Antibiotic Susceptibility Pattern And MecA Gene Detection In Methicillin Resistance Staphylococcus Aureus (MRSA) Isolated From Burn And Wound In Al-Diwaniya City.

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Abstract

This study was designed to detect the susceptibility of methicillin resistant staphylococcus aureus (MRSA) toward a group of antibiotic . Thirty six isolates were *S . aureus* were isolated from 157 clinical samples (burn and wound in Al-Diwaniya city) collected from Al-Diwaniya Teaching Hospital. Isolates were identified by traditional biochemical tests, then confirmed by VITEK-2 compact system. Antibiotic susceptibility was performed by disc diffusion method and minimum inhitory concentration(MIC) testing was done using VITEK-2. The presence of *mecA* gene was detected by conventional PCR technique. The result showed that 36 isolates(22.9%) were found as *S.aureus*. Eight isolates (22.2%) wereMRSA. All isolates 8/8 (100%) were resistant to penicillin, cefoxitin and methicillin, while moderate resistant 3/8 (37.5%) to vancomycin, erythromycin and rifampicin, lowresistant 2/8 (25%) to erythromycin and tetracycline. PCR assay revealed that 6 isolates harbored *mecA* gene (4 burn and 2 wound).

Key words: Antibiotic, MRSA, Burn, Wound, mecA gene

الخلاصة

صممت الدراسة لتحديد حساسية العنقوديات المقاومة للميثيسلين MRSA لمجموعة مختارة من المضادات البكتيرية. تم جمع ١٥٧ عينة من حالات سريرية (حروق وجروح) في مدينة الديوانية لغرض عزل وتشخيص MRSA . تم استخدام الاختبارات المظهرية والكيموحيوية التقليدية كما تم تشخيصها باستخدام جهاز VITEK-2 في تشخيص العزلات. اختبرت حساسية العزلات (MRSA) للمضادات (بنسلين, سيفوكستين, اوكساسلين, فانكومايسين, اريثرومايسين, ريفامبيسين, تتراسايكلين وجنتاميسين) باستخدام طريقتي انتشار القرص في الاكار وتم تحديد التركيز المثبط الادنى (MIC) باستخدام جهاز VITEK-2 . تم التحري عن وجود *mecA* gene المشفر لمقاومة البكتريا لمضادات (بنسيلين , اوكساسلين ,ميثسلين) باستخدام تقنية تفاعل البلمرة التسلسلي . اظهرت النتائج ان ٣٦ عزلة بنسبة (٢٢.٩%) هي بكتريا *Staphylococcus aureus* تتضمن ٨ عزلات و بنسبة (٢٢.٢%) هي MRSA . اظهرت الدراسة ان جميع العزلات ٨\١٨(١٠٠%) مقاومة لمضادات (المثيسيلين و الاوكساسلين و البنسلين)بينما اظهرت مقاومة متوسطة ٨\١٣(٣٧.٥%) لمضادات (فانكومايسين و اريثرومايسين و ريفامبيسين) بينما كانت المقاومة اقل ٨\١٢(٢٥%) لمضادات (تتراسايكلين و جنتاميسين) . كما كشفت تقنية تفاعل البلمرة التسلسلي ان ٦ عزلات فقط كانت حاوية على mecA gene. (اربعة من مرضى الحروق واثنان فقط من الجروح)

Introduction:

Staphylococcus aureus a very versatile pathogen causing diseases that range from superficial skin infections to deep-seated and serious invasive infections such as septicemia and endocarditic, along with metastatic infection of further target tissues (Foster,2004).

The frequency of infection by S. aureus has increased during the last three decades and their susceptibility pattern have changed (Mokaddas and Sanyal,1999). Moreover, the importance of S.aureus as a persistent nosocomial and community acquired pathogen has become a global healthy concern (Onwubiko and Sadiq,2011). S. aureus is the second largest nosocomial (next to E.coli) causative organism of nosocomial infections not only in the immunosuppresed that can develop an infections (Monica,2002).

