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Molecular Identification of *Staphylococcus aureus* Isolated from Hospital and Community-Acquired in Baqubah City

Qutaiba Dhiaa Hashim* **and Hadi Rahman Rasheed Al-Taai**

Department of biology – College of Science University of Diyala

scibioms2139@uodiyala.edu.iq

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Abstract

A total of 250 clinical specimens were obtained from several sources included vagina, urine, nose wounds and burns. The specimens were obtained in Baaquba Teaching Hospital in Diyala province during the period from October 2021 to February 2022. The prevalence of *Staphylococcus aureus* isolated from these sources which that were diagnosed by selective media, biochemical tests, and confirmed by VITEK 2 system. Results showed 44 (17.6%) positive growth for *S. aureus* which were distributed as 2 (4%) from urine, 5 (10%) from vagina, 12 (24%) from nose, 13 (26%) from wounds and 12 (24%) from burns. Anti-bacterial susceptibility tests showed that Ceftaroline was the most resisted antibiotic by 100% followed by Imipenem which was 93.2% Netilmicin was the most effective antibiotic with sensitivity of 81.8% followed by chloramphenicol which was sensitive in 65.9% of isolates. The resistance of the other antibiotics was 38.6% for Amikacin, 43.2% for Gentamicin, 45.5% for Tetracycline, 56.8% for Clindamycin, 59.1% for Clarithromycin, 38.6% for Ofloxacin, 43.2% for Ciprofloxacin and 88.6% for Nitrofurantoin. For the production of beta lactamase enzymes, only 3 (6.8%) isolates had the ability to produce Extended Spectrum Beta Lactamase, and 1 (2.2%) were positive for Metallo Beta Lactamase. Genetic detection results showed 19 of 24 isolates were positive for *mec*A gene while 21 of 24 isolates were positive for *coa* gene. Sequencing results for *coa* gene showed that after executing NCBI BLASTn on these PCR amplicons, the sequencing reactions confirmed their exact identification. In terms of the 812 bp

Academic Science Journal

amplicons, the NCBI BLASTn software showed up to 97% to 99% matches in nucleotides between the analyzed specimens and *S. aureus* target sequence references. By correlating the nucleic acid sequences of the examined specimens by way of the retrieved nucleic acid sequences (GenBank acc. CP038229.1). The outcomes of the 812 bp specimens exposed a total of thirty-eight nucleic acid variations in the various specimens when compared to the most closely related relevant standard nucleic acid sequences (GenBank acc. no. CP038229.1).

Keyword: *S. aureus*, ESβL, *coa* gene, sequencing.

التنميط الجزيئي للعنقودية الذهبية *Staphylococcus aureus* المعزولة من عدوى المستشفى و العدوى المجتمعية في مدينة بعقوبة

قتيبة ضياء هاشم وهادي رحمن رشيد الطائي

قسم علوم الحياة – كلية العلوم – جامعة ديالى

الخلاصة

تم جمع 250 عينة سريرية من مصادر مختلفة شملت عينات مهبلية وادرار ومسحات انف وحروق و جروح. جمعت العينات من مستشفى بعقوبة التعليمي في ديالى خلال الفترة من تشرين الاول 2021 حتى شباط 2022. كانت نسبة البكتيرية العنقودية الذهبية المعزولة من هذه المصادر والتي تم تشخيصها عن طريق الاختبارات الكيموحيوية والاوساط الانتقائية والتي اكدت بواسطة جهاز الفايتك 44 (17.6%) حيث كانت 2 (4%) من الادرار, 5 (10%) من المهبل, 12 (24%) من الانف, 13 (26%) من الجروح و12 (24%) من الحروق. اظهرت اختبارات الحساسية المضادة للبكتيريا ان Ceftaroline كان المضاد الحيوي الاكثر مقاومة بنسبة 100% يليه imipenem بنسبة 93.2%, كان ال Netilmicin اكثر المضادات الحيوية فاعلية بنسبة 81.8% يليه ال Chloramphenicol بنسبة 65.9%, اما المضادات الحيوية الاخرى فكانت نسبة المقومة لها 38.6% لل Amikacin, 43.2% لل Gentamicin, 45.5% لل Tetracycline, 56.8% لل Clindamycin, 59.1% لل Clarithromycin, 38.6% لل Ofloxacin, 43.2 لل Ciprofloxacin و 88.6% لل Nitrofurantoin. باللنسبة لانتاج انزيمات البيتالاكتام واسعة الطيف, فقط 3 (6.8%) عزلات كانت لديها القدرة على انتاج انزيمات بيتا واسعة الطيف, 2 (4.5%) منتجة ل Amp^C و 1 (2.2%) منتجة لانزيمات البيتا لاكتام المعدنية. اظهرت نتائج الكشف الجيني ان 19 من 24 عزلة كانت موجبة لجين *mec*A بينما 21 من 24 عزلة كانت موجبة لجين *coa*. %. بينت نتائج التتابع النتيوكليوتيدي لجين coa والذي يتكون من 812 زوج قاعدي حيث اظهرت النتائج في NCBI BLASTn ما يصل الى 97% الى 99% من التشابه في التسلسل بين العينات قيد الدراسة و تسلسلات الجين المرجعي للمكورات العنقودية الذهبية وذلك عن طريق مقارنة تسلسل الحمض النووي المستهدف للعينات قيد الدراسة مع

