Al-Mustansiriyah Journal of Science
ISSN: 1814-635X (print), ISSN:2521-3520 (online) Volume 33, Issue 4, 2022 DOI: http://doi.org/10.23851/mjs.v33i4.1166

Research Article **Open Access**

Study of Phenotypic and Genotypic Factors of Staphylococcus aureus Clinical Local Isolates

Ali Muhsin Ali[1], Muhannad Moeen[2]*

[1]Department of Biology, College of Science, Mustansiriyah University. Baghdad, IRAQ.
[2]Directorate General of Education Baghdad Rusafa 3. The Ministry of Education. Baghdad, IRAQ.

*Correspondent contact: mohanadalhasany@gmail.com

Article Info

Received
04/07/2022

Accepted
06/09/2022

Published
30/12/2022

ABSTRACT

The isolates of *Staphylococcus aureus* were isolated from patients with various infections in hospitals, the isolates were identified and accurately diagnosed by phenotypic examination and biochemical tests, as well Vitek-2, and then genetic detection and diagnosis of many of the pathogenic factors associated with *Staphylococcus aureus* using conventional polymerase chain reaction (PCR) and testing for association by antibiotic resistance and production of some toxins by *Staphylococcus aureus*. After performing analysis of statistical, it was set up that the correlation coefficient of the PCR technique using virulence genes, sensitivity test to antibiotics and other virulence factors were significant at $p < 0.05$, but was insignificant with the biofilm production.

KEYWORDS: *Staphylococcus aureus*; *mecA*; biofilm formation; virulence factors.

الخلاصة

تم الحصول على مائة عزلة من بكتريا *Staphylococcus aureus* من مرضى يعانون من التهابات متنوعة من المرضى في المستشفيات، وتم تحديدها العزلات وتشخيصها بصورة دقيقة بالفحص المظهري والفحوصات الكيميائية الحيوية وكذلك تم اجراء اختبارات التشخيص البكتيري Vitek-2, من ثم تم إجراء الكشف والتشخيص الجيني للعديد من عوامل الامراضية المرتبطة بالمكورات العنقودية الذهبية باستخدام تفاعل البلمرة المتسلسل التقليدي (PCR) واختبار ارتباطه بمقاومة المضادات الحيوية وإنتاج بعض السموم بواسطة *Staphylococcus aureus*. وبعد اجراء التحليل الإحصائي، تبين أن معامل الارتباط لطريقة PCR مع جينات الضراوة في هذه الدراسة واختبار حساسية المضادات الحيوية وعوامل الضراوة الأخرى كان معنويًا عند $p<0.05$، بينما لم يكن الارتباط معنويًا مع انتاج البايوفيلم.

INTRODUCTION

Staphylococcus aureus is an adaptable bacterial pathogen that can stay alive in diverse host environments. This diversity depends on its capability to obtain and use nutrients from different sources; from this time, *Staphylococcus aureus* uses nutrient obtainability as an indicator of its environment. Bacteria may have sense nutrients and react using modified gene expression containing mixture of virulence elements. As a result, the metabolic signals that bacteria encounter not only help them survive in temporary adverse conditions, but also play an important role in pathogenesis. The evolutionary benefit of this association permits bacteria to adjust to ever-changing microenvironments for the period of infection instituting and development using their virulence changeable pattern. Recent studies have provided the close linkage between metabolism and virulence synthesis [1]. Bacteria resistance to antibiotics in general takes place by altering the genetic character of an organism. Resistance genes can be decoded vertically or horizontally. Vertical resistance is produced from a modification in the

genetic code that controls penicillin-binding proteins in *Staphylococcus aureus*. This genetic mutation is a response to the antimicrobial material presented in the environment of the microorganism. Horizontal mutation take place when genes passed through the plasmid are hosted from outside the cell and lead bacteria to integrate their genetic material that is responsible for resistance [2]. *S. aureus* has many virulence elements, such as clumping factor and coagulase [3]. In addition, extracellular toxins that donates to the *S. aureus* virulence, for example staphylococcal enterotoxins and exfoliative toxins [4]. Staphylococcal enterotoxins are typically formed by coagulase positive *S. aureus*, which is present everywhere and are commonly colonized in nasal mucosae [5]. Toxins that are well known as Staphylococcus Enterotoxins (SEs) (*Sea, Seb, Sec, Sed, and See*) have been described in resents years. *mecA* gene encodes penicillin-binding protein, an enzyme in control for crosslinking the peptidoglycans in the bacterial cell wall has a little affinity for β- lactams, causing resistance to this whole group of antibiotics. Vancomycin is a glycopeptide and antimicrobial drug for treating infections but resistance to vancomycin has been described, *van* genes which is an active resistant gene translates resistance to vancomycin. These genes of resistance include *vanB,* vanA [6]. The capability to match the translated of various virulence factors participates in the wide range of infections caused by *Staphylococcus aureus*. The formation of biofilms in *S. aureus* are a communal, important in the etiology of complex infections, for example infections of pneumonia, endocarditis and bloodstream. The biofilms of bacterial accumulation set in an extracellular matrix through specific gene expression. Biofilms are responsible for chronic infections with a complex resistance to immune defenses as well as antibiotics, A result, biofilm infections exhibit harder challenges for elimination. According to the mention above, the purpose of this study was to estimate correlation of PCR genotyping technique using virulence genes in current study with resistance of antibiotics and biofilm formation.

