World Journal of Pharmaceutical Sciences
ISSN (Print): 2321-3310; ISSN (Online): 2321-3086
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Available online at: http://www.wjpsonline.org/
Original Article

Molecular detection of methicillin resistant *staphylococcus aureus* isolated from burns infection in Al-nasiriyah city

Ahmed Nihad Ibed[1] and Saad Salman Hamim[2]

[1]MSc student, Dept of Biology, Thi qar College of Science - Iraq, [2]Dept of pathological analysis Thi qar College of Science – Iraq

Received: 21-07-2014 / Revised: 04-08-2014 / Accepted: 23-08-2014

Abstract

The present study aimed to investigate the prevalence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) in the burn unit at Al-Hussein teaching hospital during the period from September, 2013 to January, 2014 in Thi-Qar province, Iraq. From a total of two hundred burn swab samples, there were 90 isolates recorded a 90 positive MRSA culture with a percentage of (45%). Identification was done depending on morphological, cultural, microscopical characterization and biochemical tests. Depending on the area of collection, the swabs were collected from 7 sites. Hands showed the highest Staph. *aureus* infections with 25 isolates (27.77%), followed by feet and necks with 20 (22.22%) and 16 (17.77%), respectively. axilla and back sites showed the lowest infections with one *Staph. aureus* isolate with a percentage of for both (1.11%). A total of 90 isolates of *Staph. aureus* was further examined using polymerase chain reaction (PCR) for detection of *mec*A gene,16s rRNA and PVL. The results revealed that of MRSA isolates yield amplification products of mecA gene 68(75.5%), 16S rRNA found in all isolates and eighty-six isolates were PVL negative (95.5%) and the remaining 4.4% (4 isolates) were PVL positive.

Key words: Methicillin-Resistant *Staphylococcus aureus*-Burn infections-Polymerase chain reaction.

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INTRODUCTION

Staphylococcus aureus is one of the most significant human pathogens which causes different infections ranging in severity from mild superficial skin infections to life threatening bacteremia and endocarditis. The occurrence and dissemination of methicillin-resistant variants (MRSAs) in clinical settings has raised the concern for the constant increase of nosocomial infections all over the world. Burns is a thermal injury of the skin, although electrical and chemical injuries may also result in burns [1]. Thermal injury destroys the physical skin barrier that normally prevents invasion of microorganisms. During the first weeks following thermal trauma, the affected sites are colonized with bacteria [2]. Following colonization, these organisms of the surface start to penetrate the burn eschar to available extent and viable sub eschar tissues become invaded [3,4]. It is now estimated that about 75% of the mortality following burn injuries is related to infections. The pattern of infection differs from hospital to hospital; the varied bacterial flora of infected wound may change considerably during the healing period [5]. When a hole is created on the skin, microorganisms, usually the opportunistic organisms, invade the holes and multiply leading to a delay in the healing process and finally infectious condition. The spectrum of infection ranges from asymptomatic colonization to bacteraemia and death [6]. Methicillin - resistance *Staphylococcus aureus* was first isolated in 1960, and for the past four decades MRSA infections have been largely associated with hospital environments and referred to as hospital-acquired MRSA (HA-MRSA). However, in the late 1990s, community-acquired MRSA (CA-MRSA) infections began to appear in healthy people who had no known risk factors for these infections [7]. MRSA has rapidly become the bacteria of the decade and significant cause of both health care–associated and community-associated infections [8]. MRSA now respond only to very advanced antibiotics that were never meant to be a first-line defence such as vancomycin. However, many researchers have been noted that MRSA resistant to Vancomycin [9,10,11,12,13]. The broad range of infections caused by *Staph. aureus* is

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***Corresponding Author Address:** *Dr. Saad S. Hamim, Pathological Analysis Department, Science College, Thi-Qar University, Nassiriyah, Iraq; E-mail:saad@sci.thiqaruni.org*

related to a number of virulence factors that allow it to adhere to surface, invade or avoid the immune system, and cause harmful toxic effects to the host [14,15]. The present study aimed to perform a molecular detection of MRSA among burn infections in Nassyriah city, Iraq.

MATERIALS AND METHODS

Samples collection: Sterile swab was moistened with sterile normal saline and was rotated at least 5 times in one directly inoculated on Mannitol Salt Agar (MSA) and incubated at 37°C for 24 hr. All colonies from primary cultures were purified by subculture on brain-heart infusion (BHI) agar and then re-inoculated onto MSA and incubated at 37°C for 24 hrs [16,17,18].

Identification of *Staphylococcus aureus:* *Staphylococcus aureus* was identified depending on the morphological features on culture media and biochemical tests according to Bergey's manual [19,20]:

A. Microscopic examination: The isolates were stained by Gram stain to detect their response to stain, shapes and their arrangement [21].

