The 3rd International Conference of (Environmental and Agricultural status in the Middle East 15-17/4/2020 Cairo/ Egypt

Novel strain MN907806 *S.aureus* expressed high production of Thrombolytic enzyme(Staphylokinase)

Nebras Rada Mohamed

AL-Turath University

Abstract

Study design of this study is Descriptive / Cross-sectional study designs for 500 isolates, Analytic study design is Case-Control study design for (1) case is Novel strain MN907806. Study seeting in Baghdad hospital at 2019/2020 including 280 (56%) Tonsils, 100(20%) Nose, 40(8%) Tumors, 17(3.2%) Urine, 27(5.4%) Skin (Acne) and 36(7.2%) Blood, identification by using Viteck2-GP and genotypic detection of conventional PCR for *16srRNA* (ribosomal units 30s) reveals all isolates are *S.aureus*. Quantitative screening of Thrombolytic enzyme (staphylokinase) of Novel strain MN907806 *S.aureus* done on plasma agar plate in order to determine fibrinolysis zone around well, results give positive result for over production of thrombolytic enzyme.

Genotypic detection of *sak* gene achieved to Novel strain MN907806 *S.aureus* with denaturation 94°C(1min), Annealing 52 °C (1min), Extension 72°C(1min) and Final extension 72°C for (10 min) by PCR (Polymerase Chain Reaction) in order to determine possess Novel strain MN907806 *S.aureus* for *sak* gene and send PCR product to NICEM/USA ABI3730XL Applied Biosystems to study DNA Sequencing of *sak* gene, the results analyzed according to NCBI (National Center for Biotechnology Information) data and Alignments of amino acid sequence were analyzed with the same software, the results give novel strain have mutation transversion in locus 156 from Guanine(G) into Adenine (A) that leading to convert amino acid in locus 148 from Glycine (G) to Serine (Ser) leading to increase production of thrombolytic enzyme compared with Control (wild type strain). Protein drawing by Mega 6 program software program for two forms Alpha helix and Beta sheets that explain found change in protein(thrombolytic enzyme), record strain in NCBI (Gene Bank in America USA) with name Nebras R. Mohammed.

Keywords: Fibrinolytic enzyme, *Staphylococcus aureus*, Genotypic detection.

Introduction

Staphylococcus aureus is a major bacterial human pathogen that causes a wide variety of clinical manifestations causing skin infection, tissue infection, deep abscess, wound infections ,sepsis, endocarditis, septic arthritis and osteomyelitis (Rasigade and Vandenesch,2014; Shagufta *et al* ., 2014).

Staphylokinase known thrombolytic enzyme and proteolytic enzyme that act to dissolve fibrin in order to help the bacteria in spreading (invasiveness) and causing damage to tissue by interact with plasminogen that convert into plasmin (proteolytic enzyme) which hydrolyses fibrin clots, then inhibiting phagocytosis(Chen *et al*.,2013) .

Mutation is any inheritance change in the base sequence of a DNA molecule that affect or not effect on the phenotype of the organism, the term “mutation” was coined by Hugo de Vries derived from Latin meaning "to change" , including substitution, addition or deletion of one or more bases(Malacinski,2003).

Mutagenesis is an important technique to DNA in laboratory to produce mutant genes, proteins, strains of bacteria or genetically modified organisms(GMOs). The mutation may produce mutant proteins with new enhanced properties or novel functions that may be important in of commercial use(Hsu *et al*.,2014).

Materials and Methods:

Study design

Descriptive (Cross-sectional study designs), Analytic (Case-Control).

Study Setting

Baghdad hospitals in 2018/ 2019.

Study population

Selection criteria and sampling method for all samples were cultivated in Nutrient broth, Nutrient agar medium and Mannitol Salt agar medium incubated at 37 ^{0}C for

24 hr. according to Lemair *et al*., (2008),sample selection and size isolation of *S.aureus* were 500 specimens from different provenance of humans possessed from, Identification of *S.aureus* achieved by Vitek2-GP with specific Card for Gram Positive bacteria and by Genotypic detection by PCR to conformative identification of tumor isolates, the Vitek2 system was advanced for affirmation the identification of bacterial isolates. The Genotypic revelation PCR as well utilized for the affirm the identification of *S.aureus* secluded from tumors by using Go Taq Master Mix Kit (Biomeruex corporation,2010).

Pilot study (Pre-Testing the methodology)

Pre –testing achieved to chemical mutagens for 20 *S.aureus* in order to determine mutagenicity of chemical mutagen.

Data collection tools

Include clinical examination from different infections ; laboratory tests for antibiotics, blood hemolysis Staphylokinase production, chemical mutagenesis and screening procedures records for chemical mutagenesis, Gene Cloning with best Cloning Vector, Screening procedures for characterization of thrombolytic enzyme in different conditions.

Quantitative Screening of Staphylokinase of Novel strain MN907806 by Plasma agar plate assay:

The plasma agar prepared in used to detect for expression of staphylokinase or thrombolytic enzyme activity for all bacterial isolates performed by inoculating 5ml of nutrient broth medium into 50µl of fresh culture incubated at 37^{o}C for 24 hours, the positive result by formation zone around wells on plasma agar plate.

Genotypic detection of *sak* gene of Novel strain MN907806 *S.aureus* by Polymerase Chain Reaction (PCR)

Direct PCR done amplification of *16SrRNA* genes (30s Subunit of ribosomal of *S.aureus*) (Kai *et al*.,2018). Primers of *16srRNA* used in this study showed in table(2-

8) and amplification of *sak* gene from mutant *S.aureus* with primers listed in table (2-9).

Forward and reverse primers of *Sak* gene used to amplify specific gene of mutant *S.aureus* that supplied from Alpha DNA Company in lyophilized form that were dissolved in free nuclease distilled water to give final concentration of 100 picomole /µl , then 10 picomole /µl of primer (10µl of primer stock solution added to 90µl of free nuclease distilled water), mixed and stored a -20°C until use.

Table(1): Primers of *16srRNA* gene of *S.aureus*.