MATERIALS AND METHODS

Sample collection

For period from September – December 2020, Peoples were invited to participate in the study and ethical approval was obtained from them to take samples, One hundred isolates of *S.aureus* were isolated from patients suffering from wound, abscess, burn infections at many hospitals in Baghdad, which includes (Imam Ali hospital, Ibn-El Balady, Welfare Teaching Hospital, Sader hospital and Gazi Alhariry hospital) that 100 of these isolates was *S.aureus*. All samples from primary cultures were purified by way of culture in subculture on blood agar and later on Mannitol Salt Agar and keep warm at 37°C for 24hrs. [8].

Identification of Staphylococcus aureus

Staphylococcus aureus samples were cultured on blood agar, Mannitol Salt Agar and all plates were incubated for 24hrs. The growth culture was examined for colony morphology and then the isolates were tested using gram stain, catalase test, oxidase test, biochemical test and API 20 E.

Identification by VITEK 2 system

All isolates were indicated by using VITEK 2, the turbidity of the bacterial suspension was modified to match 0.5 standards in NaCl and VITEK 2 Gram positive ID card and the suspension tubes were loaded inside the VITEK-2 system. (BioMerieux, Marcy L'Etoile, France).

Antimicrobial susceptibility test

The Kirby-Bauer technique was tested to examine the isolates for antibiotic resistance to 13 diverse antimicrobial agents. The suspension of bacteria was set by selection 4-5 colonies of each single isolate from the primary culture and put off within a test tube by adding 5 ml of normal saline. The turbidity was adjusted to obtain around 1.5 $x10^8$ CFU/ml (Macfarland tube), using sterile cotton swab a portion of bacterial suspension was transported with carefully and just as spread on Mueller - Hinton agar and at that time it was left for ten min. There, after the antimicrobial agents were sited on the agar using sterilized forceps pushed definitely to make sure interaction by agar. Then, the peteri-dishes were upturned and kept warm at 37 C° for 24 hr. Inhibition regions established close the discs were measured with a metric ruler. Later, isolated were read as sensitive, intermediate, or resistant to particular antibiotics according to [9].

Heamolysin

By following company guidelines and using the autoclave for sterilization, blood agar was prepared by adding human blood in concentration of 5% then and there left to cool it to 45 ºC and added to

the Petri- dishes, furthermore left to get harden and keep at 4 ºC until next step, every isolate was spread in surface of blood agar plate to notice the ability of bacterial to produce clear zone around colony for positive result [10].

Coagulase test

It was prepared by following [11]:

Slide test, the test was done to distinguish bound coagulase in which a single pure bacterial colony was suspended with a drop of normal saline on a fresh slide and a drop of human plasma was suspended and gently mixed. Clumping after a few seconds is revered as a coagulase-positive test. The control of test consisted of normal saline and bacteria without addition of plasma to make sure that the bacteria did not spontaneously clump in normal saline.

Tube test: In this test, a single colony of the bacteria was hanging in milliliter of human plasma (diluted in normal saline 1:6) within a test tube, then incubated for 24 hours at temperature 37 ℃. The tubes were tested after period 1-4 hrs. The result indicated as positive when a clot formation and the negative result was when the tube was incubated for another 24hrs and examined once more.

Biofilm Production

A-Congo red method

The Congo red test was set up by mixing 37gm of Brain heart Infusion broth, 50 gm sucrose and 10 gm Agar in 900 ml of distilled water and using autoclave for sterilizing it. 0.8 gm of Congo-red stain was dissolving in 100 ml of distilled water and using autoclave for sterilize, mix to the medium and cooling it to 55°C. The media discharged into petri-dish. The media used to examine production of biofilm, petri-dish were inoculated a single colony of isolated and incubated at 37C° for 1-2 days in aerobically condition. The positive result was identified by notice black colonies with crystalline density. The weak slime that producers in generally remain pink, however irregular grow dark in the midpoints of the plate. The blackening of the colonies with the lack of crystalline detect as indeterminate result [12].

