MOLECULAR DETECTION OF ADHESIVE MATRIX MOLECULES FOR *Staphylococcus aureus* ISOLATED FROM DIFFERENT SAMPLES

Falah. A. Jafaar **Nuha. J. Kandala**
Researcher **Prof.**
Department of Biotechnology - College of Sciences - University of Baghdad
falaah.abdulrazaq1206a@sc.uobaghdad.edu.iq

ABSTRACT

The *Staphylococcus aureus* is a ubiquitous Gram-positive bacterium, which may resident in various parts of body as a nqiormal flora, but this bacterium can also cause community and nosocomial infections, which enhanced by different virulence factors. Hence, the goal of the recent work is isolation and identification of *S. aureus* form different sources and investigate the capability of these isolates to synthase microbial surface components recognizing adhesive matrix molecules (MSCRAMs). For achieving this aim, a total of 326 samples were obtained from various clinical sources, including wounds, blood, acne, skin, vagina and gum. Among these samples, only 100 isolates were confirmed as *S. aureus*, after subjecting to conventional identification techniques as well as molecular detection of *nuc* gene utilizing PCR technique. The molecular investigation of *mecA* gene was carried out for confirmation of the methicillin-resistant *S. aureus* (MRSA). The results show 70 isolates were MRSA and the rest of these isolates were regarded as methicillin-sensitive *S. aureus* (MSSA). The investigation of virulence factor presence was carried out by molecular detection of nine genes (*fnbA, fnbB,eno, ebps, fib, bbp,clfA, clfB,* and *cna*), which encode to MSCRAMs of *S. aureus.* The findings revealed that the majority of MRSA isolates harbored *clfA* (52%% of total isolates), followed by *eno, clfB, fib, bbp, fnbA, fnbB, ebps,* and *cna* with (35, 34, 33, 25, 22, 21, 19 and 16%), respectively. This study indicates thatdifferent of MRSA isolates may harbor one or more these genes in different distribution.

Keywords: MRSA, virulence factor genes, MSCRAMs.

التحري الجزيئي عن الجزيئات المصفوفة اللاصقة لعزلات بكتيريا المكورات العنقودية الذهبية السريرية المقاومة للميثيسيلين المعزولة من عينات مختلفة

فلاح عبد الرزاق جعفر **نهى جوزيف قندلا**
باحث **أستاذ**
قسم التقنيات الاحيائية، كلية العلوم،جامعة بغداد، العراق

المستخلص

بكتريا المكورات العنقودية الذهبية هي بكتيريا موجبةلصبغة كرام واسعة الانتشار يمكن ان تتواجد في أجزاء متنوعة من الجسم كبكتريا طبيعية لكنه ممكن لهذه البكتريا ان تسبب عدوى في المجتمع والمستشفياتبوجود عوامل ضراوة مختلفة. لذا هدف هذا البحث هو عزل وتشخيص بكتريا المكورات العنقودية الذهبية من مصادر مختلفة وتحري قدرة هذه العزلات على انتاج مكونات السطح الميكروبية التي تتعرف على جزيئات المصفوفة اللاصقة (MSCRAMs). ولتحقيق هذا الهدف، تم جمع 326 عينة من مصادر سريرية مختلفة شملت الجروح والدم والجلد والمهبل واللثة, 100 عينة شخصت على انها بكتريا المكورات العنقودية الذهبية، اعتمادا على طرقالتشخيص التقليدية بالإضافة الى التحري الجزيئي للمورث*nuc* باستخدام تقنية تفاعل البلمرة المتسلسل. تم الكشف الجزيئي على المورث*mecA*لتأكيد المكورات العنقودية الذهبية المقاومة للميثيسيلين (MRSA). أظهرت النتائج أن مجموع 70 عزلة كانت حاوية على المورث المشفر للمقاومة للميثيسيلين MRSA. تم التحري عن المورثات المشفرة لبعض عوامل الضراوة من خلال الكشف الجزيئي عن تسع مورثات (*fnbA, fnbB,eno, ebps, fib, bbp,clfA, clfB, cna*) والتي تشفر الىMSCRAMs. اظهرت النتائج ان غالبية العزلات المقاومة للميثيسلينMRSA وبكتريا المكورات العنقودية الذهبية الحساسة للميثيسيلين تحمل مورث *clfA* بنسبة 23.6% تتبعه المورثات*eno* و*fib* و *clfB*و*bbp* و*fnbA* و*fnbB* و*ebps* و*cna* بنسب (12.%16 and 19, 21, 22, 25, 33, 34, 35) على التوالي. تشير هذه الدراسة إلى أن عزلات MRSA المختلفة قد تؤوي واحدًا أو أكثر من هذه الجينات في توزيع مختلف.

الكلمات مفتاحية: MRSA, جينات عوامل الضراوة، MSCRAMs.

Received:12/11/2022, Accepted:8/2/2023

INTRODUCTION

The *Staphylococcus aureus* is a ubiquitous Gram-positive bacterium, which may resident in various parts of body, especially skin and mucous membrane, as a human normal flora (8,44). In some cases, this bacterium can cause both community and nosocomial infections, including fatal pneumonia, bacteremia, osteomyelitis, infective endocarditis, mild skin and soft tissue infections (28). Cells of *S. aureus* is arranged in "clusters of grapes" as shown in microscopic field with size (approximately ~0.8µm in diameter). This bacterium can aerobically or anaerobically grow at 37◦C and neutral pH (7.4). On blood agar, β-hemolysis characteristic was observed as a clear zone around their colonies (1, 19). Methicillin resistance *Staphylococcus aureus* (MRSA)is recognized as *S. aureus* strain that possessed multi-drug resistant ability against various types of β-lactam antibiotics, including derivatives of penicillin (oxacillin, methicillin, etc.). This ability can develop via either a natural selection or acquired as a result of horizontal gene transfer (21). At molecular scale, the *mecA* gene is show its responsibility for encode the penicillin-binding protein 2a (PBPa), results in a reduction of binding affinity for β-lactam antibiotics, especially penicillinase-resistant penicillin(29). The MSCRAMMs, which are collection of virulence factors, are the bacterial cell wall-associated proteins, which aid the bacterium for its adherence to extracellular matrix componentsof host, such as bone sialoprotein, vitronectin, von Willebrand factor, elastin, collagen, fibronectin and fibrinogen (66). About twenty various potential MSCRAMMs expressed by *S. aureus* were investigated. These MSCRAMMs, including bone sialoprotein-binding protein Bbp (Zhang et al. 2015),elastin-binding protein EbpS (12), fibrinogen binding protein fib (63), collagen-binding protein(50),fibronectin-binding protein fnbA and fnbB (43), fibrinogen-binding proteins ClfA and ClfB (35), as well as α-enolase, whichfacilitate *S. aureus* for attaching to laminin of blood vessel(33). In addition, these molecules are attached to peptidoglycan via covalent links by enzymatic reaction of sortase (22). The goal of the resent workis to evaluate some virulence genes of *S. aureus* isolated from different clinical locations in order to detect the distribution of different variants of highly divergent gene encoding the MSCRAMMs.

MATERIALS AND METHODS

Collection of clinical specimens: A total of three hundred twenty-six clinical samples were supplied from cases with varied infections, including vagina, acne, nasal cavity, wounds, gums and skin, with ensure gained an approval the College of Science Research Ethics Committee based on (CSEC/0122/0045) during the period from February 2022 to April 2022. All these samples were supplied from AL – Karama Hospital in Baghdad.

Bacterial isolation and identification

All samples were isolated from vagina, acne, nasal cavity, wounds, gums and skin, were plated on varied media, including blood agar, MacConkey and mannitol salt agar plates, then incubated at 37^{o}C for 24h. The morphological and biochemical examination were carried out for identification of the bacterial isolates of *S. aureus* (11). In addition, the molecular diagnosis was carried out using PCR technique for *nuc* gene detection to ensure the genus and species of *S. aureus* (51).

Antimicrobial susceptibility test

The disk diffusion method, as specified in Clinical and Laboratory Standards Institute (CLSI) guidelines, was utilized in order to carry out this test. The antimicrobial discs that utilized in this work were supplied by Himedia/India as antimicrobial disc (symbol, µg/disc):Nitrofurantoin (NIT, 300), Erythromycin (E, 15), Clindamycin (CD, 2), Chloramphenicol (C/30), Gentamycin (GEN, 10), Doxycycline (DO, 30), Ciprofloxacin (CIP, 5), Penicillin-G (P, 10), Tigecycline (TGC, 15), Rifampicin (RIF, 5), Azithromycin (AZM, 15), Linezolid (LZ, 30), Levofloxacin (LE, 5), Tetracycline (TE, 30), Ofloxacin (OFX, 5), Norfloxacin (NX, 10), Trimethoprim-sulfamethoxazole (SXT, 23.75/1.25), Vancomycin (VA, 30) and Oxacillin (OX, 1).The results were represented as sensitive, intermediate and resistant based on (CLSI, 2014, 2022).

Molecular detection of (*nuc* and *mecA*) genes using PCR technique

DNA Extraction: For all isolates, the genomic DNA was extracted using (Geneaid/Korea) kit.

The purity and concentration of extracted genomic DNA were measured and its integrity was estimated using gel electrophoresis method.

