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Detection of Integron Classes and *Agr* Group in *Staphylococcus aureus* Isolated from Different Clinical Samples

Rasha Zaid Tariq Ahmed[1] and Rana Mujahid Abdullah[2*]
[1,2]Department of Biology College of Education Pure Science (Ibn Al-Haitham), University of Baghdad, Baghdad, Iraq.
*Corresponding Author.

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Abstract

Fifty isolates of *Staphylococcus aureus* were obtained out of 220 samples collected from clinical sources after a diagnosis of the bacteria, and then a sensitivity test was performed for the isolates, and the minimum inhibitor concentration of 15 antibiotics in the bacterial isolates under study was determined using the Vitek -2 Compact System device. All isolates showed 100% resistance to Benzylpenicillin at (>=0.5-0.12) and the resistance to Oxacillin was 76% at (>= 1-4), Ciprofloxacin and Moxifloxacin was 42, 40% at (>=2-8), Gentamycin (20%) at >=16, Erythromycin by (78%) at >=8, Clindamycin (by 74%) at (>=8-16), Tetracycline by 40% at (>=2-16), Fusidic Acid by (14%) at (>=8-32), and Rifampicin by (6%) at of (>=32 and Trimethoprim/ Sulfamethoxal by 14% at a concentration of (>=80-320). The isolates also showed less resistance to Vancomycin, Linezolid, and Teicoplanin (5, 2, and 2%), respectively, at a concentration of >=32 and all isolates were sensitive to Tigecycline (100%). Genomic DNA was extracted for the bacterial isolates under study and revealed that the bacteria possessed the integron. The results showed that *S. aureus* bacteria had the genes of the integron (*intI1*, *intI2*, *intI3*) using a specific promoter. The results showed that the most commonly found genes in *S. aureus* bacteria were the *intI1* gene by 41 (82%), followed by the *intI2* gene (5%). All isolates gave a negative result for the *intI3* gene. The result of molecular typing using the agr method showed that the percentage of the presence of the genes *agr1*, *agr2*, *and agr3* for *S. aureus* bacteria was 88, 6, and 35%, respectively, and all the isolates under study showed that they did not contain the *agr4* gene.

Keywords: *Staphylococcus aureus,* integron class I. П. III, agr group.

1.Introduction

The epidemiology of *Staphylococcus aureus* bacteria is one of the most important aspects that must be taken care of and taken care of because of its importance to the individual and population in order to control its distribution, confront the disease and prevent it, these bacteria work to settle different areas of the body such as the nose, pharynx, airways, armpits and skin and found about 25-30% endemic healthy people and this percentage increases in people working in hospitals about 80% [1]. Integron is defined as elements of animated DNA that have

the ability to implant and move in large pieces such as a plasmid and chromosome and acquire the exchange of gene bands that are mobile genetic elements that are characterized by having a recombination site and a single gene its size ranges between 500-1000 base pairs and usually gene strands merge within the integron between two genetic union sites (*attI, attC*) or exist in the form of a circular strand of DNA independently and the gene strands have the ability to move within the same genome of the organism or the genome of another organism. The gene strands have antibiotic resistance genes found within the recombination site (*attC*) that confer resistance to many antibiotics, including β-lactam antibiotics, aminoglycosides, rifampin, erythromycin, lincomycin, quinolones, chloramphenicol, trimethoprim [2]. One of the most common species found in gram-negative and gram-positive bacteria, including *Staphylococcus aureus*, which was considered important in its resistance to antibiotics through vertical gene transfer [3] is the Class 1 integron. This class is found at 9% in the genome of bacteria and is more prevalent and common among bacteria with clinical sources [2]. This class is directly related to plasmids and transposons, including (*Tn3* and *Tn402*) which play an important role in transferring class I integron within the genome of one organism or transferring it to the genome of another organism [4].

