La Prensa Medica Argentina

ha sido distinguida con el premio a la
Excelencia Empresarial edición 2019, en el rubro:

Best Medical Journal Publishing Company 2019 – Argentina

(Mejor Compañía Editora de Revistas de Medicina – Argentina – 2019)



Este premio es otorgado por *Acquisition International*
(Burton on Trent, Staffordshire, England)

RPM Nº 02-2019.indd 1 4/16/19 09:34

La Prensa Médica Argentina

Fundada
el 10 de junio de 1914

Director: Dr. Pablo A. López

Directores Asociados:
Dr. Manuel L. Martí
Dr. Eduardo Saad

Dr. Ariel P. López - Asistente del Director

CONSEJO ASESOR

Dr. Osvaldo González Aguilar
Cirugía de cabeza y cuello

Dr. Daniel Cione *Diagnóstico por imágenes*

Dr. Luis Chiappetta Porras *Cirugía*

Dr. Marcelo Corti *Infectología*

Dr. Daniel Piñeiro *Cardiología*

Dr. Carlos Luna *Neumonología*

Dr. Miguel Ángel Allevato *Dermatología*

Dr. Mario S. Palermo *Obstetricia*

Dr. Jorge D. Lemus
Epidemiología y Salud Pública

Dr. Manuel Montesinos *Cirugía General*

Dr. Hugo Said Alume *Cirugía oncológica*

Dr. Carlos Damin *Toxicología*

Dr. Miguel Ayerza *Ortopedia*

Dr. Pablo Chiaradía *Oftalmología*

Dr. Carlos Kukso *Otorrinolaringología*

Dr. Federico Micheli *Neurología*

Dr. Juan Sordá *Gastroenterología*

Dr. Ariel P. López *Genética y Biología Molecular*

Dr. Miguel L. Podestá *Urología*

Premio: MAESTRO DE LA MEDICINA ARGENTINA®
es marca registrada de Ediciones Médicas del Sur
Visite nuestro website: www.prensamedica.com.ar

Editores: EDICIONES MEDICAS DEL SUR SRL
Director Editorial: Claudio Alberto López
Gerente
email: ediciones@prensamedica.com.ar / edimedsur@hotmail.com

Junín 917 - p. 2º "D" (C1113AAA) Buenos Aires. Telefax (54-11) 4961-9213

RPM Nº 01-2019.indd 1 3/22/19 09:36

SUPERVIELLE

Crédito Express para Pymes de **Salud**

Con aprobación en **24 horas** hasta **$10.000.000**

Lo grande de ser una Pyme



#Superate

0810-333-7873
supervielle.com.ar

CARTERA COMERCIAL. OFERTA VÁLIDA DEL 13/12/2018 AL 30/04/2019. EXCLUSIVO PARA EL SEGMENTO MIPYME, SEGÚN REGULACIONES DEL BANCO CENTRAL DE LA REPÚBLICA ARGENTINA Y CALIFICACIÓN VIGENTE DE LA SECRETARÍA DE EMPRENDEDORES DE LA PEQUEÑA Y MEDIANA EMPRESA DEL MINISTERIO DE PRODUCCIÓN DE LA NACIÓN BAJO LA RES. 340/2017, SUS MODIFICATORIAS Y COMPLEMENTARIAS. OTORGAMIENTO SUJETO A CALIFICACIÓN CREDITICIA Y CONDICIONES COMERCIALES DE CONTRATACIÓN DE BANCO SUPERVIELLE S.A. - MITRE 434 – C.A.B.A.- C.U.I.T 33500005179, INSCRIPTO I.G.J. N° 23, F° 502, L. 45, T° A DE ESTATUTOS NAC. MÁS INFORMACIÓN EN WWW.SUPERVIELLE.COM.AR/EMPRESAS LA PRESENTE PIEZA SE DESTINA A AQUELLAS MIPYME CON UNA ACTIVIDAD REGISTRADA MAYOR A 18 MESES Y CUYA ACTIVIDAD SEA: CLÍNICAS, SANATORIOS Y HOSPITALES PRIVADOS. LABORATORIOS DE ANÁLISIS. CENTROS DE DIAGNÓSTICO POR IMÁGENES. CENTROS ESPECIALIZADOS. CONSULTORIOS EXTERNOS. TODOS DEBEN SER DE CARÁCTER PRIVADO. A TRAVÉS DE "CRÉDITO EXPRESS" EL CLIENTE PODRÁ SOLICITAR CUENTA CORRIENTE EN PESOS Y DÓLARES, ACUERDO EN CUENTA CORRIENTE, PRESTAMOS FINANCIERO, LEASING, TARJETA VISA BUSINESS Y DESCUENTO DE DOCUMENTOS, RESULTANDO DE APLICACIÓN A CADA TIPO DE FINANCIACIÓN EL MONTO MÁXIMO INDIVIDUAL INFORMADO EN CADA CASO POR EL BANCO Y QUE EN SU CONJUNTO NO PODRÁN SUPERAR LA SUMA DE $ 10.000.000 DE CALIFICACIÓN. ESTA IMAGEN HA SIDO RETOCADA Y/O MODIFICADA DIGITALMENTE, LEY 14953 PCIA. DE BUENOS AIRES Y LEY 4827 CIUDAD AUTÓNOMA DE BUENOS AIRES.

