Int. J. Pure Appl. Sci. Technol., 27(1) (2015), pp. 44-49

International Journal of Pure and Applied Sciences and Technology

ISSN 2229 - 6107

Available online at ***www.ijopaasat.in***

Research Paper

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

Nawfal R. Hussein[1], Ahmed Alyas[1], Marwan Majeed[1] and Mahde S. Assafi[2,*]

[1] Department of Internal Medicine, School of Medical Sciences, University of Duhok

[2] Department of Biology, Faculty of Sciences, University of Zakho, Zakho, Kurdistan Region, Iraq

* Corresponding author, e-mail: (mahdy.assafi@yahoo.co.uk)

(Received 9-2-15; Accepted: 11-3-15)

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 (SCC*mec*) (Melzer *et al.,* 2000). So far, eight SCC*mec* genotypes have been found for *S. aureus* described as types I, II, III, III, IV, V, VI, VII and VIII (David *et al.*, 2010). CA-MRSA strains have been shown to carry the SCC*mec* 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 SCC*mec* types in CA-MRSA in Duhok ciry, 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 55ºC for 1 min, and elongation at 72ºC for 2 min. Primers Luk-PV-1 (ATCATTAGGTAAAATGTCTGGACATGATCCA) and Luk-PV-2 (GCATCAAGTGTATTGGATAGCAAAAGC) were used (Chang *et al.*, 2003). Amplification for

both *mecA* and *pvl* genes started with an initial denaturation at 95ºC for 60 s and a final elongation step of 5 min at 72ºC. 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. SCC*mec* 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 94ºC. This followed by 35 cycles of 94ºC for 45 seconds, annealing for 45 seconds at 65ºC and elongation step for 90 seconds at 72ºC. PCR reaction was performed with an ending final extension step at 72ºC 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 bactraemia 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

Infection type	MRSA	
	No	%
Skin and soft (61)	35	57.4
Pneumonia (31)	14	45.2
Bacteremia and sepsis (6)	3	50.0
Total (98)	52	53.1

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 SCC*mec*. Out of 52 MRSA isolates, 3.8% (2/52), 9.6% (5/52) and 13.5% (7/52) carried SCC*mec* I, II, and III, respectively. 73% (38/52) of our isolates carried SCC*mec* type IV.

One strain which was isolated from a patient with pneumonia was found as SCC*mec* 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 bactraemia 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.

Mupirocin, which is a monoxycarbolic acid antibiotic, plays an important role in the eradication and treatment of MRSA. Resistance to this antibiotic may develop rapidly and to avoid such a resistance, the use of this antibiotic should be monitored. In our study, one high-level mupirocin resistant with MIC $\geq$ 512 mg/L was found. Two factors may explain the rarity of mupirocin resistance in this project: the rarity of mupirocin use and the small sample size used in this study. In Iraq, mupirocin is not used for the eradication of MRSA colonization as part of infection control measures. In addition, it is rarely used for the treatment of skin infections.

PVL is the most known virulence determinant is *S. aureus* that is responsible for decoding leukotoxin. This cytotoxin responsible for killing leukocytes and contributes in the increased severity of the infection. PVL genes can be carried by both MRSA and methicillin sensitive *S. aureus* strains (MSSA) (Narita *et al.,* 2001; Prevost *et al.,* 2001). In the current study, it was found that the prevalence of PVL gene, MSSA-PVL and MRSA-PVL isolates were 22%, 26% and 19 %, respectively. Previous studies have shown that the vast majority of CA-MRSA are PVL gene carriers (David *et al.,* 2010). In the United States, investigation of numerous epidemiological studies showed that the presence of the PVL genes has closely related to infections caused by MRSA isolates in the community. In Europe, 30-70% of CA-MRSA have shown to carry PVL (David *et al.,* 2010). Despite the fact that PVL plays a major role in the disease process of necrotising pneumonia, it is not likely to be the only virulence factor responsible for this syndrome (Narita *et al.,* 2001; Prevost *et al.,* 2001).

MRSA strains bear smaller SCC*mec* genes that carry the *mecA* gene which is responsible for methicillin resistance. So far, eight SCC*mec* genotypes have been found for *S. aureus* described as types I, II, III, III, IV, V, VI, VII and VIII. In CA-MRSA, the most common genotypes found to be type IV and type V (Berglund *et al.,* 2008; David *et al.,* 2010). SCC*mec* genotype IV was found to be linked with CA-MRSA infections in the community especially in children (David *et al.,* 2010). In this study, out of 52 MRSA isolates, 3.8%, 9.6% and 13.5% carried SCC*mec* I, II, and III, respectively and 73% of our isolates carried SCC*mec* type IV. In addition, one strain was SCC*mec* type IV-bearing, PVL-positive MRSA strains. This strain was isolated from patient with pneumonia. In agreement with previous studies from Iraq, Europe and the United States where the SCC*mec* type V is rare, none of our strains carried this element (David *et al.,* 2010; Hussein *et al.,* 2014).

Conclusion:

To conclude, more than half of the studied isolate were resistant to methicillin. 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 SCC*mec* 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

[1] J.W. Beam and B. Buckley, Community-acquired methicillin-resistant *Staphylococcus aureus:* Prevalence and risk factors, *J Athl Train*, 41(3) (2006), 337-340.

