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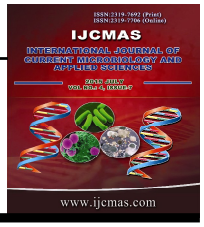

Original Research Article

Virulence factors of Methicillin Resistant *Staphylococcus aureus* (MRSA) isolated from burn patients

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Keywords

MRSA, Burn, Virulence factors, Molecular detection

A B S T R A C T

The present study aimed to investigate some of virulence factors among Methicillin Resistant *Staphylococcus aureus* (MRSA) isolated from burn wound. From a total of 126 isolates of *S. aureus*, only eighty five(67.46%) isolates of MRSA were obtained from burn patients at Al- Hussain teaching hospital during the period from July to November, 2014 in Thi-Qar province, Iraq.All MRSA isolates were examined using Polymerase Chain Reaction (PCR) for detection 16SrRNA, *mec* A gene and some virulence factors of this bacteria include *sea*, *hla*, *hlb*, and *cap* 8.The results revealed that all isolates have 16SrRNA and *mec* A genes that were used to confirm these bacteria as Staphylococci and MRSA respectively. The virulence factors detection results showed percentages of (72.941%), (82.352%) and (85.882 %) of isolates have *sea*, *hla* and *hlb* genes respectively, while only 69 (81.176%) of isolates have *cap*8 gene.

Introduction

Thermal injury destroys the skin barriers that normally prevent invasion by microorganisms (Singh *et al*., 2003). Burn patients become susceptible to various infections due to the loss of this protective barrier and decreased cellular and humoral immunity (Wong *et al*., 2002). In these patients, burn wound infections can easily escalate into sepsis (Church *et al*.,2006).

The common pathogens isolated from burn wound are *S.aureus*(75%), *Pseudomonas aeruginosa*(25%), *Streptococcus pyogenes* (20%) and various coliform bacilli (5%) (Ahmad and Iranzo, 2003).

MRSA is the most important pathogen among Staphylococi (Lee *et al*., 2007). MRSA strains are isolated in more than half of all community and hospital infections (Klevens *et al*., 2007). MRSA has become a major public health problem worldwide, and the problem of MRSA continues to rise (Nimmo *et al*.,2006 and Jarvis *et al*., 2007). MRSA has been the most commonly recognized multidrug-resistant pathogen in the universe and the emergence of MRSA strains found in increasing number of infections and often multi drug resistant in nature now pose serious therapeutic problems to clinicians (Groundmann *et*

al.,2006; Marais *et al*.,2009).

Most MRSA strains carry *mec*A encoding low affinity penicillin-binding protein PBP2a (or PBP2') (Hiramatsu *et al*., 2001). The MRSA characteristic phenotype is due to the presence of *mec* Awhich encodes a PBP2a, with degraded affinity for β-lactams (Oliveira and De Lencastre, 2011; Moellering, 2012).

The virulence factors of *Staphylococcus* include surface components, such as the capsule, peptidoglycans, teichoic acid, protein A, enzymes such as (esterases, lipases, fatty-acid modifying enzymes, various proteases, hydrolytic enzymes, catalase, betalactamase), and various toxins, such as (leukocidins, enterotoxins, TSST-1 and alpha, beta, gamma and delta hemolysins) (Vasconcelos and Cunha, 2010).

One of the virulence factors of *S. aureus* is cytolytic, pore-forming toxin (Diep and Otto, 2008), such as alphahemolysin (*Hla*) has been implicated in the pathogenesis of *S. aureus* (Labandeira-Rey *et al*., 2007). *Hla* has cytolytic activity toward a variety of host cell types, including human keratinocytes, epithelial cells and lymphocytes (Hocke *et al*., 2006; Wardenburg*et al*.,2008). Other types of hemolysin is Beta-hemolysin (*Hlb)* is a magnesium-dependent sphingomyelinase C that induces lysis of sheep erythrocytes and human monocytes **(**Walev *et al*., 1996).

S. aureus is produced one of the extracellular protein toxins, staphylococcal heat stable enterotoxin (SE) is the most im-portant virulent factors belonging to the superantigen family (Pinchuk *et al*, 2010), and many strain of *S. aureus*, especially MRSA, secreted one or more specific staphylococcal exotoxins, including staphylococcal enterotoxins (SEs), (Llewelyn and Cohen, 2002).