Lunel

PREGABALINA

Lunel 25 PREGABALINA
30 cápsulas

Lunel 75 PREGABALINA
15 y 30 cápsulas

Lunel 75 Practidosis PREGABALINA
30 comprimidos birranurados

Lunel 150 PREGABALINA
30 cápsulas

Combate el dolor y devuelve la calma





Juntos para restablecer y preservar la salud de sus pacientes

Montpellier
TRADICIÓN Y FUTURO EN LA TERAPÉUTICA ARGENTINA
www.montpellier.com.ar

RPM Nº 02-2019.indd 5 4/16/19 09:34

UCA

FACULTAD DE

CIENCIAS MÉDICAS

GRADO

Medicina

Odontología

Licenciatura en **Nutrición**

Licenciatura en **Kinesiología y Fisiatría**



DEPARTAMENTO DE INGRESO
ingreso@uca.edu.ar

ESPECIALIZACIONES Y CURSOS DE POSGRADO EN SALUD

csmedicas@uca.edu.ar

Av. Alicia Moreau de Justo 1600 PB;
4349-0419/20

RPM Nº 02-2019.indd 6 4/16/19 09:34



En Novartis, los pacientes son la razón de lo que hacemos.

NOVARTIS

RPM Nº 02-2019.indd 7 4/16/19 09:34

PRESIMAX D

Losartán 50 mg
Hidroclorotiazida 12,5 mg

Efectivo control de la Hipertensión Arterial durante las 24 h

LA RESPUESTA RACIONAL A LA HIPERTENSIÓN MODERADA Y SEVERA



- **Presentación:**
  Presimax D
  30 comprimidos recubiertos

Bagó

RPM Nº 02-2019.indd 8 4/16/19 09:34

Detection of some virulence factors genes in *Staphylococcus aureus* isolated from different clinical cases

Dheaa Ghanim Al-Naaily[1], Rana Masheel Salim[2], Aqeel Meer Al-Zamily[3*]

Pren. Méd. Argent.
Abril 2019
Vol. 105 - Nº 2
82 - 91

1 Al-Diwaniya Teaching Hospital, Ministry of Health, Iraq
2 College of Dentistry, University of Al-Qadisiyah, Iraq
3 College of Medicine, University of Al-Qadisiyah, Iraq

Corresponding author. Name: Aqeel Meer Al-Zamily. Contact: +9647801610310. E-Mail: draqeelmeer@yahoo.com

Introduction

Staphylococci include many species of pathogenic bacteria that cause infection to humans and animals, the species (*S. aureus*) is one of the most important pathogenic type to humans, and the most common species responsible for a wide range of diseases such as furuncles, various abscesses, wounds abscesses resulting from surgical operations, dermatitis, soft tissue inflammation, arthritis, bones inflammation, bronchial pneumonia, inflammation of internal parts of the heart and injuries caused by toxins such as toxic shock syndrome and *Staphylococcus aureus* syndrome and food poisoning (1). Due to a lot of Virulence factors, this bacterium has pathogenesis and ability to invade the host's tissue and spreading in it such as the production of Toxins and enzymes that help bacteria to cause infection (2). They have the ability to produce many toxins, extracellular enzymes such as coagulation enzyme of blood plasma that has the ability to inhibit phagocytosis, It also has the ability to produce other enzymes representing spreading factors such as Staphylokinase enzyme, proteinase enzyme and lipase enzyme which contribute to bacterial invasion of tissues and the spread of infection , It also works on the production of Alpha, Beta, Gama and Delta-type lipoproteins as well as the production of gastrointestinal toxins leading to food poisoning . Besides that, it has ability to produce exogenous toxins produced in cases of Toxic shock syndrome and Staphylococcal scalded skin syndrome (3). In addition to that, it has a wallet that helps bacteria to resist phagocytosis (4). This Bacteria also has cell wall, which is considered as an anti-genetic structure containing anti-genetic structures (peptidoglycan, teichoic acid and A protein). The cell wall works to resist the host's immune system and forms osmosis protection for the bacterial cell (5,6). One of the other Virulence factors is that some *S.aureus* strains is having *mecA* gene which is responsible for the resistance of the fixed penicillin group as a result of their encryption of PBPs Penicillin binding proteins which work to reduce the harmony with Meticillin antibiotic, the *Staphylococcus* resisting Meticillin are called MRSA which are characterized mainly by their institutionalization in hospitals and called HA(Hospital Acquired)-MRSA and when they take the community as a residence , they are called CA(Community acquired) MRSA. The importance of these antibiotica came through their resistance to many antibiotics such as resisting all the species of β-Lactam antibiotics and many other antibiotics (7). The first Meticillin Resistant *S.aureus*was recorded in England in 1961 and made a massive health disaster that moved to different parts of the world (8) . Due to the importance of MRSA in causing various infections of the body and the difficulty to find a medication and controlling them besides the lack of studies about their spread in the hospitals of Al-Diwaniyah city, Iraq, also we have lack of molecular studies related to pathologic factors (Virulence factors) of this bacteria. The current study

RPM Nº 02-2019.indd 82 4/16/19 09:34

aimed by finding the genes responsible for the virulence factors in *S.aureus* isolates by using the Single and Multiplex PCR mechanism (technology).