Polymerase Chain Reaction (PCR) amplification: The thermal cycler machine was utilized for amplification of all PCR reactions (labenet- USA). The reaction mixture was offered as follows: 12.5µl from the Go Taq®Green Master-Mix provided by (Biolabs-England) that contains Taq DNA polymerase, $MgCl_2$, deoxynucleotides (dNTP), reactions buffer, and two dyes (yellow and green) that allow monitoring of progress throughout electrophoresis, 0.7µl of each (10 pmol) primer, 1µl of template-DNA, and adding sterile D.W to obtain a total volume of 25 microliter. All amplification reactions were carried out in an aseptic laminar air flow cabinet. The negative control reaction had all of the components but no DNA template so that any contaminating DNA in the reaction would be amplified and detected on an agarose gel (Thermo, USA). Molecular detection of (*nuc* and *mecA*) genes utilizing PCR technique In order to conformed the isolates were *S. aureus*, the presence of *nuc* gene was investigated(17). The sequence of *nuc* gene primer and amplicon size of these primers were mentioned as in Table (1), whereas the *nuc* gene amplification steps were listed in Table (2). The detection of *mecA gene* was investigated using PCR reaction for confirmed the MRSA isolates(Zhang et al. 2005). The sequence of primer and the amplicon size of these primers were mentioned in Table (1), while the *mecA* gene amplification steps were listed in Table (2).

Table1. Oligonucleotide primers sequences and amplicon sizes of *nuc* and *mecA* genes

Primer	Sequence (5'-3')	Size of amplicon (bp)
***nuc*–R**	**AGCCAAGCCTTGACGAACTAAAGC**	**279**
***nuc*–F**	**GCGATTGATGGTGATACGGTT**	
***mecA* -R**	**ATGCGCTATAGATTGAAAGGAT**	**147**
***mecA* -F**	**GTGAAGATATACCAAGTGATT**	

Table 2. The PCR analysis program of *nuc* and *mecA* primers

The steps	Temperatures (°C)	Period	No. cycles
Initial denaturation	**95**	**5 minutes**	
Denaturation	**94**	**30second**	
Annealing	**56**	**1minutes**	**30**
Extensions	**72**	**30second**	
Final extensions	**72**	**5 minutes**	

Molecular Detection of Virulence factors

The identification of *S. aureus* virulence factors genes was performed(20), the PCR reaction was carried out using specific primers for the investigation of the genes of MSCRAMMs among 70 MRSA isolates, the sequences and amplicon sizes of primer were supplied as in Table (3) and the program of PCR analysis was mentioned in Table (4).

Table 3. Primer sequences and amplicon sizes of *nuc* and *mecA* genes

Primers	Sequences (5' to 3')	Product size(bp)
Bbp	**F: AACTACATCTAGTACTCAACAACAG-3 R: ATGTGCTTGAATAACACCATCATCT-3**	**574**
Eno	**F: ACGTGCAGCAGCTGACT-3 R: CAACAGCATCTTCAGTACCTTC-3**	**301**
Ebps	**F: CATCCAGAACCAATCGAAGAC-3 R: AGTTACATCATCATGTTTATCTTTTG-3**	**188**
cna	**F: AAAGCGTTGCCTAGTGGAGA-3 R: AGTGCCTTCCCAAACCTTTT-3**	**192**
fnbB	**F: GTAACAGCTAATGGTCGAATTGATACT R: CAAGTTCGATAGGAGTACTATGTTC**	**524**
fnbA	**F: CATAAATTGGGAGCAGCATCA R: ATCAGCAGCTGAATTCCCATT**	**128**
clfB	**R: TTCGCACTGTTTGTGTTTGCAC F: ACATCAGTAATAGTAGGGGCAAC**	**204**
clfA	**F: ATTGGCGTGGCTTCAGTGCT R: CGTTTCTTCCGTAGTTGCATTTG**	**288**
Fib	**F: CTACAACTACAATTGCGTCAACAG R: GCTCTTGTAAGACCATTTTCTTCAC**	**405**

Table 4. The PCR analysis program of virulence factor genes

The steps	Temperatures	Period	No. of cycles
Initial Denaturation	95	5 minutes	
Denaturation	94	30 second	
Annealing	(54,56*,58)	1 minutes	30
Extensions	72	30 second	
Final extensions	72	5 minutes	

*** Optimal annealing range**

RESEULTS AND DISCUSSION

Isolation and Identification of *Staphylococcus aureus:* A 326samples were obtained from AL-Karama hospital in Baghdad, as follows: 52 blood samples, 95 wound swabs, 17 acne swabs, 64 skin, 25 nasal cavity, 32 vaginal swabs, 41 gum. Primarily, all bacterial isolates were subjected to morphological examination, include gram staining, culturing on, blood agarandmannitol salt agarplates. Among these isolates, only one hundred sixty isolates were observed as gram-positive purple coccus populations in clusters with grape-like under light microscopic, given β-hemolysis on blood agar plates as well as shown their tolerance to the high concentration of NaCl, fermentation of mannitol and form yellow colonies on agar. After performing biochemical examinations, one hundred isolates were given catalase-positive, coagulase-positive and oxidase-negative) for ensuring that these isolates were *S. aureus*. Hence, the result of examinationswas revealed that only 100 (30.7%) of isolates were *S. aureus*, represented as follows: 26 blood samples, 39 wound swabs, 8 acne swabs, 7 skin, 5 nasal cavity, 4 vaginal swabs, 11 gum. All these isolates were subjected to further examinations. Various studies were reported that wounds are common source for isolate this bacterium (32,45,29). Despite the fact that most skin infections are self-limiting, they might occasionally serve as a portal for this bacterium to penetrate deeper tissues and the bloodstream. In fact, the most prevalence origin of *S. aureus* bacteremia is skin diseases (68; Yarovoy et al. 2019). Bacteremia, caused by *S. aureus*, is a main event for the onset of sepsis, a systemic inflammatory response to infection (4). Significant in vitro evidence points to a probable pathogenetic role for *S. aureus* in acne vulgaris(42). The rates of *S. aureus* skin colonization are quite low(60), and compared to other bacteria that colonize the skin, the abundance levels are hardly detectable(41). Despite this, 76% of all soft tissue and skin infections are produced by *S. aureus*(38), resulting in ten million nosocomial infections every year (25). The bacteria are typically found in the nasal vestibule of about 35% of people who appear to be in good condition (2). The bacterium can also continue to exist as a typical vaginal resident(58). It has long been known that staphylococci are part of the oral flora. Additionally, a significant number of oral infections (include staphylococcal mucositis, parotitis, angular cheilitis) are caused by these bacteria (34).

Antibiotic susceptibility test

Nineteen antibiotic discs were utilized for evaluation of the antibiotic susceptibility of *S. aureus*, as mentioned in the Table 1. Based on the results which illustrated in Figure 1.,all examined *S. aureus* isolates were sensitive to nitrofurantoin NIT, linezolid LZ and tigecycline TGC, but they were resistant to penicillin G. In addition, the majority of these isolates were sensitive to most of antibiotics (Chloramphenicol C, rifampicin RIF, gentamycin GEN, ciprofloxacin CIP, Trimethoprim-sulfamethoxazole SXT, clindamycin CD, ofloxacin OFX, norfloxacin NX, doxycycline DO and vancomycin with 88, 88, 85, 81, 77, 77, 75, 74, 67%, 96%, respectively). Conversely, 53% and 57% of these isolates were resistant to erythromycin E and azithromycin AZM. A 68% of isolates were intermediate against oxacillin *Staphylococcus aureus* has developed resistance to many antibiotics as a result of the determinants acquisition by the mobile genetic elements and the horizontal transfer of genes (30). *S. aureus* released a β-lactamase that breakdown the β-lactam bond and destroys the activity of β-lactam antibiotics, including penicillin (18).Gentamicin was first employed in the 1970s to treat major *S. aureus*-caused hospitalized diseases; however, its application was hampered by the appearance of great resistances that was encoded by mobile genetic elements. Tigecycline is significantly more

effective than tetracycline and is still effective against tetracycline-resistant bacteria(18). When treating S. aureus bacteremia or endocarditis, rifampicin has been suggested as an adjuvant treatment to be used in conjunction with a glycopeptides or beta-lactam (in MSSA)(56). In the clinic, semisynthetic macrolides, including telithromycin, clarithromycin, and azithromycin, are utilized for treatment of infectious diseases caused by bacteria other than *S. aureus*(18).Overexpression of chromosomally encoded efflux pumps is a common mechanism by which clinical isolates acquire resistances to fluoroquinolones such as norfloxacin and ciprofloxacin (27).

