



Prevalence of Methicillin-resistant *Staphylococcus aureus* Carrying *lukS-lukF* Gene in Iraqi Patients with Furunculosis

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Abstract

In this study, *Staphylococcus aureus* was found to be the causative agent of furunculosis in 64 (27.5%) out of 233 Iraqi patients presented with furunculosis. *16SrRNA* gene was located in all isolates. Nevertheless, *mecA* and *lukS-lukF* genes were located in 60% and 4% of *S. aureus* isolates, respectively. Interestingly, the *lukS-lukF* carrying *S. aureus* isolates were *mecA* positive as well.

Keywords: *Staphylococcus aureus*, *mecA*, *lukS-lukF*, furunculosis

نسبة انتشار المكورات العنقودية المقاومة للمثيسيلين الحاملة لجين *lukS-lukF* في المرضى العراقيين المصابين بداء الدمامل

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الخلاصة

وجد ان المكورات العنقودية الذهبية هي المسبب لداء الدمامل في حوالي 64 من 233 مريضا (27.5%) من المرضى العراقيين المصابين بداء الدمامل. احتوت العزلات جميعها على جين *16SrRNA* في حين احتوى 60% و 4% من العزلات على جيني *mecA* و *lukS-lukF*، على التوالي. من الاهمية بمكان، ان عزلات المكورات العنقودية الذهبية الحاملة لجين *lukS-lukF* كانت حاملة لجين *mecA* ايضا.

1. Introduction

SSTIs embrace a various array of pathological pictures including abscesses, folliculitis, furuncles, staphylococcal scalded skin syndrome and others [1]. Furunculosis is a well-known illness exemplified by the hair follicles infection alongside the pus and necrotic tissue accumulation *in situ* [2].

Staphylococcus aureus colonize the skin and mucosa with no apparent pathological effects. Also, it is the main etiological agent of the skin and soft tissue infections (SSTIs) outside the

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health care setting [3]. Roughly, 20-25% of healthy individuals are predominant carriers of *S. aureus*, particularly in their nares [4]. Chronic furunculosis patients are regularly *S. aureus* carriers, markedly the isolates of their skin and nose usually have similar features [5].

Methicillin-resistant *S. aureus* (MRSA) involves strains of *mecA*-harbouring *S. aureus*, thus, developing a resistance to methicillin trait and principally all other beta-lactams. Interestingly, these strains constitute a boundless defiance in medicine, specifically as a hospital-acquired pathogen. Yet, nosocomial variants of MRSA demonstrated a resistance to nearly all known antibiotics [6]. Consequently, treatment policies against MRSA are a problematic issue. Moreover, since the last 30 years, MRSA emerged as a challenging concern in non-hospitalized individuals or those who recently experienced invasive surgery. Therefore, these strains are referred to as community-acquired MRSA [7].

Pantone-Valentin-Leukocidin (PVL) belongs to a class of pore-forming toxins consisting of two protein moieties (*LukF* and *LukS*) that are highly efficient at disrupting the plasma membrane of leukocytes [8]. PVL is involved in the chronic or recurrent SSTIs and necrotizing pneumonia that also affects immunocompetent individuals [9]. Seemingly, PVL is the most important virulence factor related to furuncles in South America and Europe [10]. To our best knowledge, there is still no evidence of a study investigating the prevalence of PVL-producing *S. aureus* isolated from furunculosis patients in Iraq. Hence, this study aimed to determine the prevalence of *S. aureus* carrying *lukS-lukF* gene isolated from Iraqi patients with furunculosis.

2. Materials and Methods

2.1 Ethical statement

The ethics committee of the College of Science, University of Baghdad approved the present work (Ref. CSEC/1120/0061). All the participants joined in this study through an informed consent.

2.2 Isolation and Identification

A total of 233 pus swabs were collected from patients with furunculosis under the supervision of a dermatologist. For the preliminary identification of *S. aureus*, the collected swabs were cultured on blood agar. Around 40 g of blood agar base (ThermoFisher Scientific, USA) was suspended in 1 litre of distilled water, brought to the boil, sterilized by autoclaving at 121°C for 15 minutes and then cooled to 50°C. Thereafter, around 7% v/v of sterile defibrinated blood was added and incubated at 37°C for 24 h. Later, β -haemolytic colonies were re-cultured on mannitol salt agar (MSA) that was prepared in accordance with the manufacturer instructions (HiMedia, India) and incubated at 37°C for 24 h. The resultant grown colonies were submitted to Gram stain and conventional biochemical identification tests including, coagulase, acetoin production, oxidase, and catalase. Moreover, *16SrRNA* was detected to confirm the identification.