MATERIALS AND METHODS

Isolates collection

60 isolates (urine, wound swabs, burn swabs) were collected from Al-Diwaniyah Teaching Hospital, Iraq for the period from October 2016 to January 2017, the study included different clinical isolates, these isolates were collected from different parts of the dormant patient's bodies for all ages and both genders.

Isolation and identification (Genomic DNA extraction)

DNA was extracted from the *S.aureus* bacteria by using the Genomic DNA Mini Kit which is supplied by the American company (Geneaid) and due to the instructions of the supplying company .

Preparation of Agarose gel

Prepared with *Sambrook* and method and his group.

Preparation of Single PCR enzyme master mix

The Single PCR master mix was prepared by using AccuPower® PCR PreMix kit supplied by the Korean company Bioneer and according to the instructions of supplying company as follows:

1. The PCR master mix was prepared in tubes equipped with the kit and container with adding other components of the reaction mixture as in Table (1):

Table (1) Components and volumes of single PCR master mix for *coa*, *clfA*, *spa*, *luks* and *fnbA* genes.

CR master mix		Volume
DNA template		µL 5
Primer	Forward primer	1.5µL
	Reverse primer	1.5µL
PCR water		µL 12
Total		**µL 20**

2. After completing the preparation of the PCR master mix Single, the tubes were carefully sealed with the vortex carburetor for 5 seconds.
3. The tubes were transferred to the thermocycler of PCR master mix Single to conduct DNA amplification depending on the optimal conditions of the thermal cycles represented by the DNA Denaturation and primersannealing with the separate string (Annealing) with the extension of the DNA chain.

Preparation of Multiplex PCR enzyme master mix

The MultiplexPCR master mix was prepared by using KAPA2G Fast Multiplex PCR kit supplied by KAPA (South Africa) and according to the instructions of supplying company as shown in Table 2:

Table (2): Products and components of multiplex PCR mastermix for *coa*, *clfA*, *spa*, *luks* and *fnbA* genes.

CR master mix		Volume
DNA template		2.5µL
KAPA2G Fast Multiplex		12.5µL
Primer	Forward primer	0.5µL
	Reverse primer	0.5µL
PCR water		up to 25µL
Total		**µL25**

RPM Nº 02-2019.indd 83 4/16/19 09:34

DNA Primers

The genes used in the current study, which are responsible for the Virulence factors, were supplied by the Korean company (Bioneer) as shown in Table (3).

Thermal Cycles Program for the amplification of DNA

The PCR reaction was made by using the thermal amplifier of PCR thermo cycler. The device was processed for genes under study according to interaction, as shown in Table (4) and Table (5).

Table (3): DNA primers.

Genes		Sequences	Amplifies products	References
mecA	F	TAGAAATGACTGACGTCCG	154 bp	26
	R	TTGCGATCAATGTTACCGTAG		
Luks	F	CAGGAGGTAATGGTTCATTT	151 bp	27
	R	ATGTCCAGACATTTTACCTAA		
Spa	F	ATCTGGTGGCGTAACACCTG	1150-1500 bp	27
	R	C GCTGCACCTAACGCTAATG		
FnbA	F	GAG CAG CAT TAT TCT TAG	270 bp	28
	R	TTT GTG GCG CTT GTA CT		
clfA	F	GGCAACAACTCAATCAAGCA	378 bp	Designed in study
	R	GCTTGGTGCGGATAAACTGT		
Coa	F	AGCAGTTAAAGAAGCAGACG	593 bp	Designed in study
	R	CGTTGTATTCACGGATACCT		

Table (4): Thermocycler program for single PCR.

Gene	Temperature (˚C)/Time				
	Final extension	Cycling condition			Initial denaturation
		extension	annealing	denaturation	
mecA	72/5 min	72/10 sec	55/30 sec	95/30 sec	95/2 min
spa	72/5 min	72/2 min	59/30 sec	95/30 sec	95/2 min
clfA	72/5 min	72/40 sec	56/30 sec	95/30 sec	95/2 min
coa	72/5 min	72/1 min	55/30 sec	95/30 sec	95/2 min
fnbA	72/5 min	72/10 sec	50/30 sec	95/30 sec	95/2 min
luks	72/5 min	72/20 sec	52/30 sec	95/30 sec	95/2 min
mecA	72/5 min	72/10 sec	55/30 sec	95/30 sec	95/2 min

Table (5): Thermocycler program for Multiplex PCR.