Among those factors considered for typing, capsular polysaccharides expressed by *S. aureus* are one of them, since they are also important in the pathogenesis of staphylococcal infections, most *S. aureus* isolates are encapsulated and so far eleven capsular serotypes have been described, of these, types *cap* 5 and *cap* 8 predominate in approximately 75% of the clinical isolates (Murphy *et al*., 2011).

Materials and Methods

Samples collection

Two hundred and seventy six samples were collected from burn patients in burn unit of AL-Hussain Teaching Hospital of Thi-Qar province in the period from July to November, 2014 by moistened sterile swabs with normal saline, then these swabs directly inoculated on Mannitol salt agar (LAB/ United Kingdom) and incubated at 37ºC for 24 hours.

Identification of *S. aureus*

S. aureus was identified depending on the morphological properties on culture media and biochemical tests which done according to Bergeys manual (MacFaddin, 2000).API Staph system was used as identification system for *Staphylococcus* and *Micrococcus*. This test was done according to the company instructions (BioMerieux, France).

StaphyloMonotec test kit Plus

This kit is a new rapid agglutination test for differentiation between *S. aureus* and other *Staphylococcus*. The test was performed according to the directions of manufacturing

company (Fluka Analytical, Switzerland).

Antibiotic sensitivity test

The antibiotic sensitivity test was done by the agar disc diffusion method as described by (Kirby and Bauer, 1966).

Molecular Detection

DNA extraction

DNA from all MRSA isolates were extracted using Genomic DNA Extractionspin kit (Bosphore, Anatolia genewors). 16S rRNA, *mec*A, *sea*, *hla*, *hlb*, and *cap*8 genes were identified by using primers describedin Table (1).

Amplification of the *mec*A gene was done using primer described by (Jonas *et al*., 2002). The final volume of reaction tubes is 20µl, consist of 10 µl Master Mix., 1.25µl of both Forward (F) and Reverse (R) of the primer specific for the *mec*A gene, 5µl of template DNA and complete the volume by adding free water to 20µl.Amplification of the 16SrRNA, *sea, hla, hlb, hla,* and *cap*8 genes were done using primers described as above (Table1). The final volume of reaction tubes is 20µl, consist of 10 µl Master Mix., 1µl of both F. and R. of the primers specific for these genes, 5µl of template DNA and complete the volume by adding deionizing water to 20µl.

Results and Discussion

Eighty five isolates (67.460 %) of MRSA were obtained from *S. aureus* isolates that collected from burn patients. All strains identified was done by cultural, biochemical and serological tests to confirm exact identification as*S. aureus*. MRSA outbreaks are estimated in about 40-60% of *S. aureus* outbreaks, which are mainly affected by the infection control program and medical treatments leading to a wide range of hospital infections (Fatholahzadeh *et al*.,2008). Alfatemi *et al*.,(2014) showed the prevalence of MRSA among *S. aureus* isolates was (42.3%), which indicates little difference in terms of frequency with studies by Fatholahzadeh *et al*.,(2008) who reported MRSA prevalence of (36%) in Tehran.

The prevalence of MRSA in present study wasslightely, in agreement with other studies in Iraq, which recorded percentages of (65.3%), (88%) and (75%) respectively (Al-Mussawi, 2014; Yaseen *et al*.,2013 and Al-Azawi, 2013).

The molecular diagnostic of this bacteria was performing to all MRSA isolates through the amplification of 16SrRNAand *mec* A genes to confirm that the tested isolates are staphylococci and MRSA respectively. Other genes were used to detect many virulence factors of MRSA that included*hla, hlb, sea* and *cap*8genes.

All isolates were showed positive results of both 16SrRNA and *mec* A genes (100%)(Table 2). The bands were 756, 310 bp size corresponds to amplification of16SrRNA and *mec*A genes respectively, Fig(1 and 2).Al-Talib *et al*.,(2009)reported that all isolates had 16SrRNA, 82 contained *mec*A genes. Current study results agreed with Makgotlho, (2009) who showed that all isolates 97/97 (100%) have 16SrRNA gene while *mec* A gene was detected in 96/97 (99%) of the MRSA isolates, which did not show the presence of mec A gene was, however phenotypically identified as MRSA.

