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THE PRESENCE OF MEC A GENE IN METHICILLIN –RESISTANT *STAPHYLOCOCCUS AUREUS* STRAINS (MRSA) ISOLATED FROM SURFACES OF PLANTS IN AL – BEIDA HOSPITAL GARDEN

Nagah S. A. Abubaker* **and Alia G. Alythi**

Department of Botany, Faculty of Science, Omar AL-Mukhtar University.

***Corresponding Author: Nagah S. A. Abubaker**

Department of Botany, Faculty of Science, Omar AL-Mukhtar University.

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ABSTRACT

MRSA is one of the most worrisome microorganisms in hospitals, as they cause serious health problems. Detection of mec A gene of this pathogen must be used as a rapid screening method for detection of MRSA isolate. The molecular result documented that all six Staphlococcus aureus isolates showed positive results of mec A gene. And MRSA in the plant surfaces this indicates possible spread of these strains from hospital into community.

KEYWORDS: MRSA – Identification - PCR – mec A gene.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) has been noted as the main problem in many countries and appropriate identification methods were developed for rapid detection of MRSA. The method was based on the polymerase chain reaction (PCR) which is a scientific technique in molecular biology used to amplify apiece of DNA to generated thousands to millions of copies of a particular DNA sequence. (Joshi and Deshpand, 2011). (Rajamani *etal.*, 2017)

Nowadays, *S. aureus* is the cause of major health problems in many countries. Due to its ability to cause threatening infections and adapt to different environmental conditions Stefani and Gglio (2010), Yok-Ai and Philippe (2010). Moreover, their ability to develop effective mechanisms against anti-Staphylococcal drug (Navratna *etal.*, 2010). In addition, different antimicrobial susceptibility methods were used for identification of MRSA, such as oxacillin disk diffusion Mohanasoundaram and Lalitha (2008), Struelens *etal.*,(2009). Therefore, there are many reports that it is necessary to use more fast and accurate tool such as PCR assay (Hallin *etal.*, 2003).

The gene called mecA is a gene present in bacterial cells. The bacterium known as MRSA is the most widely recognized carrier of the mecA gene (Japoni *etal.*, 2004). Besides in the species *Staphylococcus*, mecA is distributed on the genetic factor SCCmec (Ghaznavi-Red *etal.*, 2010, Shore and Coleman, 2013, Padmavathi, 2019). The mec A gene which encodes penicillin – binding protein 2a (PBP 2a). a large mobile genetic variable that differs in size and genetic composition between different MRSA strains (Lim *etal.*, 2002 and Padmavathi,2019). This protein is responsible for MRSA resistance to mithicillin (Hallin *etal.* ,2003) and it can be passed to other bacteria (Zúñiga *et al*., 2019).

The mec A gene are highly conserved among Staphylococcal organisms, molecular amplification of the mecA gene is used to identify MRSA strains with mecA gene because it was found to be more sensitive in detecting the mec gene Berger-Bächi, (1999) and Nasution *et al*.,(2018)

The purpose of this work was to detect the mec A gene in *Staphylococcus aureus* (MRSA) strains were contaminated the surfaces of plants in AL – Beida hospital garden. And evaluate usefulness of amplification of mec A gene and its reliability in the identification of MRSA strains.

MATERIAL AND METHODS

Collection and identification of samples

Samples were collected by swabs from the surface plants in the hospital garden and placed on a selective Media of Mannitol salt agar and were incubated at 37C° for 24 and 48 hours. All suspected *S. aureus* colonies were plated onto blood agar. Identification of *S. aureus* suspicious grown colonies was based on Gram staining and standard biochemical reactions, including catalase, coagulase and antibiotic (oxacillin) susceptibility test.

Gram stain

The most common and useful staining procedure is the gram stain which separates bacteria into two groups according to the composition of their cell wall and was

done as described (De La Maza *etal.*, 1997)

Coagulase test
Coagulase test was done according to (De La Maza *etal.*, 1997)

Catalase test
Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile and then Place a drop of 3% H2O2 on to the slide and mix. A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling. Negative result is no bubbles or only a few scattered bubbles (De La Maza *etal.*, 1997).