Specific primer	Primers of ***16srRNA*** **gene**	**Refrences**
Forward primer	5′GGAATTCAAAGGAATTGACGGGGGC -3′	**(Ali *et al*.,2014)**
Reverse primer	′ 5′CCAGGCCCGGGAACGTATTCAC-3′	

Table(2): Primers of *sak* gene of *S.aureus*.

Specific primer	Sequences of Primers of *sak* gene encoded for thrombolytic enzyme	**References**
Forward primer	**5'-AGAGATTGATTGTGAAAGAAGTGTT -3'**	**(Liu *et al*.,2009)**
Reverse primer	**5'GAATCTAGACCCAAGCTTTTTCCTTTCTATAACAAC-3'**	

Table(3): PCR mixture for detection of *sak* gene of Novel strain MN907806 *S.aureus*.

No.	Content	Volume for single tube (µl)
1	Green master mix	**12.5**
2	Forward Primer	**1.5**
3	Reverse Primer	**1.5**
4	DNA template	**5**
5	Nuclease free water	**4.5**

Total volume	**25**

Table(4): PCR condition for detection of *sak* gene of Novel strain MN907806 *S.aureus*.

Initial denaturation	No. of cycles	Denaturation	Annealing	Extension	Final extension
95 ˚C for 5 min.	35	94 ˚C for 1min.	52 ˚C for 1min.	72 ˚C for 1min.	72 ˚C for 10 min.

DNA Sequencing (Nucleotide sequencing) of Novel strain MN907806 *S.aureus* encoded for staphylokinase

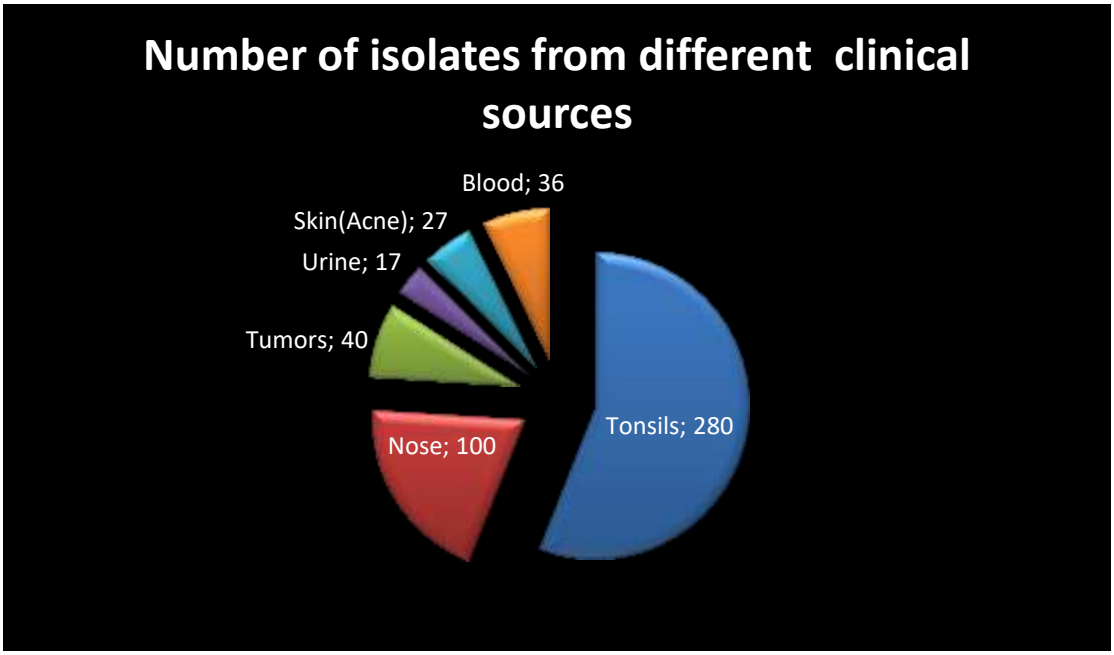
PCR products resulted from the amplification of *sak* gene were sent to NICEM/USA ABI3730XL Applied BIOSYSTEMS to determine the complete nucleuotide sequence of *sak* gene . The results were analyzed according to National Center for Biotechnology Information (NCBI) data and Alignments of amino acid sequence were analyzed with the same software.

Results and Discussion

Study design

The study design of cases are cross-sectional study in descriptive study design to 500 isolates of *S.aureus* were collected from hospitals in Baghdad through 2018/2019.

Results of isolation from different clinical sources of human are 280(56%) tonsils , 100(20%) nose, 40(8%) tumors, 17(3.4%) urine, 27(5.4%) skin(Acne) and 36(7.2%) Blood showed in figure(1).



Figure(1):Prevalence of *S.aureus* from different clinical sources with percentage of isolates.