The results showed that 69 (81.1 %) of isolates have *cap*8 gene (Table 2), the bands were (450 bp) size corresponds to amplification of*cap*8 gene, Fig (6). Udo and

Sarkhoo (2010) reported that capsular polysaccharides and types prevalence was (77.3%) and only three isolates (2.2%) yielded negative result for both*cap*5 and *cap*8.

Seventy (82.35%) of isolates have *hla* gene only, Table (2).The bands were (209 bp) size corresponds to amplification of *hla* gene, Fig (4). Most of *S. aureus* isolated from human have usually an alpha haemolytic character, because the human platelets and monocytes are more sensitive to the alpha toxin (Todar, 2005). Kateete *et al*.(2011) showed the frequency of *hla*gene was 100%. Likewise, in a study from the United States the *hla*gene frequency was reported at 100% (Shukla *et al*.,2010).The percentage of *hlb* gene in MRSA isolateswas 73 (85.88%), Table (2). The bands were (833 bp) size corresponds to amplification of *hlb* gene, Fig (5). The study performed by Rusenova *et al*.,(2013) showed that 31 MRSA isolates (42.5%) for beta toxin, 41 (56.2%) of isolates showed double hemolysis (alpha + beta hemolysins), and 1 (1.4%) was non-hemolytic. MRSA isolates have 62 (72.94%) of *sea* gene Table (2). The bands were (120 bp)size corresponds to amplification of *sea* gene, Fig (3). Alfatemi *et al*.,(2014) the frequency of the *sea* gene was 27.39%. Our study was in agreement with Udo and Sarkhoo, (2010) whomreported that 103 (76.3%) isolates yielded positive results for *sea*. However, the role of *S. aureus* superantigenic toxins in the severity of septicemia patients should not be discounted as *sea* is significantly associated with severity of sepsis caused by *S. aureus* (Ferry *et al*, 2005).

Table.1 Oligonucleotide primers sequences for PCR amplified of 16SrRNA, *mec*A, *sea*, *hla*, *hlb* and *cap* 8 genes

Genename	Primer Sequences (5´- 3´)	Length	References
16SrRNA	F: AAC TCT GTT ATT AGG GAA GAA CA R: CCA CCT TCC TCC GGT TTG TCA CC	756 bp	(McClure *et al*., 2006)
***mec*A**	F: GTA GAA ATG ACT GAA CGT CCG ATA A R: CCA ATT CCA CAT TGT TTC GGT CTA A	310 bp	(Geha*et al*., 1994)
hlb	F: GCC AAA GCC GAA TCT AAG R: GCG ATA TAC ATC CCA TGG C	833 bp	(Booth *et al*., 2001)
hla	F: CTG ATT ACT ATC CAA GAA ATT CGA TTG R: CTT TCC AGC CTA CTT TTT TAT CAG T	209 bp	(Mehrotra*et al*.,2000)
***cap*8**	F: GCG CTA CAA ACA TTA AGC AT R: TTC TTA GCC TGC TGG CAT C	450 bp	(Sau*et al*.,1997)
sea	F: TTGGAAACGGTTAAAACGAA R: GAACCTTCCCATCAAAAACA	120 bp	(Betley and Mekalanos, 1988) with modified

Table.2 The percentage of genes in MRSA isolates

Genes	Positive %	Negative %
16SrRNA	85 (100 %)	-
***mec*A**	85 (100 %)	-
hla	70 (82.352 %)	15 (17.647 %)
hlb	73 (85.882 %)	12 (14.117 %)
sea	62 (72.941 %)	23 (27.058 %)
***cap* 8**	69 (81.176 %)	16 (18.823 %)