Academic Science Journal

الحمض النووي في (GenBank acc. CP038299.1), كشفت نتائج التسلسل النيوكليوتيدي بوجود ثمانية و ثلاثين اختلافا من التتابعات النيوكليوتيدية والتي قورنت مع الحمض النووي المرجعي.

كلمات مفتاحية: العنقودية الذهبية، انزيمات بيتا لاكتام واسعة الطيف، جين التجلط، تتابع نيوكليوتيدي.

Introduction

The bacteria *Staphylococcus aureus* is classified as a Gram - positive cocci that belong to the *Staphylococcus* genus, this bacterium is divided into two major types which are: methicillin susceptible *S. aureus* (MSSA) and methicillin resistance *S. aureus* (MRSA) [1,2]. Basically, MRSA developed from MSSA by receiving SCC*mec* complex which contain the *mec* gene that is responsible for the coding of extra penicillin binding protein which is in charge of the strong resistance toward all Beta lactam antibiotics and other kinds of similar cases [3]. *S. aureus* is a major bacterium that causes nosocomial and community-acquired illnesses, and despite extensive prophylactic measures, methicillin-resistant *S. aureus* (MRSA) infections have increased [4]. Resistance to various antibiotics is common in *S. aureus*. Resistance to classic beta-lactam antibiotics (penicillin and derivates) that are beta-lactamase sensitive is nearly universal in *S. aureus* [5].

Genotype examination for MRSA resistance was performed to know antibiotic resistance gene such as *mec*A which is the gold standard to identify MRSA genotypes [6]. *S. aureus* may be molecularly typed using a variety of approaches. Numerous molecular approaches have been developed and utilized in epidemiological research over the last decade to identify and compare *S. aureus* isolates [7].

Because of the varied sequences (81 bp tandem repeats) at its 3' end, the *coa* gene producing coagulase protein is highly polymorphic, allowing differentiation of *S. aureus* species. This *coa* gene polymorphism is used as an epidemiological marker, and typing is done using primers that are homologous to a conserved area of the *coa* gene. Because the frequency of repeated sequences within the *coa* gene varies, the resultant polymerase chain reaction (PCR) products of various strains might be of varying lengths [8].

This study was aimed to the isolate and identify of *S. aureus* from Hospital-Acquired and community sources using genetic typing.

Materials and Methods

Collection of specimens

A number of 250 specimens were obtained from Baaqubah Teaching Hospital in Diyala province during the period from October 2021 to to February 2022 from people suffering of various illnesses. The sources of samples include wounds, burns, vaginal swabs, nasal swabs, and urine. These specimens were taken in the appropriate manner to avoid contamination and exposed to tests such as catalase, oxidase, and culturing in Blood agar, mannitol salt agar as well as on Baird barker, which media was also used to detect coagulase action by adding fibrinogen plasma [9].

Detection of β-Lactamase production

Detection of Metallo β-Lactamase (MBLs.):

The *S. aureus* isolate suspensions were created by transmitting one colony to five ml of normal saline and incubating it for 24 hours or till it was analogous to 0.5 McFarland. The bacterial suspension was then streaking into Muller Hinton agar plates, two Imipenem (10 µg) discs were positioned 20 mm separately on the Mueller Hinton agar plates, and 5 µl of EDTA was added to one of the Imipenem[10].

Detection of extended-spectrum β-Lactamase production:

The combined disk or double-disk diffusion test technique was used to assess the isolates capability to generate ESβLs [11]. The seeded isolates (0.5 McFarland) are streaked on the surface of Muller-Hinton plates, and an antibiotic disk containing Amoxicillin/Clavulanic acid (30µg/disk) was put in the middle of the inoculated plate. Then, 3 cm out from the core disk, an antibiotic disk of Aztreonam and third-generation cephalosporins Cefotaxime and

Ceftazidime were placed. The plates were incubated for 24 hours at 37°C. Zone inhibition of 5 mm or greater in the existence of Augmentin was considered as a favorable outcome for ESβL enzyme synthesis. The disk diffusion technique on Mueller-Hinton agar was performed to determine the antibiotic susceptibility of all isolates, as recommended by the Clinical and Laboratory Standards Institute [12].

