Detection hla and spi gene of Staphylococcus aureus and it is relation with agr system presence

Jilan Ahmed Shakur
Collage of science, microbiology, university of Baghdad, jelianahmed5@gmail.com

Rasmiya Abd Aburesha
Collage of science, microbiology, university of Baghdad, Rasmiaabd8@gmail.com

Abstract

Background: Staphylococcus aureus genomes size are almost 2.8 Mbp , these genomes are having a very similar architecture and differ in their function like accessory genome that encoded to virulence factors of Staphylococcus aureus pathogen's caused broad range of diseases .

Methods: Identification of Staphylococcus aureus bacteria that isolated from urine specimens have been done according to the cultured on the mannitol salt agar and biochemical tests, then confirm the identification by amplification 16srRNA gene, virulence factor gene hla and sbi genes amplification by conventional PCR and agr gene amplification by multiplex PCR

Result: Thirty isolates of Staphylococcus aureus diagnosed and found all this isolates have agrI,II,III,IV that related with hla gene and sbi gene

Conclusion: Urinary tract system was more infected with S. aureus bacteria and agr system was regulated virulence gene (hla gene and sbi gene).

Keywords: *S. aureus genome, 16srRNAgene, agr system, virulence factor.*

INTRODUCTION

Staphylococcus aureus genomes size are 2.8 Mbp and the genomes having a very similar architecture and difference in their function like accessory genome that encoded to Staphylococcus aureus virulence factor caused broad range of diseases, the genome contains from mobile genetic elements (transposons, plasmids, insertion sequences , pathogenicity islands and antibiotic resistance determinants, Staphylococcus aureus composed of the:

-The core genome is present with percentage 75% of 95% of Staphylococcus aureus isolates.

- The accessory component are present in (1 - 95%) of Staphylococcus aureus isolates.

-The foreign genes.

(Cheung et al ., 2021: Kadhim et al., 2022) .

Staphylococcus aureus in most carry single or multiply plasmids carry different gene in a single cell and this plasmid are divided into three groups:

1-Small multicopy plasmids that have one resistance gene.

2-Larger about (15 – 30 kb) plasmid , allow copes (4 – 6 copies per cell) which have many of resistance gene .

3-Conjugative multiresistance plasmids. (Khudher and Jabur, 2020).

Staphylococcus aureus have specific structure is agr quorum sensing contain of two-component responsible about critical toxin genes to survey Staphylococcus aureus infection and regulate number of genes (hla gene, sbi gene) in addition this complexity system of Staphylococcus aureus activation toxin production and decreased adherence genes which be as counterproductive for Staphylococcus aureus pathogenicity during chronic infection (Miller and Gilmore, 2020: Aburesha et al, 2018 : Abdrabaa and Aburesha, 2023).

Material and method

Sample collection: Collected 150 clinical samples during the period (February to May, 2022) used sterile swap for taken sample from tonsil and sterile cup for collected urine sample all they from the people return to Tuz general Hospital in the Salah al-Din Governorate and from patient broadcasts in hospitals.

Bacterial identification: Used various methods for Staphylococcus aureus identification include cultured all isolates on the Mannitol salt agar media that differentiate Staphylococcus aureus isolates by fermentation of mannitol (Obaid and Abdulwahhab, 2021: Najem and Lafta, 2020) , microscopic examination by Gram's stain, taking smears of samples and investigated under light microscopes as Gram positive cocci clustered mostly in grape-like irregular clusters circular in clusters , single, paired or short chains (Gnanamani et al., 2017 :Chotigarpa et al., 2018) and biochemical test include Catalase test was distinguished by transport some pure colonies of bacterial isolates to clean glass slide by sterilized loop, then add few drops of 3% H_2O_2 on it air bubbles production is refer to positive result that evidence enzyme production (Omran & Hussein, 2019: Mahdi et al., 2020). In coagulase test evaluated to distinguished free plasma coagulase enzyme that lead to coagulation product from interaction between blood and Staphylococcus aureus bacteria, which indicates positive result While the clotting did not emerge at room temperature that refer to negative result (Rakotovao-Ravahatra et al., 2019) at last confirmed this identification method by detection 16srRNA gene of Staphylococcus aureus bacteria by PCR .