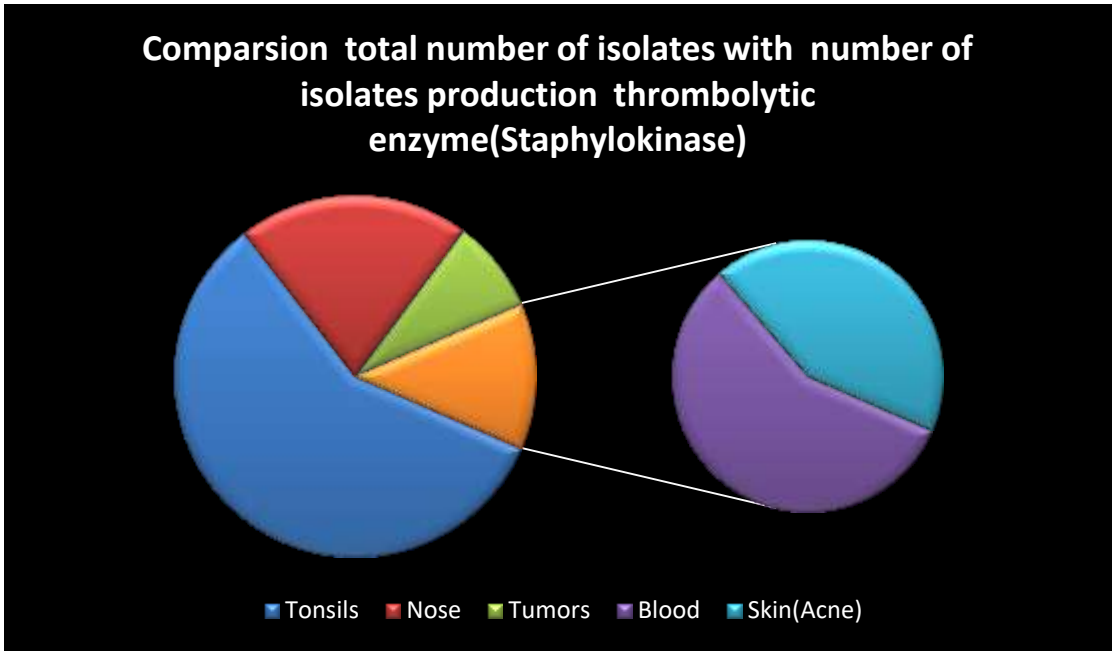
The pathogenicity of *S. aureus* related to some of virulence factor including protein A and extracellular proteins such as staphylokinase, coagulase, hemolysins, enterotoxins, toxic-shock syndrome (TSS) toxin , exfoliatins and Panton-Valentine leukocidin (PVL) have roles in invasive infections from cutaneous abscesses into severe necrotic skin infections in order to its colonization and infection (Gopal *et al*., 2015) .

Results exhibit all tumor isolates possess *16srRNA* gene (Ribosomal RNA of 30s Subunit of *S.aureus*). *16SrRNA* ribosomal RNA genes as molecular marker for bacterial classification by utilizing genotypic method is robust technology for identifying bacteria Sanderson *et al*.,2017 and Mitsuhashi *et al*.,2017). Amplification of *16S rRNA* (˜479 bp) is the utmost commonly utilizing method for identifying and classifying bacteria, inclusive staphylococci(Mohammed et al.,2007; Ghebremedhin *et al*.,2008).

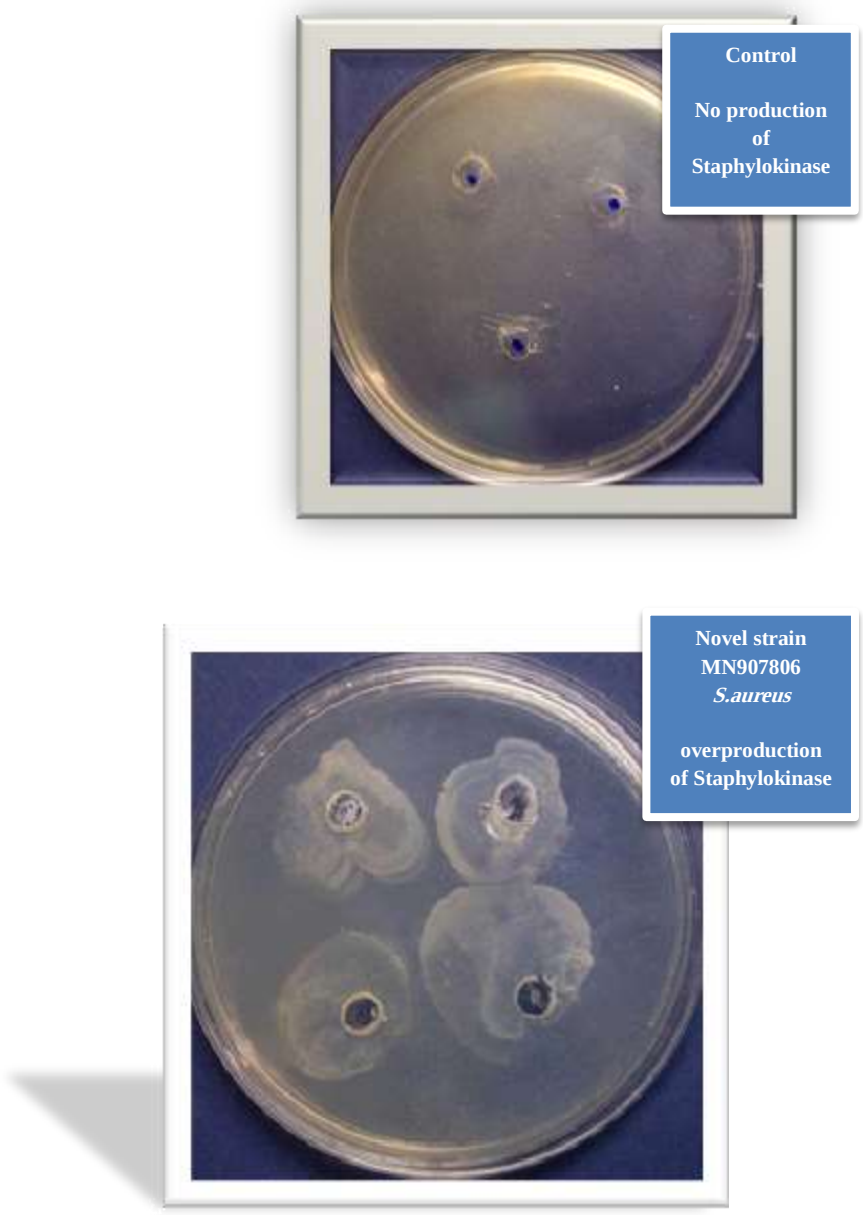
Phenotypic detection for thrombolytic enzyme (Staphylokinase) of Novel strain MN907806 *S.aureus*

Staphylokinase is one of the important virulence factors produced by *S.aureus* to resist human immunity via interact with α-Defensins a peptide secreted by host polymorphonuclear cells (PMNs) (that give antimicrobial protection mediated by disruption the integrity of cell wall leading to bacteriocidal effect)also, thrombolytic enzyme(staphylokinase) interacts with plasminogen that convert into plasmin that digest fibrin clots (Yerasi *et al* .,2014) .

