NUCLEOTIDE SEQUENCE ANALYSIS OF METHICILLIN RESISTANCE *STAPHYLOCOCCUS AUREUS* IN KURDISTAN REGION-IRAQ

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Abstract

In this study a total of 241 clinically important specimens were collected from three hospitals in Kurdistan region; from which 64 isolates of *S. aureus* were isolated representing 26.5% of the total samples. The molecular confirmation of *S. aureus* isolates were done by PCR using nuc primer as a species-specific primer producing 280bp DNA fragment for *nuc* gene. 31 of these isolates were resistance to methicillin representing 48.4% of total isolates. Resistance to Methicillin was confirmed by using mecA primer producing 310bp DNA fragment for *mecA* gene. Three isolates (SaDu5, SaEr17 and SaSul24) were subjected to partial DNA sequencing of the *mecA* gene to assess the relation of Kurdistan isolates with other global sequences from GenBank. Sequence analysis showed that the sequence identity of all three *mecA* genes among the Kurdistan isolates was ranged between 97.5-98.5%, whereas the sequence identity among the Kurdistan isolates and other global isolates selected from GenBank was ranged between 92.6-96.1%. The sequenced product of *S. aureus mecA* genes from Kurdistan found similarities and showed partial homology with *S. aureus* strains (UK/NTCC124, Turkey/HM5, South Korea/ YSSA11 and India/TN/CN/1/12) penicillin binding protein 2a *mecA* gene, partial cds, which is available in public databases. The phylogenetic tree of the aligned sequences was produced by using MEGA5 Beta version 6.1 online software, and showed a typical relatedness among Kurdistan isolates and GenBank reference strains. This study will gives insights in to the molecular diagnosis of *S. aureus* and to find better synthetic drugs or organic to control expression of antibiotic genes in different pathogenic organism in general and in particular MRSA strains.

Key words: MRSA, *mec*A gene, DNA Sequencing.

Introduction:

Staphylococcus aureus is one of the most significant and serious human pathogens, which is known as a threat to human health for more than a century. This pathogen is responsible for a wide range of diseases, causing both nosocomial and community-acquired infections (Askari *et al*., 2012). Acquisition of resistance to several antimicrobial agents by this pathogen has been a major problem for the treatment of infections in the hospital and the community settings. Resistance to β-lactam antibiotics such as methicillin and oxacillin, is named "methicillin resistance" and is considered of great importance since the multidrugresistant phenotype of MRSA strains and their intrinsic β-lactam resistance make them difficult and costly to treat (Paradisi *et. al.*, 2001). Most strains of Methicillin Resistance *S. aureus* (MRSA) are also resistant to other β-lactam antibiotics, with the exception of glycopeptide antibiotics (Tiwari *et. al.*, 2006). Methicillin resistance is mediated by the presence of PBP-2a which is highly conserved among staphylococci and is encoded by the chromosomal gene *mecA* (Malik *et al*., 2006). *mecA* is located on a genetic element called the staphylococcal cassette chromosome (SCC) in *Staphylococcus aureus*. SCC*mec* is a group of mobile DNA fragments of about 21 to 67 kb that is integrated into the chromosome of methicillin resistant *S. aureus* at a unique site (*attBscc*) located near the *S. aureus* origin of replication (Hanssen *et. al*., 2004). The incidence of MRSA has become increasingly prevalent in the world since it first reported. Subsequently, the occurrence of MRSA particularly in hospitalized patients has increased steadily and nosocomial infections caused by such strains have become a serious problem worldwide. Therefore, rapid and accurate identification of MRSA is essential (Terry *et. al*., 2011). Improved strain characterization would improve our understanding of the epidemiology of this pathogen and will allow the development of a rapid assay for monitoring and controlling of MRSA. In view of this, the detection of *mecA* gene has been used as an alternative way of detecting or confirming MRSA either by use of DNA probe, commercially available fluorescence test, latex agglutination test, and PCR (Ieven *et al*., 1995, Cavassini *et. al*., 1999 and Rohrer *et. al*., 2001). Molecular typing techniques are widely applied in studies of *S. aureus* epidemiology, especially MRSA, with the aim of distinguishing isolates that are epidemiologically related from those unrelated. *S. aureus* outbreaks in hospitals are frequently

considered as short duration events of localized epidemiology and, in these situations, most of the molecular typing methods are able to distinguish the isolates that belong to the outbreak (Shopsin and Kreiswirth, 2001).