Extraction and determine Staphylococcus aureus DNA:

genomic DNA extraction according to (Geneaid Biotech kit instruction) used agarose gel electrophoresis was stained by ethidium bromide used for determined DNA extracted presence and integrity the total DNA concentration and conserved at - 20 °C until uses in PCR process.

genomic DNA of Staphylococcus aureus before loaded inside the wells of the gel must mixed together with the loading buffer (DNA: loading 7/2 v/v) .

16srRNA gene amplification:

Commonly used 16srRNA gene for identification Staphylococcus aureus species and classification it, this genes codes for the small subunit of bacterial ribosome have some factors make it perfect target to complete phylogeny studies.

Used specific primer (Gumaa et al., 2021) in PCR program according the TRANS protocol to detection 16srRNA .

Table (1) PCR reaction components for detection 16srRNA according to Trans protocol

Primer	Type of reaction	Master mix volume	Volume of F-primer (10pmol/*m*l)	Volume of R-primer (10pmol/*m*l	Nuclease free water	DNA	Final volume
16s rRNA	uniPCR	10	1	1	6 *m*l	2 *m*l	20 *m*l

Table (2) PCR program to amplify 16srRNA

Steps	ºC	Minute-Second	Cycle	Product band
Initial denaturation	95 ºC	4 Minute	1	257
Denaturation	95 ºC	4 Minute	30	
Annealing	53 ºC	45 Second		
Extension	72 ºC	45 Second		
Final extension	72 ºC	7 Minute	1	

Detection Staphylococcus aureus hla , sbi and agrI, II, III, IV genes

hla, sbi and agr gene amplification:

Detection Staphylococcus aureus hla, sbi and agr genes that were encoding for virulence factor was carried out by the amplification the extraction DNA of Staphylococcus aureus with target gene by used PCR that include using various specific set of primers encoding for each target gene mixed with the template (DNA sample) and master mix reagent (PCR buffer, MgCl2, Taq polymerase and dNTPs) the end constituent was the deionized water, then the mixture were mixed and centrifuged for 3second to collect the drops from walls for ensure the final volume of 25ml, transferred mixture to a thermal cycler to start reaction according to the steps of the specific program (Alwash and Aburesha, 2021: Fadhil and Mohammed, 2022:).

Table (3) PCR reaction components of hla, sbi and agr genes according to (Trans protocol)

Primer	Type of reaction	Master mix volume	Volume of F-primer (10pmol/*m*l)	Volume of R-primer (10pmol/*m*l	Nuclease free water	DNA	Final volume
hla gene	uniPCR	10	1	1	6 *m*l	2 *m*l	20 *m*l
Sbi gene	uniPCR	10	1	1	6 *m*l	2 *m*l	20 *m*l
Agr gene	Multiplex PCR	10	1	1	6 *m*l	2 *m*l	20 *m*l

To amplify hla gene used specific primer (Rossato et al., 2018) in PCR program.

Table (5) PCR program to amplify hla gene

Steps	ºC	Minute - Second	Cycle	Product band
Initial denaturation	**95 ºC**	**4 Minute**	**1**	**209**
Denaturation	**95 ºC**	**30 Second**	**30**	
Annealing	**58 ºC**	**45 Second**		
Extension	**72 ºC**	**45 Second**		
Final extension	**72 ºC**	**7 Minute**	**1**	

Table (6) PCR program to amplify spi gene using specific primer (Gonzalez et al., 2015).

Table (6) PCR program to amplify sbi gene

Steps	ºC	Minute-Second	Cycle	Product band
Initial denaturation	**95 ºC**	**4 Minute**	**1**	**variable**
Denaturation	**95 ºC**	**30 Second**	**30**	
Annealing	**58 ºC**	**45 Second**		
Extension	**72 ºC**	**45 Second**		
Final extension	**72 ºC**	**7 Minute**	**1**	

PCR program to amplify agr gene using specific primer (Zhang et al., 2018).

