Detection of *mec A* gene in *Staphylococcus aureus* from Osteomyelitis infections

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المقدمة: تعد المكورات العنقودية الذهبية *Staphylococcus aureus* من اهم الممرضات المرتبطة بالأمراض المكتسبة واصابات المستشفيات. من خلال ذلك تهدف الدراسة الحالية للتحري عن بكتريا المكورات العنقودية الذهبية في اصابات العظام ومن ثم تضخم جين *mecA* لعدد من العزلات المقاومة للمضادات الحياتية.
طرق العمل: عزل بكتريا المكورات العنقودية الذهبية من اصابات العظام ومن ثم فحص جميع العينات لعدد من المضادات الحياتية لأجل التحري عن جين *mecA* .
النتائج: تسعة وعشرون عينة تم الحصول عليها من كلا الجنسين المراجعين لمستشفى السماوة التعليمي. فقط 14(48.27%) هم من المصابين ببكتريا المكورات الذهبية *Staphylococcus. aureus* و 19 عزلة تم الحصول عليها من تلك العينات. اظهرت العزلات مقاومة واضحة لعدد من المضادات الحياتية. من بينها 5 عزلات مقاومة بشكل كامل(100%) لمضادات الاوكساسلين والبنسلين و السيفوكسيتين وعلى اساس ذلك اعتبرت المكورات العنقودية المقاومة للمثيسلين (MRSA).بينت نتائج التشخيص الجيني ان خمس عزلات تمتلك الجين المشفر للمقاومة *mec A* في مادتها الوراثية.
التوصيات: بينت الدراسة الحالية حدوث اصابات العظام في كلا الجنسين وبنسب قليلة. تعد بكتريا المكورات العنقودية الذهبية *S. aureus* من اهم الممرضات التي تمتلك مقاومة لعدد من المضادات بسبب امتلاكها جين *mecA*.

Abstract

Background: *Staphylococcus aureus* is a major pathogen that associated with serious community acquired and nosocomial diseases. In view of the present problem the study aimed to isolation of *S. aureus* within osteomyelitis samples and amplified the *mecA* gene of some antibiotic resistant isolates.

Methodology: *S. aureus* were isolated from osteomyelitis and all isolates screened for antibiotic susceptibility test to detection of *mecA* gene.
Results: 29 swabs were taken from both sex patients related to Al- Sammawa Teaching hospital at period from September 2013 to march 2014. Only 14(48.27%) patients had *S. aureus* and 19 isolates yielded from all samples. These isolates showed pronounced resistance for a group of active antibiotics. Five isolates have full (100%) resistance to oxacillin, penicillin and cefoxitin so considered as Methicillin Resistant *Staphylococcus aureus* (MRSA). Genetic identification showed that five *S. aureus* isolates have *mec A* gene in their genome.
Conclusion: This study highlights the presence osteomyelitis infection in both sex with little incidence. *S. aureus* one of the important pathogens that have resistance to many antibiotics due to *mecA* gene.

Key words/ *Staphylococcus aureus*, MRSA, *mec A* gene, Antibiotic

Introduction

. Osteomyelitis is a bacterial infection of the bone that affects an alarming number of patients that are admitted to hospital (1). *Staphylococcus aureus* (*S. aureus*) is the predominant causative organism in long bone osteomyelitis while mandibular osteomyelitis is usually considered a polymicrobial disease (2).

. Over all, skin and soft tissues infection represent about 77%to 90% of Methicillin Resistant *staphylococcus aureus* (MRSA) infection and these are predominantly abscesses or cellulitis, osteomyelitis alone account for only 1% of all MRSA infection (3,4). Data from

media mannitol salt agar. All media were incubated at 37C for ver night.

Biochemical tests.

Identification *S. aureus* were done according to (12) by gram stain, catalase, coagulase, urease,hemolysis, citrate utilization and mannitol fermentation.

Antibiotic susceptibility test .

All *S. aureus* isolates were cultured on Muller Hinton agar. The zone of inhibition was determined after 24h of incubation at 37C as show in table (1). CLSI 2012(13) was used as a reference for susceptibility measurement. 9 antibiotics were used (penicillin, oxacillin, cefoxitin, gentamicin, tetracycline, erythromycin, vancomycin,amoxicillin- calvulanic acid and rifampicin).

Genotypic detection.