Genes	Temperature (˚C)/Time					Cycle No.
	Initial denaturation	Cycling condition			Final extension	
		Denaturatio	annealing	extension		
fnbA ***luks***	95/2 min	95/30 sec	52/30 sec	72/20 sec	72/5 min	30
mecA ***spa*** ***clfA***	95/3 min	95/15 sec	55/30 sec	72/30 sec	72/5 min	30

RPM Nº 02-2019.indd 84 4/16/19 09:34

Electrophoresis in Agarose Gel

Electrophoresis was made to the prepared Agarose gel with a proportion of 1.5% under 100 volts and 80 amperes within an hour, for the purpose of detecting the extracted DNA bands and amplified DNA which represents the amplifications products or PCR products in comparison with the DNA Ladder (100-1500bp)

Primers Design

The *clfA* and *coa* primers were designed in the present study by obtaining the gene serial for each primer from the genes bank, the gene serial was entered in Primer.3Plus program which is found on the website www.Primer3Plus.com. The appropriate settings were set for each primer such as the annealing temperature in a proportion G+C with product size as needed, finally the primer, who is believed to be appropriate, had been chosen.

Results

Confirmative Diagnosis to detect MRSA isolates by using method of PCR reaction

All *S.aureus* isolates were tested to make sure they have *mecA* gene (Meticillin resistant) by using method of PCR reaction , the (60 isolates) showed (according to the previous methods) that it included this gene , therefore , these isolates were considered as Meticillin resistant , as shown in figure (1) .

Mechanism of Single & Multiplex PCR enzyme reaction

Some virulence factors of (30 only) *S.aureus* were detected isolates by using the mechanism of Single & Multiplex PCR enzyme reaction as shown in figures below , present study found that all the isolates (100%) were having the genes (*Coa, AClf, Spa and fnbA*), while (13) isolates (43.3%) had (*luks*) gene as shown in table (6):

Discussion

The method of Single & Multiplex PCR reaction were used to detect some genes responsible for virulence factors in Methicillin resistant *Staphylococcus aureus* (MRSA), through the use of DNA fragments with a limited number of oligonucleotide, which work as specialized primers for virulence genes in the MRSA strain that included *coa* gene which is responsible for producing Coagulase enzyme, *clfA* gene, responsible for clumping factor, *spa* gene, responsible for A protein, *fnbA* gene, responsible for fibronectin Binding Protein, *luks* gene, responsible for the production of killer toxin of white blood cells (Panton Valentine Leukocidin), the results of the study of virulence factors genes using PCR method showed that all the 30 isolates (100%) were having *coa, clfA, spa and fnbA* genes, as shown in table 11, while the rate of MRSA isolates possession of *fnbA* and *clfA* genes in the current study was consistent with the rate of Hassnawi (23) study which was (100%) in all MRSA 24 isolates in her study distributed between 9:9 (100%) in MRSA isolates acquired from the hospital (HA-MRSA), 15:15 (100%) in MRSA isolates acquired from the community (CA-MRSA). The ratio of *clfA* and *fnbA* was also consistent with the Atshanet al (24) ratio which reached 100% in Baba-Moussaet al (25) study that showed that 17% of MRSA isolates contained. *fnbA* gene and isolated from patients infected with urinary tract infection (UTI) in Nigeria. In another study, isolates from isolated MRSA showed that patients with diarrhea had 100% *fnbA*gene , bna-Moussaet al (26). In the current study, *clfA* and *fnbA* ratio were higher than in et alEiichi (27) in Japan which reached (77.1%) and

RPM Nº 02-2019.indd 85 4/16/19 09:34

Figure (1): Products of *mecA* gene amplification for *S.aureus bacteria* (Agarose 1.5%, 100 Volt and 1 hour) by using PCR technique

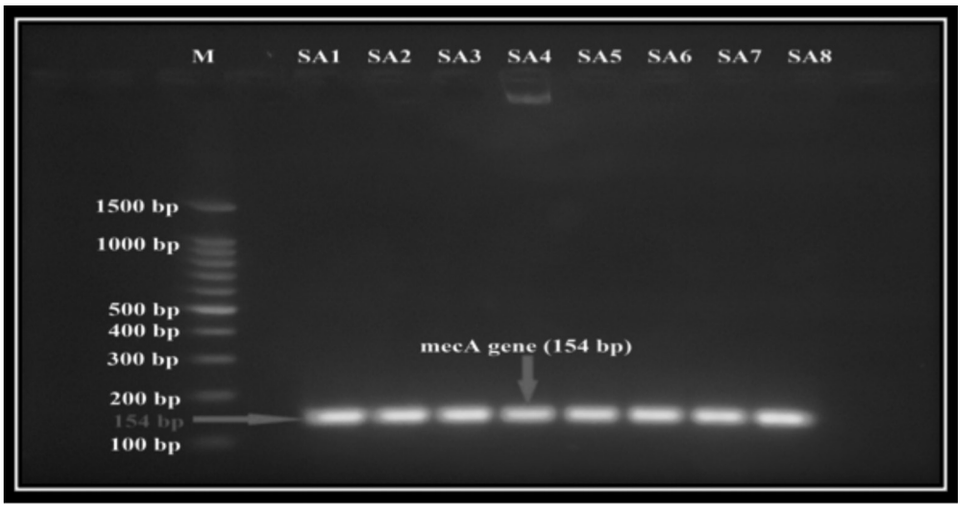


Figure (2): Products of Spa gene amplification for MRSA bacteria by using Single PCR technique