B- Micro-titer method

This test was described by [13], which known as the gold standard test for biofilm formation, all isolates from plates agar were mix to ten milliliters of Trypticase soy broth with 1% glucose w/v. The broths incubating overnight at 35 ± 2 ºC, diluted in a new sterile broth was diluted in a ratio of 1:40. Two hundred microliter of cell suspension were inoculated in a sterilized 96 well. little wells that contained inoculated sterile broth individual were considered as the negative control. Then microtiter plates were incubated in an incubator at 37ºC for a period of 24hrs. After incubation, the content washed gently through tapping and washed with de-ionized water, that isolate the floating bacteria. The biofilm formed by bacteria adhesive to micro-titer plate that picks dye (0.1%) w/v during 15 min. further dye rinse with D.W. and then drying. A stick that stained with biofilm was gain using ELISA reader at wavelength 630 nm.

The extraction of DNA

The DNA was taken out from *Staphylococcus aureus* by (Geneaid GBB100. Korea). DNA extraction was completed using company guidance. The nanodrop device was applied to determining the concentration and purity, 1µl of every single DNA samples employed in nanodrop device to measuring (O.D) at wavelength 260 nm and 280 nm [14]. Later it was put in storage at -20 ºC awaiting carry out PCR assay.

Genes election

Primers election forward and reverse oligonucleotide primers which were employed to check genes are enumerated in Table 1. The primers (Macrogene, Korea) were supply as lyophilized formula were mixed with water of Nuclease–Free to supply a final concentration of 100µl by according to guidance of provider. an amount of primers was extended in the middle of 1.1 to 2.3µl that were loaded to mix with final concentration 0.2 µM of every single primer. The solutions of the primers were kept away at -20C◦. The primers equipped to examined sea to tst1 were described by [15], the primers *sec, sed, see, seg, selk, sell* also reported by [16]. The primer that is particular to *S. aureus* was applied to detect *femA* gene [17]. The gene *vanA* and *vanB* described by [18, 19], primer that is

specific to *S. aureus* (MRSA) was applied to detect *mecA* gene [17]. To prepared a multiplex PCR method, four groups (Set 1*: sea, seb, sec, sed, see;* Set 2*: seg, femA;* Set 3*: selk, mecA;* Set 4*: sell, vanA, vanB*).

Table 1: The Oligonucleotide sequence primers

Genes	Oligonucleotides sequence 5……..3	Prod.
Sea	F-CCTTTGGAAACGGTTAAAACG R-TCTGAACCTTCCCATCAAAAAC	127
Seb	F-TCGCATCAAACTGACAAACG R-GCAGGTACTCTATAAGTGCCTGC	477
Sec	F- CTCAAGAACTAGACATAAAAGCTAGG R-TCAAAATCGGATTAACATTATCC	271
Sed	F- CTAGTTTGGTAATATCTCCTTTAAACG RTTAATGCTATATCTTATAGGGTAAAC ATC	319
femA	F-AAAAAAGCACATAACAAGCG R-GATAAAGAAGAAACCAGCAG	134
mecA	F-ACTGCTATCCACCCTCAAAC R-CTGGTGAAGTTGTAATCTGG	163
See	FCAGTACCTATAGATAAAGTTAAAACA AGC R- TAACTTACCGTGGACCCTTC	178
Seg	F- AAGTAGACATTTTTGGCGTTCC R- AGAACCATCAAACTCGTATAGC	287
Selk	F-TAGGTGTCTCTAATAATGCCA R-TAGATATTCGTTAGTAGCTG	293
Sell	F-TAACGGCGATGTAGGTCCAGG R-CATCTATTTCTTGTGCGGTAAC	383
vanA	F-GGGAAAACGACAATTGC R-GTACAATGCGGCCGTTA	732
vanB	F-AAGCTATGCAAGAAGCCATG R-CCGACAATCAAATCATCCTC	536

Multiplex PCR condition

For every single primer used using GoTaq Green Master Mix PCR according to manufacturer's guidelines. Every one reaction combined 50µl that contained 25µl of 2X PCR Master Mix, 2µl (close to 1.1 to 2.3 µl] of all primers, and 2µl template DNA (10– 100 ng]. The amplification protocol as follows: the denaturation temperature of DNA was 95 °C for 15 min and then followed by applied 35 cycles (95 °C for 30 s, 57 °C for 1.30 min, and 72 °C for 1.30 min], finally was done by extension at 72 °C for 10 min and keep at 4°C for 5 min. PCR product were resolved with applied electrophoresis in 2% agarose with 1X TBE buffer for 60 min, stained with 5µl/100ml of Ethidium Bromide, displayed by using a trans illuminator [15].

