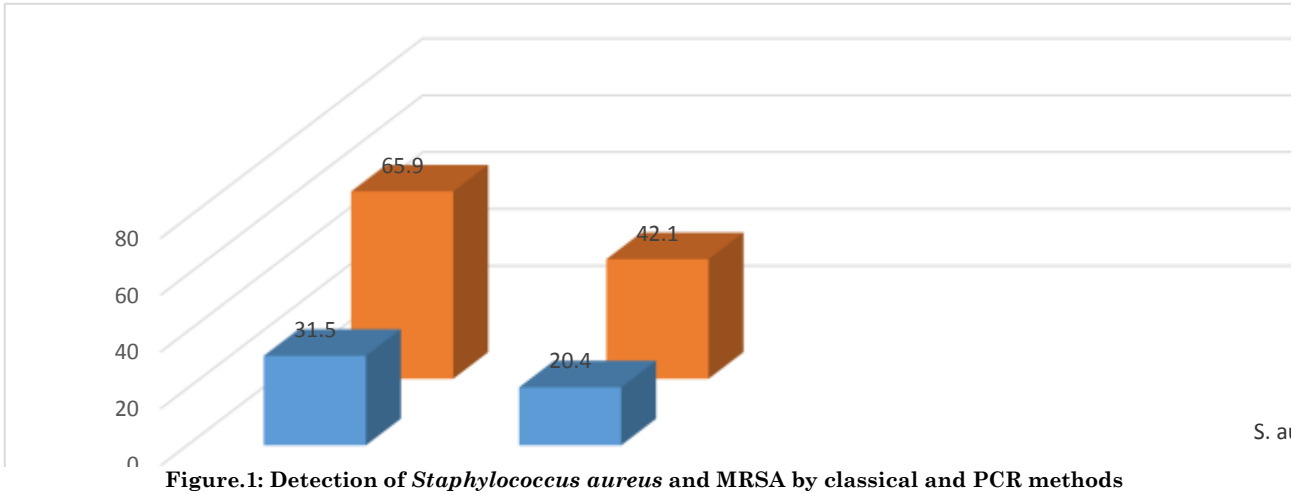

Figure.1: Detection of *Staphylococcus aureus* and MRSA by classical and PCR methods

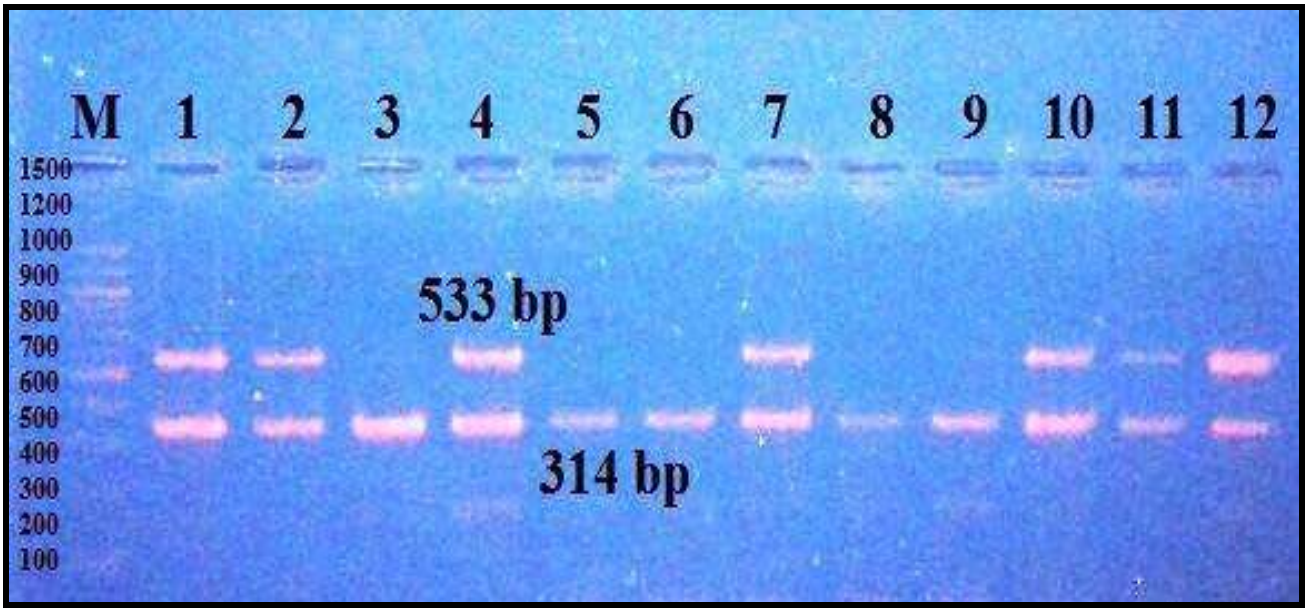

Figure 2: Represents 2% agarose gel electrophoresis of the Duplex PCR products showing lane (M) 100 bpDNA ladder, lane 1 fem A (314 bp) and *mec*A (533 bp) and lane 3 only fem A (314 bp)

There is difference in the results of the two techniques as shown in fig. 1. The PCR method in diagnosis and investigation of different genes is very accurate compared to the classical methods for the diagnosis of bacteria and its susceptibility to antibiotics using disc diffusion method, and PCR sensitivity may reaches more than 97 % [20].