Antibiotic susceptibility test
Susceptibility test was performed by streaking the colonies(about 5x 10^8 cells) on Nutrient agar (Becton Dickinson, UK) using the Kirby-Bauer disc diffusion technique. The following antibiotic at given concentration was used: Oxacillin (OX) 1mcg and incubated at 37 ◦C for overnight (Bauer *etal.*, 1966) and (Avendaño, 2010)

Detection of mecA gene by PCR technique
The standard PCR assay was performed using the DNA amplification instrument Mastercycler gradient (Enppendorf, Germany) to identify MRSA strains. Cellular DNA was obtained from *Staphylococci* colonies grown overnight on blood agar plates using DNA Extraction Kit (bioneer Co., Korea) in accordance with manufacturer's instructions. The *mecA*- specific primer pairs used for amplification of 533 base pair (bp) fragment are forward, 5'-AAAATCGATGGTAAAGGTTGGC-3', and revers, 5'-AGTTCTGGAGTACCGGATTTGC-3' (Bűhlmann *et al.*, 2008). A volume of 1 μL of prepared DNA (0.5 μg) was added to a final volume of 25 μL PCR mixture containing 10 μL of 2x Master Mix (Ampliqon, Denmark), including 1x PCR buffer, 1.5 *mmol/L* $MgCl_2$, 0.15 *mmol / L* dNTP, and 1.25 IU Taq DNA polymerase, (Ampliqon Co., Denmark), 0.7 *ul* of 0.8 *umol /L* each primer and 12.6 *ul* of sterile distilled water. The thermal cycling protocol for PCR was comprised 95 °C for 3 min, followed by 33 cycles of 94 °C for 1 min, 53 °C for 30 s and 72 °C for 1 min, with a final extension at 72 °C for 6 min. the amplified products were visualized by electrophoresis in 2% agarose gels stained with ethidium bromide (Pournajaf *et al.*, 2014)

RESULTS
Biochemical tests for the confirmation of *S. aureus*
For the confirmation of *S. aureus* in specific medium of manitol salt agar it gaves yellow collour after the incubation time (fig.1a), It contains a high concentration 7.5-10.0 % of NaCl salt (Anderson and Cindy, 2013), Gram stain (fig.1b), cogalase and catalase tests are positive so they confirm the *S. aureus* organism (Table.1).

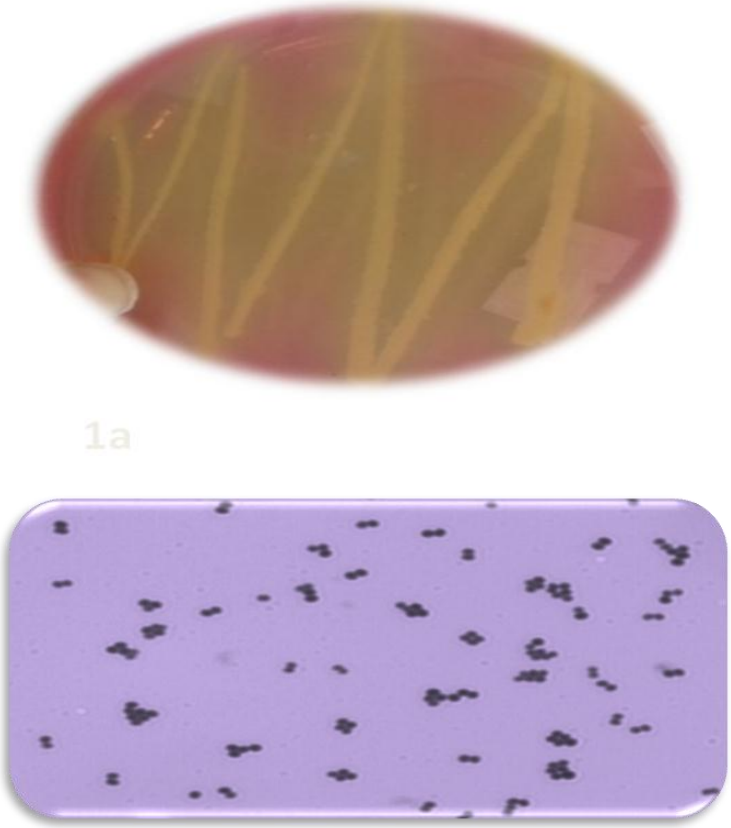

Fig.1a *S. aureus* on specific medium of manitol salt agar it gives yellow color (fig.1b positive Gram stain.