B. Growth on mannitol salt agar: The plates were streaked from a pure colony of tested bacteria and then incubated at 37°C for 24 hr. This medium was used for selective isolation and cultivation of bacteria [22].

C. Biochemical tests

1. Catalase tests: A drop of catalase reagent (3% H_2O_2) was placed on a slide. A colony of tested bacteria was mixed with the reagent on the slide, and positive results were indicated by air bubbles formation [23].

2. Coagulase test: Citrated rabbit plasma diluted 1:5 was mixed with an equal volume of BHI broth culture then incubated at 37°C. A tube of plasma mixed with sterile broth was included as a control. Formation of clots in 1-4 hr. indicates a positive test. Negative result re-examined for 24 hrs [24].

3. Api Staph system: Api Staph. is an identification system for Staphylococcus and Micrococcus. This test was done according to the company instructions (BioMerieux SA. / France).

D. Molecular detection

1. DNA extraction and purification: DNA was extracted and purified according to the company manufacturer instructions (Geneaid/ Korea).

2. 16S rRNA, mecA genes amplification: Amplification of the 16S rRNA and *mec*A genes were done by using primers described by [25] (Table 6). The amplification reaction contained 1.5µl of template DNA in a final volume of 25µl containing 0.4, 0.8 and 0.8 µm for the primers specific for the 16S rRNA, PVL and *mecA* genes respectively with 2U of Ampli-Taq Fermentas, 1.5 mmol. l-1 MgCl2, 1.6X Taq buffer, 0.2m M of each deoxynucleoside triphosphate (dNTP). The thermo cycling conditions were set at 94°C for 5 min. followed by 10 cycles of 94°C for 45s, 55°C for 45s, and 72°C for 75s and 25 cycles of 94°C for 45s, 50°C for 45s, and 72°C for 75s and finally soaked at 20°C. The expected PCR amplicons were 756, 433 and 310 bp for the 16S rRNA, PVL and *mecA* gene, respectively. The fragments were visualized by 0.2µl of ethidium bromide staining using 1.5% agarose gel using with 1x TBE buffer at 100V for 45 min.

3. Agarose gel electrophoresis: The agarose gel was prepared according to the method of [26]. Two concentrations of agarose gel were prepared (1% and 1.5%). The concentration of 1% agarose was used in the electrophoresis after DNA extraction process, while 1.5 % agarose was used after *mecA* and 16S rRNA gene by PCR detection. A 25ml of 1X TBE buffer and 0.5 µl ethidium bromide were added into a beaker, 0.25 g agarose was added to the buffer. The mixture was heated for boiling by hot plate until all gel particles were dissolved and allowed to cool down to 50-60ºC.

RESULTS AND DISCUSSION

16S rRNA, PVL and *mec*A genes amplification

All 90 *Staph. aureus* isolates were confirmed to be Staph through the amplification of the 16S rRNA gene and tested for being MSSA or MRSA and being PVL positive or not through testing *mecA* and *lukS/F-PV* genes, respectively. Accordingly, triplex PCR assay may yield 3, 2 or 1 band/s. The first band of 756 bp size corresponds to the partial amplification of the 16S rRNA and must be present for all *Staphylococcus* isolates. The second band was a 433 bp size that corresponds to the amplification product of the PVL gene. The third band was a 310 bp size that corresponds to the amplification product of the *mecA* gene (Fig.1). Fig. (2) summarizes the results of the triplex PCR for all 90 isolates, showing that 75.5% (68 samples) of the isolates were MRSA and 24.5% (22 samples) were MSSA. Eighty six isolates were PVL negative (95.5%) and the remaining four isolates (4.4%) were PVL positive. MRSA occurrence among *Staph. aureus* varies according to the geographical region, with a low frequency (~1%) in some countries in Europe (e.g. Netherlands, Denmark and Sweden) and a high frequency (>60%) in countries such as that observed in USA and Japan [27,28,29,30,31]. The prevalence of MRSA in the present study was (75.5%) in which be in agreement with other studies in Iraq, [32,33,34,35,36], who recorded a percentages of 90.9%,75%, 94.3%, 88% and 65.3% , respectively.