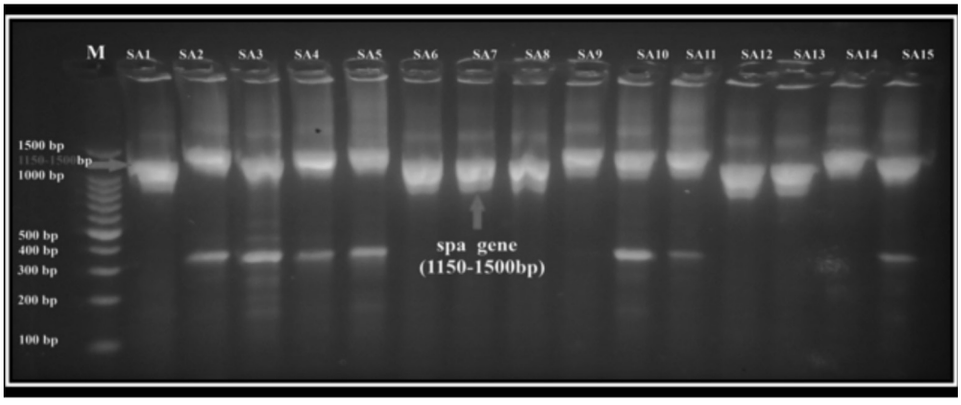
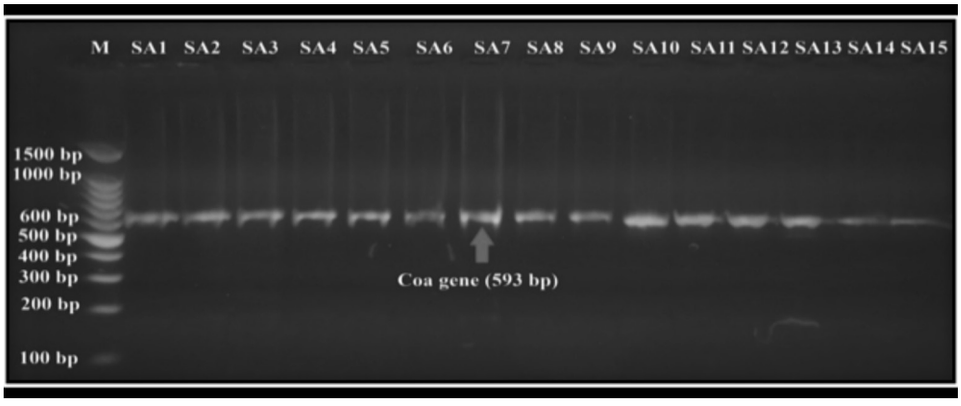


Figure (3): Products of Coa gene amplification for MRSA bacteria by using Single PCR technique



RPM Nº 02-2019.indd 86 4/16/19 09:34

Figure (4): Products of *Fnb* gene amplification for MRSA bacteria by using Single PCR technique

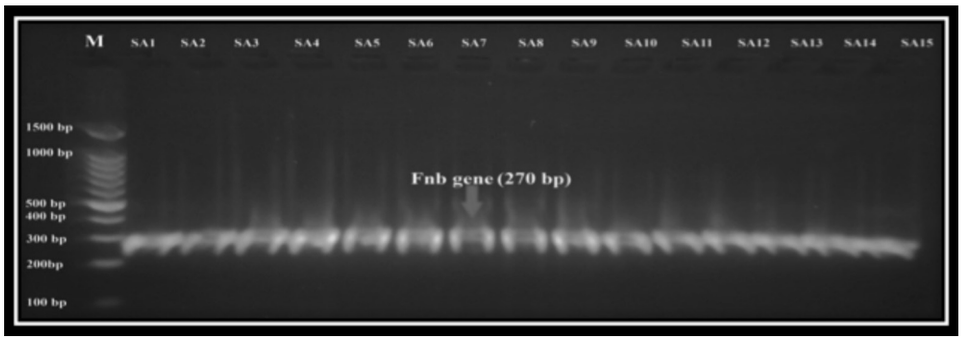


Figure (5): Products of (*Luks+Fnb*) gene amplification for MRSA bacteria by using multiplex PCR technique