Thirty one (35.2 %) isolates of the 88 isolates were misdiagnosed as *S. aureus* by the classical method. Furthermore, 34 (58.6 %) isolates of *S. aureus* were misdiagnosed as MRSA by the classical disc diffusion method. The reason for the weakness of the disc diffusion method could be due to the storage conditions of the antibiotic before and after the first use. Since, the antibiotic should be kept before use at a temperature of -20 ° C to 8 ° C.

This condition may not be available before use at some of the local companies for laboratory supplies. Moreover, the antibiotic is accompanied by poor storage conditions since its import until it reaches the local companies in Iraq. The storage conditions of the antibiotic after use should be placed in a sterile tube with a moisture-proof material equipped with antibiotic and the closure of this tube well and then stored at a temperature of -20 m, preferably not to store for a long time so as not to lose antibiotic efficiency. Another reason may also be the disturbance in incubator temperature due to various factors, such as a power outage in the Iraqi hospitals or an overheating of the laboratory room to more than 37 C° at summer, which may affect the diameter of the inhibition zone.

Percentage of *S. aureus* and MRSA Isolates among Medical Staff at Salah Al-Din Hospitals

The results showed that the proportion of *S. aureus* among the medical staff of Salah al-Din hospitals was (20.4%). The study also showed that MRSA represented 42.1 % of the total *S. aureus* isolates. The percentage of *S. aureus* nasal carriage among the medical staff was 37.5%, 22.6%, 16.3%, and 15.2% in Baiji General Hospital, Samarra General Hospital, Tikrit Educational Hospital and Salah Al-Din General Hospital respectively.

The rates of MRSA from these isolates were 52.6%, 50%, 41.1%, and 11.1% in Samarra General Hospital, Baiji General Hospital, Tikrit Teaching Hospital and Salah Al-Din General Hospital, respectively, as shown in Figure 3 and Table 4. Nasal carriage of MRSA in Iraq increased in the last years. Nawfal, 2016 reported that more than 51 % of *S. aureus* isolated from medical staff nares were resistant to methicillin [21]. Also Jawad found that *S. aureus* nasal carriage among health care workers in Iraq was 47.6 %, and 56.7 % of them were MRSA [22].

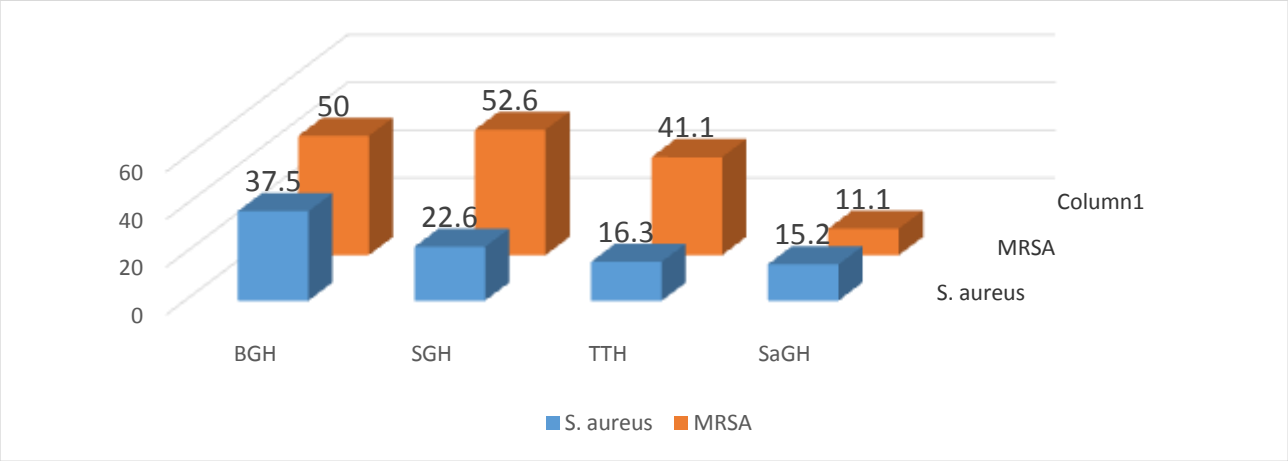

Figure 3: Percentage of *Staphylococcus aureus* and MRSA isolates among medical personnel at Salah al-Din hospitals

Table 4: Distribution of *Staphylococcus aureus* and MRSA among Salah-al-Din hospitals

Hospital	No. of swabs	No. of *S. aureus* isolates	%	No. of MRSA isolates	%
Baiji General Hospital	32	12	37.5	6	50
Samarra General Hospital	84	19	22.6	10	52.6
Tikrit Teaching Hospital	104	17	16.3	7	41.1
Salahuddin General Hospital	59	9	15.2	1	11.1
Total	**279**	**57**	**20.4**	**24**	**42.1**

The high rates of MRSA nasal carriage among medical staff may be due to their prolonged stay at the hospitals and their contact with patients [23]. Also resistance could be due to misuse and excessive of the antibiotics for both hospital and community acquired infections .Furthermore, the lack of antibiotic prescription policies, and the commercial availability of antibiotics without a medical doctor prescription.

Conclusions

The classical methods used in most Iraqi hospitals are not accurate in diagnosing S. aureus isolates and MRSA isolates. Also a high percentage (42.1%) of S. aureus isolated in this study was resistant to methicillin (MRSA). Therefore, precautions should be taken to keep that staffs (who carries MRSA strains) from the operating theaters and intensive care units.

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