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mec A *AND mec* C GENES PROFILE OF CLINICAL ISOLATES OF *STAPHYLOCOCCUS AUREUS*

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Abstract

Methicillin resistant *Staph. aureus* (MRSA) was a substantial bacteria that caused diverse hospital and systemic infections. The detection of *mec* gene of this pathogen must be used as a rapid screening technique. The current study was aimed to characterize the frequency of *mec*A and *mec*C genes in *Staph. aureus* were isolates which phenotypically were resistance to methicillin which were recovered from patients with tonsillitis that was happened at Al-Habboby teaching hospital during the period from February to November, 2016 in Thi-Qar province/Iraq by using PCR technique. From a complete of 109 (63%) *Staph. aureus* isolates, only 71 isolates were identified phenotypically as MRSA. The molecular results were documented that (62% and 31%) of isolates expressed *mec*A and *mec*C, respectively. Sixty nine percentage of all *Staph. aureus* isolates showed negative results of *mec*C gene. The current results of were established the significance of *mec*C gene in MRSA recognition than *mec*A gene and highlighted the increasing manner of its frequency in south of Iraq.

Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) is one of the greatest vital multi-resistant human pathogens universal, causing the infections in both hospitals and community and in the livestock (Paterson *et al*., 2014A). The gaining of the *mec* A gene by *S. aureus* isolates, therefore those isolates become resistant to methicillin antibiotic and recorded as MRSA, and the *mec* A gene situated on the staphylococcal cassette chromosome *mec* (SCC*mec*) (Doğan *et al*., 2016).

The SCC*mec* elements were characterized through the presence of two indispensable loci: the *mec* gene complex comprising the methicillin resistance determinant with intact copies of the *mec* regulatory genes (*mecI*,*mecR*, and *mecR2*) and the *ccr* gene complex, which encodes site- and orientation-specific recombines responsible for SCC*mec* mobilization (Katayama *et al*., 2000). The incidence of hospital associated and similarly community acquired infections triggered by *S. aureus* strains, especially MRSA which have gained worldwide notoriety as hospital 'superbugs' and that are resistant to numerous antibiotics (Nwokah *et al*., 2012).

The MRSA poses a serious problem for infection prevention, control and antibiotic treatment globally. In MRSA, resistance against almost all beta-lactam compounds in clinical use is caused by the expression of an alternate penicillin binding protein (PBP2a) that is encoded by the *mec*A gene and those genes can be found in different staphylococci (Ito *et al*., 2012).

A novel *mec* gene type was discovered in 2011, which located on a novel SCC*mec* element designated as type XI (Garcia-Alvarez *et al*., 2011; Shore *et al*., 2011). Because of its highly divergent sequence, it cannot be detected by routinely used molecular assays designed to identify *mec*A (formerly $mec_{ALGA251}$) (García-Álvarez *et al*., 2011; Laurent *et al*., 2012). This gene renamed *mec*C (Ito *et al*., 2012). *mec*C had been isolated from various animals including cattle, sheep, dogs, cats, a guinea pig, rabbits, rats, and a chaffinch as well as from humans from Ireland, England, Scotland, Germany, Denmark, Sweden, Norway, France, Switzerland, Belgium and The Netherlands (Petersen *et al*., 2013; Robb *et al*., 2013; Porrero *et al*., 2014A). The aim of the this study was to characterize a sensitivity of *S.aureus* isolates to methicillin antibiotic, to detect the attendance of *mec*C and *mec*A genes that found in isolates of *S. aureus* recovered from patients with tonsillitis.

Material and methods:

Ethical approval

This research was approved by the Medicine College Ethics Committee, Thi-Qar University, Thi-Qar Province, Iraq.

Laboratory methods

All *S. aureus* were isolated from 173 swabs which collected from tonsillitis patients whom admitted to ENT unit in AL-Habbuby Teaching Hospital of Thi-Qar province through the period from February to November, 2016 and identified depending on cultural properties (LAB/ United Kingdom), followed by biochemical tests (Brooks *et al*., 2007; Harley and Prescott, 2002). The confirmed diagnosis was performed by using API system (BioMerieux/France).