Many studies and research indicate depending on the integrase gene (*intI*) the presence of the first class integrons within three genetic link sites (*attI* 1, *attC*, Secondary site) which is associated with different genomes and this class is characterized by the ability to acquire gene bands responsible for resistance to many antibiotics and that the gene bands of the first class contain *aadA* resistance genes that code resistance to many antibiotics, including Streptomycin and Trimethoprim and have been observed in *Staphylococcus aureus* bacteria [2; 4; 5]. Studies indicated that Gram-positive bacteria, including *S. aureus* integron class 1, contain genes resistant to heavy metals and antibiotics and have the ability to move from chromosome to plasmid and vice versa, causing a genetic mutation after implantation at the target sequence site, and encoding the integron primarily in antibiotic-resistant *Staphylococcus aureus* [6]. Class 2 integron is structurally and organizationally similar to the first class and it is directly associated with the Transposon family, which includes (Tn7, Tn1825, Tn4132) that carry the region of *attI2* genetic reconnection and the promoter pc, which is found within the Transposon family and there are within the Transposon genes (*tnsD, tnsC, tnsB, tnsA, tnsE*) that mediate the transfer of the second class integron to the bacteria chromosome. Studies indicated that the percentage of amino acid homogenization within the genes of the second class *IintI2* is less than 50% compared to *intI1* genes that cannot complete the recombination process due to its loss of function and its production of an ineffective and short polypeptide [2].

Studies indicated that the gene bands of the second class *intI2* carry the genes dihydrofolate reductase (*dfrA1*), streptothricin acetyltransferase (sat1), and aminoglycoside adenyltransferase (*aadA1*), which mediate resistance to trimethoprim, streptothrivin, and streptomycin, and the gene bands of the second category integron also contain a gene *blaoxa2* resistant to β -lactam such as the oxacillin and ampicillin found in *S. aureus* bacteria [6]. It was found in gene bands *intI2* carrying the gene *ereA* mediates resistance to erythromycin and that this is a common type in gram-positive and gram-negative bacteria and is characterized by its ability to resist many antibiotics [7]. [3] explained that the genes of the integron first class are carriers of the *aadA* gene responsible for resistance to aminoglycosides, while the class 3 integron is organizationally and structurally similar to the first class and the second class, and its presence is associated with microorganisms isolated from soil and fresh water and is similar in function to the genes of the

first class *intI1*. It has the ability to acquire gene bands at the site of genetic recombination; *intI3* is less compared to the first and second-class integrons. The third class was first identified in Japan in 1993, where it was found in *Klebsiella pneumoniae* bacteria, after which it was identified in a few microorganisms such as *Acinetobacter* spp., *Escherichia coli*, *Seratia marcescens, and Salmonella* spp. isolated from the environment, and it is reported that the percentage of the presence of class III in gram-negative bacteria by 0–10% was resistant to Ceftazidime and Sulbactam-Cefoperazone. Studies have also indicated that its presence in bacteria isolated from animals is 7% [2]. The regulator of virulence factors *Agr* was first described in 1988 and codes for many virulence factors produced by *S. aureus* bacteria, such as hemolysin, toxic shock syndrome, staphylokinase enzyme, protein regulation, and biofilm [8; 9]. The helper genome, which differs from the basic genome, carries the basic genes that encode many activities such as growth and nutrition. The accessory genome includes mobile genetic elements (such as transposons, plasmids, phages, insertion sequences, pathogenicity islands, and genomic islands). Moving elements play an important role in regulating many virulence factors, such as transposons and plasmids. They carry genes that encode antibiotic resistance, and pathogenicity islands include Vsa1 and Vsa2. About 50% of the virulence factors responsible for encoding many intestinal toxin genes *(Seb*, toxic shock, and genomic syndrome) are encoded by genomic islands, which encode many virulence and antibiotic resistance factors. The bacterial bacteriophage encodes many of the virulence factors of these bacteria, such as exfoliative toxin, Panton-Valentine leucocidin (PVL), and some intestinal toxins, and the regulator of virulence factors *Agr* is the most studied virulence regulator and depends on the mechanism of quorum sensing, which works to regulate many virulence factors and toxins. The *agr* regulator consists of two units encoded by operon p1 and operon p2, where p2 encodes four types of proteins: *AgrA*, *AgrB*, *AgrC*, *and AgrD*, and the *AgrD* protein encodes the quorum sensing in *S. aureus* bacteria, which is a pentagonal structure AIP (autoinducing peptide) secretes to the outside through the phosphorylation of the *AgrC* protein and then activates *AgrA*, which works in a cooperative manner with the regulator *SarA* and encodes to produce operon p2 and p3, thus producing high levels of *RNAIII* catalyzed by the production of many virulent agents, including a-hemolycin, leukocyte toxin, lipase, and fibronectin-binding protein. At the end of the stationary phase and the logarithmic phase, as well as the production of *RNAII*, amplifying the quorum sensing signal [10, 11; 12; 13], The current study used the agr method to detect integron I, II, and III as well as genotype S. aureus bacteria with multiple antibiotic resistance.