The phenotypic assay of thromnbolytic enzyme (Staphylokinase) done on plasma agar plate in order to determine hydrolysis areas of plasma found in medium, result of phenotypic assay for Novel strain MN907806 *S.aureus* have overproduction of thrombolytic enzyme (Staphylokinase) shown in figure (2).



Figure(2): Comparsion total number of isolates(500 isolates) with number of isolates production thrombolytic enzyme by phenotypic assay on plasma agar.



Figure(3): Fibrinolysis of plasma (Lysis zone) on plasma agar plate for Novel strainMN907806 *S.aureus* to determine production of thrombolytic enzyme (Staphylokinase).

Results in figure (3) showed increase lysis zone around well on plasma agar plate that is indicates for overproduction of fibrinolytic enzyme (Staphylokinase) from Novel strain MN907806 *S.aureus*.

Genotypic detection for *sak* gene from Novel strain MN907806 *S.aureus* by PCR

Genomic DNA for Novel strain MN907806 *S.aureus* extracted by DNA extract kit and amplified by using Go Taq master mix for amplification *sak* gene , purity of genomic DNA measured by the ratio of absorbance at 260nm and 280nm (260 /280 ratio) 1.8 pure DNA. PCR technique used for amplification *Sak* gene from Novel strain MN907806 *S.aureus* with annealing temperatures in 52 °C , denaturation DNA in 95°C and extension in 72°C by using specific primers, forword primer and reverse primer explain in table(2).

Results the genotypic analysis of *sak* gene showed presence *sak* gene in Novel strain MN907806 *S.aureus* compared with control accomplished in agarose gel electrophoresis, size of *sak* gene (490bp) compared with DNA Ladder (1500bp).

Figure (4) : Gel electrophoresis for detection for amplified mutant *sak* gene (400, 450,800,900bp) on agarose gel (1%) , 50V for 1 hr. , Lane (1 – 12) represents Novel strain MN907806 *S.aureus*, (L) DNA Ladder (1500bp).

There are several types of PCR have sensitivity, especially with small numbers of bacteria, Nested PCR is one of detection only in a few bacteria found in clinical specimens (Madico *et al.*,2000; Apfalter *et al.*,2001).

DNA Sequencing (Nucleotides Sequencing) of *sak* gene from Novel strain MN907806 *S.aureus*

DNA Sequencing of Novel strain MN907806 *S.aureus* achieved by sent product of PCR (polymerase chain reaction) in order to known the nucleotide sequence by utilizing NICEM/USA ABI3730XL Applied BIO SYSTEM. DNA Sequencing determined by an automatic sequencer and the DNA sequences are anatomize and resemblance by Basic Local Alignment Search Tool (BLAST) in National Center for Biotechnology Information (NCBI). Results of DNA Sequencing shown in figure(5),(6),(7),(8) elucidate the coding region for *Sak* gene amplified from mutant VSSA and MSSA *S.aureus* compared with analogous sequence of the same gene from standard strain of *S.aureus* possessed in BLAST/ NCBI on the web site .

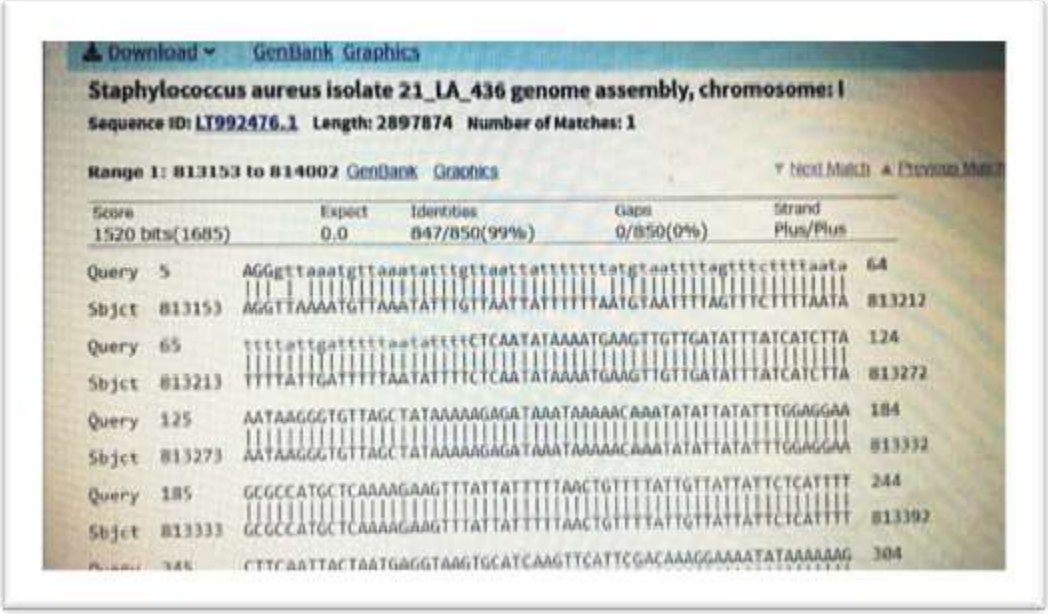


Figure (5): DNA Sequencing of Nucleotide sequence alignment of Novel strain MN907806 *S.aureus* of *sak* gene comparsion with sequence of *S.aureus* recorded in BLAST/ NCBI(Standard strain), other strain.

Results of nucleotide sequence alignment of *sak* gene for Novel strain MN907806 *S.aureus* with the nucleotide sequence of the same gene for standard strain by using BLAST program in NCBI exhibit that there are many transversion mutations in coding region of *sak* gene that leading to convert nitrogen base from Guanine(G) into Adenine (A) that leading to changing in codons, affect for amino acid from Glycin

(Gly) into Serine (Ser) in Thrombolytic enzyme (Staphylokinase) resulted by substitutions mutation of one nitrogenous base into another from purine into pyrimidine as indicated in table (6).

Substitution is a type of mutation causes by conversion one nitrogen base into anothers that leading to change in a codon encoded to another amino acid and change in created protein (Michael *et al*.,2010) .