Genetic detection

Primers and their sequencing:

Table 1 shows the primer sequencing utilized for detecting. The Macrogen Company provided the primers in lyophilized condition. Lyophilized primers were neutralized in nuclease-free water to a final volume of 100pmol/l as a stock solution. To produce a 10 pmol/µl working primer solution, blend 10 µl of primer stock solution (kept at -20° C) with 90 µl of nuclease-free water.

Table 1: The primers used in the current study [13]

Primer name	Sequence	Annealing Temp. (°C)	Product Size (bp)
coa-F	5'-CGAGACCAAGATTCAACAAG-3'	55	650-812
coa-R	5'-AAAGAAAACCACTCACATCA-3'		
*mec*A-F	5'-TGGCTATCGTGTCACAATCG-3'	56	310
*mec*A-R	5'-CTGGAACTTGTTGAGCAGAG-3'		

DNA Extraction

The DNA of the bacterial isolates was obtained utilizing ABIO (pure extraction methodology) DNA was extracted as mentioned by [13].

Quantitation of DNA:

A Quantus Fluorimeter was used to measure the amount of extracted DNA in order to assess the integrity of extraction for downstream applications.

Agarose Gel Electrophoresis and DNA Loading

The agarose (1.5 %) was prepared according to the manufactured company. The agarose was poured into the gel box and was left to set at room temperature for 30 minutes. The gel was placed in the gel box after gently discarding the comb. 1X TAE electrophoresis buffer was loaded into the box until it reached 3-5 mm above the edge of the gel. Two micro-liters of loading dye were inserted carefully in separate wells of each 5 µl DNA sample. The PCR yields were loaded immediately. Each well received 5 µl of PCR product immediately. Electrical power of the electrophoresis device was turned on 100v/mAmp for sixty minutes.

Statistical analyses:

SPSS program was used for obtain *P*-values. *P* value below than 0.05 were deemed statistically significant.

Results and Discussion

An overall of 250 specimens were collected from different clinical sources: vagina, wounds, burns, urine and nasal which were 50 specimens for each source. The specimens were collected from Baaquba Teaching Hospital in Diyala during the period from October 2021 until February 2022. The results showed that the positive growth was 198 (79.2%), *S. aureus* represented the highest percentage of bacteria isolated from the study sources by (17.6%) followed by *E. coli, Klebsiella* spp., *Pseudomonas* spp., *Streptococcus* spp., *S. epidermidis, Proteus* spp. and *E. faecalis* which were 16.8%, 15.6%, 11.6%, 8.8%, 5.2%, 2% and 1.6% respectively. The positive growth among sources was the highest in burns specimen 92% followed by wounds, urine, vagina and nose which were 90%, 80%, 70% and 64% respectively (Table 2). Blood agar, Mannitol salt, and Baird parker agar were used to culture all isolates. Catalase was found to be positive for *S. aureus*, whereas oxidase was shown to be negative. The VITEK 2 System validated the identity of the isolated micro-organisms.

Table 2: Isolation of *Staphylococcus aureus*

Bacteria / source	Urine	Wounds	Burns	Nose	Vagina	Isolation %
S. aureus	2 (4 %)	13 (26%)	12 (24%)	12 (24%)	5 (10%)	44 (17.6%)
S. epidermidis	0	0	8 (16%)	2 (1%)	3 (6%)	13 (5.2%)
E. faecalis	4 (8 %)	0	0	0	0	4 (1.6%)
E. coli	16 (32 %)	5 (10 %)	5 (10%)	0	16 (32%)	42 (16.8%)
Klebsiella spp.	8 (16%)	14 (28 %)	8 (16%)	0	9 (18%)	39 (15.6%)
Pseudomonas spp.	1 (2%)	10 (20 %)	12 (24%)	4 (8%)	2 (4%)	29 (11.6)
Proteus spp.	1 (2%)	3 (6 %)	1 (2%)	0	0	5 (2%)
Streptococcus spp.	8 (16%)	0	0	14 (28%)	0	22 (8.8%)
Total of growth	40 (80%)	45 (90%)	46 (92%)	32 (64%)	35 (70%)	198 (79.2%)

The current result was in agreement with Sapkota *et al.*, [14], who reported that the incidence of *S. aureus* among different clinical sources was 19.96%, he also reported a high frequency of *S. aureus* in wounds infections. The present outcome is comparable to the findings of the a study conducted by Shahi, *et al.*, [15] which displays that the prevalence of *S. aureus* was 14.4%.

Our findings confirmed that 44 (100%) of the strains were (CoPS), as determined by the tube or slide technique. *S. aureus* can cause serious infections and must be distinguished from opportunistic coagulase negative *Staphylococci*. The coagulase test distinguishes *S. aureus* from those other *Staphylococci*. Furthermore, not even all *S. aureus* are coagulase positive, and not all coagulase positive *Staphylococci* are *S. aureus*. Other assays, in addition to the coagulase test, should be done to enhance the diagnosis of *S. aureus* [16].