Antibiotic sensitivity test

To detect the *S. aureus* sensitivity to methicillin antibiotic (5µg /disc) (Bioanalyse, Turkey) by using the disc diffusion method described by (Kirby and Bauer, 1966). The diameters of inhibition zone were measured and interpreted according to CLSI, (2011).

Preparation of bacterial DNA

The *S. aureus* chromosomal DNA extraction was carried out on entirely *S. aureus* isolates using Genomic DNA Extraction kit (Geneaid/Korea).

PCR diagnosis of *mec*A and *mec*C genes

The specific primer pairs of *mec*A as following: forward: 5'-GTA GAA ATG ACT GAA CGT CCG ATA A-3' and reverse: 5'-CCA ATT CCA CAT TGT TTC GGT CTA A-3' (Geha *et al*., 1994). While for *mec*C gene: forward: 5'-GAA AAA AAG GCT TAG AAC GCC TC -3' and reverse: 5'-GAA GAT CTT TTC CGT TTT CAG C-3' (Stegger *et al*., 2012).

The PCR cycling conditions of *mec*A gene: initial denaturation at 94°C for 4 min, followed by 30 cycles of denaturation at 94°C for 45 sec, annealing at 50°C for 45 sec, extension at 72°C for 1 min and final extension for 2 min (Jonas *et al*., 2002). Whereas for *mec*C gene: initial denaturation at 94°C for 15 min, followed by 30 cycles of denaturation at 94°C for 30 sec, annealing at 59°C for 1 min, extension at 72°C for 1 min and final extension for 10 min after the last cycle (Doğan *et al*., 2016). Electrophoresis of PCR product was carried out in 1.4% agarose gel and the presence of a 310 bp and 138 band indicate a positive result for *mec*A and *mec*C genes, respectively.

Statistical Analysis:

The results of the present study were statistically analyzed by using SPSS program Version 16. *P* values below or equal to 0.05 was considered statically significant.

Results and Discussion:

The results of the current study presented that the prevalence of *S. aureus* was 109 isolates (63%) from completely collected swabs. The *S. aureus* was an important causer of tonsillitis infection (Jeong *et al*., 2007; Gowrishankar *et al*., 2016).

From 109 isolates of *S. aureus*, only 71 isolates (65%) exhibited resistant to methicillin disc and which recorded phenotypically as MRSA.

In similar study conducted in Thi-qar province, Hamim, (2017) recorded an approach percentage of MRSA infection outbreak (53%) in comparison with the results of the present study. On other hand, the recent results dissimilar with local studies that recorded a low percentages of MRSA among *S. aureus* isolates such as Taha *et al*., (2010) in Erbil, Abdullah, (2013) in Baghdad, reported that the rates of MRSA were 30.24% and 41.54%, respectively.

Also the present results disagreed with results of study piloted in Nigeria by Nwokah *et al*., (2016) exhibited that 25 (12.2%) out of 205 isolates of *S. aureus* were resistant to oxacillin.

The molecular detection of *mec*A gene revealed that 44/71 (62)% of isolates contained this gene with the molecular weight of approximately 310bp (Fig1). The genetic profile of *mec*A is commonly used as a reference standard for MRSA identification, and used as a main test or for validation (Chambers, 1997). The percentage of *mec*A gene in current study approached with other studies like Hamim, (2017) and Nwokah *et al*., (2016) showed that 88 (73.3%) and 17 (68%) of isolates had the targeted gene.

Sixty eight isolates from total *S. aureus* harbored *mec*A gene, the current results disagreed with studies performed by Lepainteur *et al*., (2015); Becker *et al*., (2016) showed that all 98 methicillin sensitive *S. aureus* (MSSA) strains exhibited negative results for *mec*A.

Not all *S. aureus* isolates which recorded phenotypically as MRSA had *mec*A gene, because of the resistancy to methicillin may be due to not only to the existence of the *mec*A gene alone; nevertheless by a cluster of *ica* gene with this gene (Memmi *et al*., 2008), furthermore must be due to the present of *mec*C gene that encoded to the same resistancy among human and bovine MRSA isolates (Garcia-Alvarez *et al*., 2011; Ito *et al*., 2012).

Among the examined MRSA, 22 amplified of the goal gene (*mec*C); the percentage was 30% with the molecular weight of approximately 138bp (Fig.2).