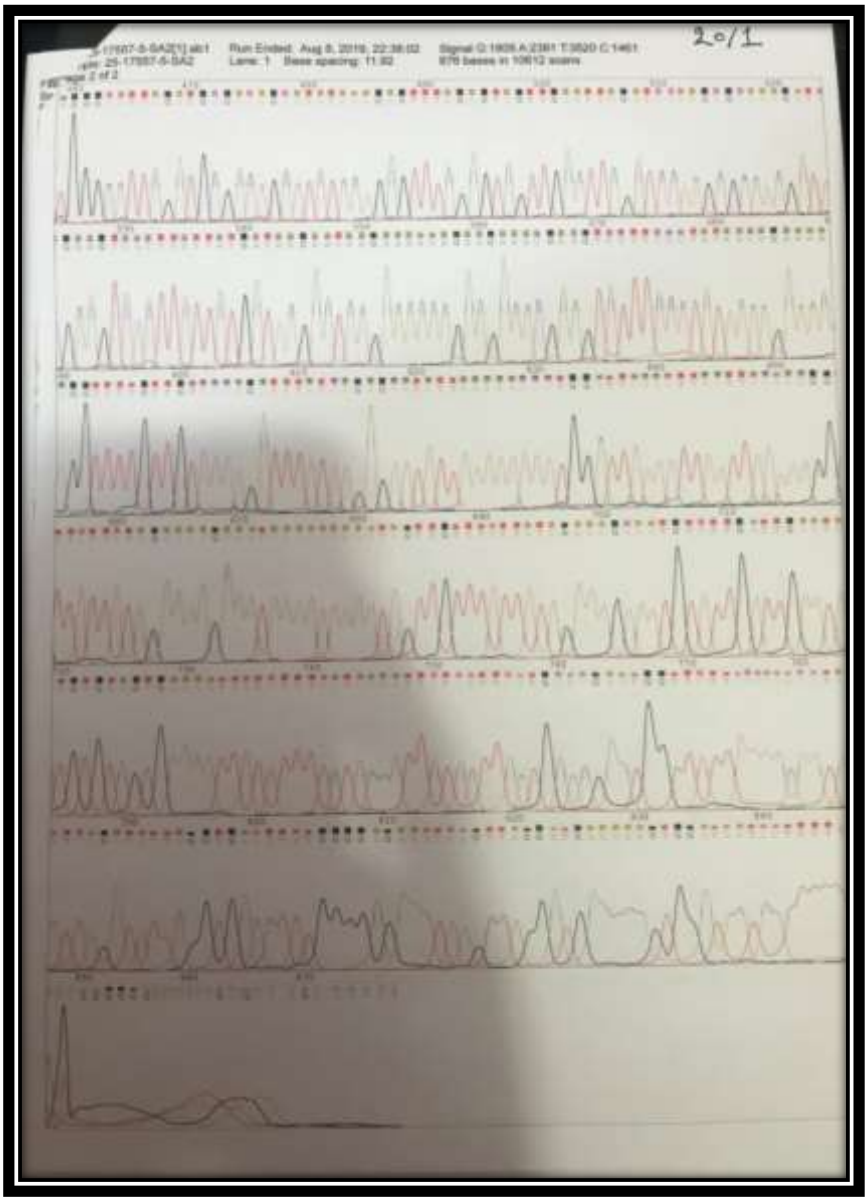


Figure (6): DNA Sequencing of Nucleotide sequence alignment of Novel strain MN907806 *S.aureus* of *sak* gene.

Analytic of Sequencing of *sak* gene of Novel Strain MN907806 *S.aureus*

***Staphylococcus aureus* strain ST20130945 chromosome, complete genome**

Sequence ID: CP033114.1Length: 2853520Number of Matches: 1

Range 1: 2067597 to 2068330GenBankGraphicsNext MatchPrevious Match

Score	Expect	Identities	Gaps	Strand
1320 bits(1463)	0.0	733/734(99%)	0/734(0%)	Plus/Minus

```
Query  1        ttttagtttcttttaatattttattgatttttaatattttCTCAATATAAAATGAAGTTG
60

                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  2068330  TTTTAGTTTCTTTTAATATTTTATTGATTTTTAATATTTTCTCAATATAAAATGAAGTTG
2068271


Query  61       TTGATATTTATCATCTTAAATAAGGGTGTTAGCTATAAAAAGAGATAAATAAAAACAAAT
120

                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  2068270  TTGATATTTATCATCTTAAATAAGGGTGTTAGCTATAAAAAGAGATAAATAAAAACAAAT
2068211


Query  121      ATATTATATTTGGAGGAAGCGCCATGCTCAAAAGAAGTTTATTATTTTTAACTGTTTTAT
180

                |||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  2068210  ATATTATATTTGGAGGAAGCGCCATGCTCAAAAGAGGTTTATTATTTTTAACTGTTTTAT
2068151


Query  181      TGTTATTATTCTCATTTTCTTCAATTACTAATGAGGTAAGTGCATCAAGTTCATTCGACA
240

                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  2068150  TGTTATTATTCTCATTTTCTTCAATTACTAATGAGGTAAGTGCATCAAGTTCATTCGACA
2068091


Query  241      AAGGAAAATATAAAAAAGGCGATGACGCGAGTTATTTTGAACCAACAGGCCCGTATTTGA
300

                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  2068090  AAGGAAAATATAAAAAAGGCGATGACGCGAGTTATTTTGAACCAACAGGCCCGTATTTGA
2068031


Query  301      TGGTAAATGTGACTGGAGTTGATGGTAAAGGAAATGAATTGCTATCCCCTCATTATGTCG
360

                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||

Sbjct  2068030  TGGTAAATGTGACTGGAGTTGATGGTAAAGGAAATGAATTGCTATCCCCTCATTATGTCG
2067971


Query  361      AGTTTCCTATTAAACCTGGGACTACACTTACAAAAGAAAAAATTGAATACTATGTCGAAT
420
```

```
                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2067970  AGTTTCCTATTAAACCTGGGACTACACTTACAAAAGAAAAAATTGAATACTATGTCGAAT
2067911


Query  421      GGGCATTAGATGCGACAGCATATAAAGAGTTTAGAGTAGTTGAATTAGATCCAAGCGCAA
480

                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2067910  GGGCATTAGATGCGACAGCATATAAAGAGTTTAGAGTAGTTGAATTAGATCCAAGCGCAA
2067851


Query  481      AGATCGAAGTCACTTATTATGATAAGAATAAGAAAAAAGAAGAAACGAAGTCTTTCCCTA
540

                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2067850  AGATCGAAGTCACTTATTATGATAAGAATAAGAAAAAAGAAGAAACGAAGTCTTTCCCTA
2067791


Query  541      TAACAGAAAAAGGTTTTGTTGTCCCAGATTTATCAGAGCATATTAAAAACCCTGGATTCA
600

                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2067790  TAACAGAAAAAGGTTTTGTTGTCCCAGATTTATCAGAGCATATTAAAAACCCTGGATTCA
2067731


Query  601      ACTTAATTACAAAGGTTATTATAGAAAAGAAATAAAACAAAATAGTTGTTTATTATAGAA
660

                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2067730  ACTTAATTACAAAGGTTATTATAGAAAAGAAATAAAACAAAATAGTTGTTTATTATAGAA
2067671


Query  661      AGCAATGTCTTGATTGAATATGTGTAGTGAAAATTATCTTTCATCAAATTCTCATTCATG
720

                ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  2067670  AGCAATGTCTTGATTGAATATGTGTAGTGAAAATTATCTTTCATCAAATTCTCATTCATG
2067611


Query  721      CACGAATGGTTCTT  734

                ||||||||||||||
Sbjct  2067610  CACGAATGGTTCTT  2067597
```