Figure.1 Agarose gel electrophoresis of 16S rRNA gene amplification

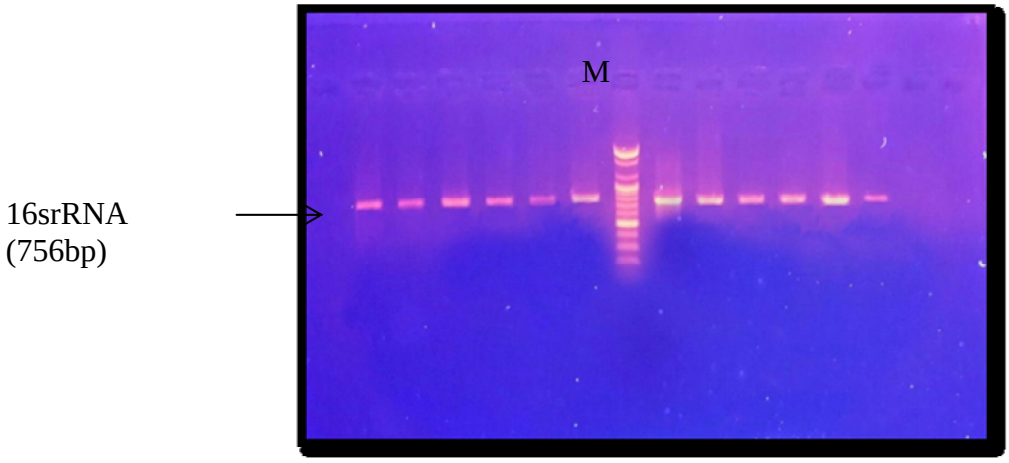


Figure.2 agarose gel electrophoresis of mec A gene amplification

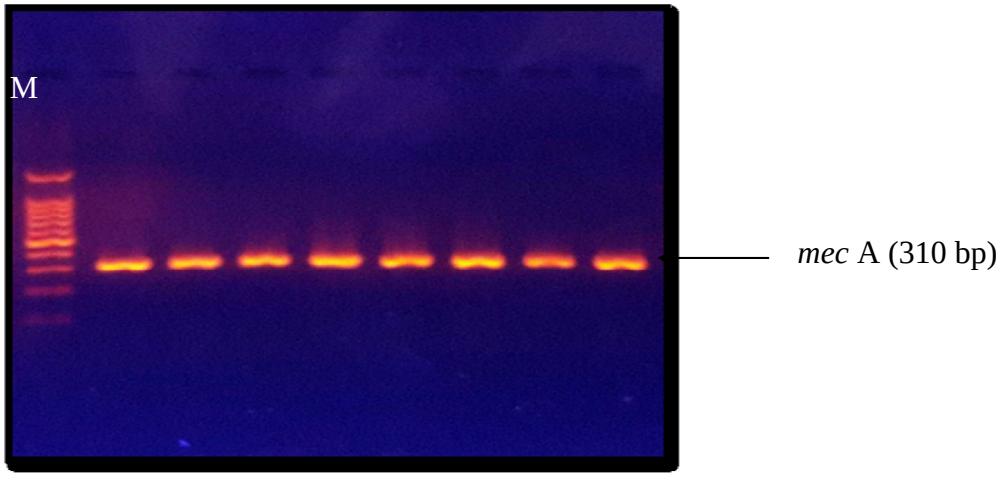


Figure.3 Agarose gel electrophoresis of sea gene amplification

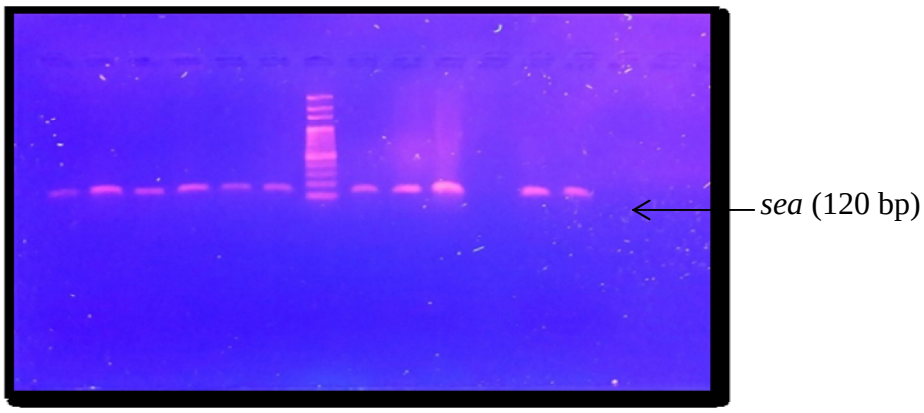


