Original Research Article

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

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Abstract

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.

Keywords

MRSA, Burn, Virulence factors, Molecular detection

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 Staphylococci (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
Most MRSA strains carry mecA encoding low affinity penicillin-binding protein PBP2a (or PBP2’) (Hiramatsu et al., 2001). The MRSA characteristic phenotype is due to the presence of mecA which 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 alpha-hemolysin (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; Wardenburget 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, staphyloccocal heat stable enterotoxin (SE) is the most important virulent factors belonging to the superantigen family (Pinchuk et al., 2010), and many strain of *S. aureus*, especially MRSA, secreted one or more specific staphyloccocal 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
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 Extraction spin kit (Bosphore, Anatolia Genewors). 16S rRNA, mecA, sea, hla, hlb, and cap8 genes were identified by using primers described in Table (1).

Amplification of the mecA 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 mecA gene, 5μl of template DNA and complete the volume by adding free water to 20μl. Amplification of the 16SrRNA, sea, hla, hlb, hla, and cap8 genes were done using primers described as above (Table 1). 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 was slightly, 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 16SrRNA and 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 cap8 genes.

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 of 16SrRNA and mecA genes respectively, Fig (1 and 2). Al-Talib et al., (2009) reported that all isolates had 16SrRNA, 82 contained mecA 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 cap8 gene (Table 2), the bands were (450 bp) size corresponds to amplification of cap8 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 cap5 and cap8.

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 hlagene frequency was reported at 100% (Shukla et al., 2010). The percentage of hlb gene in MRSA isolates was 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) whom reported 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, mecA, hla, hlb and cap8 genes

<table>
<thead>
<tr>
<th>Genename</th>
<th>Primer Sequences (5’-3’)</th>
<th>Length</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>16SrRNA</td>
<td>F: AAC TCT GTT ATT AGG GAA GAA CA</td>
<td>756 bp</td>
<td>(McClure et al., 2006)</td>
</tr>
<tr>
<td></td>
<td>R: CCA CCT TCC TCC GGT TTG TCA CC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>mecA</td>
<td>F: GTA GAA ATG ACT GAA CGT CCG ATA A</td>
<td>310 bp</td>
<td>(Gehaet et al., 1994)</td>
</tr>
<tr>
<td></td>
<td>R: CCA ATT CCA CAT TGT TTC GGT CTA A</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hlb</td>
<td>F: GCC AAA GCC GAA TCT AAG</td>
<td>833 bp</td>
<td>(Booth et al., 2001)</td>
</tr>
<tr>
<td></td>
<td>R: GCG ATA TAC ATC CCA TGG C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hla</td>
<td>F: CTG ATT ACT ATC CAA GAA ATT CGA TTG</td>
<td>209 bp</td>
<td>(Mehrotraet al., 2000)</td>
</tr>
<tr>
<td></td>
<td>R: CTT TCC AGC CTA CTT TTT TAT CAG T</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cap8</td>
<td>F: GCG CTA CAA ACA TTA AGC AT</td>
<td>450 bp</td>
<td>(Sauet et al., 1997)</td>
</tr>
<tr>
<td></td>
<td>R: TTC TTA GCC TGC TGG CAT C</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sea</td>
<td>F: TTGAAACGTTAAAACGAA</td>
<td>120 bp</td>
<td>(Betley and Mekalanos, 1988) with modified</td>
</tr>
</tbody>
</table>
Table 2 The percentage of genes in MRSA isolates

<table>
<thead>
<tr>
<th>Genes</th>
<th>Positive %</th>
<th>Negative %</th>
</tr>
</thead>
<tbody>
<tr>
<td>16SrRNA</td>
<td>85 (100 %)</td>
<td>-</td>
</tr>
<tr>
<td>mecA</td>
<td>85 (100 %)</td>
<td>-</td>
</tr>
<tr>
<td>hla</td>
<td>70 (82.352 %)</td>
<td>15 (17.647 %)</td>
</tr>
<tr>
<td>hlb</td>
<td>73 (85.882 %)</td>
<td>12 (14.117 %)</td>
</tr>
<tr>
<td>sea</td>
<td>62 (72.941 %)</td>
<td>23 (27.058 %)</td>
</tr>
<tr>
<td>cap 8</td>
<td>69 (81.176 %)</td>
<td>16 (18.823 %)</td>
</tr>
</tbody>
</table>

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 of cap8 gene amplification

References


Moellering, Jr. (2012). RC. MRSA: the first


