Prevalence Rate and Prevalent Genotypes of CA-MRSA in Kurdistan Region: First Report from Iraq

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Abstract: Community-acquired methicillin resistant Staphylococcus aureus (CA-MRSA) is responsible for a large proportion of the increased disease burden observed in the community over the last few years. The aims of current paper were to study the prevalence and prevalent genotypes of CA-MRSA in clinical samples in Iraq and study the sensitivity pattern of these strains to vancomycin and mupirocin. MRSA isolates from 98 clinical samples were studied. Sensitivity of these strains to vancomycin and mupirocin was determined and PVL and SCCmec genotyping was performed. The prevalence of MRSA was 53% among all studied strains. One strain found to be vancomycin resistance and one high-level mupirocin resistant strain was found. SCCmec type IV was shown to be the most prevalent type (73%) in the studied samples. More than half of the studied isolate were resistant to methicillin. Vancomycin resistant was rare and one of our strains was resistant to mupirocin. Further study is needed to study the MRSA in community and hospitals in Iraq.

Keywords: MRSA, Community, PVL and SCCmec, Mupirocin, Vancomycin, Iraq.

Introduction:

Staphylococcus aureus (S. aureus) is one of the major resistant pathogens and one of the earliest bacteria in which penicillin resistance developed. S. aureus is widely distributed in nature and it can be found on the skin or in anterior nares in about 25-33% of normal individuals. Since 1970s, S. aureus have emerged resistant to the penicillinase-stable pencillins and historically these resistant strains known as methicillin resistant S. aureus (MRSA). Hospital-acquired MRSA (HA-MRSA)
infection is defined as infection with such a strain acquired after the use or visit of hospitals or other healthcare facilities (e.g. dialysis or rehabilitation centers). On the other hand, community-acquired MRSA (CA-MRSA) infections is defined as infection with MRSA strains without prior experience of healthcare related procedures or hospitalized. CA-MRSA infections have increased steadily since 1980s (Lindenmayer et al., 1998; Graham et al., 2006). They have become the major sources of significant morbidity, mortality and healthcare costs in many countries (Gorak et al., 1999; Beam et al., 2006). Modified penicillin-binding protein (PBP-2a), which is responsible for methicillin resistance, is encoded by the mecA gene. This gene is located on a chromosomal region called the staphylococcal cassette chromosome (SCCmec) (Melzer et al., 2000). So far, eight SCCmec genotypes have been found for S. aureus described as types I, II, III, IV, V, VI, VII and VIII (David et al., 2010). CA-MRSA strains have been shown to carry the SCCmec type IV element and the Panton-Valentine leukocidin (PVL) genes (Zetola et al., 2005; David et al., 2010). PVL gene is responsible for the secretion of leukocidin that may play a role in the increased severity of infection with PVL-carrier isolates (David et al., 2010). The aims of this work were to determine the prevalence of CA-MRSA in clinical samples and to study the prevalence of PVL-containing isolates and the SCCmec types in CA-MRSA in Duhok city, Kurdistan region, Iraq.

Materials and Methods:

The clinical strains isolated from 61 skin and soft tissue infection; 31 pneumonia and 6 bactremia and sepsis subjects were included in this study. Exclusion criteria were (1) Previous admission to hospital, undergoing surgical procedures, renal dialysis and long-term stay in a care unit within a year of the sample collection, (2) Using of a catheter or intravenous line at the time of sample collection and (3) Former detection of MRSA from the subject. The isolates were recognized as S. aureus based on Gram stain, catalase test, coagulase test, and mannitol salt agar fermentation. Antimicrobial susceptibility testing to oxacillin and vancomycin was carried out according to the recommendations of the Clinical Laboratory Standards Institute (CLSI) (NCCLS 2004; Tenover et al., 2007).

BHI agar plates containing 6 µg/ml of vancomycin were used to screen strains for vancomycin resistance. After correcting the bacterial suspension to the concentration of 0.5 McFarland, a 10µl inoculum was spread on the agar plate (final concentration=10^6 CFU/ml). In addition, agar dilution assay was used to determine vancomycin minimum inhibitory concentration (MIC). Strains for which vancomycin MICs were less than 2 were classified as sensitive, MIC of 2-8 µg/mL were considered as vancomycin-intermediate, and strains for which vancomycin MICs were ≥16 µg/mL were considered as vancomycin-resistant. All experiments were repeated in triplicate and in case of vancomycin resistance, the isolates were sent to an independent lab for confirmation.

Mupirocin sensitivity was assessed using E-test® mupirocin strips (Biomerieux, Durham, NC, USA) according to the manufacturer’s instructions. Following incubation at 35°C for 24 h, strains were classified as susceptible if the MIC was ≤4 mg/L and if MIC was between MIC 8–256 mg/L, the strains were classified as low-level resistance. The strains were considered to be highly resistant, if the MIC was ≥512 mg/L (Mohajeri et al., 2012).