The rapidly expanding number of sequenced microbial genomes has served as a catalyst for the development of a variety of molecular typing approaches that focus on either single or multiple chromosomal loci. Single loci sequence typing (SLST) Sequence data for specific loci (genes for virulence, pathogenicity, drug resistance …etc.) from different strains of the same species have revealed variability in a specific gene, such as single-nucleotide polymorphisms and areas with repetitive sequence that demonstrate potential for epidemiologic application. SLST shows potential for a typing and clearly represents an important and promising sequenced-based approach to epidemiologic analysis (Singh *et. al*., 2006). Thus the objectives of this study were to molecular identification of *S. aureus* by using nuc primer as a species-specific primer and to determine the nucleotide sequence of *mecA* gene. Furthermore, analysis and determine the phylogenetic diversity and nucleotide sequence comparison of *S. aureus* isolates depending on the sequence of *mecA* gene in Kurdistan Region-Iraq.

Materials and Methods:

Bacterial isolation

Isolates of *S. aureus* used in this study were collected from Azadi, Dr. Khalid and Rizgary General Hospitals in Duhok, Koya and Erbil cities respectively in Kurdistan Region-Iraq. These samples were taken from patients, healthcare staffs and hospital environment. Basing on the source of infections, the isolates were obtained from: urine, nose, throat, wounds, burns, skin infections and hospital environments. These isolates were confirmed at molecular level as *S. aureus* by species-specific PCR technique using nuc primer.

Antimicrobial Susceptibility Testing

Disk diffusion testing was performed as recommended for staphylococci by Clinical and Laboratory Standards Institute (CLSI, 2012). Strains were considered susceptible to oxacillin if the zone of inhibition around the 1µg disks was >13 mm, intermediate if 11-12 mm, and resistant if the zone was < 10 mm.

Oligonucleotides sequences

Oligonucleotides of *nuc* gene as species-specific primer and *mec*A for Methicillin Resistant gene detection were used in this study provided by MWG Company, Germany (Table: 2) (Merza 2009).

PCR amplification:

The PCR amplification was carried out in 50-µl reaction mixture volumes containing 4µl of genomic DNA, 2 µl of each primer (50 pmol/µl), 5 µl of 10× PCR buffer (100mM Tris-base pH8.3, 500mM KCl, 20mM $MgCl_2$ and 0.001 gelatin), 5 µl of 100 mM deoxynucleoside triphosphates, and 2 units of *Taq* DNA polymerase (Gold Taq). DNA amplification was carried out in Applied Biosystem (USA) thermocycler, with the following thermal cycling profile for nuc gene: initial denaturation at 94°C for 4 min was followed by 30 cycles as follows: denaturation at 94°C for 45sec, annealing at 50°C for 45sec, and DNA extension at 72°C for 1 min. The reaction was achieved with a final extension at 72°C for 2 min. (Tiwari *et. al*., 2006).

The reaction condition for detection *mecA* gene was programmed with the initial denaturation, 4 min at 94°C; 30 cycles with a 45-s denaturation step at 94°C, a 45-s annealing step at 56°C and a 30-s extension step at 72°C and 2 min extension step at 72°C (Geha *et. al*., 1994). Five microliters of the amplified PCR products were used for detection of amplified products by electrophoresis in a 2.0% agarose gel and visualization with UV illumination after staining with ethidium bromide for 15 min. Standard DNA ladder 1500-100bp promega Company (USA) were included in each run.

DNA sequencing:

The amplification products were purified by using Illustra ™ GFX ™ PCR DNA and GeL Band Purification Kit (GE Healthcare Company, UK) following the manufacturers protocol before being used in a sequencing reaction. Sequencing was carried out on each DNA strand with BigDye Terminator Ready Reaction Mix v3.1 (AB Biosystems, HITACHI) by using the same primer set for *mecA* gene under the following conditions: initial denaturation at 96°C for 1 min, 30 cycles of 10 sec at 96°C, 5 sec at 51°C and 2 min at 60°C. Unincorporated dye terminators were removed by precipitation with 95% alcohol. The reaction products were separated and detected on an Applied Biosystem

genetic analyser 3130 (AB Biosystems) using a standard sequencing module with a Performance Optimised Polymer 7 (POP7) and 36 cm capillary array.