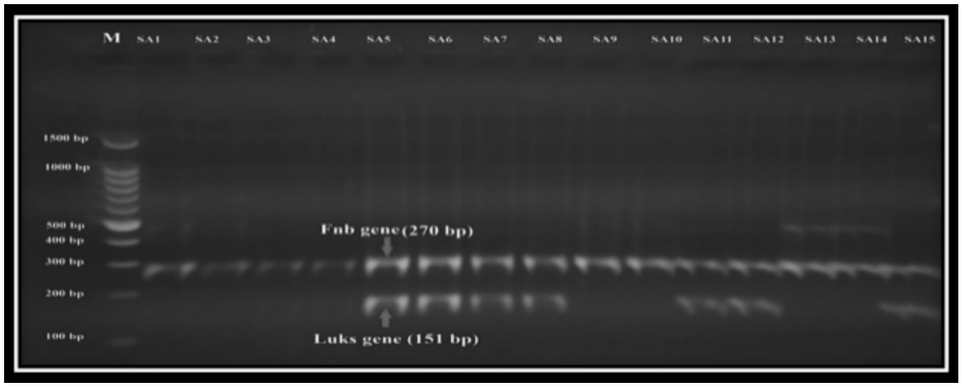
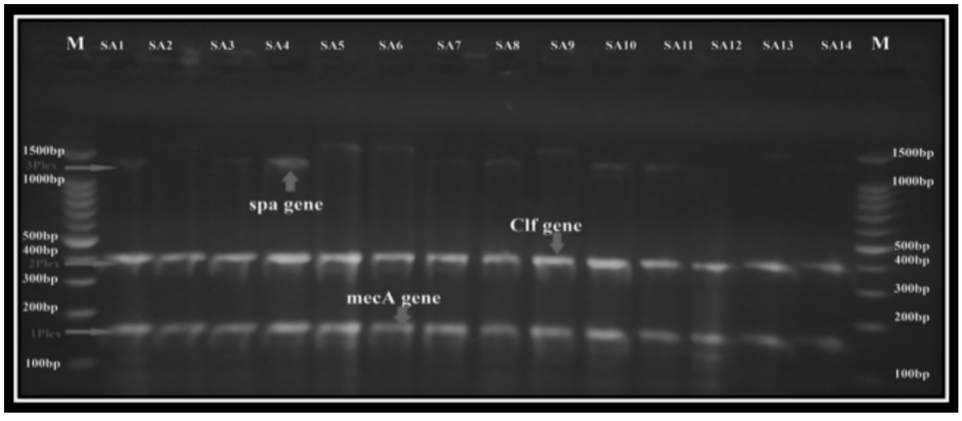


Figure (6): Products of (*ClfA+Spa+mecA*) gene amplification for MRSA bacteria by using multiplex PCR technique



(72.5%) respectively. While the ratio of *coa* gene in the current study was higher than that of Mlynarczyket al (28), which was (92.8%), 39 isolates of the 42 isolates of MRSA were having this gene in his study. While in the current study the prevalence of *spa* gene was (100%) higher than in the Fatemeh *et*

RPM Nº 02-2019.indd 87 4/16/19 09:35

al.,2006 study which was (96.6%) in Gorgan, the northern region of Iran. The results of the current study on virulence factors showed that 13 isolates out of 30 isolates of MRSA isolates had *luks* gene (43.3%). This ratio was close to Al-Hassnawi (23), which was 42.1% of the MRSA isolates acquired from the hospital (HA-MRSA). The ratio of *luks* gene in the current study was higher than in the Gulmez et al (29) and Marimonet al (41) in Spain during the period 2007 and 2009 which reached (2.2% and 4.4% respectively). While the ratios were (27.2%) in the study of Al-Mohana *et al* in MRSA isolates acquired from the community (CA-MRSA). The high rate of the encoded gene for the production of lethal toxin of white blood cells *luks* in the present study may be due to the type of isolates where its higher ratio was (33.3%) in pyogenic skin infections isolates compared to other sources shown in table (11-4) because of the close link between the *luks* gene and this type of infections, *Lina et al* (30). This is not surprising, because of the horizontal transfer of virulence genes among MRSA strains, which include toxin genes including the *luks*gene Diep et al (31). In Iraq, there is no interest in the prevalence of *luks*gene in MRSA isolates except in few studies in Al-Najaf and Babylon provinces (Al-Mohanaet al (32), Al-Hassnawi (23). Besides that, there is no specialized center for study and research about the prevalence, distribution and purification of this toxin. The presence of *luks* gene in the current study was lower than the proportion in the study of Havaeiet al (33) which reached (61.8%) in MRSA isolates acquired from the hospital (HA-MRSA).