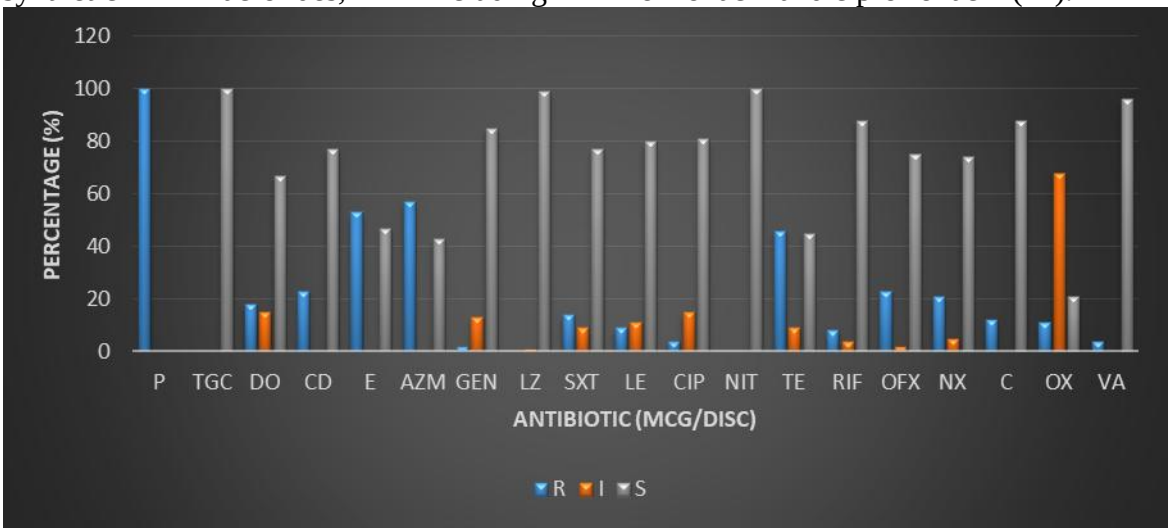

Figure 1. The antibiotic susceptibility testing for the examined isolates

Although linezolid resistance is still fairly uncommon in clinical isolates, MRSA, including the pandemic ST22-MRSA-IV clone, has been shown to acquire plasmids that encode the resistance determinant of Cfr(59).Linezolid's binding site is highly similar to that of chloramphenicol and probably other drugs with similar structures(67). In the case of conjunctivitis, chloramphenicol is applied topically only. In patients with penicillin hypersensitivity or infections caused by MSSA, clindamycin is an essential treatment agent (55). It is becoming increasingly apparent that SXT, one of many understudied alternatives for MRSA decolonization, may be useful in reducing MRSA infections in some situations. In the case of less severe, community-acquired infections, the scant evidence on its treatment efficacy is promising. It's possible that SXT can be utilized as an alternative, low-cost weapon in the fight against MRSA. Additional clinical trials are necessary to clarify its efficacy against this rapidly evolving pathogen(48). In comparison between the phenotypic and genotypic detection of methicillin-resistance ability of isolates in the current study, only 11 (15.7%) isolates from 70 (100%) of MRSA were resistant to oxacillin, while the rest of these isolates were intermediate. Proper patient therapy and application of institutional programs to recognize and manage MRSA outbreaks and cross-infection rely on precise and fast detection of MRSA isolates(10). Despite carrying the *mecA* gene, the vast majority of hospital-acquired MRSA strains exhibit homogenous methicillin resistance.(53,13).Some strains of NMDR MRSA have troublesome borderline MICs and show a great heterogeneous resistance to methicillin/oxacillin *in vitro*. A minor, rapid diagnostic test that could reveal the expression of oxacillin/methicillin resistances in MRSA would greatly facilitate patients in the selection of efficient antibiotic therapy plans and the monitoring of infectious disease outbreaks(13).

Molecular identification and characterization of *S. aureus:* After the morphological and biochemical examinations of *S. aureus*, further tests required to confirmed these isolates were *S. aureus*. In this context, PCR technique was utilized for detection of *nuc* gene which is present only in this species. One of the most distinctive and useful traits that could be used to distinguish*es. aureus* from other

staphylococcus spp. is the production of an extracellular thermostable nuclease, encoded by the *nuc* gene, which is an effective marker gene for identifying *S. aureus* by PCR (Zhang et al. 2004). The results revealed that all isolates were carried this gene, which in turn ensure that these isolates were *S. aureus*, as in following Figure (2). It was reported that all *S. aureus* isolates harbored *nuc* gene 70).

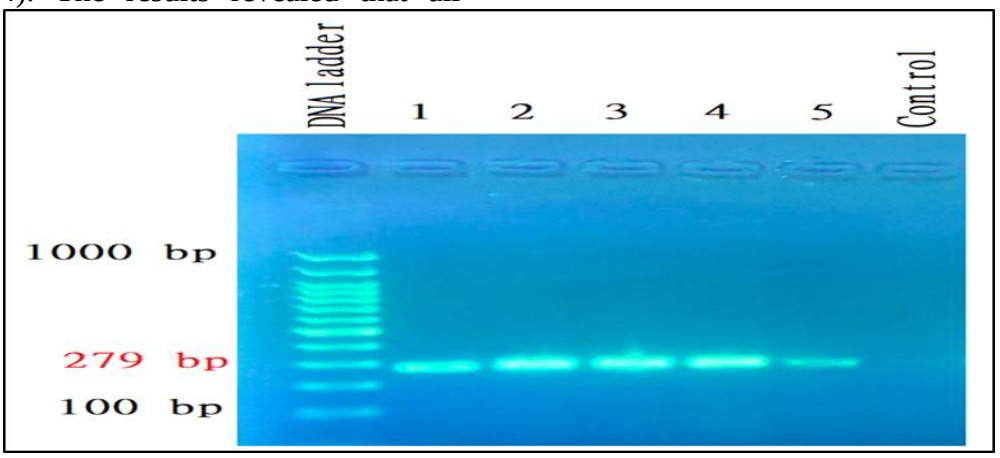

Figure 2. Gel electrophoresis in agarose for (279 bp) for *nuc* gene amplification using PCR among *S. aureus* isolates in in agarose (2%), for 90minutes under 70 volts

Molecular detection of *mecA* gene for identification of MRSA: The best detection for accurate identification of MRSA is detection of methicillin resistant gene (*mecA*) in the examined isolates using PCR with certain primer sequences as listed in Table (2). Among 100 isolates of *S. aureus*, 70 isolates (70%) were MRSA (*mecA* positive) while 30 isolates (30%) were MSSA (*mecA* negative) (Figure -3). This finding is agreed with (54)who reported that *mecA* gene were presented in 98.7% of MRSA. Hence, any *S. aureus* possessed this gene, indicated as MRSA. β-lactam resistance was observed due to existence of *mecA* gene according to (7) who reported that this gene existed in all MRSA, which cause the resistance of bacteria by encode to production of protein-binding proteins (PBPs) which have low affinity for β-lactams. In addition, it was reported that all *S. aureus* contain *mecA* gene (2).

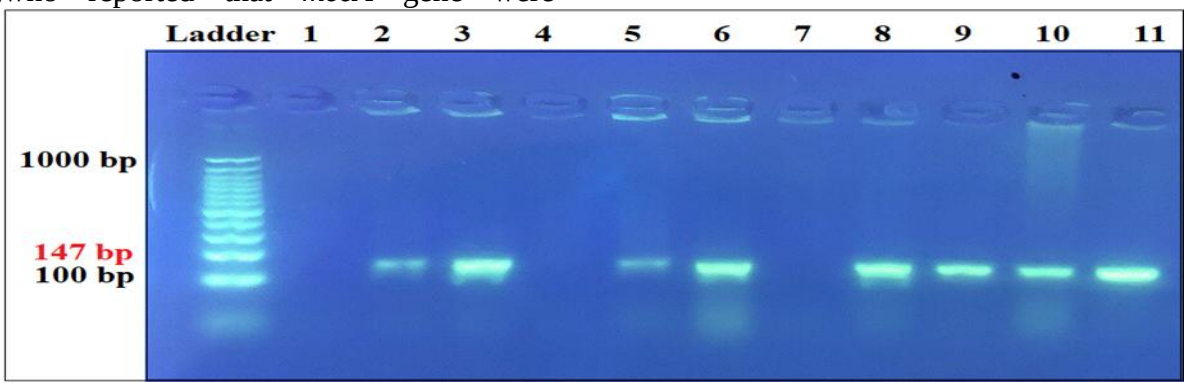

Figure 3. Gel electrophoresis for *mecA* gene (147bp) amplification using PCR among isolates of *S. aureus* in agarose (2%) for 90minutes under 70 volts. Lane (1): negative control, lanes (2,3,5,6,8,9,10 and 11): isolates with positive of *mecA* gene and lanes (4,7): isolates with negative result of *mecA* gene

Molecular analysis of virulence factors

According to the findings of this study as showed in Table (5) and figures (4,5,6,7,8,9,10), the most prevalent virulence genes from MRSA isolates detected was *clfA*(52%), followed by *eno*, *clfB*,*fib*, *bbp*, *fnbA*, *fnbB*, *ebps*, and *cna* with (35, 34, 33, 25, 22, 21, 19 and 16%), respectively (figure4. there are variations in distribution of virulence factor genes among the isolates of MRSA,one wound isolate among 70 MRSA isolates were positive to all virulence genes (*fnbA*, *fnbB*,*eno*, *ebps*, *fib*, *bbp*,*clfA*, *clfB*, and *cna*), while two isolates (from wound and blood) harbored eight of these genes., but one acne isolate harbored seven of these genes. whereas 8

isolates showed negative results among all virulence factor genes. *S. aureus* can attach and colonized with existence of the clumping factor. Several researches were reported the prevalence of these clumping factors in *S. aureus* which isolated from various sites. Klein et al. revealed 91.8% of bacteria contain *clfB* (31). Whereas Atshan *et al*. detected *clfA, B* genes in all strains(6). Furthermore, it was observed that *clfA* gene presented in approximately 20% of mastitis-causing *S. aureus*(37). In addition, Ghasemian *et al.* reported that regardless of where an infection may have occurred, every MRSA strain possessed *clfA and clfB.* Indeed, *clfAB* and *fnbAB* genes are common in all *S. aureus* which isolated from Sepsis and the strong-biofilm producing bacteria. The *fib* gene was observed in all strains of *S. aureus*(9). Both of *eno* and *can* genes be prevalent in bacteria with 82% and 63%, respectively(20). Another study showed that *cna* gene present in 78.4% of isolates (40) and in 46% of isolates(5). By contrast, only 9% of bacteria (isolated from catheters and blood infection) shows the presence of *bbp* gene. The most common virulence genes were *ica*, *ebps*, *bbp*, *cna*, *clfB* and *clfA*, in isolates of *S. aureus* that related with catheter(74). Only one isolate was harbored *bbp* as what reported by (62). It has been shown that the infection source can have a major impact in determination of the patterns and expressions of the most important types of genes required for *S. aureus* attachment (20).