Table (7) PCR program to amplify agr gene

Steps	ºC	Minute-Second	Cycle	Product band
Initial denaturation	**95 ºC**	**4 Minute**	**1**	**1.441**
Denaturation	**95 ºC**	**30 Second**	**30**	**2.575**
Annealing	**54 ºC**	**45 Second**		**3.323**
Extension	**72 ºC**	**1 Minute**		**4.659**
Final extension	**72 ºC**	**10 Minute**	**1**	

Result and discussion:

Identified 30 isolates of Staphylococcus aureus from 150 clinical sample are growth on the mannitol salt agar and appearance with golden yellow colonies because it was fermented mannitol salt in media, Gram-positive cocci, catalase positive, coagulase positive, and oxidase, motility negative. More strain hemolysis it is lysis the red blood cell and then confirmed this methods of identification by 16srRNA genes amplification through PCR technique.

extraction Staphylococcus aureus DNA: Extracted DNA and purified from all Staphylococcus aureus using the manufacturer's DNA purification kit (DNA extraction kit) used agarose gel electrophoresis and stained by adding ethidium bromide for determined DNA extracted presence and integrity the optimum DNA concentration,

figure (1) showed the result the result showed and used PCR technique to amplifying the 16srRNA gene that helped to identify bacterial genome it was as developed and effective tool helped to recognize the specific bacterial strains this step applied on the 30 isolates taken from urinary tract and found all isolates are Staphylococcus aureus bacteria. figure (2) clarify 16srRNA amplification.

Figure (1) showed Staphylococcus aureus DNA extraction used 1 % of agarose gel electrophoresis to visualized that stained with ethidium bromide by 1xTBE and used UV light transilumiator at 350nm.

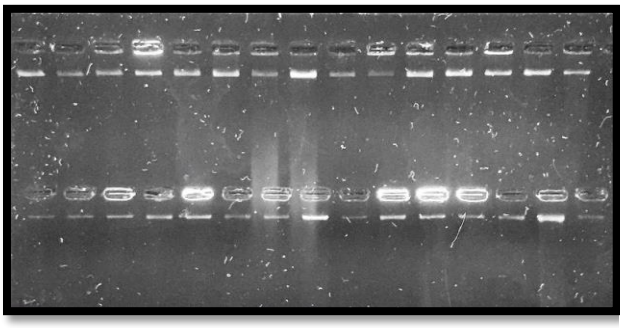
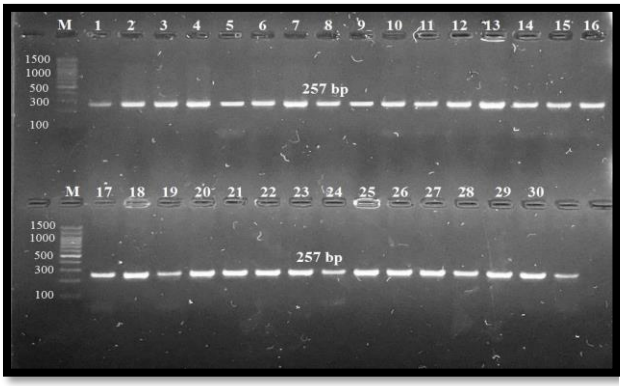


Figure (2) PCR amplification of 16srRNAgene (257bp) of Staphylococcus aureus, used 1 % of agarose gel electrophoresis to visualized that stained with ethidium bromide by using TBE, for 30 min at 90 Volt. Lane M: Marker. Lane (1-30): Urinary tract sample from people return to Hospital.



In last year's found the amplification of 16srRNA gene be a common genetic marker to identify Staphylococcus aureus bacteria and determine the reason of taxonomy increasing prevalent, that return to the increasing multidrug resistant Staphylococcus aureus infections number all that lead to research about the relation between genetic variation and bacterial antibiotic resistance.

In fact, Staphylococcus aureus interfamilial transmission jointed with local communities and another important cause the mutations of 16srRNA lead the research uses the 16srRNA technique to identify this bacteria (Gumaa et al., 2021).