In mannitol salt agar medium, all isolates caused fermentation of mannitol and changed the color of colonies to yellow due to the generation of acids that contribute to reducing the medium's pH and transforming the color of phenol red to yellow, *S. aureus* might ferment the mannitol sugar and generate yellow zones in the reddish agar., this test differentiates between *S. aureus* and *S. epidermidis*, which has the capability to procedure colonies with red zones on mannitol salt agar [17].

Interestingly, 66.6 % of the isolates have an opaque zone due to coagulase activity when cultured on Baird parker agar, according to the data of the detection of *coa* gene, all of isolates that surrounded by an opaque zone due to coagulase activity were positive for *coa* gene while

isolates that didn't have this character on Baird parker agar were negative for *coa* gene, this result was in agreement with Jasim and Alzubaidy [13] who reported that on Baird Parker agar, overall strains showed black, convex, and glossy colonies, suggesting the presence of *S. aureus* with a clear zone 34 (68%), confirming coagulase-positive, but some strains without a clear zone 16 (32%), demonstrating coagulase-negative.

Antibacterial susceptibility tests:

In the present study (Table 3), two cell wall synthesis inhibitors were tried, and the findings showed that the *S. aureus* strains were resistant to Ceftaroline by 100 % which was the most antibiotic resisted, this result was disagree with Urbán and Stone [18] who reported that the resistance for Ceftaroline was 4.5%. Imipenem was the second most resisted antibiotic by *S. aureus* which was 93%, this result was disagree with Al-Taey [19] who found that only 10% of *S. aureus* isolates were resist to Imipenem while Jasim and Alzubaidy [13] found that the resistance was 44%.

Seven agents of protein synthesis inhibitors, Netilmicin was the most effective antibiotic against *S. aureus* isolates, *S. aureus* isolates were resist to Netilmicin by 81% and resist in 13%, this result was in agreement with Jasim and Alzubaidy [13] who reported that 14% of *S. aureus* isolates were resist to Netilmicin. Chloramphenicol was resist in 25% and sensitive in 65% of *S. aureus* isolates, these findings were a somewhat close to AL-Zengena [20] and Noaman [21] who reported that the resistance to Chloramphenicol was 34% and 31% respectively. The resistance to Chloramphenicol in the current study was lower than that revealed by a local research done in Erbil hospitals by Rafiq and Hamad [22] and Sahm [23] who found that 94% and 88% of *S. aureus* isolates were resistant to chloramphenicol, respectively. Other earlier research conducted in Iran by Jafari-Sales *et al*., [24] demonstrated that the resistance rate to Chloramphenicol was (16.75%). Amikacin was resist in 38% and sensitive in 43% of *S. aureus* isolates, according to local study, AL-Zengena, [20] and Sahm, [23] reported that the resistance to Amikacin was 60%, 83% and 88% respectively while Al-Taey, [19] reported high sensitive rate to Amikacin which was 2.5%. In the present study, the current study indicate that

Gentamicin was resist in 43% and sensitive in 47% of *S. aureus* isolates, this findings was in agreement with AL-Zengena, [20], Al-Taey, [19] and Jasim and Alzubaidy [13] who reported that *S. aureus* isolates were resist to Amikacin by 40%, 37% and 56% respectively while it was disagree with Bobai, [17] who found that the resistance to Amikacin was 18%. Tetracycline was resist in 45% and sensitive in 45% of *S. aureus* isolates, this findings was in agreement with Noaman, [21] who report that *S. aureus* isolates were resist to Tetracycline by 41%, while it was disagree with Sahm, [23] who found a high rate of resistance to tetracycline which was 100%. A local study done in Baghdad hospitals by Saber and Kandala [25] who showed that resistance rate of these isolates to tetracycline was (62.8%). Clindamycin was resist in 56% and sensitive in 15% of *S. aureus* isolates, this result was in agreement with Noaman, [21] who found that *S. aureus* isolates were resist to Clindamycin by 47%, while Sahm, [23] reported that 72% of *S. aureus* isolates were resist to Clindamycin. Clarithromycin was resist in 59% and sensitive in 34% of *S. aureus* isolates, this result was disagree with Jasim and Alzubaidy [13] who reported that *S. aureus* isolates were resist to Clarithromycin by 74%.

Three agents of DNA synthesis inhibitors, Ofloxacin was resist in 17% and sensitive in 54% of *S. aureus* isolates, this result was disagree with AL-Zengena, [20] and Jasim and Alzubaidy [13] who reported that *S. aureus* isolates were resist to Ofloxacin by 32% and 40% respectively. Ciprofloxacin was resist in 43% and sensitive in 38% of *S. aureus* isolates, this result was in agreement with Al-Taey, [19] who reported that 40% of the *S. aureus* isolates were resist to Ciprofloxacin. The result were also approximately similar to another local study done by Saber and Kandala, [25] who monitored in their study in Baghdad hospitals that the resistance rate of Ciprofloxacin was (37.19%). Nitrofurantoin was resist in 88% and sensitive in 9% of *S. aureus* isolates, Jasim and Alzubaidy [13] found that 100% of *S. aureus* isolates were resist to Nitrofurantoin.