Table 5. The distribution ofthe studied virulence factors in MRSA isolates

Gene Source	*clfA*	*clfB*	*fnbA*	*fnbB*	*ebps*	*fib*	*bbP*	*cna*	*Eno*
Wound	**23**	**17**	**9**	**14**	**10**	**19**	**17**	**6**	**16**
Blood	**14**	**11**	**5**	**6**	**3**	**8**	**4**	**6**	**11**
Acne	**5**	**1**	**4**	**1**	**1**	**3**	**2**	**2**	**3**
Skin	**1**	**2**	**2**	**-**	**2**	**1**	**2**	**2**	**2**
Nasal Cavity	**3**	**3**	**2**	**-**	**3**	**2**	**-**	**-**	**2**
Vagina	**2**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**1**
Gum	**4**	**-**	**-**	**-**	**-**	**-**	**-**	**-**	**-**
Total No. (%)	**52**	**34**	**22**	**21**	**19**	**33**	**25**	**16**	**35**

70 samples

The initial stage in any infection is for the pathogen to attach to components of the host surface (like epidermal keratinocytes, fibrinogen or fibronectin). The virulence proteins of *S. aureus* attach to collagen and bone matrix with the facilities of a group of wall-anchored adhesins of staphylococcal cell, known as MSCRAMMs(14). Staphylococci stick to the extracellular matrix of host via MSCRAMMs on the surface of *S. aureus*(66). *Staphylococcus aureus* adheres to fibrinogen, elastin, and fibronectin (Fn) via FnBPA and B. By adhering to Fn in the extracellular matrix of the tissues, FnBPs aid *S. aureus* in colonizing and infecting the host. The bacterium *S. aureus* adheres to and then invades a broad ranges of mammalian cells, like keratinocytes (36),osteoblasts (3), endothelial cells (52), fibroblasts (65) and epithelial cells (16). In addition, it is well established that the attaching of *S. aureus* to platelets is a key event in the progression of endocarditis infection. There are two distinct phases to this procedure. In the first step, staphylococci engage with resting platelets, activating the integrin GPIIb/IIIa to stimulate intracellular signaling. Furthermore, vegetations and thrombi on the heart valve's surfaces are formed in part by active platelets aggregating together. From the exponential phase of growth onward, the FnBPs expressed on the Staphylococcal surfaces are the primary platelet activating factors(61). It is hypothesized that FnBPs cause biofilm development by facilitating the creation of many low-affinity, hemophilic, Zn^{2+}-dependent connections between the A domains of FnBPB or FnBPA on neighboring cells(24). It has been reported that part A of FnBPs appears to be a fruitful target for the development of staphylococcal vaccines due to its strong immunogenicity of this part. In addition, role of these FnBPs in several diseases, include infective endocarditis,

pneumonia, bacteremia and Sepsis, has been reported in different studies (61). Figure (4) show both of *fnbA* and *fnbB* genes in several *S. aureus* isolates.

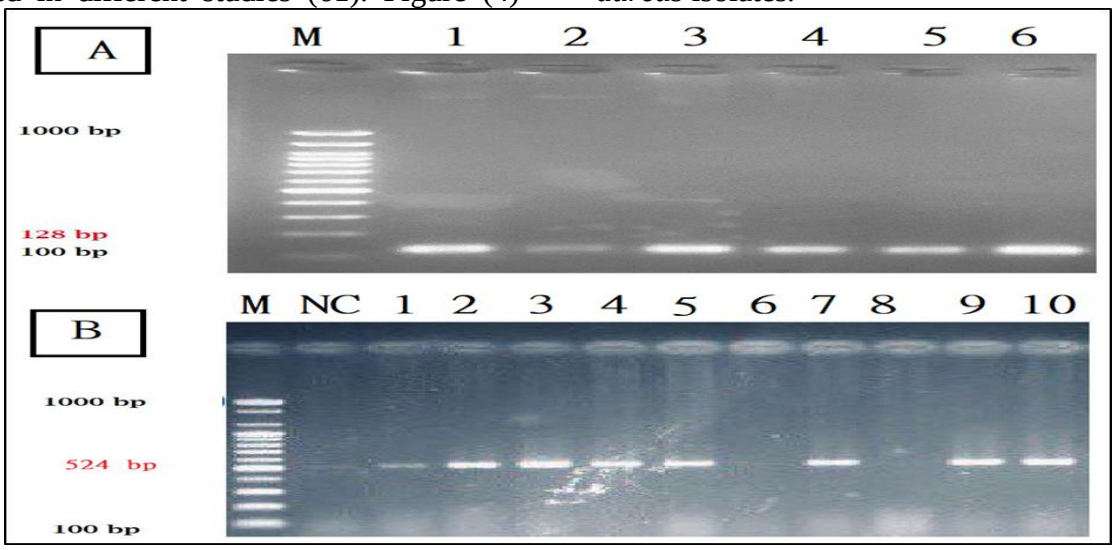

Figure 4. Gel electrophoresis for genes amplification utilizing PCR among isolates of *S. aureus* in agarose (2%), for 90minutes under 70 volts. A- M: ladder of DNA, NC: Negative control, lanes (1-5) positive result for *fnbA gene* (128 bp) bands B-M: ladder of DNA, NC: Negative control, Lane (6 and 8):isolates show negative result for *fnbB*, lanes (1,2,3,4,5,8,9,10): isolates show positive result of *fnbB gene*(524 bp) bands

The MSCRAMMs, ClfA and ClfB are two proteins from *S. aureus*(35). Clumping factor A is an essential virulence factor in several infection models, including rat experimental endocarditis, murine sepsis and septic arthritis and rabbit infective endocarditis. It is possible that ClfA acts as a virulence factor in certain infections by inhibiting phagocytosis, as well as promoting attachment to fibrin and fibrinogen. It appears that being coated with either immunoglobulin or fibrinogen, or a mixture of both, is sufficient to impede phagocytosis (26). By binding host ligands cytokeratin 10 and loricrin in the nasal mucosa, ClfB was thought to play a key role in enabling *S. aureus* nasal colonization(39). The figure 5 include *clfA* and *clfB* genes among *S. aureus* isolates.

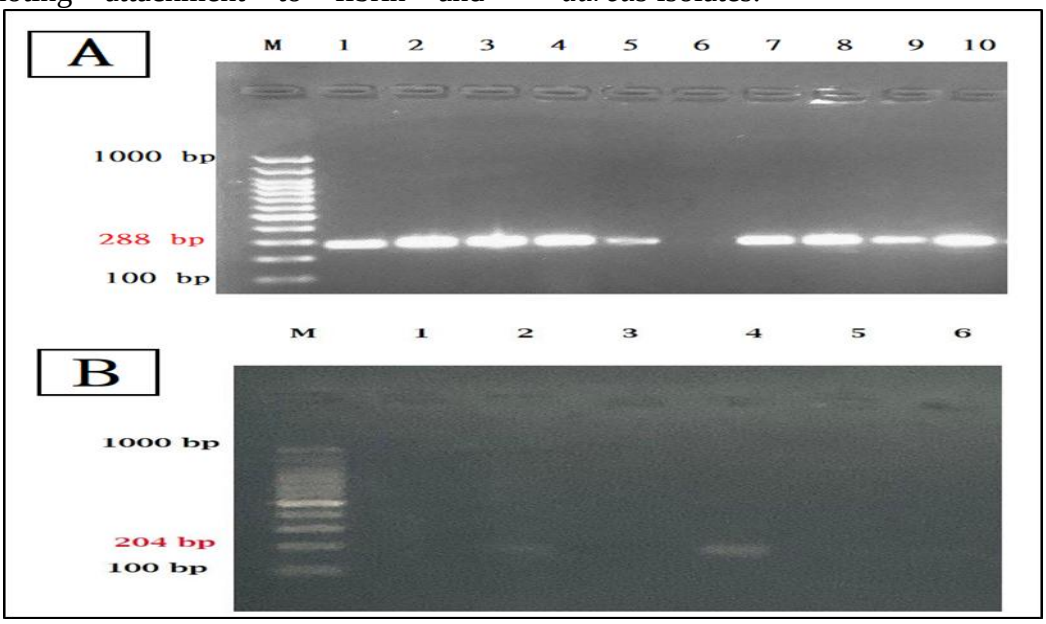

Figure 5.Gel electrophoresis in agarose for gene amplification utilizing PCR among isolates of *S. aureus* in agarose (2%), for 90minutes under 70 volts. A-M: ladder of DNA, NC: Negative control, lanes (1-5): *clfA* (288 bp) bands, lane (6): isolate negative result for *clfA*, B- M: ladder of DNA, NC: Negative control, lanes (2,4) isolates harbored *clfB* gene, (1,3,5): isolate not harbored *clfB gene*

Moreover, elastin-binding protein (EbpS), on surface of *S. aureus*, attached into elastin, which is an important structural protein whose primary physiological role is to confer the property of reversible elasticity to tissues and organs(49). Laminin-binding proteins are important bacterial adhesins, since this protein is located in all tissue's types as networks, conferring a greatly cross-linked property to ECM.S. aureus exhibits elastin-mediated adhesion, which is mediated by fibronectin-binding protein A (FnBPA) and elastin-binding protein S (EbpS)(57). In Figure 6, some of *S. aureus* harbored *ebps* genes.