Statistical analysis

The analyses were completed by SPSS program (NY, USA). Chi-square were applied to differentiate between measures of categorical collections (Fisher's exact where suitable). Statistical analysis tests, including Kendall's tau-b non-parametric association coefficients, were set with two significance level for correlation of genotyping system with virulence factors, capsule types and antibiotics resistance, the product of correlation test by *p* values a lesser amount of than 0.05 were estimated statistically significant.

RESULTS AND DISCUSSION

Identification of *S. Aureus*

Further than 176 samples, one hundred were isolated as *S. aureus* such as presented in Table 2 based on characters of colony, catalase test, biochemical test, Gram stain [20]. *S.aureus* are a communal kind of bacteria that found on the mucous membranes and skin [21]. In humans' body [22]. MRSA is hardly issue for each hospital and nursing homes, where persons with open wounds, invasive devices, and lower immunity are at high threat of contamination than the other persons [23]. It can also produce a variety of infections, for example skin infections, which are the common type of disease produced by *Staphylococcus spp*. bacteria of the skin can development to inflammation or cellulitis which infected of the lower layers of skin and connective tissue under the skin also can lead to cause enlargement and redness. In uncommon cases, a severe infection identified as scalded skin syndrome [24].

Table 2. Isolates of clinical samples.

Clinical sources	No. of isolates	Percentage %
Wounds	52	52
Abscess	32	32
Burns	8	8
Nose	8	8
Total number	100	100

Staphylococcus aureus in women may result in inflammation of the mammary gland then in swelling of the breast. The inflammations can discharge bacteria into the milk of mothers. [25]. *Staphylococcus aureus* may development significantly during 24–48 hours of first detect of signs. During 72 hours, MRSA can take part in tissues of human and finally come to be resistant to medication [26]. The initial infection of MRSA is small red bumps that look like pustules, boils or spider bites. Another symptom such as occasionally, rashes and fever. During little days,

the lumps grow into larger and further painful, they finally spread into low layers, boils loaded with pus [27]. The bacteria require enter a wound to initiate infection. A people with well immunity does not generally get sick, sufficient to deal with flesh-eating disease and while the wound has been immaculate. On the other hand, for the cases of persons have immune- compromised suffering of Hepatitis, diabetes, cancer or aging can infect with disease easily, precisely if the injure careless [28]. When the bacteria enter and poisons are produced, every well tissue, such as skin, fat and muscles are receiving damaged that causing of necrosis. The inflammation wounds processed to be necrotizing fasciitis show observable signs for example swelling, redness and pain accompanied by warm skin, blisters, violet skin color. Last stages develop symptoms for example scaling, gangrene, skin peeling and discoloration, it also leading to failure and ulcers, Later, eventually the meat-eating infection if remained without treated, this infection cause toxic shock and finally death during 24 hours [29]. Bacteremia, or as identified as the infection which progress when bacteria enter the blood stream and initiate to colonize. A few patients, case is restricted and without symptoms, in contrast in some patients is cause a shock, which is lethal. The infection of blood may possibly be distinguished by these symptoms which: nausea, vomiting, fever, abdominal pain, diarrhea, chills, malaise, anxiety, shortness of breath, and confusion [30].

Biochemical tests

All isolates indicated negative results for oxidase test, positive for urease production, Coagulase, Mannitol fermentation, while all isolates indicated as A positive results to catalase test and results of biochemical tests are listed in Table 3.

Staphylococcus aureus is Gram positive test, found as singly, sometimes cluster, facultative anaerobic and cultivates well in media including 10% sodium chloride, on the other hand weak contain 15% NaCl, indicated positive for alkaline phosphatase, catalase, coagulase, steady to hemolysis and hyaluronidase, in contrast for oxidase, B-glucuronidase were show a negativity.

Table 3. Results of biochemical tests.

Test	Result (100%)
Oxidase	0
Catalase	100
Coagulase (rabbit plasma)	100
Coagulase (human plasma)	100
Mannitol fermentation	100
Hemolysis on blood agar	100

Creates acid in an aerobic condition by using fructose, maltose and sucrose. The negative for acid building from salicin, xylitol, arabinose and xylose. Novobiocinliable, immotile, do not spore forming and only occasionally capsulate.