Detection Staphylococcus aureus hla, sbi and agr genes

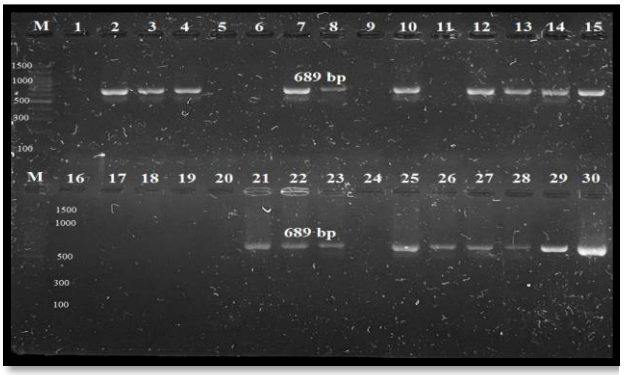
Used conventional PCR to Investigated about 2 genes include (hla gene and sbi gene) and used multiplex PCR to investigated about (agrI,II,III,IV gene) all this gene responsible of virulence factor to 30 isolates of Staphylococcus aureus bacteria. After DNA extraction by use (Mini gDNA Bacteria Kit) from Staphylococcus aureus isolated then determine virulence gene.

hla gene of Staphylococcus aureus bacteria responsible about red blood cell lysis which causes a-hemolysis or B-hemolysis where as sbi gene responsible about bacterial adhesion on the host cell surface, these genes function controlled by accessary gene regulator (agr) system controls the expression number of virulence factors in Staphylococcus aureus (Khan et al., 2021: Kretschmer et al., 2021: Hamdan-Partida et al., 2022).

hla gene were detected in this study with percentage, 63.3% from all stains of Staphylococcus aureus bacteria, (Afzal et al., 2022) showed in their study, hla gene percentage 94% they were more proportion of the result obtained, In(Wang and Zhang , 2022 : Ali and Maaroof, 2020) get in their study hla gene with percentage 98.0%, 90.0% respectively these result were approach to Afzal result but more from obtained result and

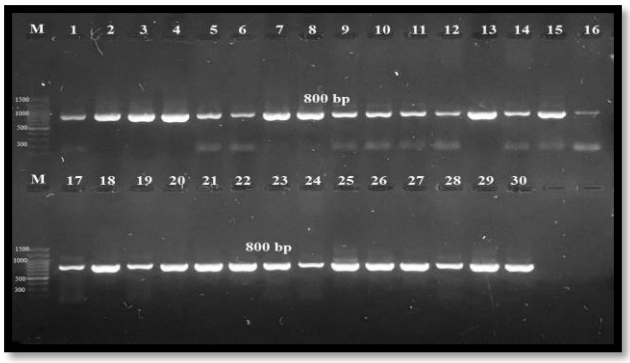
in (Rasmi et al., 2022) study isolated hla gene from Staphylococcus aureus with percentage 37.3% it was lowest from this study . (Khan et al., 2022) showed the hla gene were responsible about antibiotic resistant that lead to evolution and development has hyper virulent and spread in hospitals and community. figure (3) showed hla gene in Staphylococcus aureus bacteria isolated.

Figure (3) PCR amplification of hla gene (689bp) of Staphylococcus aureus, used 1 % of agarose electrophoresis to visualized that stained with ethidium bromide by using TBE, for 30 min at 90 Volt. Lane M: 1500 Marker



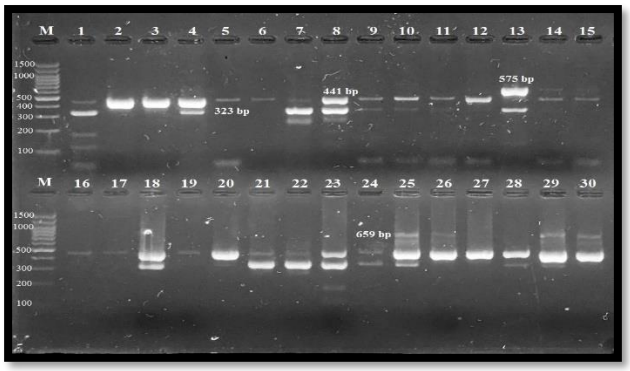
sbi gene detected in this study with percentage 96.7% figure (4) showed sbi gene in Staphylococcus aureus isolated, in (Lin et al., 2021) study get approach result was 81.82% of sbi gene helps bacteria to induction inflammatory avoid innate immune defenses and play an important role in the induction of inflammatory (Gonzaleza et al., 2019).