The tendency of S. aureus to acquire antibiotic resistance has led to the global dissemination of clone expressing multiple antimicrobial resistance including some that express intermediate or full resistance to the glycopeptides vancomycin (Hiramatsu,1997).

Resistance mechanism most commonly observed in MRSA is the synthesis of penicillin binding protein 2a (PBP2a) which is encoded by the mecA gene and show low affinity for B-lactam antibiotic(Babel and Decker,2008).

The conventional methods to detect MRSA in the laboratory include oxacillin agar screen, disk diffusion using one microgram oxacillin disk as well as oxacillin MIC. Polymerase chain reaction (PCR) is considered the gold standard (Sakoulas et al.,2001; Obasuyi,2013).

The infection caused by MRSA are serious and are difficult to treat. Only a few antimicrobial agents are available for treatment of such infections and none of these possesses ideal characteristics (Pulimood et al.,1996;Mathur et al.,1994). Hence accurate and rapid identification of MRSA in a clinical specimen is essential for timely decisions on isolation procedures and effective antimicrobial therapy (Kohner et al.,1999).

The frequency of MRSA depends on a region and is less than 1% in Nordic countries, and more than 30% in Spain, France, Italy and India (Herwalt,1999). In Iraq, the percentage of MRSA was reported to accounted for Al-Sahlawi (2002), Al-Fudi (2010) and Al-Hasseny (2011). MRSA is the most common in the departments of resuscitation, burns, and traumatology.

Burns remain a significant public health problem in terms of morbidity, long-term disability and mortality through world, especially in developing countries (Othman and Kendrich,2011). Burn patients become susceptible to infection due to the loss of the protective barrier and decreased cellular and humoral immunity (Wong et al.,2002). Infection remains a major complication in burn patients after initial period of shock and the chance of infection persist until complete wound healing (Kaushik et al.,2001).

Also, MRSA cause different type of wound infection due to endogenous or exogenous. Wound infections, however, account for about 24% approximately 2 million nosocomial infections are seen yearly in hospitalized patients, these rates may vary according to surgeon hospital, and patients conditions (Colle et al.,1996).

In this research, S.aureus isolated from the wound and burn were biochemically characterized. Antibiotic susceptibility pattern were determined phenotypically and genotypically.

Materials and Methods

Specimen collection

The collection of specimen for this research was done under aseptic conditions. The organisms used were isolated from swab sample collected from 63 patients with infected burn and wound with irrespective of age and sex. The patient was on admission at the Al-Diwaniya Teaching hospital.

Isolation

A total of 157 burn and wound swabs were collected. These swabs were immersed in transport medium, transported to laboratory, cultured on Blood agar, Nutrient agar, and Manitol salt agar. Cultured media were incubated at 37℃ for over night.

Biochemical tests

Characteristics *S. aureus* colonies were identified by gram stain, catalase, coagulase, urease, indol, methyl-red, vogas-proskaur, citrate utilization, haemolysis and manitol salt fermentation (Forbes *et al*.,2007). The definitive identification made by Vitek 2-compact.

Antibiotic susceptibility tests

a- Phenotypic detection

All *S. aureus* isolates were cultured on Muller-Hinton agar without NaCl supplementation. The zone of inhibition was determined after 24 h of incubation at 37℃. This test used as a screening test (CLSI,2012). The disks were used listed in (Table-3). Moreover, Vitek 2 was used to identify antibiotic susceptibility test.