Antibiotic susceptibility test

The test result showed that all selective strains were resistance to oxacillin by using oxacillin disk diffusion method (Table.1). Oxacillin is the best indicator antibiotic (De La Maza *etal.*, 1997). *S. aureus* were resistance to methicillin. (Al-Alak and Qassim, 2016) and (Kader *et al.*, (2011) reported that most of isolates were resistant to methicillin and Oxacillin discs. Isolated bacteria showed resistance against oxacillin and erythromycin in most cases (Naghavi-Behzad *et al*., 2015 and Panliliom *etal.*, 1992)

Table. 1 Biochemical tests for the confirmation of *S. aureus.*

Strain No.	Gram stain	Coagulase	catalase	manittol fermentation	Resistance to oxacillin
1	+	+	+	+	R
2	+	+	+	+	R
3	+	+	+	+	R
4	+	+	+	+	R
5	+	+	+	+	R
6	+	+	+	+	R

Detection of mecA gene by PCR technique

The present study, the polymerase chain reaction (PCR) was used to detect the methicillin resistance determinant by amplifying a 533 – bp region of the mec A gene. The results indicated that all selective six isolates were confirmed as MRSA based on the presence of mec A gene in *S. aureus s* strains, which were resistance to oxacillin (Fig.1). Amplification of DNA by PCR is rapid, specific, sensitive and accurate and valuable diagnostic tool for identification of MRSA, particularly in clinical microbiology laboratories (Kumurya, 2015). (Al-Alak and Qassim, 2016) reported that the staphylococcus isolated from patient were confirmed as *S. aureus* by using mecA gene. Moreover the *S. aureus* (MRSA) were detected in all samples of west water treatment plant by using PCR (mec A gene) Börjesson *etal*., 2009.

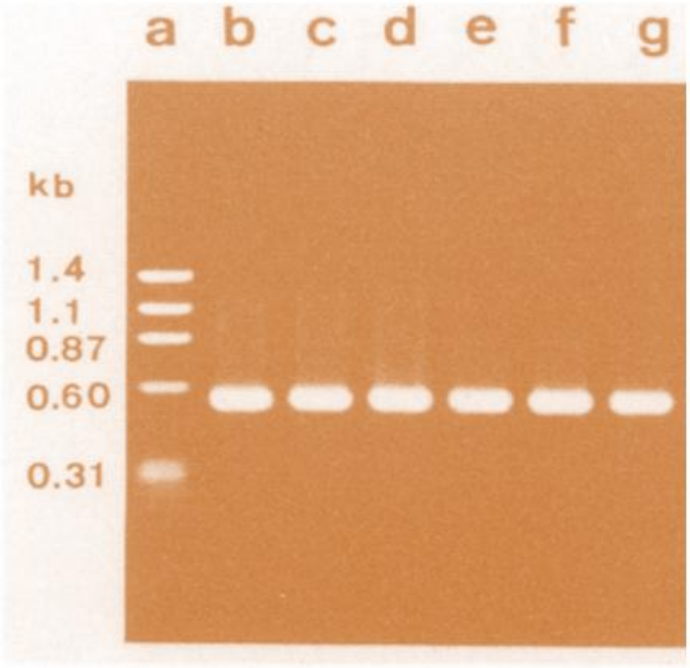

Fig.1 PCR amplification of mec A gene in six selected isolates of *S.aureus* Lane a: marker Lane b - g PCR product of mec A gene (533bp).

IN CONCLUSION

our findings indicate that PCR based detection of MRSA is highly recommended. Moreover, the samples were collected from plants were nearest windows in hospital. MRSA on the plant surfaces indicate possible spread of these strains from hospital into community.

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