Detect of Biofilm by Congo red agar

The biofilm formation Resulting in formed (81%) isolates of *S. aureus* using Congo red media. The formation of biofilm was indicated by showed black colonies with a dry crystalline uniformity. Whereas other isolates showed a little formation or did not formed biofilm on Congo red as maintained to weak slime producers typically keep on pink color, however irregular blackening at the middles of colonies. A black color of the colonies that lack of crystalline characters point to an indeterminate formation as listed in Figure 1.

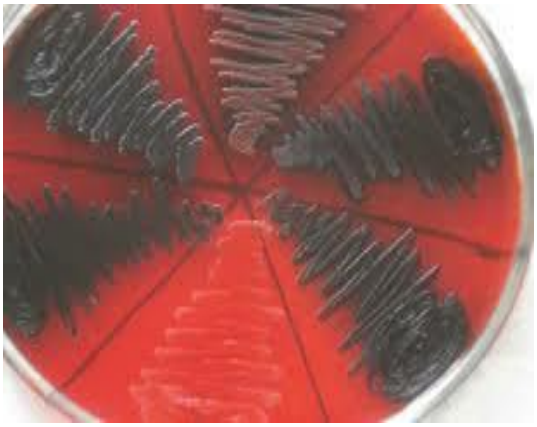

Figure 1. Biofilm of *S.aureus* result on congo red agar plate.

Biofilm test by micro titer plate

One hundred isolates of *S.aureus*, biofilm formation was detected as following: (48%) percentage of isolates as maximum value of biofilm production due to strong adherent, even though (32%) produced intermediate result biofilm formation and (20%) percentage did not form biofilm. These results based on the density in the ELISA system [28] which numerate in Table 4 and Figure 2.

Table 4. Biofilm results in ELISA reader according to O.D.

Biofilm formation	No. of Isolates	Range of O. D
Strong produce	48	> 4x ODc
Moderate produce	32	2x ODc < ~ ≤ 4x ODc
No produce biofilm	20	≤ OD /ODc < ~ ≤ 2x ODc

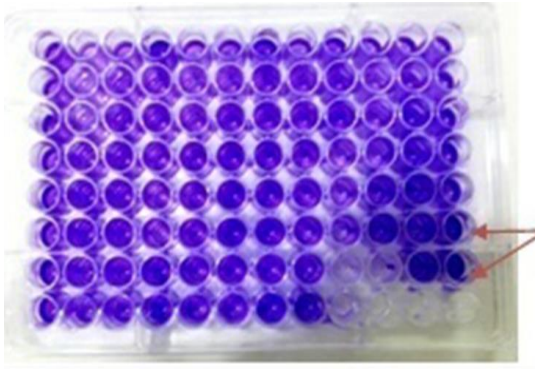

Figure 2. Detection of Biofilm formation using microtiter plate method.

Antibiotic sensitivity

Antibiotic sensitivity was done performed the agar disc diffusion on every single isolates of *S.aureus*. sensitivity was confirmed to thirteen antibiotics: methicilline trimethoprime, cloxacillin, gentamicin, cephalexine clindamycin, rifampicin teicoplanine, cefoxitine, lincomycine, levofloxacine, Azithromycine and vancomycin and results were illustrated following to the guidance of [9]. *S.aureus* showed the maximum resistance to Cloxacillin and Methicilline with resistance (100%), As a result, this resistance causes a significant threat in medical care.

The result indicated that every single isolate was resistant to antibiotic: (13%) for each of clindamycin and levofloxacine, torifampicin (22%), gentamycin (18%), vancomycin (17%), lincomycin (26%), Azithromycine (24%), trimethoprim (23%), cephalexin (51%) and, cefoxitin (86%). The current research showed that Teicoplanine was the most effective antibiotic, with resistance (4%) against isolates of *S. aureus*.

Genotyping of *S.aureus*

S.aureus isolates were detected genotypically by PCR amplification, Multiplex and Uniplex PCR technique. Magnification of genes by PCR systems was achieved for one hundred of *S.aureus* isolates to examined virulence features in which involved. The results of PCR technique indicated genes of sea was detected in *femA* was (88%) and (73%) for *mecA*, whereas the further result of genes showed that following: *sec* and *vanA* (61%), *seb* was (63%), *see* (51%), *seg* (46%), *sed* and *vanB* (58%), *sea* (55%), *selk* (41%) and *sell* (39) as shown in Figures 3, 4, 5, and 6.