b- Genotypic detection

PCR was used for detection *mec A* gene as recommended by (Kaya *et al*.,2009). A 533-bp fragment of *mec A* gene was amplified using the primers 5' AAA ATC GAT GGT AAA GGT TGG C-3' and 5' AGT TCT GCA GTA CCG GAT TTG C-3' (Intron, Korea). For DNA extraction, bacterial suspension adjusted to 0.5 McFarland was prepared in 1ml sterile water from a fresh subculture of *S. aureus*. Microcentrifuge tubes containing this suspension were centrifuged at 13,000 rpm for 1 min and the supernatant was removed. The remaining solution was vortexed after adding 100 µl sterile distilled water and 50µl of this solution was transferred to a clean microcentrifuge tube. Then 50 µl of digestion buffer (50 mM KCl, 10 mM Tris, 1% NP40, 1% Triton x100,1 mg/ml Proteinaz K) was added onto the suspension. The tubes were incubated in water bath for 2 h at 65°C and then for 10 min at 95°C. Following this procedure, the tubes were centrifuged at 13,000 rpm for 2 min. The supernatant was used for amplification. 5µl of the supernatant was added to 45 µl of the PCR reaction.

Conditions were as follows: first denaturation at 94°C for 5 min, followed by 30 cycles of denaturation at 94°C for 30 s, annealing at 55°C for 45 s, extension at 72°C for 1 min and final extension at 72°C for 5 min. The amplified products were detected by 1% agarose gel electrophoresis after staining with ethidium bromide (1 µg/ml) and examined under UV transilluminator.

Results

Among 63 patients 157 swabs were taken. Only24 (38.1%) patients were infected by *S. aureus*. A total 36 isolates of *S.aureus* were isolated from different sites from these patients, some of them with wound 24(66.7%) and the other with burn 12(33.3%) as shown in (Table-1).

Table-1 Summary of the patients and isolates

Total patients	Total swabs	Patients infected by *S.aureus* n (%)	Total *S.aureus* n (%)	*S.aureus* in wound n (%)	*S.aureus* in burn n (%)
63	157	24 (38.1%)	36(22.9%)	24 (66.7%)	12 (33.3%)

S. aureus isolated (No.36) were identified using traditional morphological and biochemical diagnostic tests according to the Goldman and Lorrence (2009) and were in agreement as shown in (Table-2).

Table-2 Morphological and biochemical tests for identification of S.aureus.

Test	Gram stain	Clustase	Coagulase	Oxidase	Catalase	Manitol fermentation	Vogesproskour	Mr-test	Indole	Urease	Citrate	Haemolysis
Result	+	+	+	-	+	+	**3-v**	+	-	**2+v**	-	+

The conformational identification of *S.aureus* was performed using VITEK2 system (VITEK-2 GN kit).

Identification of MRSA

The MRSA strains were selected according to their phenotypic characteristics (resistance to cefoxtine and oxacillin) by disk-diffusion and VITEK system. Out of 36 *S.aureus* isolates, only 8(22.2%) diagnosed as MRSA, 6(75%) isolates from burn and 2 (25%) isolates from wound.

Antibiotic susceptibility tests were studied to 8 antibiotics (penicillin, cefoxitin, oxacillin, vancomycin, gentamicin, tetracycline, erythromycin, and rifampicin). The MRSA showed a high level of resistance to all antimicrobials, 8(100%)isolates were resistance to penicillin, cefoxitin, and oxacillin, 3 (37.5%) were resistance to vancomycine, erythromycin, and rifampcin, 2(25%) were resistance to tetracycline and gentamycine as shown in (Figure-1).