2.3 Molecular Study

2.3.1 DNA Extraction and Preparation

ABIOpure™ Total DNA kit (USA) was utilised to extract DNA from study isolates. Whereas Quantus Fluorometer (USA) was employed to measure the DNA concentration.

2.3.2 Detection of *16SrRNA*, *mecA* and *lukS-lukF* Genes

Conventional PCR (Bio-Rad T100, USA) was employed to detect *16SrRNA*, *mecA* and *lukS-lukF* genes in *S. aureus* isolates using the primers listed in Table 1.

Table 1: Primers used in the present study

Target Gene	Primer Name	Sequence 5' - 3'	Amplicon Size (bp)	Reference
<i>16SrRNA</i>	Sa442-1	AATCTTTGTCGGTACACGATATTCTTCACG-	108	[11]
	Sa442-2	CGTAATGAGATTTTCAGTAGATAAATACAACA		
<i>mecA</i>	MecA1	AAAATCGATGGTAAAGGTTGGC	533	[12]
	MecA2	AGTTCTGCAGTACCGGATTTGC		
<i>lukS-lukF</i>	Luk-PV-1	ATCATTAGGTA AAAATGTCTGGACATGATCCA	433	[13]
	Luk-PV-2	GCATCAAGTGTATTGGATAGCAAAAAGC		

After several trials, the thermocycling conditions for *16SrRNA*, *mecA* and *lukS-lukF* genes were summarized in Tables 2, 3 and 4 respectively. PCR products were resolved in 2% agarose gel stained with ethidium bromide (Promega, USA).

Table 2: Thermocycling conditions of *16SrRNA* gene [11]

Step	Temperature (°C)	Time (min:sec)	Cycles
Initial denaturation	94	03:00	1
Denaturation	94	00:30	35
Annealing	56	00:30	
Extension	72	00:15	1
Final extension	72	03:00	

Table 3: Thermocycling conditions of *mecA* gene [12]

Step	Temperature (°C)	Time (min:sec)	Cycles
Initial denaturation	95	10:00	1
Denaturation	94	00:15	35
Annealing	55	00:30	
Extension	72	00:30	1
Final extension	72	05:00	

Table 3: Thermocycling conditions of *lukS-lukF* gene [13]

Step	Temperature (°C)	Time (min:sec)	Cycles
Initial denaturation	94	10:00	1
Denaturation	94	00:30	30
Annealing	57	00:30	
Extension	72	01:00	1
Final extension	72	03:00	

3. Results

3.1 *Staphylococcus aureus* Isolation and Identification

The present findings revealed that 64 *S. aureus* isolates developed pink colonies on MSA. When subjected to Gram stain, they appeared as Gram positive cocci. Furthermore, they gave positive results for the coagulase, acetoin production and catalase tests. However, all these isolates were oxidase negative.

3.2 Molecular Study

3.2.1 DNA Extraction and Preparation

Out of 64 *S. aureus* isolates, the DNA was extracted from only 50 isolates. The extracted DNA concentration was 20 ± 6 ng/ μ l. The purity was 1.93 ± 0.06 . Furthermore, single bands were noticed in gel electrophoresis images denoting the integrity of the extracted DNA (Figure 1).