Nucleotide sequence analysis:

The sequences were compared to the nucleotide sequence database from the National Center for Biotechnology Information (NCBI) using the BLAST tool (http://www.ncbi.nlm.nih.gov/sites/entrez). Restriction map was performed by using ChromasPro (version 1.5) online software. Multiple sequence alignments were performed by using ClustalW (http://align.genome.jp) software (www.ebi.ac.uk/clustalw). Phylogenetic tree was performed by using MEGA5 Beta version 6.1 online software (www.megasoftware.net).

GenBank accession numbers:

GenBank accession numbers of *S. aureus* sequences used in the analysis are: Turkey/HM5 (EU790490.1), Iran/M795 (GU301104.1), Egypt/1 (EF600988.1), India/TN/CN/1/12 (KC243783.1), South Korea/ YSSA11 (EF596937.1) and UK/NTCC124 (HQ686324.1).

Results and Discussions

In this study a total of 241 clinically important specimens were collected; from which 64 isolates of *S. aureus* were isolated representing 26.5% of the total samples. These samples obtained from three hospitals (Dr. Khalid General Hospital in Koya city, Rizgary General Hospital in Erbil city and Azadi General Hospital in Duhok city) in three districts in Kurdistan-Iraq. These samples collected from patients, healthcare staffs and hospital environment in hospitals mentioned above (Table: 1). Isolates were obtained from urine, nose, throat, wounds, burns, skin infections and Hospital environments. Healthcare staffs included physicians, nurses and workers. Hospital environment samples were obtained from different sites in surgical, intensive care, burn, emergency, laboratory units and delivery ward. The molecular confirmation of *S. aureus* isolates was done by using nuc primer as a species-specific primer for *S. aureus*. The results showed that all isolates were amplified and producing 280bp DNA fragment for *nuc* gene as illustrated in (Figure: 1.a). All 64 isolates of *S. aureus* were searched for methicillin resistant by using Oxacillin (1μg) disc diffusion method according to the Clinical and Laboratory Standards Institute guidelines (Antimicrobial Susceptibility Testing Standards) (CLSI, 2012). The results revealed that the prevalence of methicillin resistant *S. aureus* in Kurdistan was very high, which found that 31 of these isolates were resistance to methicillin representing 48.4% of total isolates as illustrated in (Table:2). The high prevalence of MRSA immerged in our hospitals may reflect failures in strategies to control infection and/or differences in antibiotic use, the poor hygienic conditions of the patients. Resistance to methicillin is mediated by the acquisition of penicillin binding protein PBP-2a encoded by the *mec*A gene, which exhibits a low affinity for β-lactam antibiotics (Davoodi *et al*., 2012). Resistance to Methicillin was confirmed by using mecA primer producing 310bp DNA fragment for *mecA* gene as shown in (Figure: 1.b).

Three methicillin-resistant staphylococcal isolates (SaDu5, SaEr17 and SaSul24) were subjected to partial DNA sequencing of the *mecA* gene to assess the relation of Kurdistan isolates with other global sequences from GenBank and to find similarity between them for cataloging mutation patterns and to provide useful strain resolution. Sequence analysis shows that the sequence identity of all three *mecA* genes among Kurdistan isolates was ranged between 97.5-98.5%, whereas the sequence identity among Kurdistan isolates and other global isolates selected from GenBank was ranged between 92.6-96.1% (Table: 3). Identical sequences among Kurdistan isolates and other global isolates obtained from the National Center for Biotechnology Information (NCBI) database, supporting the hypothesis that horizontal transfer plays an important role in the dissemination of the *mecA* gene in the *S. aureus* population. Only a few restriction enzymes can be used to differentiate between these closely related isolates (Fig. 2), these enzymes can be used for restriction fragment length polymorphism (RFLP) to identify and differentiate between them. Multiple sequence alignment of three Kurdistan isolates with other 6 global isolates from GenBank has been performed using ClustalW 2.0.12 multiple sequence alignment software (Fig. 3). The sequenced product of *S. aureus mecA* genes from Kurdistan when compared with *mecA* gene of *S. aureus* strain from GenBank found