2. Materials and Methods

2.1. Isolation of bacteria

220 samples were collected from different clinical sources: 30 samples of urinary tract infection, 50 samples of wounds, 40 samples of burns, 30 samples of sputum, and 70 samples of the cervix from several hospitals in the city of Baghdad: educational laboratories, Baghdad Teaching Hospital, Al-Saheed Ghazi Al-Hariri Hospital for Specialized Surgery, Burns and Wounds Hospital, and Medical City, during the period from 1/10/2022 to 30/1/2023.

2.2. Diagnosis of isolates

2.2.1. Culture diagnosis

Bacterial isolates were cultured on the Mannitol salt agar and Blood agar [14].

2.2.2. Gram Stain

Smears from bacterial isolates growing on mannitol salt agar were prepared at 24 hours of age, stained with gram stain, to determine whether the bacteria were Gram-positive or Gram-negative, and examined with light microscopy to observe the shape of the cells, their interaction, and their arrangement with the gram stain [15].

2.2.3. Biochemical test

Including: the Oxidase test [16], Catalase test [15], Coagulase test [17] and the final diagnosis of the isolates was done using the Vitek-2 device and according to the manufacturer's instructions.

2.3. Bacterial sensitivity testing was carried out using the Vitek-2 system

This is to determine the resistance of *Staphylococcus aureus* to 15 antibiotics, which included Linezolid, Ciprofloxacin, Gentamicin (Benzylpenicillin, Oxacillin, Fusidic Acid, Ciprofloxacin, Moxifloxacin, Erythromycin, Teicoplanin, Rifampicin Clindamycin, Vancomycin, Tetracycline, Tigecycline, Trimethoprim/ Sulfamethoxazole).

2.4. DNA isolation

A Genomic DNA Extraction kit prepared by Zymo (USA) was used to extract DNA for the bacterial isolates under study according to the manufacturer's instructions.

2.5. DNA Examination

The concentration and purity of DNA extracted from isolated samples using a nanodrop spectrophotometer are detected by determining the concentration of DNA, measuring its purity, and reading its absorption at a wavelength between 260 and 280 nm.

2.6. Molecular diagnosis of the integron genes of *S.aureus* bacteria

This study was conducted using PCR technology to detect the integron genes (intI1, *intI2*, *intI3*) and the *agr* genes (agr1, *agr2*, *agr3*, *agr4)*, as shown in **Table 1**.

Table 1. Oligonucleotide primer sequence and Size amplicon.

Target gene	Initial Sequence From 5′ to 3′	Product Size(bp)	Reference
IntiI	F-5′- CCTCCCGCACGATGATC-3 R-5′- TCCACGCATCGTCAGGC -3′	280	Ye *et al*. [6]
IntiII	F-5′- TTATTGCTGGGATTAGGC -3 F-5′- ACGGCTACCCTCTGTTATC -3′	233	Ye *et al*. [6]
IntiIII	R-5′-AGTGGGTGGCGAATGAGTG-3 F-5′-TGTTCTTGTATCGGCAGGTG-3	600	Ye *et al*. [6]
Agr1	R-5′-ATGCACATGGTGCACATGC -3 F-5′- GTCACAAGTACTATAAGCTGCGA-3	441	Gilot *et al*. [18]
Agr2	R-5′-ATGCACATGGTGCACATGC -3 F-5′-TATTACTAATTGAAAAGTGGCCATAGC -3′	575	Gilot *et al*. [18]
Agr3	R-5′-ATGCACATGGTGCACATGC -3 F-5′-GTAATGTAATAGCTTGTATAATAATACCCAG -3	323	Gilot *et al*. [18]
Agr4	R-5′-ATGCACATGGTGCACATGC -3 F-5′-GTAATGTAATAGCTTGTATAATAATACCCAG -3 R-5′-CGATAATGCCGTAATACCCG -3	659	Gilot *et al*. [18]