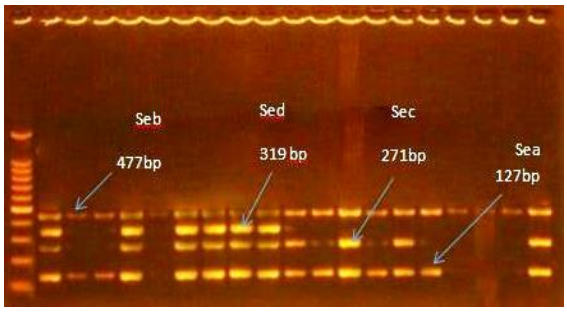

Figure 3. PCR products represent: seb, sed, sec and sea genes.

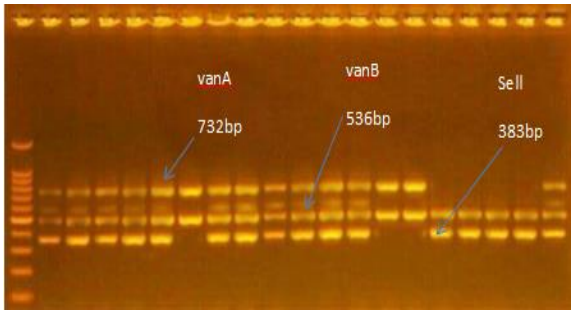

Figure 4. PCR products represent: vanA, vanB and sell genes.

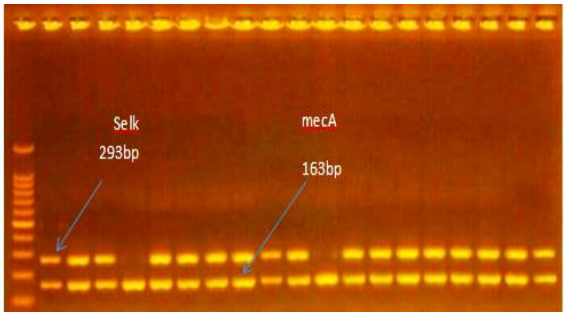

Figure 5. PCR products represent: selk and mecA genes.

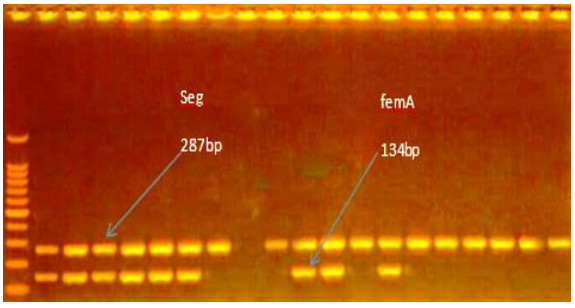

Figure 6. PCR products represent: seg and femA genes.

This diversity in genes spreading may be related to source of isolates, as well as variety in infection sites and environmental region, the genes has been horizontally moved in *S. aureus* additional regularly than may be developed from other

Staphylococcus species and others local pathogens. The troublesome of *S. aureus* that causing blood infection which mainly methicillin-resistant, in relationships of spending and supply in height. The hazard of infectious endocarditis and spreading to other raises the threat of death rate and increases the risk factor for initial, suitable medication [31]. The presence of the gene in 100 isolates which detected (100%) were positive result for every virulence genes in this test with diverse percentage as revealed in Table 5.

Table 5. virulence factors genes of *S. aureus*.

Genes	No. of Isolates	Percentage %
Sea	55/100	55%
Seb	63/100	63%
Sec	61/100	61%
Sed	58/100	58%
femA	88/100	88%
mecA	73/100	73%
See	51/100	51%
Seg	46/100	46%
Selk	41/100	41%
Sell	39/100	39%
vanA	61/100	61%
vanB	58/100	58%

The statically analysis indicated correlation coefficient of PCR technique together virulence genes in current survey with antibiotics resistance test and other virulence features was showed significant at $p < 0.05$(two-tailed), although link the biofilm was not significant as revealed in Table 6. In comparing with recent study [30] indicates to that most genes less presence from the results *femA* (100%), *mecA* (100%), (2.24%), *sea* (48.31%), *seb* (44.94%), *sec* (6.74%), *sed* (3.37%) and *see* (16.85%).

Table 6. Kendall's tau-b correlation coefficient of PCR genotyping method versus antibiotic test, Biofilm formation and virulence genes of *S. aureus* isolates.