Methicillin, is a modified penicillin expressly designed to resist the destructive action of the staphylococcal penicillinase, became available for therapeutic use in 1959, but its success was short lived. After only 2 years, the first case of MRSA was reported [37]. This time, the resistance was not due to a hydrolysing enzyme, but to a more sophisticated mechanism. Methicillin, like all other penicillins, exerts its action by blocking the proteins called penicillin binding protein (PBPs), which are responsible for the construction and maintenance of the bacterial cell wall. *Staph. aureus* resistant strains produce a new protein, called PBP2a, which was not affected by methicillin and could replace the other PBPs, thus allowing the survival of *Staph. aureus* in the presence of methicillin. PBP2a is encoded by the gene *mec*A, which is the hallmark of MRSA. As opposed to the penicillinase gene, *mec*A does not reside on a plasmid but on the chromosome, embedded in a large mobile genetic element called Staphylococcal Chromosome Cassette *mec* or SCCmec [38]. The frequency of MRSA depends on a region and is less than 1% in Nordic countries, and more than 30% in Spain, France, Italy and India [39]. In Iraq, the percentage of MRSA was reported to accounted for [40,41,42]. MRSA is the most common in the departments of resuscitation, burns, and traumatology. Although the role of PVL in *Staph. aureus* pathogenicity remains controversial, a number of studies showed its association with primary skin infections and necrotizing pneumonia, while others reduced its importance as a virulent factor [43,44,45,46,47,48]. The present study results showed that, PVL genes were detected in 1.4% of MRSA and 13.6% of MSSA. Several conducted studies showed that the prevalence of PVL genes among MRSA compared to MSSA isolates from infections and colonization [49,50] with an increase in the severity of infections in PVL positive MSSA strains being detected [46,51,52,53]. Only about five percent of methicillin-resistant strains of *Staph. aureus* and HA-MRSA carry the PVL gene [54].

CONCLUSION

There were high rates of burn infections due to *Staph. aureus*. These infections are significantly increased as the frequency of hospital admission increased and as the duration of hospital stay prolonged. The DNA amplification of *mec*A gene for the isolates demonstrated a positive results for with high percentages in burns unit under study.

Table (1): Oligonucleotide primer sequences for PCR amplified of mecA and 16S rRNA genes.

Primer	**Orientation**	**Oligonucleotide sequence (5'→3')**	**Product size (bp)**
staph 756	Forward	AACTCTGTTATTAGGGAAGAACA	756
	Reverse	CCACCTTCCTCCGGTTTGTCACC	
***Mec*A**	Forward	GTAGAAATGACTGAACGTCCGATAA	310
	Reverse	CCAATTCCACATTGTTTCGGTCTAA	
PVL	Forward	ATCATTAGGTAAAATGTCTGGACATGATCCA	435
	Reverse	GCATCAAGTGTATTGGATAGCAAAAGC	

Figure (1): Agarose gel electrophoresis showing representative PCR products after 16S rRNA, PVL and mecA genes amplification. The first lane shows 100 bp DNA marker (Fermentas), lanes 1-8 show PCR products of the three genes of different isolates. Lane 9 is the negative control.

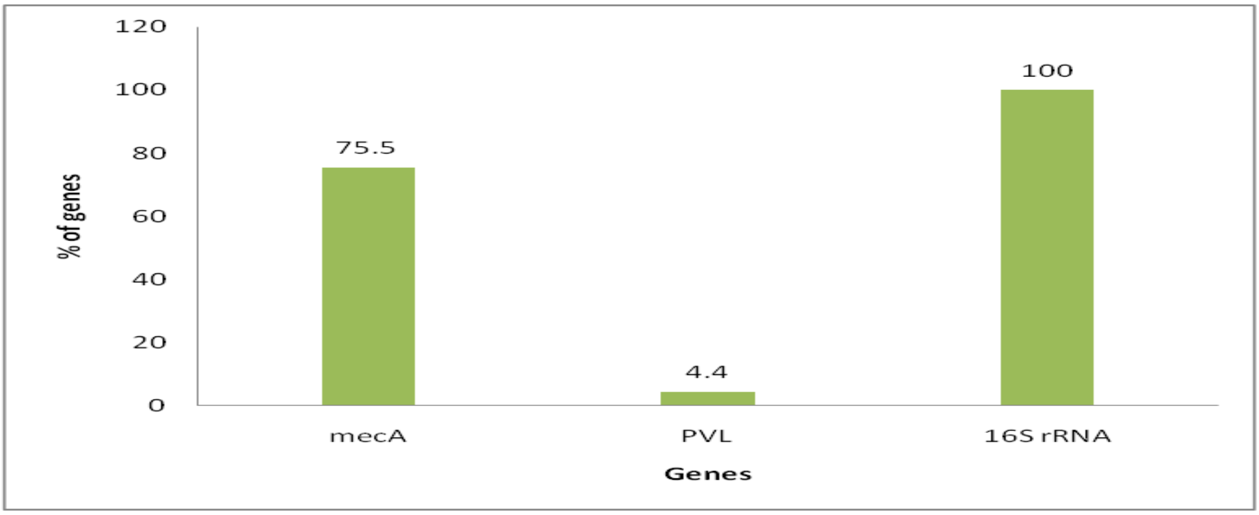

Fig (2): The percentage of genes that appears in 90 sample.

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