Table 3: Antibacterial susceptibility tests

Antimicrobial agent	Resistance pattern						*P*Value
	R	%	S	%	I	%	
CFT	44	100 %	0	0 %	0	0 %	0.000
IMI	41	93.2 %	3	6.8 %	0	0 %	0.035
NET	6	13.6 %	36	81.8 %	2	4.5 %	0.000
C	11	25.0 %	29	65.9 %	4	9.1 %	0.000
AK	17	38.6 %	19	43.2 %	8	18.2 %	0.096
GM	19	43.2 %	21	47.7 %	4	9.1 %	0.003
T	20	45.5 %	20	45.5 %	4	9.1 %	0.003
CD	25	56.8 %	7	15.9 %	12	27.3 %	0.003
CLA	26	59.1 %	15	34.1 %	3	6.8 %	0.000
OFX	17	38.6 %	24	54.5 %	3	6.8 %	0.000
CIP	19	43.2 %	17	38.6 %	8	18.2 %	0.096
NI	39	88.6 %	4	9.1 %	1	2.3 %	0.000

CFT= Ceftaroline, IMI= Imipenem, NET= Netilmicin, C= Chloramphenicol, AK= Amikacin, GM= Gentamicin, T= Tetracycline, CD= Clindamycin, CAL= Clarihromycin, OFX= Ofloxacin, CIP= Ciprofloxacin, NI= Nitrofurantoin.

Extended spectrum beta lactamase production

All of the 44 isolates of *S. aureus* were tested for the production of ESβL and MβL enzymes, the result of the present study displayed that 6.8% of *S. aureus* have the ability to produce ESβL (Table 4), 2.2% of the isolates were MβL producer with significant difference (P <0.001). The outcome of the present study was in compatible with Jasim and Alzubaidy, [13] who found that 2% of *S. aureus* have the ability to ESβL and MβL enzymes, while it was disagree with Aziz, [26] who showed that 34.8% of *S. aureus* have the ability to produce ESBL enzyme and Al-Taey, [19] who indicated that 32.5% of *S. aureus* were ESβL producers and 90% were MβL producers.

Table 4: Extended spectrum beta lactamase production

β- lactamase	No. of positive	percentage	*P* Value
ESβL	3	6.8%	< 0.001
MβL	1	2.2%	< 0.001

Distribution of isolates between Hospital acquired and Community acquired infection:

The outcomes of the present study exhibited that the majority of isolates were belonging to hospital acquired 72.72% while the community acquired infections caused by *S. aureus* were

27.27%. Over the last decade, the spread of community-acquired MRSA (CA-MRSA) has begun to spread more rapidly due to the increased transmission of resistant strains of MRSA [27]. For persons living in the community that have frequent contact with the healthcare system, nosocomial strain types of MRSA are a frequent cause of infection [28]. Lack of hand washing can also lead to the spread of bacteria more easily and can make persons more susceptible to CA-MRSA [29].

Genetic detection of *mec*A and *coa* genes and sequencing of *coa* gene:

A total of 24 isolates were selected to conduct gene detection for *mec*A gene and *coa* gene (Figure 1). Twelve isolates of *S. aureus* were selected as community acquired and 12 isolates of *S. aureus* as hospital acquired *S. aureus*, the isolates were selected according to resistance classifications which were PDR, XDR and MDR. Genetic detection results showed 19 of 24 isolates were positive for *mec*A while 21 of 24 isolates were positive for *coa* gene. Eight isolates were selected to conduct gene detection for *mec*A gene and *coa* gene. Four isolates of were selected as *S. aureus* community acquired (1, 29, 40 and 42) and 4 isolates as *S. aureus* hospital acquired (4, 6, 15 and 21). All the 8 isolate were positive for mecA gene and *coa* gene (Figure 1). In this study, we obtained different types of *coa* gene based on sizes, ranging from (650-812) bp, The reason of this variation among isolates might be deletion or insertion mutations in a portion of the 3 end area. Based on these findings, it is hypothesized that there is no universal primer for the *coa* gene, particularly in the 3 end region [30].

