Methicillin-Resistance *Staphylococcus Aureus* (MRSA) in Hospitals: The Unwanted Guest

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Abstract

Background: Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most frequent causes of Nosocomial infections around the world.

Aims: This study aimed to detect some diagnostic genes and some virulence factor genes for MRSA isolates.

Method: During the period of from August to December 2019, 46 MRSA were isolated from different clinical samples such as Urinary Tract Infections (UTIs), Wounds infection's, Diabetics foot patients, Burn patients and Otitis media in Al- Hussain Teaching Hospital in Thi-qar province, Iraq. All MRSA isolates were subjected to conventional Polymerase Chain Reaction to detect 16SrRNA and *mec*A genes and some virulence factors genes *hla* and *tst*-1 genes. Six PCR product were selected and subjected to partial DNA sequencing for the 16SrRNA gene to follow up their possible relationship between them and what recorded globally in Genbank.

Results: The results revealed that all isolates 46(100%) have 16SrRNA, *mec*A, and *hla* genes, While only 23 isolated (50%) have *tst*-1 gene. The six PCR product of 16SrRNA was registered in Genbank under official accession numbers of (MT605393.1, MT605385.1, MT605394.1, MT605386.1, MT605387.1, and MT605388.1). The phylogenetic tree that was constructed by MEGA10 software showed that there were different molecular relationships among the local *Staph. aureus* isolates with analogous ones around the world.

Keywords: *MRSA;Gene sequencing, Phylogenetic tree, Diagnostic genes, Virulence factors.*

Introduction

One of the main public health problem worldwide, especially in developing countries Nosocomial infections (NIs) [1]. As these infections can occur through hospital admission, they cause long stay, incapacity, and economic load. Commonly prevalent infections include central line-associated bloodstream infections, catheter-associated urinary tract infections, surgical site infections and ventilator-associated pneumonia. There are several pathogens cause nosocomial infections, these pathogens include bacteria, viruses,fungal and parasites[2]. Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the most common reasons of hospital- and community-associated infections. The ability of this bacteria to resist to the entire class of β-lactam antibiotics, such as methicillin and penicillin, therefore makes MRSA infections difficult to treat[3]. For instance, when the starter of penicillin to the market for the treatment of hospitalized patients, the resistant strains to the penicillin rapidly appeared[4]. Presently, MRSA strains account for various of Staphylococcal infections and increasing reports of MRSA strains in the worldwide[5].

Material and Method

Samples Collection: A total of 46 MRSA were isolated from patients of both gender of different ages who suffered from symptom-based Urinary Tract Infections (UTIs), Wounds infection's, Diabetics feet patients, Burn patients and Otitis media. A patients took

care and medication at AL-Hussain Teaching Hospital in AL-Nasiriya City, Southern Iraq from August to December 2019.

Isolation and identification of *Staphylococcus aurues:* The collected specimens were inoculated onto blood agar, mannitol salt agar,and MacConkey agar according to standard method. *Staph. aureus* was identified depending on the morphologicalfeatures (colony size, shape, color, hemolysis, translucency, edge, elevation, and texture) on culture media[6]. The biochemical tests were used include Catalase, Coagulase, Oxidase and Novobiocin [7]. The diagnostic of bacteria were confirmed by API system and Vitek2 compact (biomerieux, France).

Molecular Detection of *Staph. aureus:* 1.Genomic DNA was extracted from MRSAisolates by using Genomic DNA Mini Bacteria Kit (Anatolia/Turkey). **2.** All MRSAisolates were subjected to the detection of 16SrRNA,*mec*A, *hla*,and *tst*-1 genes by conventional PCR technique using specific primers pairs for every gene (Table1). The amplification genes were put into the thermo cycler (ABM Canada) and the right PCR cycling program parameters conditions were adjusted according to each primer.

Table 1:Specific primers of *Staph. aureus*.

Gene	Primer sequences (5˚ – 3˚)		Product size (bp)
16SrRNA	F	AGAGTTTGATCCTGGCTCAG	1500
	R	GGTTACCTTGTTACGACTT	
*mec*A	F	TGAGTTGAACCTGGTGAAGTT	855
	R	TGGTATGTGGAAGTTAGATTGG	
tst-1	F	ACCCCTGTTCCCTTATCATC	326
	R	TTTTCAGTATTTGTATCGCC	
hla	F	GAAAACACGTATAGTCAGCTCAGTAAC	951
	R	GTCATTTCTTCTTTTTCCCAATCG	

Sequencing Analysis: The PCR product of six MRSA isolated from different infected sites were subjected to partial sequencing of 16SrRNA gene and blasted in NCBI against standard strains of *Staph. aureus*. The samples sequences which assigned primarily as (N STQ1,NSTQ2,NSTQ3,NSTQ4,NSQ5,and NSTQ6). A phylogenetic tree for genes sequence was constructed by using (MEGA10) software[8].