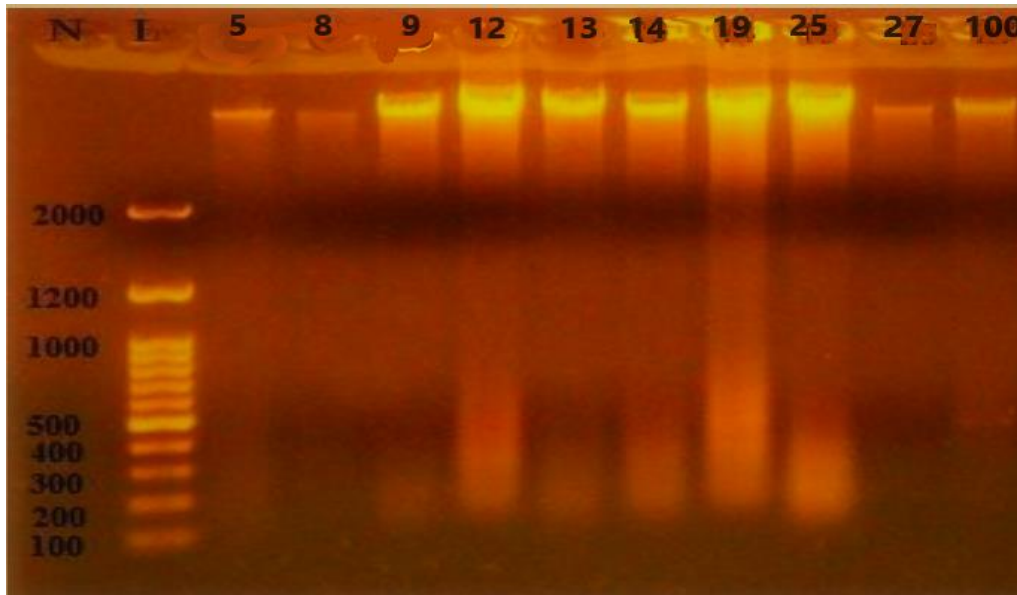


Figure 1: Analysis of *S. aureus* genomic DNA isolates on 1% agarose gel at 5 V/cm for 1hr, stained with ethidium bromide. Lane 1, 5, 8, 9, 12, 13, 14, 19, 25, 27 and 100 represent selected *S. aureus* isolates. L: 100-2000 bp DNA ladder. N: negative control.

3.2.2 Detection of *16sr RNA*, *Mec A* and *lukS-lukF* Genes

This study demonstrated that *16srRNA* was existed in all the 50 isolates (Figure 2a). Nevertheless, 30 isolates (60%) were methicillin resistant as they harboured the *mecA* gene (Figure 2b). However, *lukS-lukF* was detected in only two (4%) isolates (Figure 2c).