Figure (4) PCR amplification of spi gene (800bp) of Staphylococcus aureus , used 1 % of agarose electrophoresis to visualized that stained with ethidium bromide by using TBE, for 30 min at 90 Volt. Lane M: 1500 Marker



All isolated showed has agr gene with percentage 100%, because it is play important role in Staphylococcus aureus pathogenesis and antibiotic resistance (Bernabe et al., 2021). Also (Adeyanju et al., 2022) studied was also showed all isolated of Staphylococcus aureus bacteria won agr gene. figure (5) showed agr gene in all isolated,

Figure (5) PCR amplification of agr gene (323bp, 441bp, 575:323, 659bp) of Staphylococcus aureus , used 1 % of agarose electrophoresis to visualized that stained with ethidium bromide by using TBE, for 30 min at 90 Volt. Lane M: 1500 Marker.



hla and sbi genes of Staphylococcus aureus relation with agr system

The accessory gene regulator (agr) system acts as regulator of staphylococcus aureus virulence factors, it activates some gene to production several extracellular toxins and enzyme helped bacteria to invasive the host tissue like α-, β-, γ-hemolysis enzyme, lipases, leukotoxins and toxic shock syndrome toxins(Mahdally et al., 2021) that explained the result the agr found in all isolates with percentage 100% regulated sbi gene found with percentage 96.7% helped Staphylococcus aureus isolates to evade the immune system by different mechanisms , hla gene found with percentage 63.3 % is located on the Staphylococcus aureus chromosome cause different disease like sepsis ,pneumonia and lethal propertise (Butrico, and Cassat 2020 : Divyakolu et al., 2019).

Table (8) the relation between hla , sbi genes and agr system in Staphylococcus aureus isolates

Virulence gene	Positive	%100	Negative	%100
***agr*gene**	**30**	**100**	**0**	**0.0**
***Sbi* gene**	**29**	**96.7**	**1**	**3.3**
***Hla* gene**	**19**	**63.3**	**11**	**36.7**

Conclusion:

1-Urinary tract system was more infected with Staphylococcus aureus bacteria.

2-agr system was regulated virulence gene (hla gene and sbi gene).

3- The diagnosis method 16sRNA polymerase chain reaction was a confirmation method for distinction Staphylococcus aureus strain from other type of bacteria.

Reference

1- Cheung, G.Y, C., Bae, J.S and Otto, M. (2021). Pathogenicity and virulence of Staphylococcus aureus. Taylor&Francis, 12.1,547-569.

2- Kadhim, B.A., Essa, M.A., Sabbah, M.R. (2022). Prevalence of enterotoxin type A in Staphylococcus aureus isolated from different sources. Iraqi Journal of agriculture sciences, 53(6):1325-1330.

3- Khudher, K.K and Jabur, S.G. (2020). THE PREVALENCE OF SOME VIRULENCE PLASMID GENES IN STAPHYLOCOCCUS AUREUS INFECTION. Plant Archives, 20. ISSN:0972-5210.

4- Aburesha, R.A. Hussein, H., Khalaf, Z.Z., Mohamed, B.J. (2018). Extraction of methicillin resistant Staphylococcus aureus (Mrsa) golden pigment and study it antioxidant activity on macrophage killing pathway. ISOR Journal of Pharmacy and biological Sciences (ISOR-JPBS), 13(4):40-43.

5- Miller, C and Gilmore, J. (2020). Detection of Quorum-Sensing Molecules for Pathogenic Molecules Using Cell-Based and Cell-Free Biosensors. Antibiotics;9, (259) ; doi:10.3390/antibiotics9050259.

6- Abdrabaa, M.K and Aburesha, R.A.(2023). Gene expression evaluation of intracellular adhesion and regulatory genes among biofilm producing MRSA isolates. Iraqi Journal of Science, 1(64):75-83.10.2499/ijs.2023.64.1.8.

7- Najem, M.J and Lafta, I.J. (2020). Characterization of Mannitol fermenter and salt Tolerant Staphylococci from breast tumor biopsies of Iraqi women. Baghdad Science Journal,17(2):415-425.

8- Obaid, W.A and Abdulwahhab, A.S. (2021). Impacts of starvation stress on biofilm formation and expression of virulence genes in mono-and mixed – species cultures of Pseudomonas

aeruginosa and Staphylococcus aureus . Biochem.Cell.Arch. 1(21):685-693.

9- Gnanamani, A., Hariharan, P., & Paul-Satyaseela, M (2017). Staphylococcus aureus: Overview of bacteriology, clinical diseases, epidemiology, antibiotic resistance and therapeutic approach. Academic Press: Cambridge, MA, USA, .4, 28-36.