Genotype method	PCR
Antibiotic test	0.92 **(p < 0.001)
Phenotypic virulence factors	0.77* (p < 0.01)
Biofilm formation	0.9(NS, P=0.51)
Virulence genes	0.70*(p < 0.01)

CONCLUSIONS

The significant result between PCR genotyping and antibiotics resistance test, we concluded that the increase in virulence is due to acquisition of antibiotic resistance as a result of the wrong or random use of antibiotics by patients without consulting; thus, the antibiotic should be used physician's prescription. The accomplishment of a local and global system to monitoring antibiotic resistance among physicians is essential for controlling experimental therapy-particular measures counter to a specific pathogen.

Disclosure and conflict of interest: The authors declare that they have no conflicts of interest.

REFERENCES

[1] M. Ghahremani, NH. Jazani and Y. Sharifi, "Emergence of vancomycin-intermediate and resistant Staphylococcus aureus among methicillin-resistant S. aureus isolated from clinical specimens in the northwest of Iran", J Glob Antimicrob Resist.; 14:4-9, 2018. https://doi.org/10.1016/j.jgar.2018.01.017

[2] S.X. Li, P.P.Wang, J.L. Zhao, L.H. Zhou, , P.F. Zhang, C.Y. Fu, J. Meng, and X.Wang,"Characterization of toxin genes and antimicrobial susceptibility of Staphylococcus aureus from retail raw chicken meat," J. Food Prot, 2018.

[3] C. Jenul, and A.R. Horswill, "Regulation of Staphylococcus aureus virulence," Microbiol, 2018.

[4] H. K. Ono, Y. Sato, K. Narita, I. Naito, S. Hirose, J. Hisatsune, K. Asano, D.L. Hu, K. Omoe, M. Sugai, and A. Nakane, "Identification and characterization of a novel staphylococcal emetic toxin", Appl. Environ. Microbiol.81:7034-7040, 2015.

[5] C. Jenul, and A. R. Horswill, "Regulation of Staphylococcus aureus virulence, "Microbiol.GPP3-0031, 2018.

[6] E.L. Fisher, M. Otto and G. Y. C. Cheung, "Basis of virulence in enterotoxin-mediated staphylococcal food poisoning", Microbiol.9:436, 2018. https://doi.org/10.3389/fmicb.2018.00436

[7] M. Otto, "Staphylococcal biofilms," Microbiol.GPP3-0023, 2018.

[8] H. Sharma, C.E. Turner, M.K. Siggins, M. El-Bahrawy, B. Pichon, A. Kearns and S. Sriskandan, "Toxic shock syndrome toxin 1 evaluation and antibiotic impact in a transgenic model of staphylococcal soft tissue infection", mSphere. 4:e00665-19, 2019. https://doi.org/10.1128/mSphere.00665-19

[9] Clinical and Laboratory Standards Institute (CLSI), "Performance standard for antimicrobial susceptibility testing, "Twenty seventh informational supplement. M100-S21.3 (1), 2020.

[10] SA Rice, S. Wuertz and S. Kjelleberg, "Next-generation studies of microbial biofilm communities,"Microb Biotechnol. 9:677-80, 2016. https://doi.org/10.1111/1751-7915.12390