Figure.4 Agarose gel electrophoresis of hla gene amplification

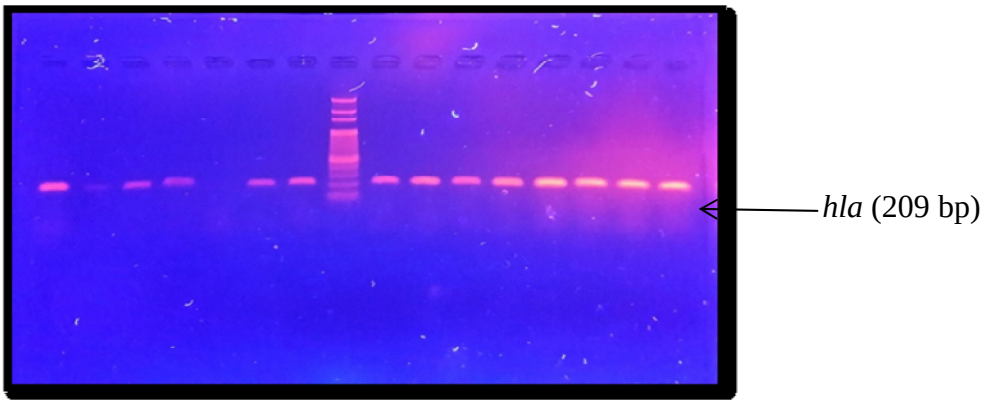


Figure.5 Agarose gel electrophoresis of hlb hemolysin gene amplification

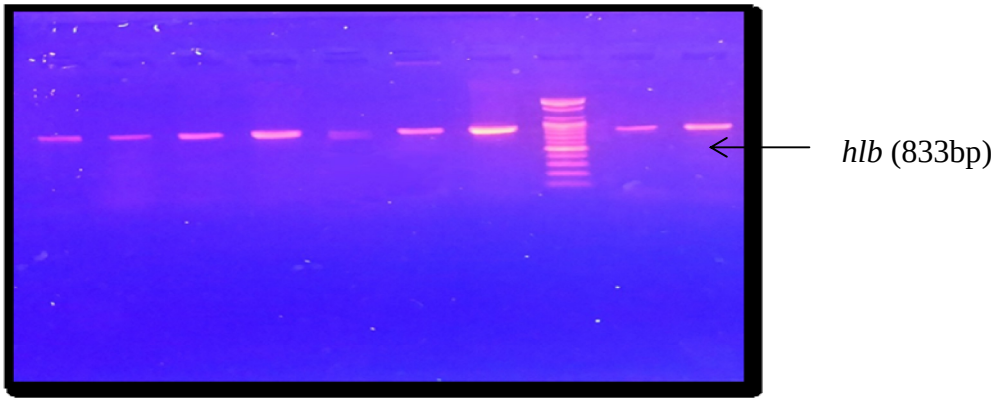
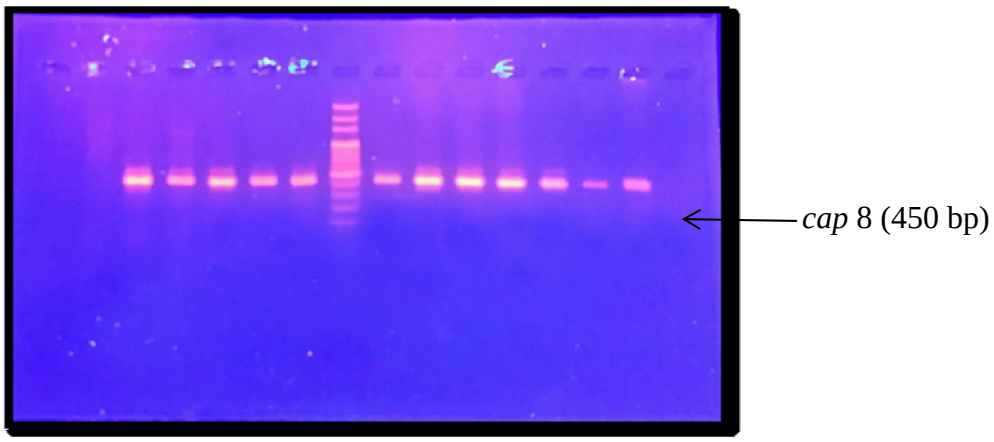


Figure.6 Agarose gel electrophoresis ofcap8 gene amplification



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