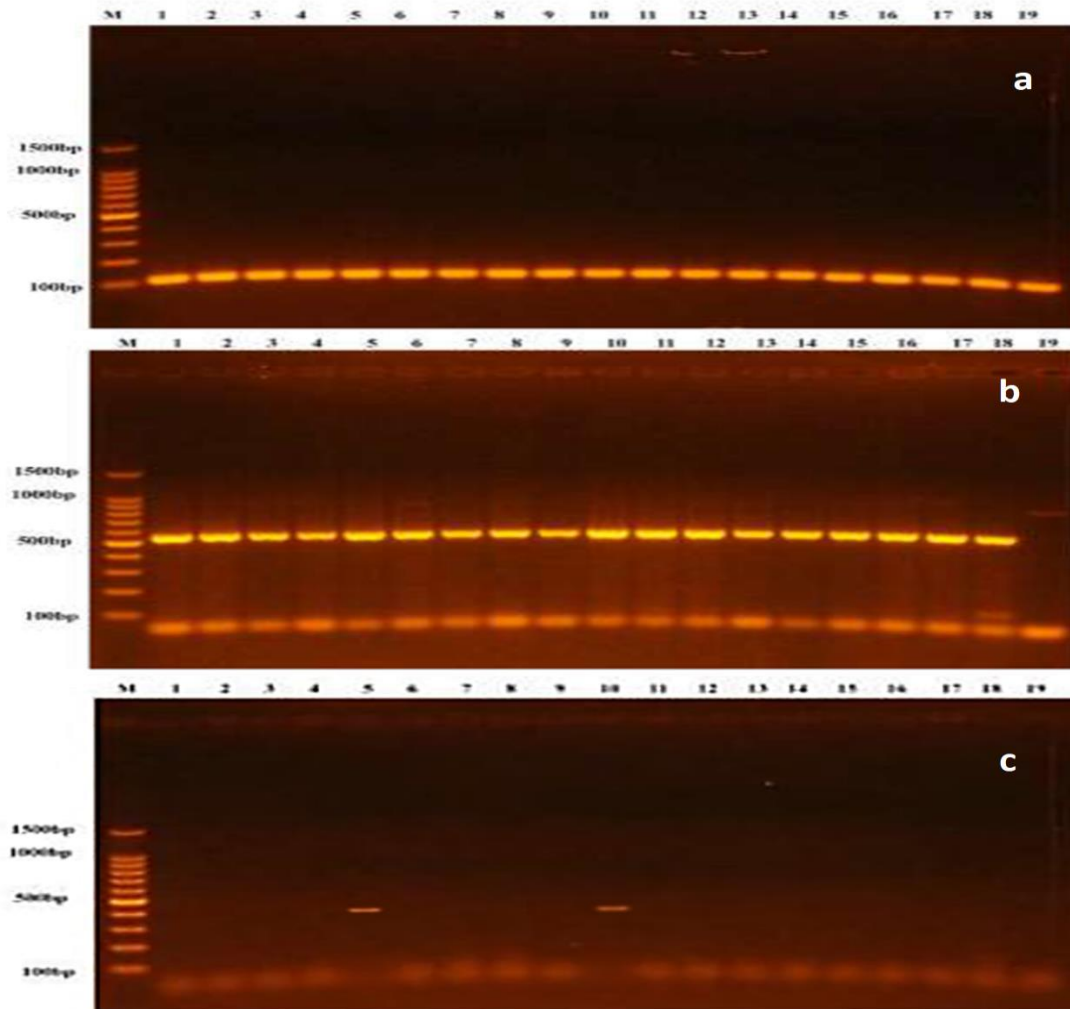


Figure 2: Agarose gel electrophoresis (1.5%) of *Staphylococcus aureus* genes a) *16SrRNA* (108 bp), b) *mecA* (533 bp), and c) *lukS-lukF* (433 bp). Lane M: 100 bp DNA ladder. Lanes 1-19 denote PCR products of selected isolates stained with ethidium bromide and run at 5V/cm.

4. Discussion

Many authors reported the use of conventional biochemical vs molecular methods to identify bacterial isolates. Karmakar, *et al.* [14] identified 60.60% (100/165) of *S. aureus* isolates depending on cultural and biochemical test batteries. Similarly, Rusenova and Rusenov [15] depended on biochemical test to identify a total of 156 isolates as *S. aureus*. However, they stated that accurate identification of *S. aureus* was achieved via a conventional alongside molecular technique. Nevertheless, Ibrahim and Al-Mathkhury [16] referred to the exactitude of conventional assays in the *S. aureus* identification. Upon that, authors claimed that the phenotypic identification should be authenticated by molecular assays, thereby, false-positive results might be avoided.

Locally, MRSA was isolated at a high level from Baghdad hospitals; nevertheless, none of them isolated this group from patients with furunculosis. Hantoosh [17] indicated that MRSA was detected in 24% (72/300) of Al-Muthanna province students. Remarkably Mussa and Al-Mathkhury [6] reported that all the 160 *S. aureus* isolates were MRSA. In Duhok province, Hussein, *et al.* [18] stated that 50.4% of *S. aureus* were MRSA, among which 3.7% were PVL-producing MRSA. However, in Baghdad province, 80% of *S. aureus* isolates were *mecA*-carriers (MRSA), of interest, six (10%) isolates were *pvl* positive [16].

Ascribed to pointless or uncontrolled antibiotics use, multidrug resistance was developed by many pathogens, which led to a huge limitation in the therapeutic choices [17]. nosocomial MRSA should have a great attention as a potential pathogen with an outstanding ability to cope with various antibiotics [12].

Baba-Moussa, et al. [10] established that 96% of furuncles-causing *S. aureus* isolated from HIV patients were PVL-producers. Similarly, Masiuk, et al. [19] highlighted that the PVL-encoding genes were a distinctive feature of the furuncles-causing strains. Yet, these genes were nearly undetectable (detected in only one isolate) in the isolates of nasal origin; given that, 85.1% (64/74) of isolates were furunculosis strains carrying PVL genes.

Considering that PVL gene (*lukS-lukF*) is recognized worldwide as an epidemic marker of CA-MRSA [20-22] and due to the low level of PVL gene prevalence in the current investigated isolates, it can be suggested that the origin of *S. aureus* in this study is health-associated-MRSA.

Conflict of Interest: No conflict of interests to declare.

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