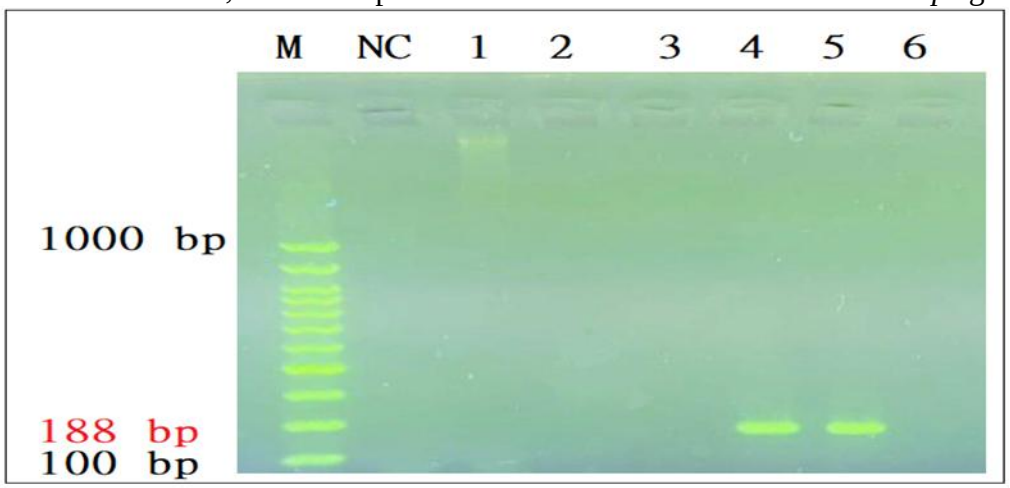

Figure 6. Gel electrophoresis in agarose for (188bp) of *ebps* gene amplification using PCR among isolates of *S. aureus* .NC: negative control, lanes (4,5): positive result ; lanes (1,2,3,6): negative result(no band).

α-enolase is a multifunctional protein that serves as crucial glycolytic enzymes in the cytoplasm of both eukaryotes and prokaryotes. Multiple cell types express alpha-enolase, which serves as a receptor for plasminogen and thereby concentrates proteolytic plasmin action at the cell surface. While their roles as plasminogen receptors and glycolytic enzymes are well-established, α-enolase also possess other cellular activities and subcellular localizations. In addition, a variety of diseases, including rheumatoid arthritis, Alzheimer's and cancer, have been linked to a-enolase expression differences(15). alpha-enolases are plasminogen- and laminin-binding proteins in *Staphylococcus aureus*(33). In this study, several isolates harbored *eno* gene was investigated using PCR as shown in Figure

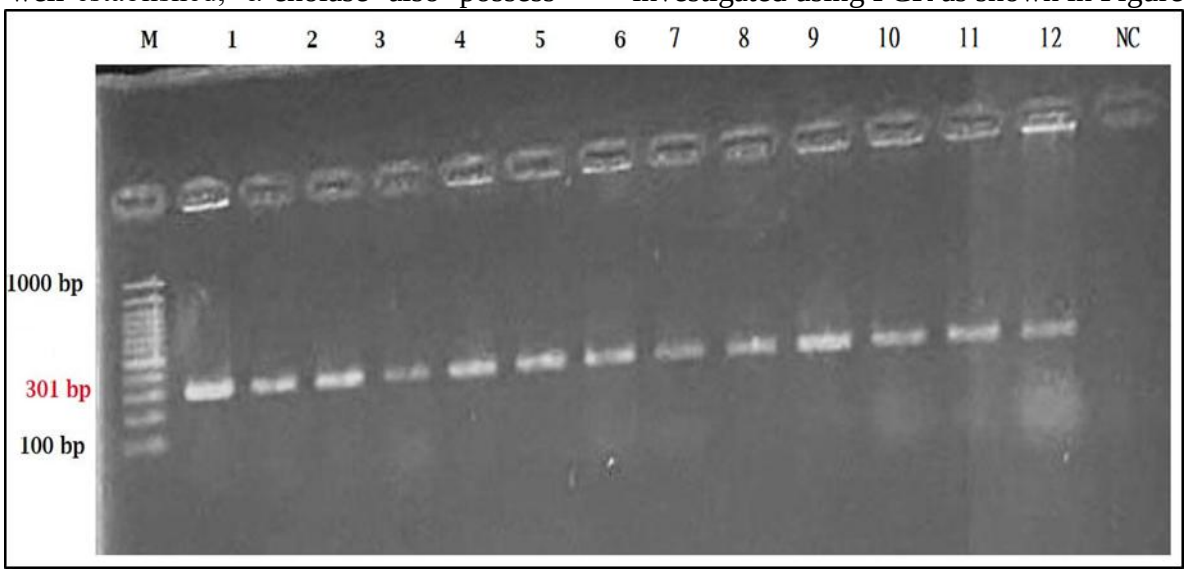

Figure 7. Gel electrophoresis in agarose for (301 bp) of *eno* gene amplification using PCR among isolates of *S. aureus* in agarose (2%), for 90minutes under 70 volts.M: ladder of DNA, NC: negative control, lanes (1-12): positive result of *S. aureus isolates*

Moreover, the collagen (Cn)-binding protein Cna is a prototype of the MSCRAMMs that has an essential function in staphylococcal pathogenesis, both as an immune evasion

factor and as adherence factor. Septic arthritis has been shown to have cna as a virulence factor, and the degree of adhesion to collagen is correlated with the development of the disease. In addition, Cna binds to complement protein C1q and block the classical pathway of complement fixation(23).*can* genes be investigated in the examined isolates of *S. aureus* in this study as observed in the figure (8).

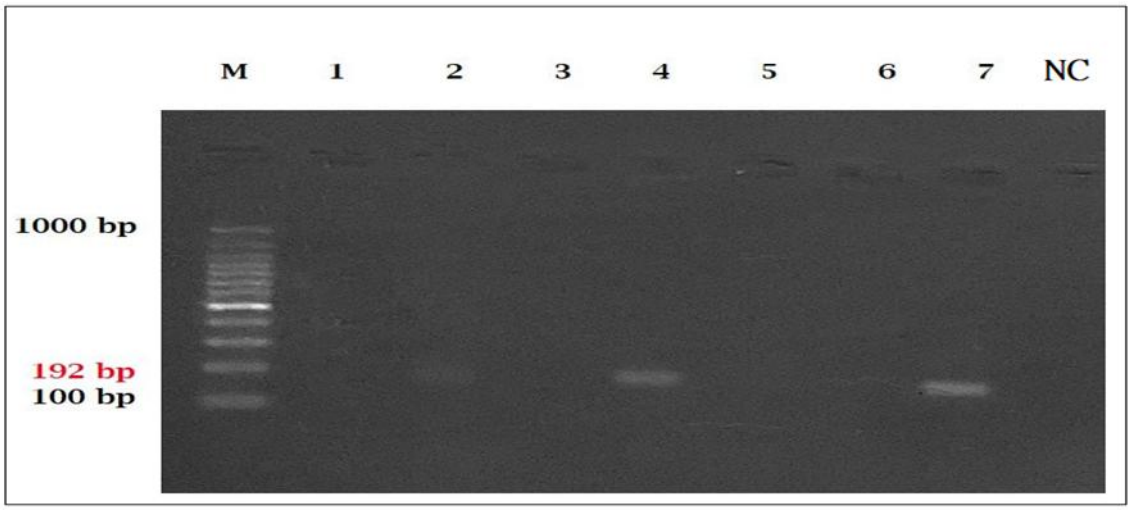

Figure 8. Gel electrophoresis in agarose for (192bp) of *cna* gene amplification using PCR among isolates of *S. aureus*in agarose (2%), for 90minutes under 70 volts. M: ladder of DNA, NC: negative control, lanes (4,7): positive result (bands of *can* genes), lanes (1,2,3,5,6): negative result (no *cna* band).

During vascular trauma, different enzymes stimulate clotting of blood through a cascade reaction which converts fibrinogen to fibrin. *S. aureus* interacts with fibrinogen in several ways, producing different proteinous components that attach specifically to this 340-kDa plasma protein. Several different fibrinogen-binding proteins (fibs) from *S. aureus* are synthase. Cell surface-related proteins, the clumping factors of 92 kDa, are the major mediators of adherence to fibrin (46). The figure (9) shows the *fib* genes in *S. aureus* isolates in the current study.