[11] G. F Brooks, K. C Carroll, J. S. Butel, and S. A. Melnick, "Medical Microbiology," 24th.ed. the McGraw-Hill Companies, Inc., New York: 224-232, 2007.
[12] R. Page and W. Peti, "Toxin-antitoxin systems in bacterial growth arrest and persistence", Nat. Chem. Biol. 12 208-14, 2016. https://doi.org/10.1038/nchembio.2044
[13] A. Omar, J. B. Wright, G. Schultz, R. Burrell, and P. Nadworny, "Microbial Biofilms and Chronic Wounds", Microorganisms.5 pii:E9, 2017. https://doi.org/10.3390/microorganisms5010009
[14] W. Abdulkareem, N.Hussein, A. Mohammed, S. Arif, and I. Naqid, "Risk Factors Association for MRSA Nasal Colonization in Preoperative Patients in Azadi Teaching Hospital-Duhok, Kurdistan Region", Iraq. Science Journal of University of Zakho, 8(3), 88-9, 2020. https://doi.org/10.25271/sjuoz.2020.8.3.712
[15] V. Mascaro, M. S. Capano, T. Iona, C. G. A. Nobile, A. Ammendoila and M. Pavia, "Prevalence of Staphylococcus aureus carriage and pattern of antibiotic resistance, including methicillin resistance, among contact sport athletes in Italy", Infect Drug Resist, 12, 1161-1170, 2019. https://doi.org/10.2147/IDR.S195749
[16] Lakhundi, S., and K. Zhang, "Methicillin-resistant Staphylococcus aureus: molecular characterization, evolution, and epidemiology", Clinical microbiology reviews, 31(4), 2018. https://doi.org/10.1128/CMR.00020-18
[17] S. Ansari, R. Gautam, S. Shrestha, S. R. Ansari, S. N. Subedi and M. R. Chhetri, "Risk factors assessment for nasal colonization of Staphylococcus aureus and its methicillin resistant strains among pre-clinical medical students of Nepal", BMC research notes, 9(1), 214, 2016. https://doi.org/10.1186/s13104-016-2021-7
[18] F. Sultan and S. Al Meani, "Prevalence of Staphylococcus aureus toxins genes in clinical and food isolates in Iraq", Journal of Pharmaceutical Sciences and Research, 11(2); 636-642, 2019.
[19] R. Parastan, M. Kargar, K. Solhjoo, and F. Kafilzadeh, "A synergistic association between adhesion-related genes and multidrug resistance patterns of Staphylococcus aureus isolates from different patients and healthy individuals", Journal of Global Antimicrobial Resistance, 22, 379-385, 2020. https://doi.org/10.1016/j.jgar.2020.02.025
[20] M. H. Al-Khafaji and M. T. Flayyih, "Relationship between methicillin resistance and the presence of femA and mecA genes in coagulase positive and negative staphylococci isolated from milk and cheese", World J Exp Biosci, 3, 50-56, 2015.
[21] A. M. Alalem Antibiotic, "Resistant Staphylococcus aureus Infection Studies in Hospitals," Doctoral Thesis. Middle East Technical University.p.157, 2008.
[22] N. E. Onwubiko and N. M. Sadiq, "Antibiotic sensitivity pattern of Staphylococcus aureus from clinical isolates in a tertiary health institution in Kano," Northwestern Nigeria. J. of Pan Afri. Med.40(7): 1937- 8688, 2011. https://doi.org/10.4314/pamj.v8i1.71050
[23] A. Vainio, "Molecular Methods for the Epidemiological Analysis of Methicillin-Resistant Staphylococcus aureus (MRSA) and Streptococcus pneumoniae", Juvenes Print-Tampere University Print Tampere, Finland. ISBN 978-952-245-555-0, 2012.
[24] A. Antony, "Study of Biofilm forming capacity of pathogens involved in Chronic Rhinosinusitis", Master Thesis. Auckland University of Technology. Auckland, 2011.
[25] T. Iwase, Y. Uehara, S. Hitomi, T. Akiko, S. Hiromi, T. Koji, T. Agata and M. Yoshimitsu, "Staphylococcus epidermidis Esp inhibits Staphylococcus aureus biofilm formation and nasal colonization", Nature 465 (7296): 346-9, 2010. https://doi.org/10.1038/nature09074
[26] B. A. Lipsky, Y. P. Tabak, R. S. Johannes, L. Vo, L. Hyde and JA. Weigelt, "Skin and soft tissue infections in hospitalized patients with diabetes: culture isolates and risk factors associated. with mortality, length of stay and cost", US National Library of Medicine National Institutes of Health53(5):914-23, 2010. https://doi.org/10.1007/s00125-010-1672-5
[27] J. L. Raygada, and D.P. Levine,"Managing CA-MRSA Infections: Current and Emerging Options", Infections in Medicine 26 (2), 2009.
[28] A. Jain, A. Varma, Mangalanandan, P.H Kumar, and A. Bal, "Surgical outcome of necrotizing fasciitis in diabetic lower limbs", Journal of Diabetic Foot Complications 1 (4): 80-84, 2009.
[29] A. Schwartz, Robert, "Dermatologic Manifestations of Necrotizing Fasciitis," Medscape.com. June 9, 2011.
[30] J. G. Hendriks, E. van Horn, J R.; van der, H. C. Mei and H. J Busscher, "Backgrounds of antibiotic-loaded bone cement and prosthesis-related infection", Biomaterials.25 (3): 545-556, 2004. https://doi.org/10.1016/S0142-9612(03)00554-4
[31] L. H. Chen, Z. H. Xiong, L. L. Sun, J. Yang and Q. Jin, "toward the genetic diversity and molecular evolution of bacterial virulence factors", Nucleic. Acids Res. 40(Database issue): D641-D645, 2012. https://doi.org/10.1093/nar/gkr989

How to Cite

A. M. Ali and M. M. Abdallah, “Study of Phenotypic and Genotypic Factors of Staphylococcus aureus Clinical Local Isolates”, *Al-Mustansiriyah Journal of Science*, vol. 33, no. 4, pp. 49–56, Dec. 2022.