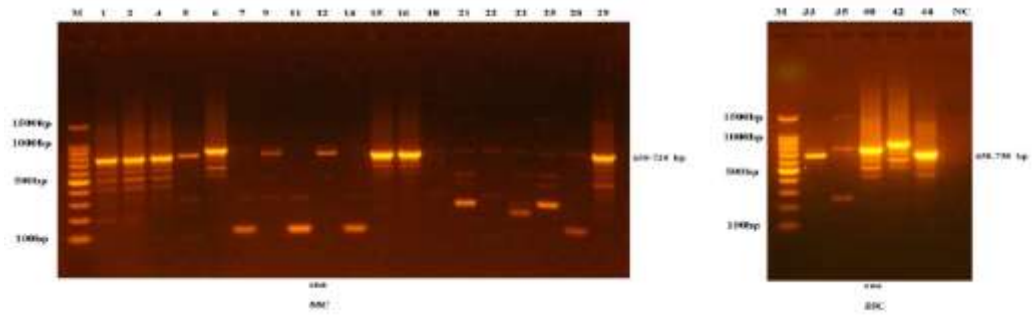

Figure 1: Results of the amplification of *coa* primers in *Staphylococcus aureus* specimens fractionated on 1.5% agarose gel electrophoresis stained with Eth.Br. M: 100bp ladder marker, NC: negative control at 100v/mAmp for 60min.

Within the targeted locus, eight specimens (assigned S1 to S8) were involved in the existing study. These specimens were screened using PCR fragments made of 812 bp to partially amplify the *coa* sequences, intergenic sequences located downstream of the *coa* gene, and all the coding sequences of the E4J95 gene. Thus, the variation of these *coa* sequences can be used for the description of this bacteria because of its potential capacity to adapt to changing genetic diversity, as demonstrated in many bacterial kinds. The NCBI BLASTn engine revealed 97% to 99% sequence similarities between the sequenced specimens and *S. aureus* reference target sequences for the 812 bp amplicons.

Two comprehensive phylogenetic trees were generated, which were based on the nucleic acid sequences found in the analyzed bacterial specimens In addition to the other DNA sequences that have been deposited, this phylogenetic tree contained the presently examined specimens (S1 to S8) aligned with its highly related sequences in a neighbor-joining mode. The total number of aligned nucleic acid sequences in the currently created trees was sixty-three. This complete tree included only one species, *S. aureus*, which contains the tree's only integrated nucleic acid sequences. Our examined sequences were grouped into many neighboring phylogenetic clades based on the investigated genetic sequences of *S. aureus*, suggesting the presence of a wide range of variety in this organism in relation to our analyzed sequences. (Figure 2, A and B). These nearby groups were represented by *S. aureus*, which included the recently examined S1 - S8 specimens.

The biggest clade consisted of the majority of incorporated specimens of *S. aureus* sequences as 14 sequences of variable strains of *S. aureus* sequences were incorporated within the same phylogenetic position in this major clade. Within this clade, variable strains were found to be deposited from variable Asian, American and European sources, such as China (GenBank CP038229.1, CP076028.1, CP076026.1, CP076029.1, CP076025.1, CP076027.1, and CP038229.1), USA (CP049374.1 and CP051912.1), and Denmark (GenBank CP047021.1). In the vicinity of this major clade, a small clade was also incorporated. Within this clade, the S5 specimen was incorporated with no observed close homology with any incorporated sequence. However, the reason behind such positioning of the S5 specimen away from the clade of S3,

S7, and S8 was due to the absence of any detectable variations in these three specimens. As well, eleven variants were detected in the S5 specimen that made it occupy this unique position within the currently generated phylogenetic tree. Besides the S5 specimen, several sequences were suited to constitute another clade of the variable distribution of *S. aureus* strains, such as GenBank AB436973.1, CP007659.1, LR130513.1 that were respectively deposited from Japan, Belgium, and Australia.