similarities and showed partial homology with *S. aureus* strains (UK/NTCC124, Turkey/HM5, South Korea/ YSSA11 and India/TN/CN/1/12) penicillin binding protein 2a *mecA* gene, partial cds, which is available in public databases. The phylogenetic tree of the aligned sequences was also produced using MEGA5 Beta version 6.1 online software (www.megasoftware.net), and showed a typical relatedness among Kurdistan isolates and GenBank reference strains (Fig. 4). As a result, the structure of the same gene reported by different labs can be different! Multiple sequence alignment and phylogenetic tree analysis separate the isolates into different groups which are genotypically related to each other. DNA sequence analysis of the *mecA* gene provides an unambiguous, portable dataset that simplifies information sharing between laboratories and facilitates creating a large scale database for studying global and local epidemiology. Amplification of specific gene and sequencing of *mecA* gene gives insight into pharmaceutical aspects to design new effective drugs for treatment of methicillin resistance *S. aureus*. In conclusion the current study of isolation, molecular identification and sequencing of the *mecA* gene from MRSA strain will give insight in to the molecular diagnosis of *S. aureus* and to find better synthetic drugs (analogs) or organic molecules by computational biologist in pharmaceutical companies to control expression of antibiotic genes in different pathogenic organism in general and in particular MRSA strains.

Table 1: Source, location, number of isolates and methicillin resistant isolates.

Hospital	Location	Samples	Isolates	MRSA
Dr. Khalid	**Koya**	62	16	7
Rizgary	**Erbil**	93	27	13
Azadi	**Duhok**	86	21	11
Total		241	64	31

Table 2: Oligonucleotides used in this study.

Primer name	Target gene	Forward 5'----------3'	Reverse5'----------3'	Size(bp.)
mecA	PBP2a	GTAGAAATGACTGAACGT CCGATGA	CCAATTCCACATTGTTTCG GTCTAA	310 (Merza 2009)
nuc	Nuclease	GCGATTGATGGTGATACG GTT	AGCCAAGCCTTGACGAACT AAAGC	280 (Merza 2009)

Table 3: Sequence Identity Matrix of *S. aureus mecA* gene partial sequence.

Seq->	Kurdistan/ Du5	Kurdistan/ Er17	Kurdistan/ Sul24	Turkey /HM5	Iran/ M795	Egypt/1	India/TN/CN/1/12	S.Korea/YSSA11	UK/NTCC12493
Kurdistan/Du5	ID	0.975	0.985	0.961	0.957	0.933	0.954	0.961	0.961
Kurdistan/Er17	0.975	ID	0.971	0.957	0.961	0.926	0.943	0.957	0.957
Kurdistan/Sul24	0.985	0.971	ID	0.961	0.957	0.933	0.950	0.961	0.961
Turkey/HM5	0.961	0.957	0.961	ID	0.996	0.939	0.967	1.000	1.000
Iran/M795	0.957	0.961	0.957	0.996	ID	0.935	0.964	0.996	0.996
Egypt/1	0.933	0.926	0.933	0.939	0.935	ID	0.967	0.939	0.939
India/TN/CN/1/12	0.954	0.943	0.950	0.967	0.964	0.967	ID	0.967	0.967
S.Korea/YSSA11	0.961	0.957	0.961	1.000	0.996	0.939	0.967	ID	1.000
UK/NTCC12493	0.961	0.957	0.961	1.000	0.996	0.939	0.967	1.000	ID