10- Chotigarpa, R., Na Lampang, K., Pikulkaew, S., Okonogi, S., Ajariyakhajorn, K., and Mektrirat, R. (2018). 'Inhibitory effects and killing kinetics of lactic acid rice gel against pathogenic bacteria causing bovine mastitis', Scientia pharmaceutica. Multidisciplinary Digital Publishing Institute, 86(3), 1-10.

11- Kadhum, H.H and Abood, Z.H .(2022). Staphylococcus aureus Incidence in some Patients with atopic Dermatitis in Baghdad city. Iraqi Journal of Biotechnology, University of Baghdad, 21.2.

12- Omran, A. S., and Hussein, A. N. (2019). Molecular investigation of mec gene Among coagulase negative Staphylococcus isolated from different cases. Journal of Physics: Conference Series, 1234(1), 12074.

13- Mahdi, E.A., Al-Shaibani, A., Zbar, N.S.(2020). The impact of glucse and chloride on the biofilm formation of Pseudomonas aeruginosa& Staphylococus aureus.Ibn Al-Haitham Jour. For Pure& Appl.33(4).

14- Rakotovao-Ravahatra, Z. D., Randriatsarafara, F. M., Milasoanjara, R. N., Ranaivosoa, M. K., Rakotovao, A. L., and Rasamindrakotroka, A. (2019). Assessment of the Coagulase Test in the Identification of Staphylococcus aureus Strains. Journal of Biotechnology and Biomedicine, 2(3), 105-111.

15- Gumaa , M. A., Idris, A. B., Bilal , N. E and Hassan, M. A. (2021). First insights into molecular basis identifcation of 16 s ribosomal RNA gene of Staphylococcus aureus isolated from Sudan. Gumaa et al. BMC Res Notes , 14:240 , doi.org/10.1186/s13104-021-05569-w.

16-Alwash, S.J and Aburesha, R.A. (2021). The difference in antibiotic-resistance among several Staphylococcus aureus strains in Iraq. Medico-legal Update,3(21).10.37506-2li3.3034

17- Fadhil, H and Mohammed , J. (2022). Molecular and Classical identification of Staphylococcus aureus, isolated from Iraqi Patients with recurrent tonsillitis. Medico-legal Update, 22(1).

18- Gonzalez, C.D., Ledo, C., Giai, C., Garófalo, A and Gómez, M. (2015). The Sbi Protein Contributes to Staphylococcus aureus Inflammatory Response during Systemic Infection. PLOS ONE, | DOI:10.1371/journal.pone.0131879.

19- Zhang, Y., Xu, D., Shi, L., Cai, R., Li, Ch and Yan, H. (2018). Virulence factors, biofilm formation and antibiotic resistance of Staphylococcus aureus isolates from prok production. Frontiers in microbiology, 9:1876.

20-Khan, S., Marasa, B.S., Sung, K., Nawaz, M. (2021). Genotypic Characterization of Clinical Isolates of Staphylococcus aureus from Pakistan. Pathogens, 10: 918. https:// doi.org/10.3390/pathogens10080918.

21- Kretschmer, D., Breitmeyer, R., Gekeler, C., Lebtig, M., Schlatterer ,K., Nega, M., Stahl, M., Stapels, D., Rooijakkers, S., Peschel, A .(2021). Staphylococcus aureus Depends on Eap Proteins for Preventing Degradation of Its Phenol-

Soluble Modulin Toxins by Neutrophil Serine Proteases. Front Immunol, 6;12:701093. doi: 10.3389/fimmu.2021.701093. PMID: 34552584; PMCID: PMC8451722.

22-Hamdan-Partida , A., González-García, S., Martínez-Ruíz, F.J., Zavala-Sánchez , M.Á., Bustos-Hamdan, A., Bustos-Martínez, J.(2022). Molecular Characterization of Staphylococcus aureus Strains Isolated from Mobile Phones. Microorganisms, 10(3):669. doi: 10.3390/microorganisms10030669. PMID: 35336244; PMCID: PMC8950573.