Results

Detection of 16SrRNA and *mec*A genes: All MRSA isolates (n=46) were diagnosed by conventional PCR technique through the amplification of 16SrRNA and *mec*A genes to confirm that the verified isolates are *Staph. aureus* and MRSA, respectively. The results showed that all isolates were positive for both the targeted genes. the size of products were approximately 1500 bp for 16SrRNA gene and approximately 855 bp for *mec*A gene.

Detection of virulence genes: Two of virulence genes of MRSA were detected through the amplification of *hla* and *tst*-1 genes. The results revealed that all isolates 46(100%) had *hla* gene and only 23 isolated (50%) had *tst*-1 gene a with a product sizes of approximately 951 bp and 326 bp, respectively (Fig.1, 2).

Phylogenetic analysis: The six selected MRSA strain granted the official Genbank accession numbers of MT605393.1, MT605385.1, MT605394.1, MT605386.1, MT605387.1 and MT605388.1. The phylogenetic tree that was constructed by MEGA10 software showed that there were different molecular relationships among the local *Staph. aureus* isolates with analogous ones around the world (Fig. 3).

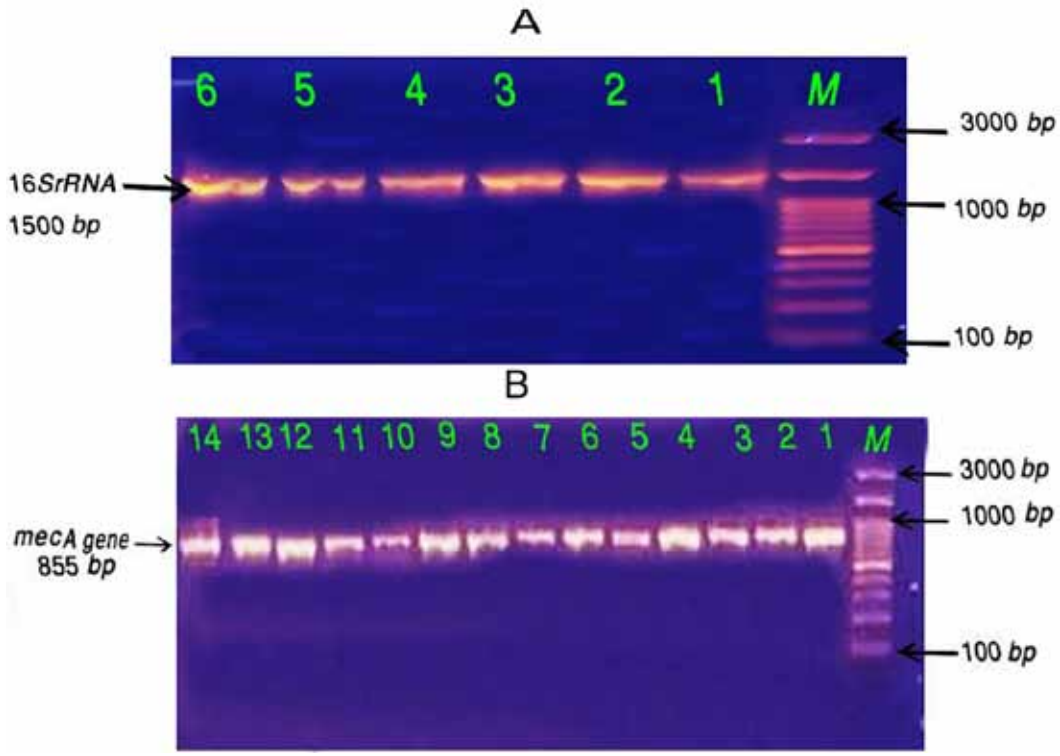

Figure 1 A: Agarose gel electrophoresis of 16SrRNA gene. M:3000 bp ladder; Lane [1-6] were positive with a product size of approximately 1500 bp. B: Agarose gel electrophoresis of *mec*A gene. M:3000 bp ladder; Lane [1-14] were positive with a product size of approximately 855 bp.