Conflict of Interest

The authors declare no conflict of interests**.**

References

1. Omoe, K.; Ishikawa, M.; Shimoda, Y.; Hu, D. L.; Ueda, S.; and Shinagawa, K. Detection of *seg*, *seh*, or *sei* genes in *Staphylococcus aureus* isolates and determination of the enterotoxin productivities of *S. aureus* isolates harboring *seg*, *seh*, or *sei* genes. J. Clin. Microbiol. 2002; 40: 857-862.
2. Brooks, G.F., Butel, J.S. and Morse. S.A. Jawetz, Melinck&Adelberg's Medical Microbiology .21st ed. Appleton &lange, Asimonand Schuster Co.,1988. California.
3. Ryan, K. J.; and Ray, C. G. Sherris Medical Microbiology 4th ed. 2004; McGraw-Hill-NewYork.
4. O'Riodan, K.; and Lee, J. C. *Staphylococcus aureus* capsular polysaccharides. J. Clin. Microbiol. Infect. Dis. 2004; 17 (1): 218-234.
5. McKinney, T. K.; Sharma, V. K Craig, W. A.; and Archer, G. L. Transcription of the gene mediating methicillin resistance in *Staphylococcus aureus* (*mecA*) is co repressed but not co induced by cognate *mecA* and ß-lactamase regulators. J. Bacteriol. 2001; 183:6862-6868.
6. Fuda, C.; Hesek, D.; Lee, M.; Heilmayer, W.; Novak, R.; Vkulenko, S. B.; and Mobashery, S. Mechanistic basis for the action of new cephalosporin antibiotics effective against methicillin-and vancomycin-resistant Staphyloccocusaureus. J. Biol. Chem. 2006; 281: 10035-10041.
7. Al-Rawahi, G. N.; Schreader, A. G.; Porter, S. D.; Roscoe, D. L.; Gustafson, R.; and Bryce, E. A. Methicillin-resistant *Staphylococcus aureus*nasal carriage among injection drug users: six years later. J. Clin. Microbiol.2008; 46: 477-479.
8. Suzuki, J.; Komatsuzawa, H.; Sngai, M.; Suzuki, T.; Kozai, K.; Miyake, Y. Suginaka, H. &Nagasaka, N. A Long-Term Survey of Methicillin-

RPM Nº 02-2019.indd 88 4/16/19 09:35

Resistant *Staphylococcusaureus* in the Oral Cavityof Children. Microbiol. Immunol.,1997; 41:681-686.
9. Al-hassnawi, H.H. Molecular Characterization of Antibiotic Resistance and Virulence Factors of Methicillin Resistance *Staphylococcus aureus* (MRSA) Isolated from Clinical Cases in Babylon Province. PhD. thesis. College of Medicine. University of Babylon. 2012.
10. Al-Fu'adi, A.H.H. (2010). Phenotypic and Genotypic (*mecA*gene) of Methicillin Resistant *Staphylococcus aureus* (MRSA) Isolates in Al-Diwaniyah City. M.Sc. thesis. Babylon University. College of medicine. 2010.
11. Zhang, H. Z.; Hackbarth, C. J.; Chansky, K. M.; and Chambers, H. F. A proteolytictransmembrane signaling pathway and resistance to beta lactams in staphylococci. Science. 2001; 291: 1962-1965.
12. Derek, F.J. Brown, David, I. Edwards, Peter, M. Hawkey, D. Morrison, L. Ridgway, Kevin, J. Towner, and Michael, W . D. Guidelines for the laboratory diagnosis and susceptibility of methicillin-resistant Staphylococcus aureus (MRSA). Oxford Unversity Press. J. Antimicrob. Chemother., 2005; 56:1003.
13. Weigelt, J.A. MRSA Informa Healthcare. USA, Inc. 2007.
14. Clinical and Laboratory Standards Institute (CLSI). Performance standards for Antimicrobial Disks Susceptibility tests. 2011; Vol.31 No.1 Jan.
15. Taguchi *et al* . J. Jap. Ass. Infec. Dis. 2004; Jan. 54-58.
16. Huletsky, A.; Gagnon, F.; and Rossbach, V. Less than 1-h detection of methicillin-resistant *Staphylococcus aureus* directly from nasal swabs by real-time polymerase chain reaction (PCR) using the smart cycler. J. Clin. Microbiol. Infect. Dis. 2002; 8(1): 85.
17. Huletsky, A.; Giroux, R.; and Rossbach, V. New real-time polymerase chain reaction (PCR) assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. J. Clin. Microbiol. Infect. Dis. 2004; 42: 1875-1884.
18. Panhotra, B. R.; Saxena, A. K.; and Al-Mulhim, A. S. Chloramphenicol susceptible methicillin-resistant *Staphylococcus aureus* in Eastern region of Saudi Arabia. J. Saudi. Med. 2005; 26(7): 1149-1151.
19. Atshan, S.S., Shamsudin, M.N., Lung, L.T.T., Sekawi, Z.,Ghaznavi-Rad ,E., and Pei,C.P. Comparative Characterisation of Genotypically Different Clones of MRSA in the Production of Biofilms . J. Biomed Biotechnol. 2012.
20. Baba-Moussa L, Anani L,Scheftel JM, Couturier M, Haikou N, Hounsou F, Monteil H, Sanni A, Prevost G (2008). Virulence factors produced by strains of *Staphylococcus aureus* isolated from urinary tract infections, J. Hosp. Infect., 2008; (968): 32-38.
21. Baba-Moussa L., Ahissou, H., Azokpota, P., Assogba, B., Atindehou, M. M., Anagonou, S. Toxins and adhesion factors associated with *Staphylococcus aureus* strains isolated from diarrhoeal patients in Benin. Afri. J. Biotech., 2009; 9(5): 604-611.
22. Eiichi, A.; Koichi, M.; Ritsuko, M.; Reiko, K.; and Hiromi K. Biofilm Formation among methicillin-resistant *Staphylococcus Aureus* Isolates from Patients with Urinary Tract Infection. Acta med. Okayama.,58(4): 2004; 207-214.
23. Młynarczyk, A; Młynarczyk, G; Szymanowska, A; Stańczak, J; Luczak, M; and Jeljaszewicz J. Use of PCR for evaluating detection of