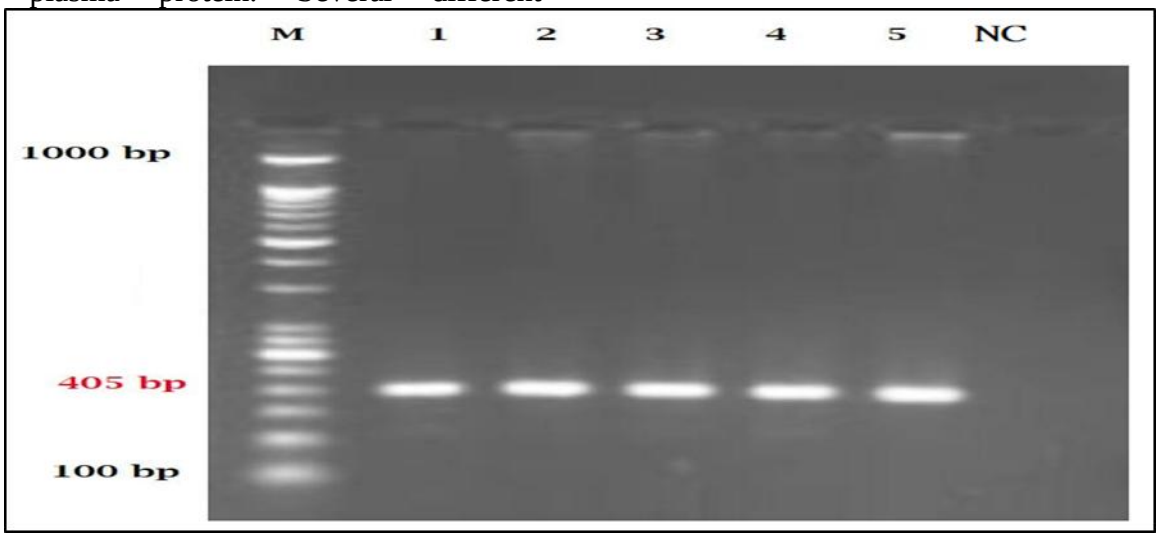

Figure 9. Gel electrophoresis in agarose for (405bp) of *fib* gene amplification using PCR among isolates of *S. aureus* in agarose (2%), for 90minutes under 70 volts. M: ladderof DNA, NC: negative control, lanes (1-5): positive result (bands of *fib* gene).

Additionally, it has been reported the specific interaction between bone sialoprotein-binding protein (BbP), an extracellular matrix (ECM) glycoprotein of 59 000 Da, found only in bone and dentine and *S. aureus* cells. This interaction is may related with the understanding of pathogenetic mechanisms in osteomyelitis. This is explained by some facts: (i) The S. aureus strains that cause infections in bone and joint tissue primarily bind

selectively to BbP; and (ii) particularly high concentrations of BSP are found in the osteoid, the newly formed bone tissue of growing bone, which is considered as the most prevalent location for osteomyelitis. S. aureus cell-surface-associated proteins that interact with ECM components are structurally and organizationally similar to the Bbp. FnBPA/B, ClfB, ClfA and Can, are all examples(64). The figure 10 show *bps* genes of *S. aureus* in this study.

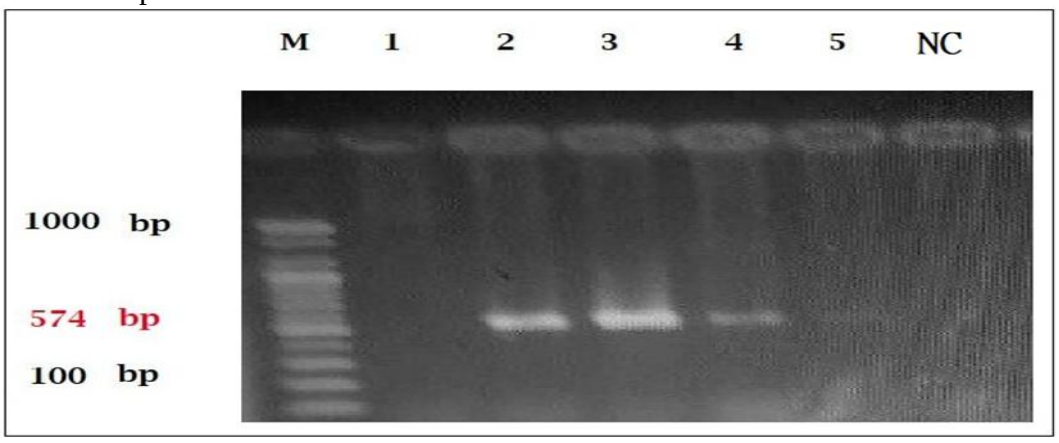

Figure 10. Gel electrophoresis in agarose for (188bp) of *bps* gene amplification using PCR among isolates of *S. aureus* in (agarose (2%), for 90minutes under 70 volts. M: ladderof DNA, NC: negative control, lanes (2,3,4): positive result (bands of *bps* gene), lanes (1,4): negative result (no band for *bps*).

The current study concluded that the majority of *S. aureus* was MRSA. The prevalence of *clfA* gene was higher than others genes. Moreover, different of MRSA isolates may harbor one or more these genes in different distribution.

REFERENCES

1. Al-Hayali, O.Z., M.F. Al Marjani, and A., Maleki, 2024. Eradication of heterogeneous vancomycin intermediate *Staphylococcus aureus* (hVISA) using *Peganum harmala* L. seeds extracts. Iraqi Journal of Agricultural Sciences, 55(2):757-768.
DOI: https://doi.org/10.36103/fg0kcs57

2. Adesida, S.A. 2007. Associated risk factors and pulsed field gel electrophoresis of nasal isolates of *Staphylococcus aureus* from medical students in a tertiary hospital in Lagos, Nigeria. Brazilian Journal *of* Infectious Diseases 11, 63–69.
DOI: 10.1590/s1413-86702007000100016.

3. Ahmed, S.,S, S., Meghji. R.J, Williams, B. Henderson, , J. H. Brock, and S.P. Nair, 2001. *Staphylococcusaureus* fibronectin binding proteins are essential for internalization by osteoblasts but do not account for differences in intracellular levels of bacteria. Infection and immunity 69(5), 2872–2877.
DOI: 10.1128/IAI.69.5.2872-2877.2001.

4. Angus, D.C. and T. van der Poll, 2013. Severe sepsis and septic shock. *N Engl J Med* 369, 840–851.DOI: 10.1056/NEJMra1208623

5. Arciola, C.R., D, Campoccia.S.Gamberini, L. Baldassarri, and L. Montanaro, 2005. Prevalence of cnafnbA and fnbB adhesin genes among *Staphylococcus aureus* isolates from orthopedic infections associated to different types of implant. *FEMS* microbiology letters 246(1), 81–86.
DOI: 10.1016/j.femsle.2005.03.035

6. Atshan, S.S. 2012. Prevalence of adhesion and regulation of biofilm-related genes in different clones of *Staphylococcus aureus.* Journal of BioMedicine and Biotechnology,30(4),155-158.
DOI: 10.1155/2012/976972

7. Becker, R. 2018. *The new S language*. Crs Press.33(3),134-145. URL:
https://2u.pw/6Dfn17Pg

8. Bien, J.O.Sokolova. and B. Bozko. 2011. Characterization of virulence factors of *Staphylococcus aureus*: novel function of known virulence factors that are implicated in activation of airway epithelial proinflammatory response. Journal of Pathogens 2011. DOI: 10.4061/2011/601905

9. BodénWästfelt, M.K. and J.-I, Flock. 1995. Incidence of the highly conserved fib gene and expression of the fibrinogen-binding (Fib) protein among clinical isolates of