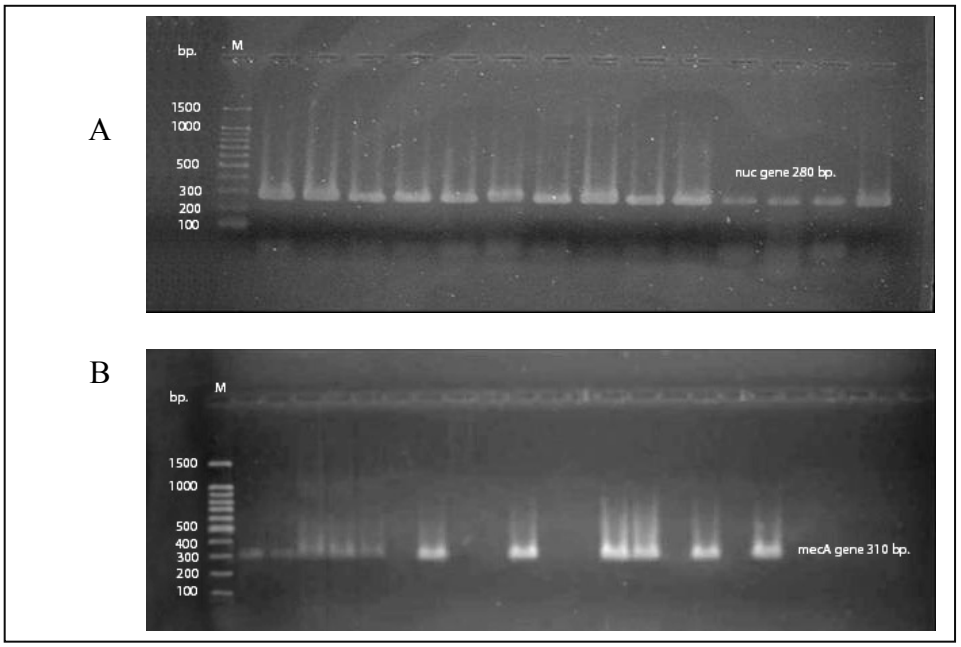

Figure (1): A. Illustrates the gel electrophoresis of amplified DNA fragments generated by species-specific PCR amplification (nuc primer), and (**B**) illustrates the amplified DNA fragments for detection of *mecA* gene.

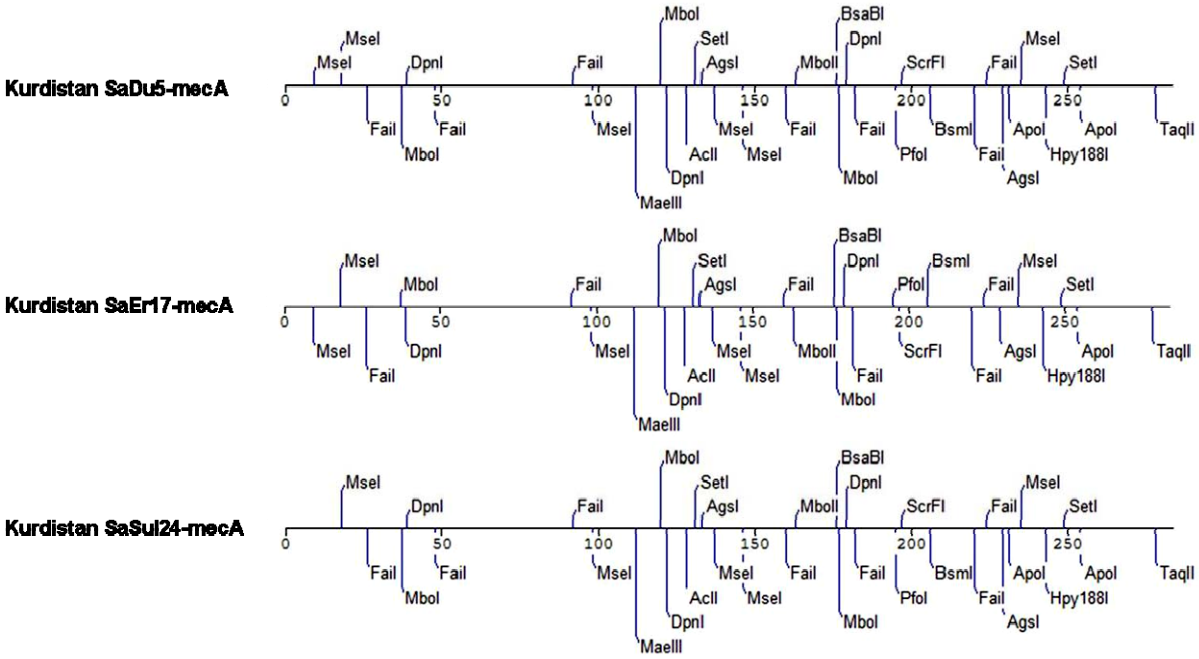

Figure: 2. Restriction map of Kurdistan isolates of *S. aureus* (*mecA*) gene partial sequence that show the restriction enzymes which can be used to differentiate between these isolates. The process was done by using ChromasPro (version 1.5) online software.