RPM Nº 02-2019.indd 89 4/16/19 09:35

coa and nuc genes in methicillin-resistant, coagulase-negative strains of*Staphylococcus aureus* (MRSA-CN).Med DoswMikrobiol., 2000; 52(3):217-222.
24. Gülmez, D;Sancak, B;Ercis, S;Karakaya,J;andHasçelik, G. Investigation of SCCmec types and Panton-Valentine leukocidin in community-acquired and nosocomial *Staphylococcus aureus* strains: comparing skin and soft tissue infections to the other infections.Mikrobiyol Bul. 2012; 46(3):341-351.
25. Marimón, JM;Villar, M;García-Arenzana, JM;Caba Ide,L;andPérez-Trallero, E. Molecular characterization of *Staphylococcus aureus* carrying the panton-valentine leucocidin genes in northern Spain.J Infect. 2012; 64(1):47-53.
26. Al-Mohana, A. M., Al-Kudhieri, M. K.,and Al-Charrakh, A.H. Community-Acquired Methicillin-Resistant *Staphylococcus Aureus* Carrying *mecA* and Panton-Valentine leukocidin*PVL* Genes That Isolated From Holy shrine In Najaf, Iraq. J.Bacteriol.Res.June 2012; (in press).
27. Lina, G; Piémont, Y; Godail-Gamot, F; Bes, M; Peter, MO; Gauduchon, V; Vandenesch,F;and Etienne; J. Involvement of Panton-Valentine leukocidin-producing *Staphylococcus aureus* in primary skin infections and pneumonia.Clin Infect Dis., 1999; 29(5):1128-1132 .
28. Diep, B., Carlton, H., Chang, B., Senabaugh, G. and Predreau-Remington, F. Roles of 34 Virulence Genes in the Evolution of Hospital- and community – Associated Strains of methicillin-resistant *Staphylococcusaureus*. J.Infect. Dis.,2011; (193): 1495-1503.
29. Santos, K. R. N.; Teixera, L. M.; Leal, G. S.; Fonseca, L. S.; and Filho, P. P. G. DNA typing of methicillin-resistant *Staphylococcus aureus*: isolates and factors associated with nosocomial acquisition in two Brazilian university hospitals. Med. Microbiol. 1999; 48: 17-23.
30. Al-Talib, H., Yean, C.Y., Al-Khateeb, A., Hassan, H., Singh, K.K., Al-Jashamy, K., andRavichandran, M. A pentaplex PCR assay for the rapid detection of methicillin-resistant *Staphylococcusaureus*and Panton-Valentine Leucocidin. *BMC Microbiology*, 2009; 9 :113.
31. Fatemeh, S; Shojai, A; Golalipour, M; RahimiAlang, S; Vaez,H;andGhaemi, EA. Spa Diversity among MRSA and MSSA Strains of *Staphylococcus aureus* in North of Iran.Int J Microbiol.2010.
32. Moore, N. Examination of Virulence genes in Nasal Isolates and Community-Acquired Strains of Methicillin-Resistant *Staphylococcus aureus*. UW-L Journal of Undergraduate Research IX .2006.
33. Havaei, S.A.; Moghadam, S. O.;Pourmand, M.R.;andFaghri, J. Prevalence for Genes Encoding Bi-Component Leukocidins among Clinical Isolates of Methicillin-Resistant *Staphylococcusaureus*.Iran.J. Publ. Health, 2010; 39(1):8-14.

SUMMARY

Staphylococcus aureus *is one of the most important pathogenic type to humans, and the most common species responsible for a wide range of diseases such as furuncles, various abscesses, wounds abscesses resulting from surgical operations, dermatitis, soft tissue inflammation, arthritis, bones inflammation, bronchial pneumonia, inflammation of internal parts of the heart and injuries caused by toxins such as toxic shock syndrome and* staphylococcus aureus *syndrome and food poisoning. The current study aimed by finding the genes responsible for the virulence factors in*

RPM Nº 02-2019.indd 90 4/16/19 09:35

S.aureus *isolates by using the Single and Multiplex PCR mechanism (technology). A total of 60 specimens (urine, burn swabs, wound swabs) from different clinical cases were collected from patients (in different age groups) who admitted to several health centers in Al-Diwaniyah Teaching Hospital, Iraq, during a period extending from October 2016 to January 2017. Some virulence factors were investigated for 30 isolate only of MRSA using Single and Multiplex PCR for detection virulence factor genes which both* coa *gene encoding production of coagulase,* clfA *gene encoding for clumping factor,* spa *gene encoding for protein A,* fnbA *gene encoding for fibronectin binding proteins,* luks *gene encoding production of Panton Valentine Leukocidin(PVL). Results of current study showed that all isolates 30 (100%) were possess* coa,clfA,spa *and* fnbA *genes, 13 (43.3%) were possess* luks *gene.*

Keyword*: Virulence Factors;* Staphylococcus Aureus; spa *gene;* clfA *gene*

RPM Nº 02-2019.indd 91 4/16/19 09:35