The reaction mixture was prepared to diagnose the genes: 25 µl for each gene (GO Taq Green Master Mix µl, 1.5 µl from DNA template, 1 µl from Primer Forward, and 1 µl from Reverse, 16 µl from ion-free distilled water), according to the manufacturer, Integrated DNA Technologies Company, Canada. The reaction conditions were determined according to the following program with modifications, as shown in **Tables 2** and **3**.

Table 2. The optimum condition of detection integrons gene

No	Stage	Temperature	Time	No. cycles
1	Initial Denaturation	°C94	5 min	1
2	Denaturation	°C 94	Sec45	
3	Annealing			
	IntI1	°C58	45sec	35
	IntI2	°C60		
	IntI3	°C58		
4	Extension	°C72	1min.	
5	Extension -2	°C72	7 min.	1

Table 3. The optimum condition of detection *agr* gene.

No.	Phase	Tm (°C)	Time	No. of cycle
1	Initial Denaturation	94°C	5 min.	1 cycle
2	Denaturation -2	94°C	30 sec.	
3	Annealing	57 °C	90 sec.	
4	Extension-1	72°C	60 sec.	35 cycle
5	Extension -2	72°C	7 min.	1 cycle

2.7. The reaction products were separated using Agarose:

Bio Basic INC (Canada) with a concentration of 2% containing 5 µl of Red Safe Nucleic Acid Staining Solution and using DNA ladder (100-1500) base pairs with a voltage difference of 5 volts for an hour was imaging using UV light Optima (Japan).

2.8. Phylogenetic tree Construction

Photo captions were used to calculate the molecular size to detect bands resulting from PCR reactions and compare them to the size of the DNA Ladder [19].

3. Results and Discussion

Identification was made based on the morphological characteristics of isolates when cultured on blood agar. The bacterial colonies appeared convex in shape, shiny with smooth, rounded edges, and white , tending to lead to a golden yellow and fully β-hemolysis type [20] when grown on mannitol salt agar containing 7.5% NaCl. Bacterial colonies appear with a non-zigzag edge, a rounded shape, and are yellow. They are fermented for mannitol with a diameter ranging from 0.1–0.8 mm, which acts to decrease the pH and thus leads to the appearance of a yellow color. This is considered a differential examination between the species of staphylococci [21].
Microscopic examination of slides prepared colonies stained with gram-stain showed that gram-positive form clusters resembling bunches of grapes with a dark purple color, and non-spores-forming [22], and when performing biochemical tests approved for the diagnosis of *S.aureus* bacteria, Oxidase test, Catalase test and Coagulase test were used, all isolates gave a negative result for the Oxidase test, all isolates showed a positive result for the Catalase test and all isolates were given initially diagnosed as a positive result of the Coagulase test conducted by the tube method when a layer appears inside the tube indicates the ability of bacteria to produce the plasma coagulant enzyme and works to convert fibrinogen to fibrin [23]. After conducting laboratory tests, culture diagnosis, and using the Vitek-2 Compact System, 50 isolates of *S. aureus* bacteria were obtained out of a total of 220 isolated samples from different clinical cases, both genders and different ages, and from several hospitals in Baghdad. The results of the study showed that the highest percentage of *S. aureus isolates* was from burns (15 isolates) at 30%, followed by wounds (12 isolates) at 24%, urine (10 isolates) at 20%, and the lowest percentage

of infection isolated from the cervix (8 isolates) at 16% and sputum (5 isolates) at 10%, as shown in **Table 4.**

Table 4. Source, Numbers and Percentages of *S.aureus* isolates**.**

Source of isolate	Number of isolates	Percentage%
Urine	10	20
Wounds	12	24
Cervicitis	8	16
Burns	15	30
Sputum	5	10
Total number	50	100

The results were in agreement with the findings of local studies [24] and [25] in their study conducted in China that the highest rate of injury from burns is 60%, and the result is also consistent with the results of [26], who showed that the highest percentage of injury was isolated from burns at 51 (53.13%), and the result was close to the findings of [27] and [28], who showed that the percentage of these bacteria isolated from burns and wounds was 40.36%, respectively.