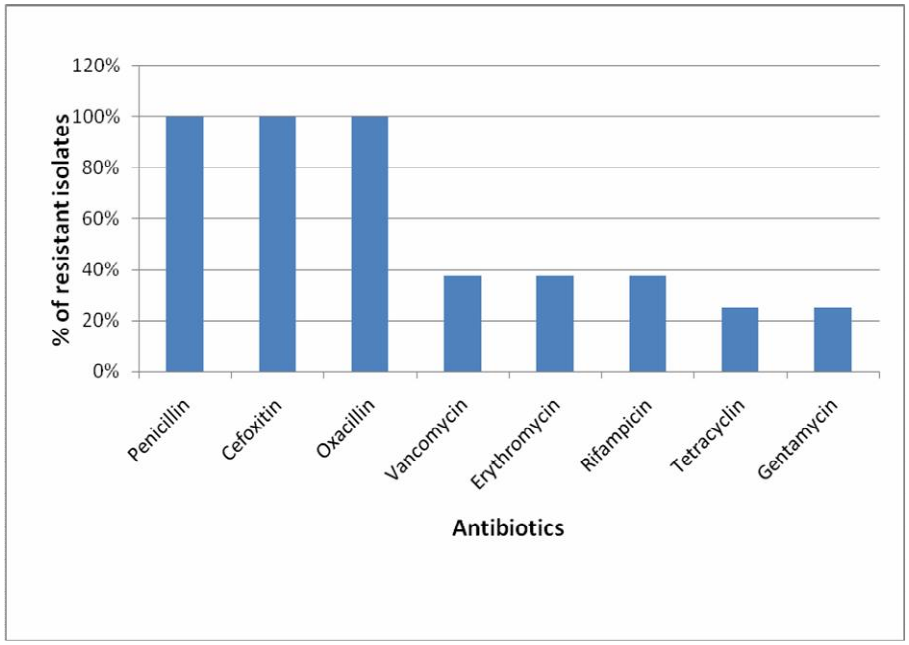

Figure -1 Percentage of resistant of MRSA isolates against antibiotics

Moreover, a high level of resistance (≥ 32µg/ml) was noted for vancomycin and rifampicin in 3(37.5%) isolates, and in 2(25%) by cefoxtine. The isolates showed moderate resistance to gentamycine and tetracycline (≥ 16 µg/ml) in 2 (25%) isolates. In this study were actually resistant to many classes of antimicrobials at the same time and thus qualify as multiply drug resistant *Staphylococcus aureus* (MDR-MRSA)as shown in (Table-3).

Table-3 The MIC of different antibiotics (mg/L) using VITEK2 automated System.

Strain	Specimen	PEN	FOX	OX	VA	GM	TE	EM	RA
Sa 3B	Burn	>=0.5 (R)	>=32 (R)	>=4 (R)	1 (S)	>=16 (R)	>=16 (R)	>=8 (R)	>=32 (R)
Sa 6W	Wound	>=0.5 (R)	>=8 (R)	>=4 (R)	<=0.5 (S)	<=0.5 (S)	<=1 (S)	<=0.25 (S)	<=0.5 (S)
Sa 10B	Burn	>=0.5 (R)	>=8 (R)	>=4 (R)	1 (S)	<=0.5 (S)	<=1 (S)	<=0.25 (S)	<=0.5 (S)
Sa 14B	Burn	>=0.5 (R)	>=8 (R)	>=4 (R)	>=32 (R)	<=0.5 (S)	>=16 (R)	>=8 (R)	>=32 (R)
Sa 38B	Burn	>=0.5 (R)	>=8 (R)	>=4 (R)	<=0.5 (S)	<=0.5 (S)	<=1 (S)	<=0.25 (S)	<=0.5 (S)
Sa 41B	Burn	>=0.5 (R)	>=8 (R)	>=4 (R)	>=32 (R)	<=0.5 (S)	<=1 (S)	<=0.25 (S)	<=0.5 (S)
Sa 49W	Wound	>=0.5 (R)	>=8 (R)	>=4 (R)	2 (S)	<=0.5 (S)	<=1 (S)	<=0.25 (S)	<=0.5 (S)
Sa 51B	Burn	>=0.5 (R)	>=32 (R)	>=4 (R)	>=32 (R)	>=16 (R)	4 (S)	>=8 (R)	>=32 (R)

Abbreviation:- PEN, penicillin; FOX, cefoxitin; OX, oxacillin; VA, vancomycin; GM, gentamicin; TE, tetracycline; EM, erythromycin; RA, rifampicin.

Genotypic testing was consistent with these 8 isolates revealing that only 6 isolates contained a mecA gene, these are (1,2,4,6,7,and 8) as shown in (Figure 1 and 2).