[2] C. Berglund and B. Soderquist, The origin of a methicillin-resistant *Staphylococcus aureus* isolate at a neonatal ward in Sweden-possible horizontal transfer of a staphylococcal cassette

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

[3] F.Y. Chang, B.B. MacDonald, J.E. Peacock, Jr., D.M. Musher, P. Triplett, J.M. Mylotte, A. O'Donnell, M.M. Wagener and V.L. Yu, A prospective multicenter study of *Staphylococcus aureus* bacteremia: Incidence of endocarditis, risk factors for mortality and clinical impact of methicillin resistance, *Medicine (Baltimore)*, 82(5) (2003), 322-332.

[4] R.S. Daum, Clinical practice: Skin and soft-tissue infections caused by methicillin-resistant *Staphylococcus aureus*, *N Engl J Med*, 357(4) (2007), 380-390.

[5] M.Z. David and R.S. Daum, Community-associated methicillin-resistant *Staphylococcus aureus*: Epidemiology and clinical consequences of an emerging epidemic, *Clinical Microbiology Reviews*, 23(3) (2010), 616-687.

[6] J.T. Gerard, R.F. Berdell and L.C. Christine, Microbiology: An Introduction with Mastering Microbiology, San Francisco, Pearson Benjamin Cummings, 2010.

[7] E.J. Gorak, S.M. Yamada and J.D. Brown, Community-acquired methicillin-resistant *Staphylococcus aureus* in hospitalized adults and children without known risk factors, *Clin Infect Dis*, 29(4) (1999), 797-800.

[8] P.L. Graham 3rd, S.X. Lin and E.L. Larson, A U.S. population-based survey of *Staphylococcus aureus* colonization, *Ann Intern Med*, 144(5) (2006), 318-325.

[9] A. Habeeb, N.R. Hussein, M.S. Assafi and S.A. Al-Dabbagh, Methicillin resistant *Staphylococcus aureus* nasal colonization among secondary school students at Duhok City, Iraq, *J Microbiol Infect Dis*, 4(2) (2014), 59-63.

[10] M. Habibi, H. Saderi, P. Owlia and M. Karam, Detection of methicillin resistance in *Staphylococcus aureus* by disk diffusion and PCR methods, *Iranian Journal of Pathology*, 3(1) (2008), 11-14.

[11] N.R. Hussein, A.H. Muhammed, S. Al-Dabbagh, W.L. Abdulkareem and M.S. Assafi, The prevalence of USA 300 CA-MRSA in Kurdistan region, Northern Iraq, *Int. J. Pure Appl. Sci. Technol.*, 21(2) (2014), 8-10.

[12] J.M. Lindenmayer, S. Schoenfeld, R. O'Grady and J.K. Carney, Methicillin-resistant *Staphylococcus aureus* in a high school wrestling team and the surrounding community, *Arch Intern Med*, 158(8) (1998), 895-899.

[13] M. Melzer, D. Goldsmith and W. Gransden, Successful treatment of vertebral osteomyelitis with linezolid in a patient receiving hemodialysis and with persistent methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococcus bacteremias, *Clin Infect Dis*, 31(1) (2000), 208-209.

[14] P. Mohajeri, B. Gholamine, M. Rezaei and Y. Khamisabadi, Frequency of mupirocin resistant *Staphylococcus aureus* strains isolated from nasal carriers in hospital patients in Kermanshah, *Jundishapur J Microbiol*, 5(4) (2012), 560-563.

[15] S. Narita, J. Kaneko, J. Chiba, Y. Piemont, S. Jarraud, J. Etienne and Y. Kamio, Phage conversion of Panton-Valentine leukocidin in *Staphylococcus aureus*: Molecular analysis of a PVL-converting phage, phiSLT, *Gene*, 268(1-2) (2001), 195-206.

[16] NCCLS, Performance standards for antimicrobial susceptibility testing, *14th Informational Supplement*, NCCLS document M100-S14, National Committee for Clinical Laboratory Standards, Wayne, PA, (2004).

[17] G. Prevost, L. Mourey, D.A. Colin and G. Menestrina, Staphylococcal pore-forming toxins, *Curr Top Microbiol Immunol*, 257(2001), 53-83.

[18] F.C. Tenover and R.C. Moellering, The rationale for revising the clinical and laboratory standards institute vancomycin minimal inhibitory concentration interpretive criteria for *Staphylococcus aureus*, *Clinical Infectious Diseases*, 44(9) (2007), 1208-1215.

[19] F. Vandenesch, T. Naimi, M.C. Enright, G. Lina, G.R. Nimmo, H. Heffernan, N. Liassine, M. Bes, T. Greenland and M.E. Reverdy et al., Community-acquired methicillin-resistant *Staphylococcus aureus* carrying Panton-Valentine leukocidin genes: Worldwide emergence, *Emerg Infect Dis*, 9(8) (2003), 978-984.

[20] U.M.D. Zafar, M.D. Leonard, Â.B.Â. Johnson, M.D. Michel, Â. Hanna, B.M. Kathleen, Â. Riederer, M.D. Mamta, Â. Sharma, M.D. Mohamad, Â.G.Â. Fakih, M.D. Muthayipalayam, Â.C.Â. Thirumoorthi, M.D. Rand, Â. Farjo and M.D. Riad and Â. Khatib, Prevalence of nasal

colonization among patients with community associated methicillin resistant *Staphylococcus aureus* infection and their household contacts, *Infection Control and Hospital Epidemiology*, 28(8) (2007), 966-969.

[21] N. Zetola, J.S. Francis, E.L. Nuermberger and W.R. Bishai, Community-acquired meticillin-resistant *Staphylococcus aureus*: An emerging threat, *Lancet Infect Dis*, 5(5) (2005), 275-286.