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                                10        20        30        40        50        60        70        80
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
Kurdistan SaDu5-mecA            AAATAATTTTAAGGCGTTTAAAGATATAAACATTCAGGATCGTAAATATAAAAAAAAGTATCTAAAAATAAAAAACGAGTAGATGCTC
Kurdistan SaEr17-mecA           AAAAAGATTTTAAGCCGTTTAAAAATATAAACATTCAGGATCGTAAAAATAAAAAAAAGTATCTAAAAATAAAAAACGAGTAGATGCTC
Kurdistan SaSul24-mecA          AAAAGTTTTTAGGGCGTTTAAAGATATAAACATTCAGGATCGTAAATATAAAAAAAAGTATCTAAAAATAAAAAACGAGTAGATGCTC
Turkey SaHM5-mecA               TATAATAGTTTAGGCGTT-AAAGATATAAACATTCAGGATCGTAAA-ATAAAAAAA-GTATCTAAAAATAAAAAACGAGTAGATGCTC
Iran SaM795-mecA                TATAATAGTTTAGGCGTT-AAAGATATAAACATTCAGGATCGTAAA-ATAAAAAAA-GTATCTAAAAATAAAAAACGAGTAGATGCTC
Egypt Sa-mecA                   --------------GTT-AA-GATATAAACATTCAGGATCGTAAA-ATAAAAAAA-GTATCTAAAAATAAAAAACGAGTAGATGCTC
India SaTN/CN/1/12-mecA         --------TTTAGGCGTT-AAAGATATAAACATTCAGGATCGTAAA-ATAAAAAAA-GTATCTAAAAATAAAAAACGAGTAGATGCTC
S.Korea SaYSSA11-mecA           TATAATAGTTTAGGCGTT-AAAGATATAAACATTCAGGATCGTAAA-ATAAAAAAA-GTATCTAAAAATAAAAAACGAGTAGATGCTC
UK SaNTCC12493-mecA)            TATAATAGTTTAGGCGTT-AAAGATATAAACATTCAGGATCGTAAA-ATAAAAAAA-GTATCTAAAAATAAAAAACGAGTAGATGCTC

                                110       120       130       140       150       160       170       180
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
Kurdistan SaDu5-mecA            AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT
Kurdistan SaEr17-mecA           AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT
Kurdistan SaSul24-mecA          AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT
Turkey SaHM5-mecA               AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT
Iran SaM795-mecA                AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT
Egypt Sa-mecA                   AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT
India SaTN/CN/1/12-mecA         AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT
S.Korea SaYSSA11-mecA           AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT
UK SaNTCC12493-mecA)            AAACAAACTACGGTAACATTGATCGCAACGTTCAATTTAATTTTGTTAAAGAAGATGGTATGTGGAAGTTAGATTGGGATCATAGCGT

                                210       220       230       240       250       260       270       280
                                ....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|...
Kurdistan SaDu5-mecA            AATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGGA
Kurdistan SaEr17-mecA           AATGCAGAAAGACCAAAGCATACATATTGAAAAATTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGGA
Kurdistan SaSul24-mecA          AATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGGA
Turkey SaHM5-mecA               AATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGGC
Iran SaM795-mecA                AATGCAGAAAGACCAAAGCATACATATTGAAAAATTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGGC
Egypt Sa-mecA                   AATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGGA
India SaTN/CN/1/12-mecA         AATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGG-
S.Korea SaYSSA11-mecA           AATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGGC
UK SaNTCC12493-mecA)            AATGCAGAAAGACCAAAGCATACATATTGAAAATTTAAAATCAGAACGTGGTAAAATTTTAGACCGAAACAATGTGGAATTGGC
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Figure: 3. Multiple sequence alignment of the sequenced part of *S. aureus* (mecA) gene of Kurdistan isolates with other selected isolates from GenBank using (CLUSTAL 2.0.12 multiple sequence alignment) showing partial homology to *S. aureus* strains penicillin binding protein 2a (mecA) gene, partial cds. Gaps indicate in (-).