Staphylokinase [*Staphylococcus aureus*]

Sequence ID: WP_103144213.1Length: 163Number of Matches: 1

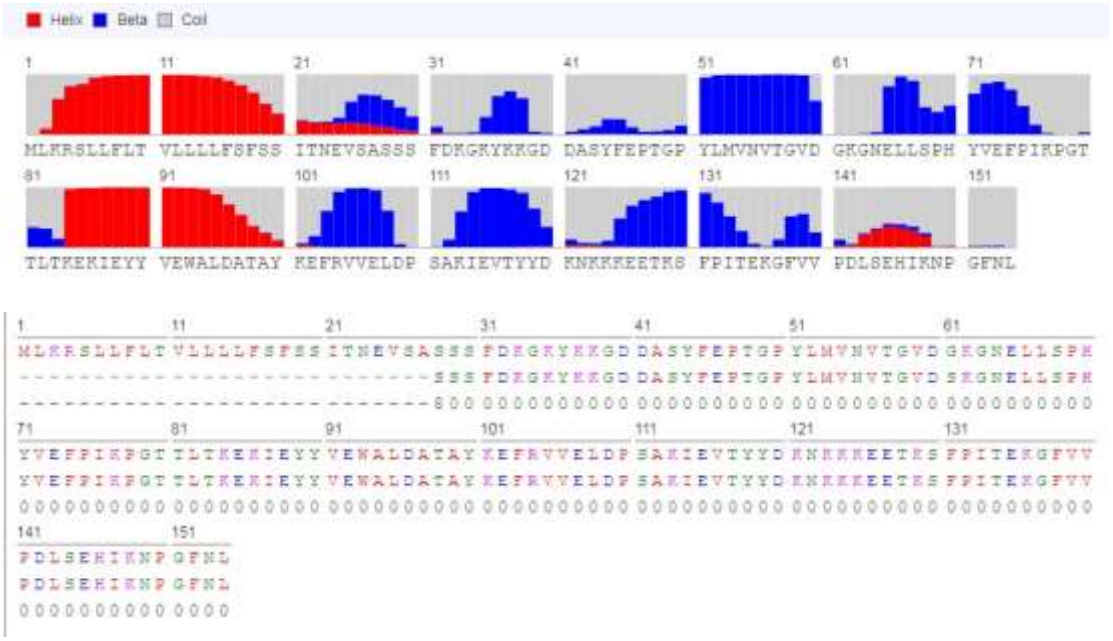
Range 1: 1 to 154GenPeptGraphicsNext MatchPrevious Match

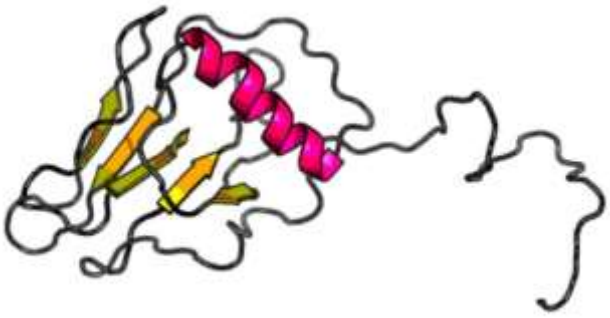
Score	Expect	Method	Identities	Positives	Gaps	Frame
273 bits(699)	5e-93	**Compositional matrix adjust.**	153/154(99%)	**153/154(99%)**	0/154(0%)	**+3**

```
Query  144  MLKRsllfltvllllfsfssITNEVSASSSFDKGKYKKGDDASYFEPTGPYLMVNVTGVD
323

             MLKRLLFLTVLLLLFSFSSITNEVSASSSFDKGKYKKGDDASYFEPTGPYLMVNVTGVD
Sbjct  1     MLKRGLLFLTVLLLLFSFSSITNEVSASSSFDKGKYKKGDDASYFEPTGPYLMVNVTGVD  60


Query  324  GKGNELLSPHYVEFPIKPGTTLTKEKIEYYVEWALDATAYKEFRVVELDPSAKIEVTYYD
503

             GKGNELLSPHYVEFPIKPGTTLTKEKIEYYVEWALDATAYKEFRVVELDPSAKIEVTYYD
Sbjct  61    GKGNELLSPHYVEFPIKPGTTLTKEKIEYYVEWALDATAYKEFRVVELDPSAKIEVTYYD
120


Query  504  KNKKKEETKSFPITEKGFVVPDLSEHIKNPGFNL  605

             KNKKKEETKSFPITEKGFVVPDLSEHIKNPGFNL
Sbjct  121  KNKKKEETKSFPITEKGFVVPDLSEHIKNPGFNL  154
```

Analytic of Sequencing of *sak* gene of Novel Strain MN907806 *S.aureus* explain there are substitutions mutation, transversion mutations of one nitrogenous base into another from purine into pyrimidine in coding region of *sak* gene leading to convert nitrogen base from Guanine (G) into Adenine (A) in locus 156 that leading to changing in codons, affect for amino acid from Glycin (Gly) into Serine (Ser) in locus 148 in Thrombolytic enzyme (Staphylokinase) that leading to overproduction of this enzyme higher than control strain(wild type strain) because conversion non functional amino acids into functional amino acids, as indicated in table (7).