Staphylococcus aureus.Journal of Clinical Microbiology 33(9), 2347–2352.
DOI: 10.1128/jcm.33.9.2347-2352.1995
10. Boyce, J. M. 1998. Are the epidemiology and microbiology of methicillin-resistant *Staphylococcus aureus* changing? *Jama* 279(8), 623–624.
DOI: 10.1001/jama.279.8.623
11. Brakstad, O. G., K, Aasbakk,. and J. A. Maeland, 1992. Detection of *Staphylococcus aureus* by polymerase chain reaction amplification of the nuc gene. Journal of Clinical Microbiology 30(7), 1654–1660.DOI: 10.1128/jcm.30.7.1654-1660.1992
12. Campoccia, D. L. Montanaro. S. Ravaioli, I. Cangini, P. Speziale, P and C.R.Arciola, 2009. Description of a new group of variants of the *Staphylococcus aureus* elastin-binding protein that lacks an entire DNA segment of 180 bp. The International Journal of Artificial Organs 32(9), 621–629.
DOI: 10.1177/039139880903200911
13. Chambers, H. F. 1997. Methicillin resistance in staphylococci: molecular and biochemical basis and clinical implications. Clinical microbiology reviews 10(4), 781–791.DOI: 10.1128/CMR.10.4.781
14. Cunningham, R., A, Cockayne,. and H. Humphreys, 1996. Clinical and molecular aspects of the pathogenesis of *Staphylococcus aureus* bone and joint infections. Journal of medical microbiology 44(3), 157–164.
DOI: 10.1099/00222615-44-3-157
15. Díaz-Ramos, À., A, Roig-Borrellas., A. García-Melero, and R. López-Alemany, 2012. α-Enolase, a multifunctional protein: its role on pathophysiological situations. Journal of Biomedicine and Biotechnology 2012.
DOI: 10.1155/2012/156795
16. Dziewanowska, K., J.M, Patti. K.W. C.F.Deobald, Bayles, W.R. G.A. Trumble, and Bohach, 1999. Fibronectin binding protein and host cell tyrosine kinase are required for internalization of *Staphylococcus aureus* by epithelial cells. Infection and immunity 67(9), 4673–4678.
DOI: 10.1128/IAI.67.9.4673-4678.1999
17. Eliezer, D., S. S. M. Townsend, P. J Sawyer, B. Major, and W.B Mendes, 2011. System-justifying beliefs moderate the relationship between perceived discrimination and resting blood pressure. *Social Cognition* 29(3), 303–321.
DOI: https://doi.org/10.1521/soco.2011.29.3.303
18. Foster, T.J. 2017. Antibiotic resistance in *Staphylococcus aureus.* Current status and future prospects. FEMS Microbiology Reviews 41(3), 430–449.
DOI: 10.1093/femsre/fux007
19. Gardete, S. and A, Tomasz,. 2014. Mechanisms of vancomycin resistance in *Staphylococcus aureus*. The Journal of Clinical Investigation 124(7), 2836–2840.
DOI: 10.1172/JCI68834
20. Ghasemian, A., S.N.Peerayeh, B. Bakhshi, and M. Mirzaee, 2015. The microbial surface components recognizing adhesive matrix molecules (Mscramms) genes among clinical isolates of *Staphylococcus aureus* from hospitalized children. Iranian journal of pathology 10(4), 258. URL: https://pubmed.ncbi.nlm.nih.gov/26351495/
21. Gurusamy.K.S., R. Koti.,C.D. Toon, P, Wilson. and B. R. Davidson, 2013. Antibiotic therapy for the treatment of methicillin-resistant *Staphylococcus aureus* (Mrsa) infections in surgical wounds. *Cochrane Database of Systematic Reviews* (8).
DOI: 10.1002/14651858.CD009726.pub2
22. Heilmann, C. 2011. Adhesion mechanisms of staphylococci. *Bacterial adhesion* , 105–123.DOI: 10.1007/978-94-007-0940-9_7
23. Herman-Bausier, 2016. Mechanical strength and inhibition of the *Staphylococcus aureus* collagen-binding protein Cna. *Mbio* 7(5), 1529-16.DOI: 10.1128/mBio.01529-16
24. Herman-Bausier, P.S. El-Kirat-Chatel,. T. J. Foster, J. A. Geoghegan, and Y.F. Dufrêne, 2015. *Staphylococcus aureus* fibronectin-binding protein A mediates cell-cell adhesion through low-affinity homophilic bonds. MBio 6(3), 413-15.DOI: 10.1128/mBio.00413-15
25. Hersh, A. L., H. F. Chambers, J. H. Maselli, and R. Gonzales, 2008. National trends in ambulatory visits and antibiotic prescribing for skin and soft-tissue infections. Archives of Internal Medicine 168(14), 1585–1591.
DOI: 10.1001/archinte.168.14.1585
26. Higgins, J., A. Loughman., van K.P.M. Kessel, J.A.G. van Strijp, and T.J. Foster, 2006. Clumping factor A of *Staphylococcus aureus* inhibits phagocytosis by human

polymorphonuclear leucocytes. *FEMS* microbiology letters 258(2), 290–296.
DOI: 10.1111/j.1574-6968.2006.00229.x
27. Hooper, D.C. and G.A. Jacoby, 2015. Mechanisms of drug resistance: quinolone resistance. Annals of theNew York Academy of Sciences 1354(1), 12–31.
DOI: 10.1111/nyas.12830
28. Humphreys, H. 2012. *Staphylococcus aureus*: the enduring pathogen in surgery. the surgeon 10(6), 357–360.
DOI: 10.1016/j.surge.2012.05.003
29. Jabur, E.Q. and N. Kandala, 2022. The production of biofilm from methicillin *resistant Staphylococcus*aureus isolated from post-surgical operation inflammation. Iraqi Journal of Science , 3688–3702.
DOI: https://doi.org/10.24996/ijs.2022.63.9.3
30. Jensen, S.O. and B.R. Lyon, 2009. Genetics of antimicrobial resistance in *Staphylococcus aureus*. Future microbiology 4(5), 565–582.DOI: 10.2217/fmb.09.30
31. Klein, R.C., M. H. Fabres-Klein, Brito, M.A.V.P., L.G. Fietto, and A. Ribon, de O.B. 2012. *Staphylococcus aureus* of bovine origin: genetic diversity, prevalence and the expression of adhesin-encoding genes. Veterinary microbiology 160(1–2), 183–188.DOI: 10.1016/j.vetmic.2012.05.025
32. Koosha, R.Z., E.M., Hosseini,Aghdam, S.G. Tajandareh, and A.A.I. Fooladi, 2016. Distribution of tsst-1 and mecA genes in *Staphylococcus aureus* isolated from clinical specimens. Jundishapur Journal of Microbiology 9(3). DOI: 10.5812/jjm.29057
33. Kot, B., H. I. Sytykiewicz,Sprawka, and Witeska, M. 2020. Effect of manuka honey on biofilm-associated genes expression during methicillin-resistant *Staphylococcus aureus* biofilm formation. Scientific reports 10(1), 1–12. DOI: 10.1038/s41598-020-70666-y
34. McCormack, M.G., A.J. Smith, A.N., M., AkramJackson,D. Robertson, and G.Edwards, 2015. *Staphylococcus aureus* and the oral cavity: an overlooked source of carriage and infection? American Journal of Infection Control 43(1), 35–37.
DOI: 10.1016/j.ajic.2014.09.015
35. McDevitt, D., P. Francois, P. Vaudaux, .and T.J. Foster, 1994. Molecular characterization of the clumping factor (fibrinogen receptor) of *Staphylococcus aureus*. Molecular microbiology 11(2), 237–248.
DOI: 10.1111/j.1365-2958.1994.tb00304.x
36. Mempel, M. *et al.* 2002. Invasion of human keratinocytes by *Staphylococcus aureus* and intracellular bacterial persistence represent haemolysin-independent virulence mechanisms that are followed by features of necrotic and apoptotic keratinocyte cell death. British Journal of Dermatology 146(6), 943–951.DOI: 10.1046/j.1365-2133.2002.04752.x
37. Momtaz, H., E. Rahimi, and E. Tajbakhsh, 2010. Detection of some virulence factors in *Staphylococcus aureus* isolated from clinical and subclinical bovine mastitis in Iran. African Journal of Biotechnology 9(25), 3753–3758.
DOI:10.5897/AJB2010.000-3243
38. Moran, G.J., A. Krishnadasan, R.J. Gorwitz, G.E.,Fosheim, L.K., McDougal, R.B. Carey, and D.A. Talan, 2006. Methicillin-resistant *S. aureus* infections among patients in the emergency department. *New* England Journal of Medicine 355(7), 666–674.DOI: 10.1056/NEJMoa055356
39. Mulcahy, M.E., J.A. Geoghegan, I.R., Monk, K.M., O'Keeffe, E.J, Walsh, T.J. Foster, and R.M. McLoughlin, 2012. Nasal colonisation by *Staphylococcus aureus* depends upon clumping factor B binding to the squamous epithelial cell envelope protein loricrin. PLoS pathogens 8(12), 1003092.DOI: 10.1371/journal.ppat.1003092
40. Nizami, D., D. Yunus, O., Burcin, D. Cemil, and K., Aydiner, 2010. Detection of adhesin genes and slime production among staphylococci in orthopaedic surgical wounds. African Journal of Microbiology Research 4(9), 708–715. URL: https://2u.pw/1TEFZVOt
41. Oh, J., A.L., Byrd, M., Park, H.H, Kong, J.A, Segre, and N.C.S., Program, 2016. Temporal stability of the human skin microbiome. Cell 165(4), 854–866.
DOI: 10.1016/j.cell.2016.04.008
42. Onanuga, A. and T.C. Temedie, 2011. Multidrug-resistant intestinal *Staphylococcus aureus* among self-medicated healthy adults in Amassoma, South-South, Nigeria. Journal of health, population, and Nutrition 29(5), 446.DOI: 10.3329/jhpn.v29i5.8898
43. O'Neill, E., H. Humphreys, and J. P. O'Gara, 2009. Carriage of both the *fnbA* and *fnbB* genes and growth at 37 C promote FnBP-