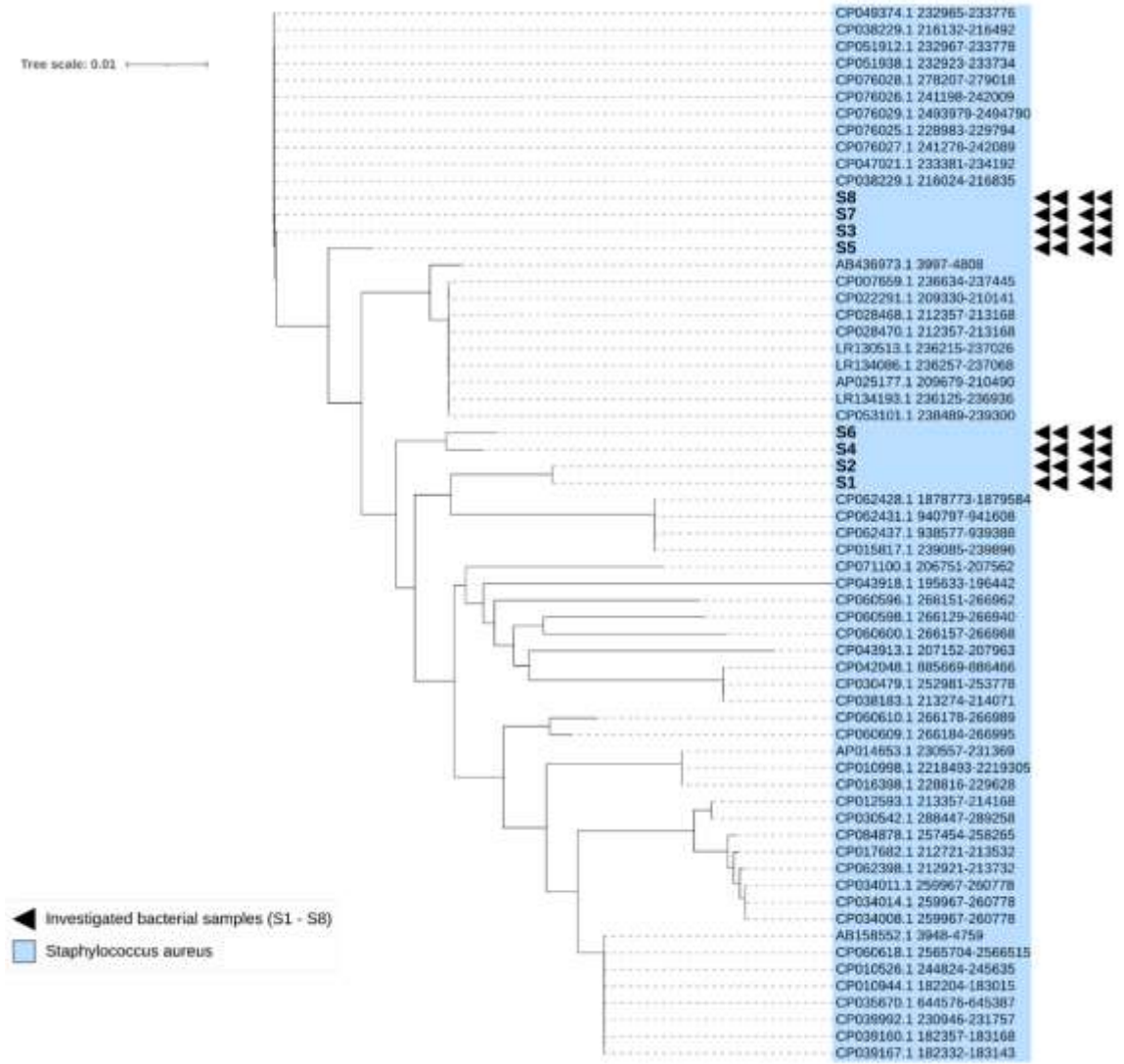

Figure 2: A) Traditional rectangular phylogenetic tree

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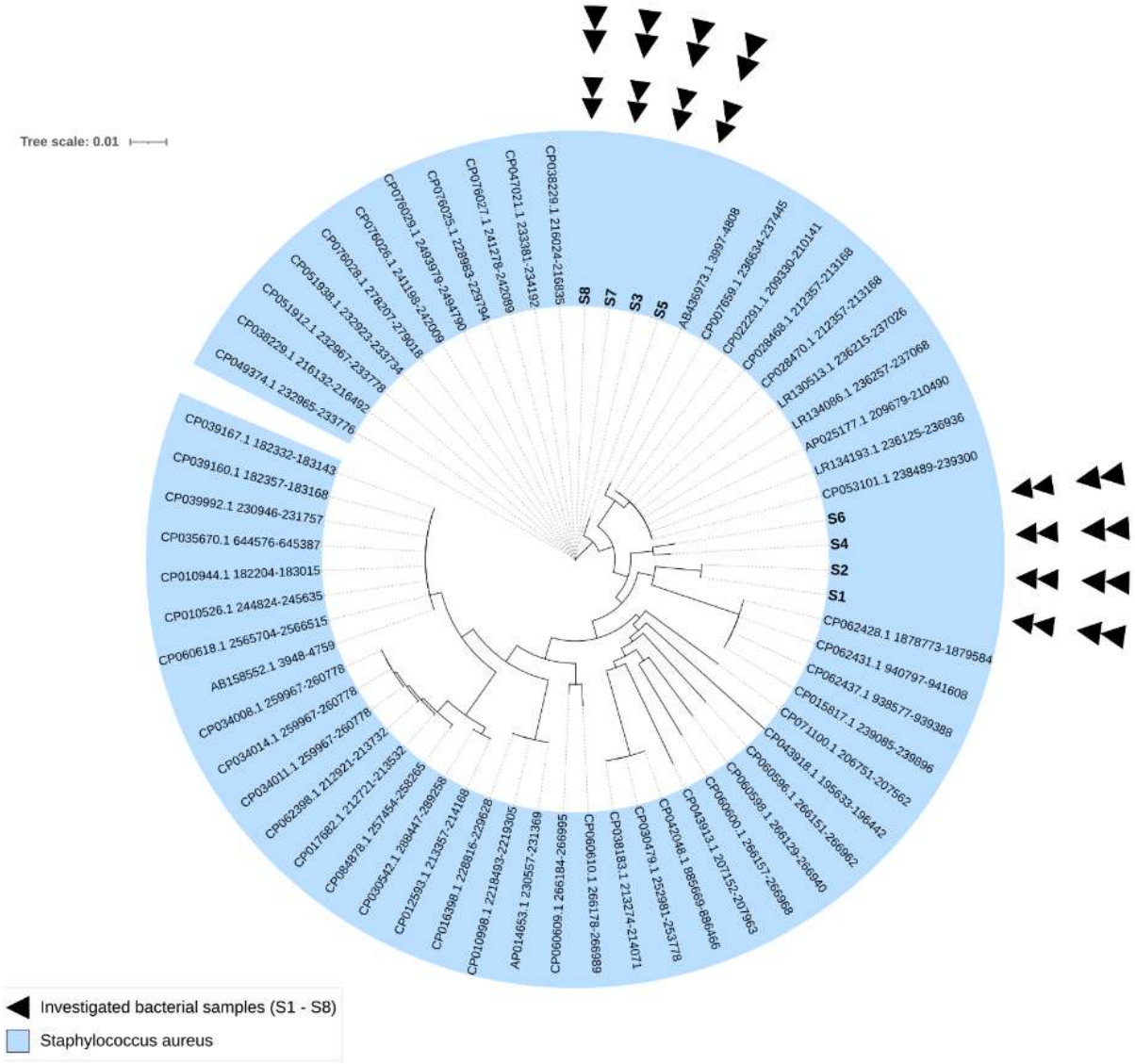