Protein drawing of Thrombolytic enzyme (Saphylokinase) of Novel strain MN907806 *S.aureus*





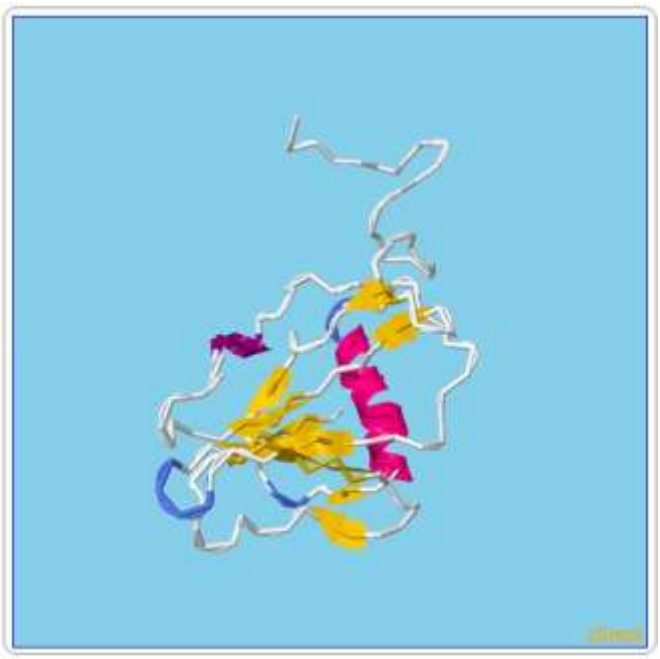


Figure (7): Protein drawing of thrombolytic enzyme (Staphylokinase) of Novel strain MN907806 *S.aureus* of *sak* gene, Partial cds Gen Bank: MN907806.1, convert nucleotide sequence to protein by using Mega 6 program software.

Recording Novel strain in Gene Bank NCBI(National Center for Biotechnology Information) in name Nebras R.Mohammed

Staphylococcus aureus **strain NeSa260 Staphylokinase (*sak*) gene, partial cds**

GenBank: MN907806.1

FASTA Graphics

LOCUS **MN907806** 616 bp DNA linear BCT 27-JAN-2020

DEFINITION *Staphylococcus aureus* strain NeSa260 staphylokinase (sak) gene, partial cds.

ACCESSION **MN907806**

VERSION **MN907806.1**

SOURCE *Staphylococcus aureus*

ORGANISM *Staphylococcus aureus*

Bacteria; Firmicutes; Bacilli; Bacillales; Staphylococcaceae; Staphylococcus.

REFERENCE 1 (bases 1 to 616)

AUTHORS Nebras R. Mohammed.

TITLE Direct Submission

JOURNAL **Submitted** (07-JAN-2020) University, **Iraq, Baghdad 00964, Iraq**

COMMENT ##Assembly-Data-START##

Sequencing Technology :: Sanger dideoxy sequencing

##Assembly-Data-END##

FEATURES Location/Qualifiers

source 1..616

/organism="*Staphylococcus aureus*"

/mol_type="genomic DNA"

/strain="NeSa260"

/isolation_source="patient"

/host="Homo sapiens"

/db_xref="taxon:1280"

/country="Iraq"

/collection_date="2019"

/collected_by="Nebras R. Mohammed"

gene 146..>616

/gene="sak"

CDS 146..>616

/gene="*sak*"

/codon_start=1

/transl_table=11

/product="staphylokinase"

/protein_id="QHN63794.1"

/translation="MLKRSLLFLTVLLLLFSFSSITNEVSASSSFDKGKYKKGDDASY

FEPTGPYLMVNVTGVDGKGNELLSPHYVEFPIKPGTTLTKEKIEYYVEWALDATAYKE

FRVVELDPSAKIEVTYYDKNKKKEETKSFPITEKGFVVPDLSEHIKNPGFNLITK"

ORIGIN

1 aattttagtt tcttttaata ttttattgat ttttaatatt ttctcaatat aaaatgaagt

61 tgttgatatt tatcatctta aataagggtg ttagctataa aaagagataa ataaaaacaa

121 atatattata tttggaggaa gcgccatgct caaaagaagt ttattatttt taactgtttt

181 attgttatta ttctcatttt cttcaattac taatgaggta agtgcatcaa gttcattcga

241 caaaggaaaa tataaaaaag gcgatgacgc gagttatttt gaaccaacag gcccgtattt

301 gatggtaaat gtgactggag ttgatggtaa aggaaatgaa ttgctatccc ctcattatgt

361 cgagtttcct attaaacctg ggactacact tacaaaagaa aaaattgaat actatgtcga

421 atgggcatta gatgcgacag catataaaga gtttagagta gttgaattag atccaagcgc

481 aaagatcgaa gtcacttatt atgataagaa taagaaaaaa gaagaaacga agtctttccc

541 tataacagaa aaaggttttg ttgtcccaga tttatcagag catattaaaa accctggatt

601 caacttaatt acaaag//

Conclusions:

1- Found Novel strain of *S.aureus* with Accession number MN907806 *S.aureus*.
2- Overproduction of Thrombolytic enzyme (Staphylokinase) from Novel strain MN907806 *S.aureus*.
3- Causes of overproduction because transversion mutation in locus 156 from Guanine(G) into Adenine (A) leading to convert amino acid in locus 148 from Glycine (G) to Serine (Ser) leading to increase production of thrombolytic enzyme compared with Control (wild type strain).
4- Novel strain MN907806 *S.aureus* preference from wild type strain in production thrombolytic enzyme because higher production of thrombolytic enzyme than wild type strain.
5- Recording strain in NCBI(Gene Bank) very important to keep sequences in Gene Bank with your name and with country.
6- Protein drawing very important in order to determine change found in sequences of gene in novel strain.