PCR primers specific for *mec A* gene 5-GTAGAAATGACTGAACGTCCGATAA-3 and 5-CCAATTCCACATTGTTTCGGTCTAA-3 were manufactured by Bonier kit, Korea and selected from (14).The PCR mixture containing :10x PCR buffer, taq DNA polymerase 0.5u/µl , each primer 1.6 µM, MgCl2 1.2 µM and each dNPT 0.64 µM (all the materials provided by Bioneer kit , Korea). .

The PCR cycles for the isolation were as following: an initial denaturation at 94C° for 5 min with 30 cycles of denaturation at 94 C° for 15 S , annealing at 61 C° for 15 S and elongation at 72 C° for 30 S and final extension at 72 C° for 5 min (14). PCR products was electrophoresed on 2% agarose gel in a 0.5x Tris Borate EDTA(TBE) buffer and stained in ethidium promide (14).

2003 indicate that resistance rates for MRSA are approximately 60% in united states intensive care units, a 12% increase from 1998-2002 (5).

The development of resistance to a wide rang of antibiotics in *S. aureus* is diversified, such as resistance to methicillin that takes the account of *S. aureus* to most B-lactam, Macrolides and Aminoglycoside (6). The specific genetic mechanism of its resistance has been identified as a mobile genetic element (Staphylococcal cassette chromosome mec)(*SCC mec*)integrated in to the *S. aureus* chromosome (7). Within which, the mec A gene that encodes penicillin binding protein(PBP2a) has low affinity to B-lactam antibiotic(8).

The *mec A* gene is a part of a 21-60 kb staphylococcal chromosome cassette mec (*SCC mec*)(9). There are four pencillin binding protein : PBP1, PBP2, PBP3 and PBP4, all have high affinity to B-lactam antibiotic except PBP2 (10). Molecular study of antibiotic resistance gene from *S. aureus* amplification and sequencing of *mec A* gene which resbonsible for most of B-lactams (11). In this study, investigation occurs for the presence of *mec A* gene in osteomyelitis *S. aureus* samples.

Materials and Methods.

Samples Collection.

The collection of samples for this work was done under aseptic condition. The used organisms were isolated from swab specimen collected from 29 patients suspected with osteomyelitis with physician aid. The patient was on admission at the Al- Sammawa Teaching hospital orthopedic unit during September 2013 to march 2014

Isolation.

A total 29 Osteomyelitis swabs were collected. These swabs were immersed in transport medium until reached to laboratory, cultured on enrichment media Blood agar, Nutrient agar and selective

sites infected bones of these patients.*S. aureus* isolated and identified using traditional morphological and biochemical tests according to (12) as shown in table (1).

Results

Among 29 swabs were taken from both sex. only 14(48.27%) patients were infected by *S. aureus*. A total 19 isolates of S. aureus were yield from different

Table (1): Biochemical tests for *S. aureus* diagnosis: + positive /- : negative /var: variable

test	Catalase	oxidase	Gram stain	coagulase	Mannitol fermentation	urease	hemolysis	Citrate utilization
result	+	-	+	+	+	var	β	-

All the isolates of *S. aureus* showed different resistance values, 3(60%) isolates were resistance to amoxillin-calvulunic acid, 2(40%) were resistance to erythromycin and vancomycin, 1(20%) were resistance to gentamicin, tetracycline and rifampicin figure (1).

After culturing all the 19 isolates of *S.aureus* on Muller Hinton agar, the phenotypic characteristic was selected according to their resistance to oxacilline and cefoxitin by disk diffusion test. Among 19 *S. aureus* isolates only 5 (26.31%) diagnosed as MRSA by their full resistance (100%) to oxacilline and cefoxitin.