23- Afzal, M., Vijay, A.K., Stapleton, F., Willcox, M. (2022). Virulence genes of staphylococcus aureus associated with keratitis, conjunctivitis, and contact lens–associated inflammation. Transl Vis Sci Technol. 11(7):5, https://doi.org/10.1167/tvst.11.7.5

24- Wang, M. and Zhang, Q. (2022). Characteristics of Virulence Genes of Clinically Isolated Staphylococci in Jingzhou Area. Contrast Media Mol Imaging. doi: 10.1155/2022/8804616.

25- Ali, S.F and Maaroof, M.N. (2020). DETERMINATION THE INHIBITION ACTIVITY OF BIOLOGICAL PRODUCTS OF CYANOBACTERIA AGAINST MULTI ANTIBIOTICS RESISTANT BACTERIA ISOLATED FROM DIFFERENT INFECTIONS AND MOLECULAR DETECTION OF SOME VIRULENCE FACTORS. Biochem. Cell. Arch, Vol. 20, No. 1, pp. 679-690.

26- Rasmi,A. H., Ahmed, E. F., Abdullah Darwish, A. M and Gad, G. F. M.(2022). Virulence genes distributed among Staphylococcus aureus causing wound infections and their correlation to antibiotic resistance. BMC Infectious Diseases, 22(1):652

27- Khan, S., Marasa, B.S., Sung, K., Nawaz, M. (2021). Genotypic Characterization of Clinical Isolates of Staphylococcus aureus from Pakistan. Pathogens, 10: 918. https:// doi.org/10.3390/pathogens10080918.

28- Lin, T., Li, Q. , Jin, D., Liu, W., Tang, Ch and Zhang, X. (2021). Investigation of Virulence Genes of Staphylococcus aureus Isolated from Sterile Body Fluid Samples and Their Correlation with Clinical Symptoms and Outcomes. Hindawi Canadian Journal of Infectious Diseases and Medical Microbiology, 8 : doi.org/10.1155/2021/5354747.

29- Gonzaleza, C. D., Ledoa, C., Celac, E., Stellae,I., Xuf, Ch., Ojedah, D. S., Frenettef,g,i , PS. And Gómeza, M . (2019). The good side of inflammation: Staphylococcus aureus proteins SpA and Sbi contribute to proper abscess formation and wound healing during skin and soft tissue infections. HHS Public Acce, 01; 1865(10): 2657–2670. doi:10.1016/j.bbadis.

30- Bernabè, G., Dal Pra, M., Ronca, V., Pauletto, A., Marzaro, G., Saluzzo, F., et al. (2021). A novel Aza-derivative inhibits quorum sensing signaling and synergizes Methicillin-Resistant Staphylococcus aureus to clindamycin. Front. Microbiol. 12:610859. doi: 10.3389/fmicb.2021.610859.

31- Adeyanju, A., Schaumburg, F., Onayade, A., Akinyoola, K., Adeyemi, T., Ugbo, O ., Köck , R., Amusa, Y., Lawal,O ., Adeyanju , T., Torimiro, N., Akinpelu, D., Kolawole, D., Kohler, CH and Becker, K . (2022). Local Epidemiology of Nosocomial Staphylococcus aureus Infection in a Nigerian University Teaching Hospital. Antibiotics, 11, 1372. https://doi.org/10.3390/ antibiotics11101372.

32- Mahdally, N.H., George, R.F., Kashef ,M.T., Al-Ghobashy, M., Murad, F.E., Attia, A.S. (2021) .Staquorsin: A Novel Staphylococcus aureus Agr-Mediated Quorum Sensing Inhibitor Impairing Virulence in vivo Without Notable Resistance Development. Front. Microbiol. 12:700494. Doi: 10.3389/fmicb.2021.700494.

33- Butrico, C. E and Cassat ,G.E. (2020). Quorum Sensing and Toxin Production in Staphylococcus aureus Osteomyelitis: Pathogenesis and Paradox. Toxins, 12, 516; doi:10.3390/toxins12080516.

34- Divyakolu, S., Chikkala, R., 1 Ratnakar, K.S and Sritharan, V. (2019). Hemolysins of Staphylococcus aureus—An Update on Their Biology, Role in Pathogenesis and as Targets for Anti-Virulence Therapy. Advances in Infectious Diseases , 9 (2), DOI: 10.4236/aid.2019.92007.