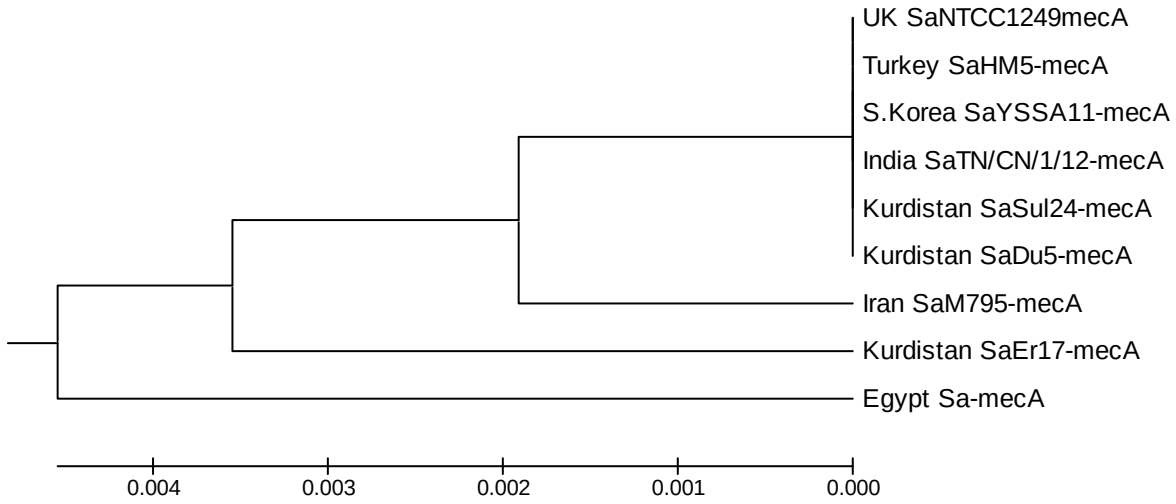

Figure: 4. Phylogenetic tree of Kurdistan isolates and other selected strains from GenBank representing different geographic regions shows the relationship among the *mecA* gene sequence of Kurdistan isolates with other selected isolates from GenBank.

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شیتەڵکردنی زنجیرەی ترشی ناوکی بۆ بەکتریای گۆیی هێشویی *Staphylococcus aureus* ی بەرهەڵستکاری Methicillin لە کوردستان–عێراق

پـوخـــتە

لەم توێژینەوەیەدا کۆی گشتی ٢٤١ نمونەی سەرجێیی گرنگ کۆکرانەوە لە سێ نەخۆشخانەی جیاوازی هەرێمی کوردستان کە تیایدا ٦٤ جیاکراوەی بەکتریای گۆیی هێشویی *Staphylococcus aureus* جیاکرانەوە کە دەکاتە ڕێژەی ٢٦,٥٪ی کۆی گشتی نمونە وەرگیراوەکان. ئەنجامەکان پشتڕاست کرانەوە بە بەکارهێنانی تەکنیکی PCR و دەستپێکەریnuc ی تایبەت بە ناسینەوەی ئەم بەکتریایە بە دوهێندکردنی گورزەی ٢٨٠ نیوکلیۆتایدی بۆ بۆهیلی *nuc*. ئەنجامەکان دەریانخست کەوا ڕێژەی جیاکراوەی بەکتریای گۆیی هێشویی بەرگریکاری Methicillin لە کوردستاندا بەرزەو٣١ جیاکراوە بەرگریان پەیداکردبوو بۆ دژەبەکتریای Methicillin کە دەکاتە ڕێژەی (٤٨,٤٪)ی کۆی گشتی جیاکراوەکان. پەیداکردنی بەرگری بۆ Methicillin پشتڕاست کرایەوە بە بەکارهێنانی دەستپێکەری mecA ی تایبەت بە بۆهێڵی *mecA* بە دوهێندکردنی گورزەی ٣١٠ نیوکلیۆتایدی بۆ بۆهیلی *mecA*. سێ جیاکراوەی بەرگریکاری Methicillin کە بریتی بوون لە (SaDu5، SaEr17 وSaSul24) دەستنیسانکران بۆ شیتەڵکردنی زنجیرەی ترشی ناوکی بۆهێڵی *mecA* بۆ هەڵسەنگاندنی پەیوەندی بۆماوەیی نێوان جیاکراوەکانی کوردستان لەگەڵ جیاکراوەی ولاتانی تر لە بانکی بۆهێڵی جیهانی و دۆزینەوەی لێکچونی نێوانیان. ئەنجامەکانی شیتەڵکاری نێوان جیاکراوەکانی کوردستان دەریانخست کەوا ڕێژەی لێکچونی زنجیرەی ترشی ناوکی بۆهیلی *mecA* لە نێوان ٩٧,٥–٩٨,٥٪ بوو. لەکاتێکدا ڕێژەی لێکچونی زنجیرەی ترشی ناوکی بۆهیلی *mecA* لە نێوان جیاکراوەکانی کوردستان و جیاکراوەی ولاتانی تر لە بانکی بۆهێڵی جیهانی لە نێوان ٩٢,٦–٩٦,١٪ بوو. ئەنجامەکانی ریزکاری و بەراوردکاری زنجیرەی ترشی ناوکی ئاشکرایانکرد کەوا لێکچون و چۆنیەکی هەیە لە نێوان جیاکراوەکانی کوردستان و جیاکراوەکانی (Turkey/HM5, UKNTCC124, South Korea/YSSA11 و India/TN/CN/1/12) کە لە داتابەیسی بانکی بۆهێڵی جیهانی دەست دەکەون. بە بەکارهێنانی ئەنجامەکانی ریزکاری و بەراوردکاری و شیتەڵکاری زنجیرەی ترشی ناوکی بۆهیلی *mecA* و لەڕێگەی بەکارهێنانی بەرنامەی کۆمپیوتەری MEGA5 بێتا ڤێرشنی ٦,١ لەسەر ئەنتەرنێت پەیوەندی بۆماوەیی نێوان جیاکراوەکان دۆزرایەوەو پۆلێن کران بۆ چەندین گروپی بۆماوەیی. ئەم توێژینەوەیە بیرکردنەوە دەدات بە دەستەوە لەسەر ناسینەوەی گەردیلەیی بەکتریای *S. aureus* و دۆزینەوەی دژە بەکتریای دروستکراو یان ئەندامی باشتر بۆ کۆنترۆل کردنی دەربڕینی بۆهێڵی بۆ دژە بەکتریاکان لە زیندەوەری توشکەری نەخۆشی جۆراوجۆر بەشێوەیەکی گشتی و بەتایبەتیش بۆ وەچەکانی MRSA.