DNA was extracted from S. aureus isolates using the Qiagen DNA Purification kit as per manufacturer’s instructions (DNAEASY, Qiagen). All MRSA isolates were tested for the presence of mecA gene using PCR as previously described (Zafar et al., 2007). Thermal cycling conditions for amplifying mecA were 95 ºC for 30 s, 58 ºC for 1 min, and 72 ºC for 2 min, for a total of 35 cycles. PCR amplification of mecA used previously described primers (MR1: GTG GAA TTG GCC AAT ACA GG and MR2: TGA GTT CTG CAG TAC CGG AT primers), which can amplify a 1399 base pair fragment specific for mecA (Daum, 2007). The pvl gene was amplified using standard protocols with the following thermal cycling conditions: 35 cycles, each consisting of denaturation at 95°C for 30 s, annealing at 55°C for 1 min, and elongation at 72°C for 2 min. Primers Luk-PV-1 (ATCATAGGTAAATGTCTTGACATGATCCA) and Luk-PV-2 (GCATCAAGTGTATTGGATAGCAAAAGC) were used (Chang et al., 2003). Amplification for
both mecA and pvl genes started with an initial denaturation at 95°C for 60 s and a final elongation step of 5 min at 72°C. PCR reactions were carried out in 25 µl volumes including 1 µl of DNA, 1 µl primer, 0.5 µl of Taq DNA polymerase, 0.5 µl dNTPs, and 2.5 10× PCR buffer. SCCmec genotyping was conducted as previously described protocol (Vandenesch et al., 2003). The PCR was run under the following conditions: DNA denaturing was carried out for 5 minutes at 94°C. This followed by 35 cycles of 94°C for 45 seconds, annealing for 45 seconds at 65°C and elongation step for 90 seconds at 72°C. PCR reaction was performed with an ending final extension step at 72°C for 10 min.

The PCR products were electrophoresed using a UV light box on a 2% wt/vol agarose gel containing 0.5 µg/ml ethidium bromide. The products were run for 40 minutes at 80 V in 1× TAE buffer and 100bp DNA ladder (Gibco, Paisley, UK) was used as a size marker (M) in all gels.

**Results:**

First, the prevalence of MRSA in clinical samples was studied. It was found that the prevalence of MRSA was 53% (52/98) among all studied strains, highest among S. aureus isolated from skin and soft tissue 57.4% (35/61) followed by 45.2% (14/31) for pneumonia as shown in table 1. 50% (3/6) of the strains isolated from patients with bacteraemia were resistant to methicillin. Then, the susceptibility of these strains to vancomycin and mupirocin was investigated. One strain isolated from pneumonia was found to be vancomycin resistant with a MIC of 64. One high-level mupirocin resistant with MIC ≥ 512 mg/L was found.

**Table 1:** The prevalence of MRSA in clinical samples

<table>
<thead>
<tr>
<th>Infection type</th>
<th>MRSA</th>
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<tbody>
<tr>
<td></td>
<td>No</td>
</tr>
<tr>
<td>Skin and soft (61)</td>
<td>35</td>
</tr>
<tr>
<td>Pneumonia (31)</td>
<td>14</td>
</tr>
<tr>
<td>Bacteremia and sepsis (6)</td>
<td>3</td>
</tr>
<tr>
<td>Total (98)</td>
<td>52</td>
</tr>
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</table>

The prevalence of PVL gene was studied, the prevalence of PVL gene, MSSA-PVL and MRSA-PVL isolates were (22/98) 22%, (12/46) 26% and (10/52) 19 %, respectively. Also, our strains were typed for SCCmec. Out of 52 MRSA isolates, 3.8% (2/52), 9.6% (5/52) and 13.5% (7/52) carried SCCmec I, II, and III, respectively. 73% (38/52) of our isolates carried SCCmec type IV.

One strain which was isolated from a patient with pneumonia was found as SCCmec type IV-bearing, PVL-positive MRSA strains.

**Discussion:**

Previously, MRSA was almost exclusively a problem in hospitals however; it is now the cause of frequent outbreaks in the general community and become the most frequent cause of skin and soft-tissue infections (Gerard et al., 2010). The prevalence of MRSA in this study found to be 53% among all studied strains, highest among S. aureus isolated from skin and soft tissue followed by strains isolated from pneumonia. 50% of the strains isolated from patients with bacteraemia were resistant to methicillin. The prevalence of MRSA is comparable to that previously found in Iraq, Iran, United States, and European countries, but the percentage of MRSA isolates is about the double of that reported in Japan (Zafar et al., 2007; Habibi et al., 2008; David et al., 2010; Habeeb et al., 2014).
Invasive infection by MRSA in the community, such as pneumonia, usually needs to be managed in the hospital with intravenous antibiotics (Daum, 2007). Vancomycin, which is a glycopeptide antibiotic, is the drug of choice for the treatment of such conditions (Chang et al., 2003). Fortunately, it was found that vancomycin resistant rate is low in Duhok. This result was in agreement with previous results showing that all MRSA isolates in Iraq were sensitive to vancomycin (Habeeb et al., 2014) and this might be attributed to that vancomycin had been used rarely in Iraq during the UN sanction period from 1990 to 2003. This is because the drug was expensive and was not imported by the health authority at the time.

Vancomycin resistant was rare and one of our strains was resistant to mupirocin. In agreement with other studies from Europe and the USA, the majority of strains isolated from clinical samples carried SCCmec type IV. Contact precaution, for prevention of skin, soft tissue and pneumonia should be applied. Further study is needed to study the MRSA in community and hospitals in Iraq.

References


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chromosome mec between methicillin-resistant *Staphylococcus haemolyticus* and *Staphylococcus aureus*, *Clin Microbiol Infect*, 14(11) (2008), 1048-1056.