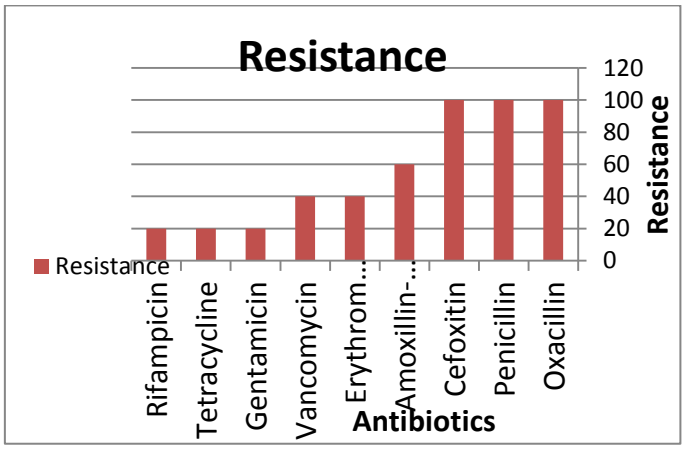

Figure (1): Percent's Resistance of *S. aureus* to chosen Antibiotics

In PCR, *mec A* gene was detected in all (5) *S. aureus* isolates (MRSA). Figure (2). Which confirmed the presence of *mec A* gene in 310 bp.

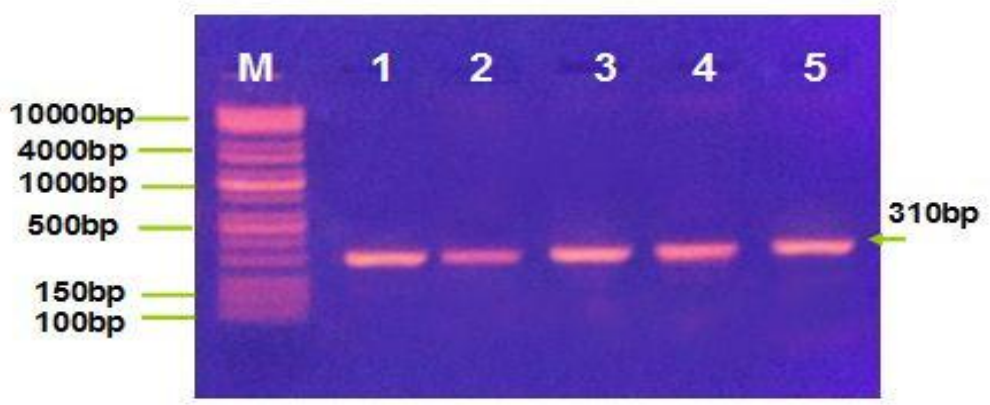

Figure (2): *mecA* gene gel electrophoreses stained with Ethidium bromide: M: Ladder(10000bp),1,2,3 ,4,5*:mecA* gene(310bp).

Discussion

Infections due to *S. aureus* are very common and MRSA contains to be a serious and formidable challenge to health care providers as their prevalence is reported to be increasing exponentially (15). *S. aureus* is an important pathogen both in community and in hospital settings, infections ranging from superficial to deep seated and systemic such as Osteomyelitis (16). Approximately 60% of clinical isolates are MRSA, the majority of the isolates were resistant to oxacilline and associated with four other antibiotics(17). Other studies similar with this work reported MRSA resistant clinical isolates in Jordan (57%) (18), 58% in Jaban (19), 61% in Tiawan (20) and 65% in Kuwait (21).

The transmission of MRSA occurs commonly in hospitals and in people who live in crowded setting (22). The less susceptibility of *S. aureus* to erythromycin, vancomycin, gentamicin, tetracycline and rifampicin in this work were agreement with (23) and (24) . MRSA has reported to possess genes encoding to protein made thicker cell wall which will cause more vancomycin molecules to be trapped in the peptidoglycan layer before reaching the cytoplasmic membrane where peptidoglycan occure resulting in a thickened cell wall of VRSA and VISA strains (23).

The PCR was used to identify *mec A* gene *S. aureus* showed five isolates only expressed. However, the PCR technique has many advantages over the convential technique and it used by workers world wide to detect *mec A* gene(25) (26), because some *S. aureus* have resistant to oxacilline , methicillin and cefoxitin in presence of another gene like *mec B* which responsible for it, furthermore, the phenotypic assay is subject to variation in inoculums size, media pH, media salt concentration(27).

The *mec A* gene, which lies in the *Scc mec* resistance islands, is carried by 95% of the isolates that display a phenotype of methicillin resistance and was detected in all multiresistant *S. aureus* isolates (28).

Conclusions.

In this work, Osteomyelitis infections can occurs in both sex but in little incidence in area of study specially with septic condition in cure unites. *S. aureus* one of the most important pathogens causing bone inflammation. Penicillin group should not prescript as an agent of choice for avoid multiresistance strains. PCR is a delicate and useful technique for early detection of new resistance strains.

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