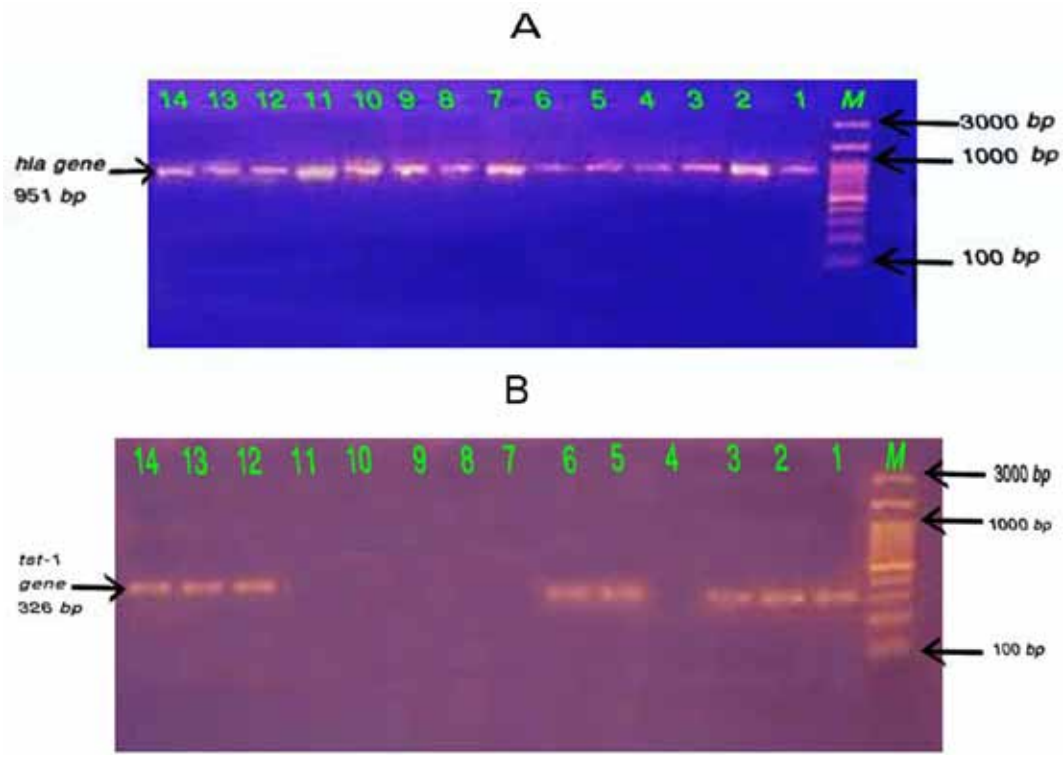

Figure 2 A:Agarose gel electrophoresis of *hla* gene. M:3000 bp ladder; Lane[1-14] were positive with a product size of approximately 951bp. B: Agarose gel electrophoresis of *tst*-1 gene. M:3000 bp ladder; Lane[1,2,3,5,6,12,13 and 14] were positive; Lane (4,7,8,9,10,11) were negative, the a product size of approximately 326 bp.