Figure 2: B) Circular phylogenetic tree

This notion indicated the little contribution of the observed variation in inducing any evolutionary effect on the incorporated sequences. Besides this clade, four sequences were equally positioned in a relatively distinct position. These sequences were CP062428.1, CP062431.1, CP062437.1, and CP015817.1. All these sequences were deposited from the USA. This is not the only phylogenetic distribution observed in the tree. Alternatively, other variable phylogenetic distributions were observed due to the presence of several other GenBank accession numbers deposited from various *S. aureus* strains worldwide. This observation

indicated the high variability of the amplified fragment. However, no obvious effect of the origin of isolation whether being nosocomial or community was seen in the positioning of both observed clades in the current tree.

However, the observed clades, in which all specimens were incorporated, were found to give clear discrimination for a broad distribution of community-based as well as nosocomial-based isolates in the current phylogenetic distribution. However, there is no remarkable deviation with respect to the original positioning of these bacterial sequences occupied within these two clades. Furthermore, the aggregation of all incorporated bacterial specimens within the clade of *S. aureus* possibly will mention to the occurrence of relatively close forms of the phylogenetic distribution of these sequences within this clade (tree scale 0.001). The existing statement of this tree has confirmed sequencing reactions because it explained the actual neighbor-joining-based positioning in such investigated sequences. In total, in addition to the Asian origins of these specimens, several American and European origins were also observed. This, the multiple origins of our investigated specimens could not be excluded from the explanation.

Interestingly, the unique positions occupied by all the variable specimens (S1, S2, S4, S5, and S6) have provided a further confirmation for the novelty of the observed variants, which entails a remarkable diversity in the investigated *coa* gene sequences. Despite the extremely high biological diversity observed in the currently investigated *coa* gene, all the observed variants in this locus were found to exhibit mixed localizations in both community and nosocomial acquired specimens. In contrast to the *coa* gene, the utilization of E4J95 sequences ensured extremely high specific localization of community and nosocomial acquired specimens despite its lower biological diversity. However, the utilization of these amplified sequences has given additional indication for the occurrence of the accurate identification of the actual description of these bacterial organisms. This 812 bp-based comprehensive phylogenetic tree has provided an inclusive tool for the higher capability of such fragments to efficiently discriminate among the currently investigated bacterial isolates. This, in turn, gives a further indication of the ability of the currently utilized sequence in the discrimination of the currently investigated human-infecting *S. aureus* and its accurate phylogenetic positions. Irrespective of the inability of these

sequences to discriminate among *S. aureus* isolates, the great capability of such genetic fragments to powerfully identify bacterial sequences. However, it is highly recommended to increase the number of specimens to discover further details about this interesting fragment.

Conclusions

The current study concluded a high resistance of *S. aureus* against different classes of antibiotics. HA-MRSA isolates were the predominant by 3:1 to CA-MRSA. Thirty-eight variations were found when the *coa* gene was sequenced by comparing this sequence with the reference sequence.

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