Refrences

Ali, R.; Al-Achkar,k.; Al-Mariri,A. and Safi,M. (2014). Role of Polymerase Chain Reaction (PCR) in the detection of antibiotic-resistant *Staphylococcus aureus*. *Egyptian Journal of Medical Human Genetics.* Vol.15, Issue 3, PP: 293-298.

Apfalter, P., F.; Blasi, J. ;Boman, C. A. ;Gaydos, M. ;Kundi, M.; Maass, A. ;Makristathis, A. ;Meijer, R. ;Nadrchal, K. ;Persson, M. L. ;Rotter, C. Y. ;Tong, G. ;Stanek, and A. M. Hirschl **(** 2001). Multicenter comparison trial of DNA extraction methods and PCR assays for detection of *Chlamydia pneumoniae* in endarterectomy specimens. *J. Clin. Microbiol*. 39**:**519-524.

Biomeruex corporation . (2010) . VITEK . USA .

Chen, C.J, ;Unger, C.; Hoffmann. W.; Lindsay, J.A.; Huang, Y.C.and Gotz, F. (2013).Characterization and comparison of 2 distinct epidemic community-associated methicillin-resistant *Staphylococcus aureus* clones of ST59 lineage. *PLoS One; 8(9): e63210.*

Ghebremedhin, B.; Layer, F.; Ko¨nig, W. and Ko¨nig, B.(2008). Genetic classification and distinguishing of Staphylococcusspecies based ondifferent partial gap, 16S rRNA, hsp60, rpoB, sodA, and tuf gene sequences. *J Clin Microbiol* . 46(3):1019–25.

Gopal , T. ; Nagarajan , V. and Elasri , M.O. (2015). SATRAT: *Staphylococcus aureus* transcript regulatory network analysis tool. *PeerJ* 3:e717.

Hsu, P.D.; Lander, E.S. and Zhang, F. (2014). "Development and applications of CRISPR-Cas9 for genome engineering". *Cell J.* 157 (6): 1262–78.

Kai, Y. M. ; Nakagawa,S.; Kryukov,K.; Matsukawa,S.; Tanaka,T.; Iwai,T.; Imanishi,T. and Hirota,K. (2018). Direct PCR amplification of 16S rRNA genes offers accelerated bacterial identification using the MinIONTM nanopore sequencer Shinichi. *bioRxiv preprint first* .Japan.

Lemaire , S. (2008) . Intracellular *Staphylococcus aureus*, an emerging links to persistent and relapsing infections: factors influencing the activity of antimicrobials against intracellular *S. aureus*. Doctoral Thesis. Université Catholique de Louvain.14:766-777.

Liu, S., Maheshwari, R. and Kiick, KL. (2009). Polymer-based therapeutics. *Macromolecules*, Vol.42, No.1, pp.3-13, ISSN: 0024-9297.

Madico, G.; Quinn, T. C. ; Boman,J. and Gaydos,C.A.(2000). Touchdown enzyme time release-PCR for detection and identification of *Chlamydia trachomatis*, *C. pneumoniae*, and *C. psittaci* using the 16S and 16S-23S spacer rRNA genes. *J. Clin. Microbiol.* 38**:**1085-1093.

Malacinski,G.M.(2003). Essentials of Molecular Biology. Fouth edition. Jones and Bartlett publishers.Printed in the united states of America.

Michal , K. ; George, B. ; Magdalena, B. ; Katarzyna, O. ; Ewa, C. ; Tadeusz, P. ; Zofia, S. ; Eusebio, M. ; Jacek, B. and Janusz, S. (2009). Cloning and expression of a new recombinant thrombolytic and anthithrombotic agent - a staphylokinase variant . *Act Biochimica Polonica*, Vol. 56 No. 1, 41–53.

Mitsuhashi, S.; Kryukov, K.; Nakagawa, S.; Takeuchi, J.S.; Shiraishi, Y.; Asano, K. and Imanishi, T.(2017). A portable system for rapid bacterial composition analysis using a nanopore-based sequencer and laptop computer, *Sci Rep* 7: 5657 14.

Mohammad, M.S.; Hirotoshi, I.; Makiko, N.; Sun, X.S.; Pham, H.N. and Kiyofumi,O.(2007). DnaJ gene sequence-based assay for speciesidentification and phylogenetic grouping in the genus Staphylo-coccus. *Int J Syst Evol Microbiol*, 57(Pt 1):25–30.

Rasigade, J.P. and Vandenesch,F.(2014). *Staphylococcus aureus*: a pathogen with still unresolved issues. *Infect. Genet. Evol*. Vol. 21, ppt: 510-4.

Sanderson, N.D.; Street, T.L.; Foster, D.; Swann, J.; Atkins, B.L.; Brent, A.J.; McNally, M.A.; Oakley, S.; Taylor, A.; Peto, T.E.A.; Crook, D. and Eyre, D.W. (2017) Real-time analysis of nanopore-based metagenomic sequencing from orthopaedic device infection.

Shagufta, N. B. ; Ravi, M. ; Subhashchandra, M. G. ; Jayaraj, Y.M. (2014) . Screening of Staphylokinase producing *Staphylococcus aureus* from Clinical Samples . *International Journal of Research in Biological Sciences , 4(2): 46-48* .

Yerasi ,G. P. R. ; Rajendran, P. ; Singaram, A.K**.** (2014). Isolation, cloning and expression of recombinant staphylokinase gene against thrombosis. *Int. J .Pharm. Sci. Vol 6, Issue 4, 266-270* .