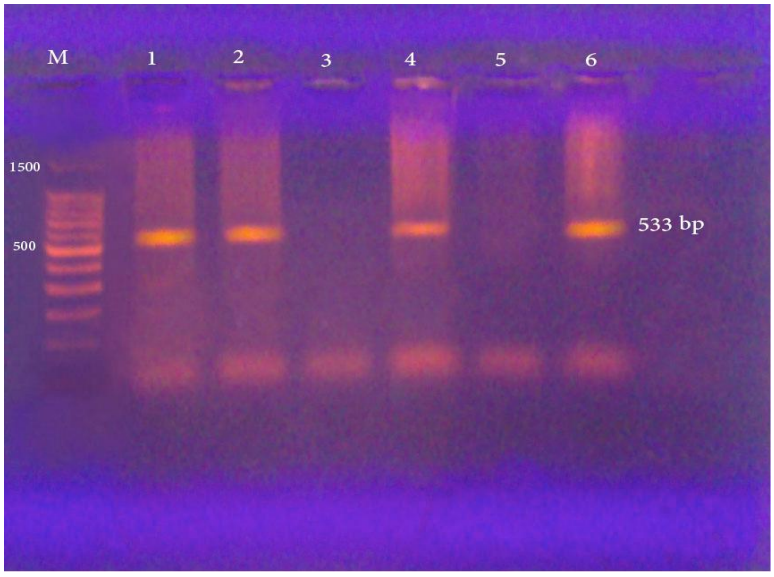

Figure-1 : Ethidium bromide stained agarose gel showing PCR amplification products with *mecA* gene(533Bp) primers for *S.*aureus . M 100 bP standerd size reference marker. Lane1 , 2 , 4 and 6 shows positive result with *mec A* gene. Lane 3and 5 shows negative result with mec A

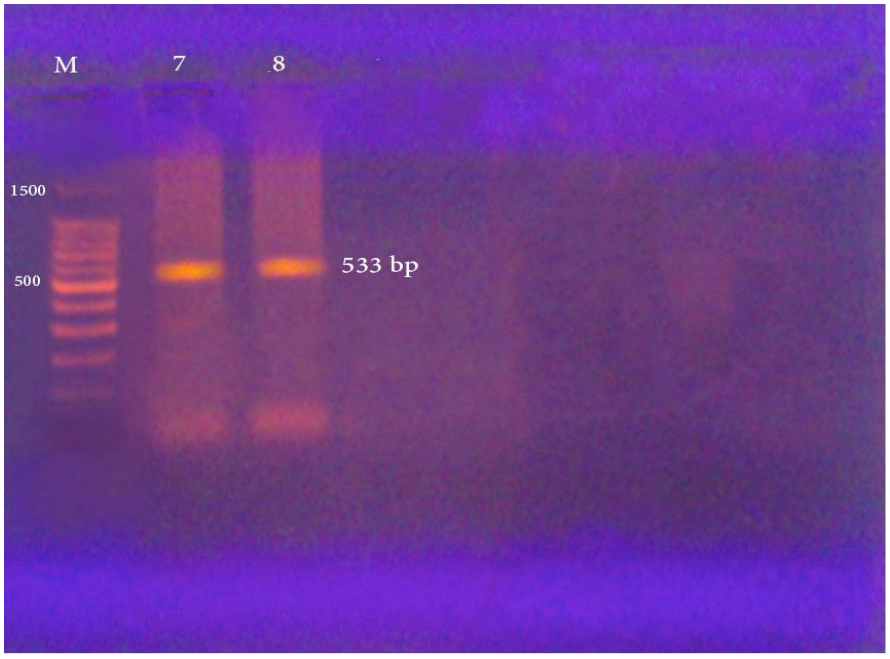

Figure-2 : Ethidium bromide stained agarose gel showing PCR amplification products with *mecA* gene(533Bp) primers for *S.*aureus . M 100 bP standard size reference marker. Lane 7 and 8 shows positive results with mec A gene.