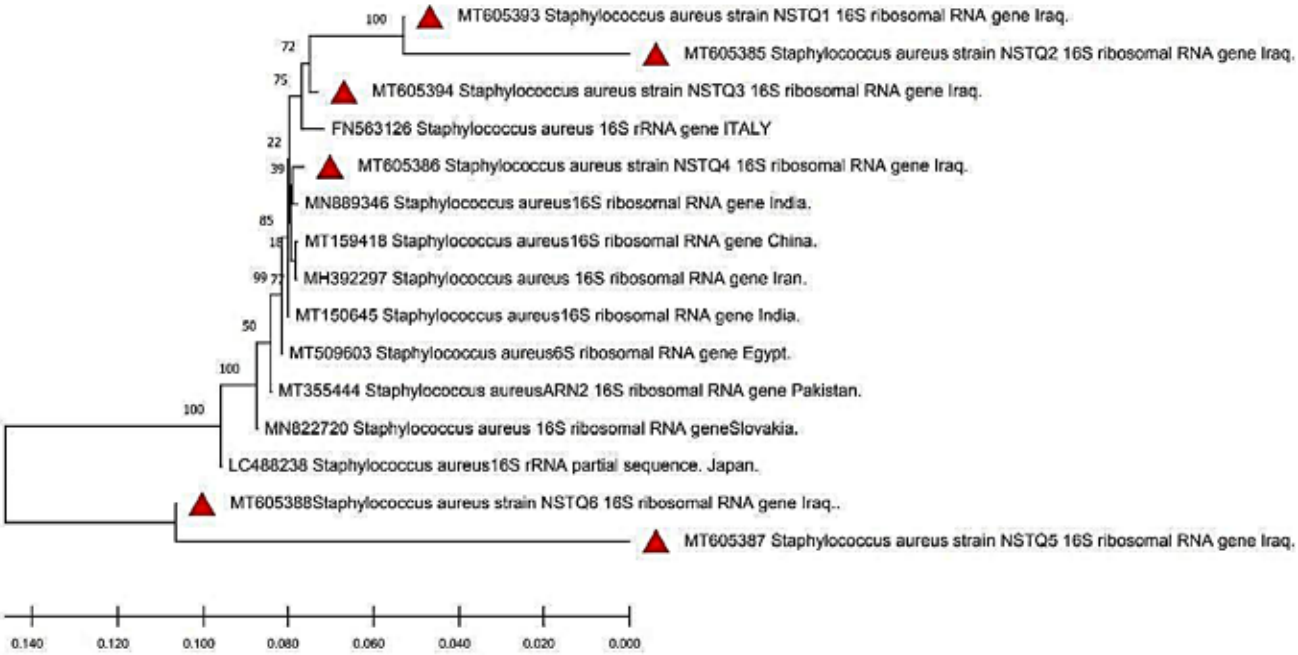

Figure 3: Phylogenetic tree analysis based on the (16sRNA) gene partial sequence that used for genetic relationship analysis of local *Staph. aureus*.

Discussion

Nosocomial infections, is an infection established at some point in hospital care which develops no longer present or incubating at the time of admission, the infections which arise further than 48 hours after admission are also taken into consideration nosocomial [9]. All MRSA isolates were diagnosed by PCR technique through the amplification of 16SrRNA and *mec*A genes to confirm that the verified isolates are *Staph. aureus* and MRSA, respectively. These results agreed completely with previous local studies like[10,11,12]. The drug resistance of MRSA is mainly because that the gene encoding regulates the expression of Penicillin Binding Protein (PBP2a), which is encoded by *mec*A gene, and arisen on the surface, which lies on the *Staphylococcus* gene cassette of SCC *mec*. Thus, the detection of *mec*A gene can be used to confirm diagnosis of MRSA isolates[13]. Other studies inside and outside Iraq revealed variable MRSA occurrence with a rates of 15% and 77.33%, respectively[14,15]. Different MRSA isolation among studies may be explained by the differences of samples or the different PCR assays. The current study tried to amplify *tst*-1 and *hla* genes as a virulence genes in MRSA isolates. All isolates of MRSA have *hla* gene which agreed with the local study of [16] who noted highly frequency of *hla* gene 70(82.35%) between MRSA isolated from burn patients. Other worldwide studies were closely compatible to the present results such as what performed in Uganda and united states whom recorded (100%) frequency of *hla* gene in MRSA isolates[17,18]. While, in Iran[19] results were incompatible with the current results who noted that frequency of *hla* gene was (51.8%). The highly frequent of *hla* gene maybe because that the most of *Staph. aureus*isolated from human have usually a *hla*, since the human platelets and monocytes are more subtle to the alpha toxin[20]. The current study revealed that a half of MRSA isolates contain *tst*-1 gene, which seems not supported with other similar local studies in Iraq who showed a variable frequency of *tst*-1 gene among MRSA isolates from clinical samples with 30% and 84%, respectively[21,22]. A closely related results to the present results were recorded in Iran who noted that frequency of *tst*-1gene was (51.4%) among MRSA isolates from intensive care units patients [23]. The relatively high rate of *tst*-1 positive *Staph. aureus* isolates coupled with the low incidence of TSS strongly suggests that sufficient *tst-1* expression causes disease only under the appropriate environmental and/or genetic regulation control. Since the virulence of microbe may be dependent on the amount of toxins production [24]. Sequencing technique is one of the modern advanced development technique in molecular biology. In this way mutation and genetic relationship can be detected between bacterial isolates

rapidly[25]. The DNA sequencing analysis results for 16SrRNA *Staph. aureus* gene isolates were genetically identical by 99% with those found in the gene bank. Six isolates are shown for the 16SrRNA gene genetically far from the genes taken from the gene bank because they appeared in the out-group. The phylogenetic tree showed that there were different molecular relationships among the local *Staph. aureus* isolates with analogous ones around the world (Fig. 4).

Conclusions

Methicillin-resistance *Staphylococcus aureus* (MRSA) were increased and become more prevalent, the molecular assay and gene sequencing were a significant tools in pathogenesis and evolutionary relationships of nosocomial infections.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

Conflict of Interest: The authors declare that they have no conflict of interest.

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