mediated biofilm development in meticillin-resistant *Staphylococcus aureus* clinical isolates. Journal of Medical Microbiology 58(4), 399–402.
DOI: 10.1099/jmm.0.005504-0
44. Organization, W. H. 2019. Antimicrobial Resistance: Grlobal Report on Surveillance. Geneva: World Health Organization; 2014. Google Scholar, 232. URL: https://www.who.int/publications/b/31459
45. Osinupebi, O.A. *et al*. 2018. Prevalence of methicillin-resistant *Staphylococcus aureus* in Abeokuta, Nigeria. South Asian Journal of Research in Microbiology 1(1), 1–8.
DOI: 10.9734/SAJRM/2018/39801
46. Palma, M., S., Nozohoor, T. Schennings, A. Heimdahl, and J.I. Flock, 1996. Lack of the extracellular 19-kilodalton fibrinogen-binding protein from *Staphylococcus aureus* decreases virulence in experimental wound infection. Infection and immunity 64(12), 5284–5289.
DOI: 10.1128/iai.64.12.5284-5289.1996
47. Paniagua-Contreras, G., E., Monroy-Pérez, R. Gutiérrez-Lucas, T. Sainz-Espuñes, T., J. Bustos-Martínez, and S. Vaca, 2014. Genotypic characterization of methicillin-resistant *Staphylococcus aureus* strains isolated from the anterior nares and catheter of ambulatory hemodialysis patients in Mexico. Folia Microbiologica 59(4), 295–302.
DOI: 10.1007/s12223-013-0300-4
48. Pappas, G., A. P. Athanasoulia, D. K. Matthaiou, and M. E. Falagas, 2009. Thertrimethoprim- sulfamethoxazole for Methicillin-resistant *Staphylococcus aureus*: A Forgotten Alternative? Journal of Chemotherapy 21(2), 115–126.
DOI: 10.1179/joc.2009.21.2.115
49. Park, P.W., J. Rosenbloom, W.R. Abrams, J. Rosenbloom, and R.P. Mecham, 1996. Molecular cloning and expression of the gene for elastin-binding protein (ebpS) in *Staphylococcus aureus*. Journal of Biological Chemistry 271(26), 15803–15809.
DOI: 10.1074/jbc.271.26.15803
50. Patti, J.M., H. Jonsson, B., Guss, L.M. Switalski, K., Wiberg, M. Lindberg, and M. Höök, 1992. Molecular characterization and expression of a gene encoding a *Staphylococcus aureus* collagen adhesin. Journal of Biological Chemistry 267(7), pp. 4766–4772. URL: https://pubmed.ncbi.nlm.nih.gov/1311320/
51. Paul, D. v et al. 2009. Bergey's Manual of Systematic Bacteriology. Williams, Georgia, *USA* , 470–471. URL: https://link.springer.com/book/10.1007/978-0-387-68489-5
52. Peacock, S.J., T.J. Foster, B.J. Cameron, and A.R. Berendt, 1999. Bacterial fibronectin-binding proteins and endothelial cell surface fibronectin mediate adherence of *Staphylococcus aureus* to resting human endothelial cells. Microbiology 145(12), pp. 3477–3486.DOI: 10.1099/00221287-145-12-3477
53. Quintiliani Jr, R. and P. Courvalin, 1995. Mechanisms of resistance to antimicrobial agents. Manual of Clinical Microbiology.44(5)124-128. URL: https://clinicronow.org/doi/book/10.1128/9781683670438.MCM
54. Rasheed, M. S. A., U. P. Tiwari, J. C. Jespersen, L. L. Bauer, and R. N. Dilger, 2020. Effects of methylsulfonylmethane and neutralizing anti–IL-10 antibody supplementation during a mild Eimeria challenge infection in broiler chickens. Poultry science 99(12), 6559–6568.
DOI: 10.1016/j.psj.2020.09.046
55. Rayner, C. and W.J. Munckhof, 2005. Antibiotics currently used in the treatment of infections caused by *Staphylococcus aureus*. Internal Medicine Journal 35, S3-16.
DOI: 10.1111/j.1444-0903.2005.00976.x
56. Russell, C.D., A.L. McLean, C. Saunders, and I.F. Laurenson, 2014. Adjunctive rifampicin may improve outcomes in *Staphylococcus aureus* bacteraemia: a systematic review. Journal of Medical Microbiology 63(6), 841–848.
DOI: 10.1099/jmm.0.072280-0
57. Salazar, N. 2014. *Staphylococcus aureus* manganese transport protein C (MntC) is an extracellular matrix-and plasminogen-binding protein. PloS one 9(11), e112730.
DOI: 10.1371/journal.pone.0112730
58. Senok, A.C., H., Verstraelen, M. Temmerman, and G. A. Botta, 2009. Probiotics for the treatment of bacterial vaginosis. Cochrane Database of Systematic Reviews (4).
DOI: 10.1002/14651858.CD006289.pub2

59. Shore, A.C. et al. 2016. First report of cfr-carrying plasmids in the pandemic sequence type 22 methicillin-resistant *Staphylococcus aureus* staphylococcal cassette chromosome mec type IV clone. Antimicrobial Agents and chemotherapy 60(5), 3007–3015.
DOI: 10.1128/AAC.02949-15
60. Sollid, J.U.E., A.S. Furberg, A.M. Hanssen, and M. Johannessen, 2014. *Staphylococcus aureus*: determinants of human carriage. Infection, genetics and evolution 21, 531–541.
DOI: 10.1016/j.meegid.2013.03.020
61. Speziale, P. and G. Pietrocola, 2020. The multivalent role of fibronectin-binding proteins A and B (FnBPA and FnBPB) of *Staphylococcus aureus* in host infections. Frontiers in microbiology 11, 2054.
DOI: 10.3389/fmicb.2020.02054
62. Tang, J., J. Chen, Li, H., P. Zeng, and J, Li, 2013. Characterization of adhesin genes, staphylococcal nuclease, hemolysis, and biofilm formation among *Staphylococcus aureus* strains isolated from different sources. Foodborne Pathogens and Disease 10(9), 757–763.DOI: 10.1089/fpd.2012.1474
63. Tristan, A., L. Ying, M., Bes, J. Etienne, F. Vandenesch, and G. Lina, 2003. Use of multiplex PCR to identify *Staphylococcus aureus* adhesins involved in human hematogenous infections. Journal of clinical microbiology 41(9), 4465–4467.
DOI: 10.1128/JCM.41.9.4465-4467.2003
64. Tung, H., B. U. Guss, L. Hellman, , K. C. Perssonrubin, and Ryden, 2000. A bone sialoprotein-binding protein from *Staphylococcus aureus*: a member of the staphylococcal Sdr family. Biochemical Journal 345(3), 611–619. URL: https://pubmed.ncbi.nlm.nih.gov/10642520/
65. Usui, A., M.,Murai, K., Seki, J. Sakurada, and S. Masuda, 1992. Conspicuous ingestion of *Staphylococcus aureus* organisms by murine fibroblasts in vitro. Microbiology and Immunology 36(5), 545–550.
DOI: 10.1111/j.1348-0421.1992.tb02054.x
66. Vazquez, V., X. Liang, J.K. Horndahl, V.K, Ganesh, E. Smeds, T.J. Foster, and M. Hook, 2011. Fibrinogen is a ligand for the *Staphylococcus aureus* microbial surface components recognizing adhesive matrix molecules (Mscramm) bone sialoprotein-binding protein (Bbp). Journal of Biological Chemistry 286(34), 29797–29805.
DOI: 10.1074/jbc.M110.214981
67. Wilson, D.N. 2014. Ribosome-targeting antibiotics and mechanisms of bacterial resistance. Nature Reviews Microbiology 12(1), 35–48.DOI: 10.1038/nrmicro3155
68. Wilson, J., R. Guy, S., Elgohari, E., Sheridan, J., Davies, T. Lamagni, and A. Pearson, 2011. Trends in sources of meticillin-resistant *Staphylococcus aureus* (Mrsa) bacteraemia: data from the national mandatory surveillance of Mrsabacteraemia in England, 2006–2009. Journal of Hospital Infection 79(3), 211–217.
DOI: 10.1016/j.jhin.2011.05.013
69. Yarovoy, J.Y., A.A. Monte, B.C. Knepper, and H.L. Young, 2019. Epidemiology of Community-Onset *Staphylococcus aureus* Bacteremia. Western Journal of Emergency Medicine 20(3), 438.
DOI: 10.5811/westjem.2019.2.41939
70 .Yassin, H.Y., A.K. Melconian, and S.S, Mahmood, 2022. Prevalence Of exfoliative toxin genes Among Clinical Isolates of *Staphylococcus aureus* In Iraq. Iraqi Journal of Agricultural Scienes 53(2): 465–470.
DOI: https://doi.org/10.36103/ijas.v53i2.1554
71. Zhang, K. et al. 2004. New quadriplex PCR assay for detection of methicillin and mupirocin resistance and simultaneous discrimination of *Staphylococcus aureus* from coagulase-negative staphylococci. Journal of Clinical Microbiology 42(11), 4947–4955.
DOI: 10.1128/JCM.42.11.4947-4955.2004
72. Zhang, K., J.-A., McClure, S., Elsayed, T., Louie, and J.M. Conly, 2005. Novel multiplex PCR assay for characterization and concomitant subtyping of staphylococcal cassette chromosome mec types I to V in methicillin-resistant *Staphylococcus aureus*.Journal of ClinicalMicrobiology 43(10), 5026–5033.
DOI: 10.1128/JCM.43.10.5026-5033.2005
72. Zhang, X., M., Wu, W., Zhuo, J., Gu, S., Zhang, J. Ge, and M. Yang, 2015. Crystal structures of Bbp from *Staphylococcus aureus* reveal the ligand binding mechanism with Fibrinogen α. Protein and cell 6(10), 757–766.
DOI: 10.1007/s13238-015-0205-x