The recent percentage of *mec*C gene differenced from the results of Stegger *et al*., (2012) showed that 12 (6%) isolates harbored $mec_{ALGA251}$ identified amongst 203 isolates. The recent results documented the slightly percentage of *mec*C gene in MRSA isolates, similarly Peterson *etal*., (2013) described that *mec*C gene established in 1.5% of *S. aureus* isolates, while the frequency of goal gene increased and reached to 1.9% in 2010 and 2.8% in 2011 in the Denmark.

In spite of the *mec*C gene was more detected in *S. aureus* isolated from animal samples and less frequently detected in humans, but Doğan *et al*., (2016) showed that all MRSA isolates harbored *mec*A gene, whereas a *mec* C gene was not presence in completely isolates of *S. aureus*.

The molecular detection of *mec*A gene in staphylococci was usual mode, also Paterson *et al*., (2014A) suggested the demonstration of this gene by PCR as gold standard method, but *S. aureus* had *mec*C gene cannot detect via specific PCR through the discovery of *mec*A gene (Paterson *et al*., 2014B), resulted from insufficiencies of phenotypic methods to the finding of *mec* C gene, therefore the methods based on DNA used to limit the goal gene.

The *mec*C gene source was not tacit adequately (Basset *et al*., 2013), but Figueiredo and Ferreira, (2014) strongly suggested that the associations between humans and livestock had been maintained, and an incidence of *mec*C gene cross-transmission between the last populations.

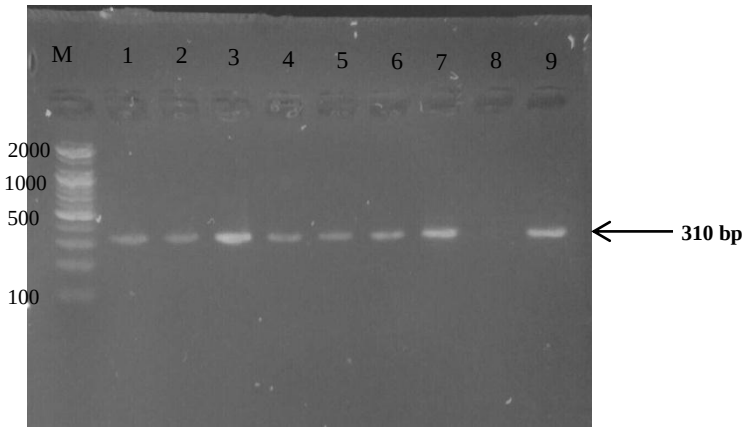

Fig. (1): Agarose gel electrophoresis of *mec* A gene amplification, M: ladder, 1-7, 9 : positive results, 8: negative result.

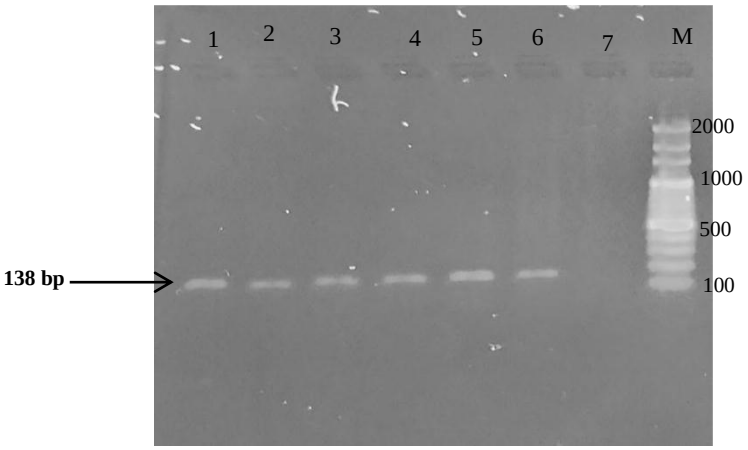

Fig. (1): Agarose gel electrophoresis of *mec* C gene amplification, M: ladder, 1-6: positive results, 7: negative result.

Conclusion:

The results of present study established the significance of *mec*C gene in MRSA recognition than *mec*A gene and highlighted the increasing manner of its frequency in south of Iraq.

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