تحليل تسلسل النكليوتيدي لبكتريا *Staphylococcus aureus* المقاومة ل Methicillin في كوردستان العراق

الخلاصة

في هذه الدراسة تم جمع ٢٤١ عينة سريرية مهمة من ثلاث مستشفيات مختلفة في اقليم كوردستان وتم حصول على ٦٤ عزلة لجرثومة المكورات العنقودية الذهبية *Staphylococcus aureus* التي تمثل ٢٦,٥% من عدد العينات. تم تاكيد الجزيئي للنتائج باستخدام تقنية PCR و بواسطة بادئ nuc المتخصص لجين*nuc* الذي انتج حزمة متضاعفة بوزن جزيئي ٢٨٠ نكليوتيد لجين*nuc*. أظهرت النتائج أن معدل انتشار مقاومة *S.aureus* في كوردستان كانت مرتفعة جدا، حيث وجدت ان ٣١ من هذه العزلات (٤٨,٤٪) كانت مقاومة ل Methicillin. تم تاكيد مقاومة هذه العزلات ل Methicillin باستخدام بادئ mecA المتخصص لجين *mecA* الذي انتج حزمة متضاعفة بوزن جزيئي ٣١٠ نكليوتيد لجين *mecA*. تم اختيار ثلاث عزلات (SaDu5، SaEr17 وSaSul24) المقاومة ل Methicillin لتحليل تسلسل النكليوتيدي لجين *mecA* لتقييم العلاقة بين هذه العزلات والعزلات العالمية المختارة من بنك الجينات و تحديد التشابه بينهم. بينت نتائج التحليل أن التشابه تسلسل جين *mecA* بين العزلات كوردستان تراوحت بين ٩٧,٥-٩٨,٥٪، في حين كان التشابه تسلسل جين *mecA* بين العزلات كوردستان والعزلات العالمية المختارة من بنك الجينات تراوحت بين ٩٢,٦-٩٦,١٪. كشفت نتائج المقارنة بان تسلسل النكليوتيدي لجين *mecA* لعزلات كوردستان وجدت التشابه والتماثل الجزئي مع عزلات (Turkey/HM5, UKNTCC124, South Korea/YSSA11 و India/TN/CN/1/12) المتوفرة في قاعدة البيانات العامة. تم إنتاج شجرة الوراثية من اصطفاف السلسلة المتعددة من تسلسل باستخدام MEGA5 بيتا الإصدار ٦,١ عبر الإنترنت، وأظهر ارتباط نموذجي بين العزلات كوردستان والعزلات العالمية المختارة من بنك الجينات. هذه الدراسة تعطي أفكارا في التشخيص الجزيئي للبكتريا *S. aureus* وللعثور على أفضل المضادات الحيوية الاصطناعية أو العضوية للسيطرة على التعبير الجيني للمضادات الحيوية في الكائنات الحية المسببة للأمراض المختلفة بشكل عام وعلى وجه الخصوص سلالات MRSA.