Discussion

Infection is the most important problem in the treatment of burn and wound. The bacteriology of burn and wound is often poly-microbial in nature, and the S.aureus was found to be a frequent cause of burn and wound sepsis (Onwubiko and Sadiq,2011). The presence of multidrug-resistant organism is often associated with more severe clinical manifestation and poor response to antimicrobial therapy. Antibiotic sensitivity patterns served as a useful guideline for choosing an appropriate antibiotic. Staphylococcal resistance to either oxacillin or methicillin occurs when the organism including an altered penicillin binding protein(PBP2A) that is coded the mecA gene. Oxacillin is stable under storage conditions, and methicillin actually is an excellent inducer of the mecA gene (Weigelt,2007). According to that, oxacillin and methicillin resistant isolates were initially interpretation as MRSA. In this study 8 isolates of MRSA were obtained, 6(75%) from burn patients, and 2(25%) from wound, may be due to that burn patients spend more time in the hospital, and undergo to more frequent dressings (Rokas et al.,2003). Moreover, all MRSA isolates were multi-drug resistant. This could be due to continuous usage of broad-spectrum antibiotic policy. Additionally, selective pressure in the hospital wards could also be takes as the most probable factor for the increased resistance in isolates from the patient (Chini et al.,2006). The results shown in (Figure-1) indicate the MRSA was fully resistance to oxacillin, cefoxtin and penicillin, because all Staphylococcal strains produce B-lactamase which destroy the B-lactam ring resulting in inactive products .This results can be attributed to the structural gene for penicillin-binding protein which is responsible for the intrinsic resistance of MRSA (Suzuki et al.,1992). Also the transmission of MRSA occurs commonly in hospitals and in people who live in crowded setting (Proctor,2006). Moreover, MRSA in this study was less susceptibility for vancomycin, erythromycin and rifampicin These results are in

agreement with Norazah et al(2009) and Al-Hasseny(2011). The resistance of MRSA to vancomycin can be attributed to the resistance genes vanA, vanB, vanC1, vanC2, and vanC3 genes(Tenover et al.,1998). MRSA has reported to possess genes encoding to protein mode thicker cell wall which will cause more vancomycin molecules to be trapped in the peptidoglycan layer before reaching the cytoplasmic membrane where peptidoglycan synthesis occurs resulting in a thickened cell wall of VRSA and VISA strains (Norazah et al.,2009). Resistance of MRSA to erythromycin confers cross-resistant to other macrolides antibiotics this may be due to mutation of the ribosomal receptor site or modification of receptor (Katzuny,2004).

The PCR was used to detect mecA gene revealed it present in 6 isolates and 2 isolates did not reveal the presence of mecA gene even thought these were resistance to oxacillin and cefoxitin in phenotypic assay, this may be due to present another gene like mecB which responsible from resistance also, and the phenotypic assay is subject to variation in inoculums size, medium pH, medium salt concentration (Mohansondara and laitha,2008). The PCR technique has many added advantages over the conventional techniques, and it used by many workers worldwide to detect mecA gene (Kaya et al.,2009; Al-Fuadi,2010).

Conclusion

Conclusively, from this study, it is evident that the MRSA strains found in the burn and wound exhibited multiple resistances to antibiotics, exploiting the immunocompromised state of the patients. This may lead to multiple infections or disease such as septicemia, pneumonia, osteomyelitis. These would increase hospital stay and additional financial cost of treatment.

S.aureus infection in wound and burn can be treated using dressings and antiseptic solution alone, without the use of systemic antibiotic.

Penicillinase resistant penicillins (methicillin, oxacillin). And their combination should not be prescribed (for treatment of S.aureus infections) without medical instructions and testing for susceptibility against these antibiotics in addition to other types of antibacterial agents in – vitro.

PCR is a good confirmatory test, but its use could be limited to reference laboratories due to its cost.

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