The result differed from the [29] study; the percentage of staphylococcal bacteria isolates from wound samples was 57.1%, the lowest rate of infection, which amounted to 7% of urinary tract infections, and the result agreed with the results [30], who recorded the highest rate of infection in males at 90% compared to females at 60%. *Staphylococcus aureus* is one of the most pathogenic bacteria that can infect the community and cause infections in hospitals because of its ability to transmit infection and its multiple resistances to antibiotics [31].

The isolates showed varying antibiotic resistance when using 15 antibiotics using the Vitek-2 system. All isolates showed 100% resistance to benzylpenicillin at the minimum inhibitor concentration (>=0.5–0.12). The resistance to Oxacillin was 76% at concentration (>=1-4), and for each of the two antibiotics Ciprofloxacin and Moxifloxacin by 42%, 40% at concentration (>=2-8), Gentamycin by 20% at a concentration of >=16, Erythromycin by 78% at a concentration of >=8, and Clindamycin by 74% at concentration of (>=8-16), Tetracycline by 40% at concentration of (>=2-16), Fusidic acid by 14% at a concentration of (>=8-32), Rifampicin by 6% at a concentration of (>=32), and Trimethoprim/Sulfamethoxal by 14% at a concentration of (>=80-320). The isolates showed the least resistance to Vancomycin, Linezolid, and Teicoplanin (5, 2, and 2%), respectively, at the minimum inhibitor concentration (>=32), as shown in **Table 4**. The results of the current study agreed with many studies, including [32] study which recorded the percentage of resistance *o4. aureus* to Benzyl penicillin (100%) and the study differed with the results of [33] who found resistance of *S. aureus* isolates to benzyl penicillin by 66.8%. The current study showed that the percentage of resistance to oxacillin was 76%, and it was closely compatible with the studies of [34], [35], [36], and [37], who recorded the percentages (90, 81.92, 62.7, 90%), respectively. The result of the current study agreed with the results of [39] Abdlel-Mongy *et al.* (2018), who found that the resistance rate of erythromycin was 78%, and the result of the current study differed from the results reached by [40] that they conducted on 200 isolates of *Staphylococcus aureus.* The resistance rate for this antibiotic was 29.5%, and the current study showed that the resistance rate of Clindamycin was 74%. The result agreed closely with the study carried out by [41] and the study carried out by [42], who showed that the percentage of resistance to Clindamycin reached 68.8% and 80%, respectively, and studies indicated that Clindamycin is used to treat skin injuries in people who

are allergic to penicillin [43]. S. aureus isolates showed resistance to Gentamicin by 20%. [40] stated that the percentage of resistance to Gentamicin was 7.5% and the results of the study disagreed to the study carried out by [44], they found a resistance rate to Gentamicin was 48.48%. The study showed that the percentage of resistance to Ciprofloxacin and Moxifloxacin reached 42 and 40%, respectively, The result of the study showed that the resistance rate of *S. aureus* to tetracycline was 40%. [37] indicated in their study that the percentage of resistance to tetracycline reached 46%. The percentage of resistance to fusidic acid, trimethoprim, and sulfamethoxal in the current study was 14%, and the result was fairly close to the result of the study carried out by [49], who found a resistance rate of 6.70%.

The current study showed that the percentage of resistance to Rifampicin was 6%, and [27] and [52] found the percentage of resistance to Rifampicin to be (47, 61.8%), respectively, which is higher than the findings of this study. Rifampicin combined with vancomycin, is used to treat MRSA-resistant *Staphylococcus*. The reason for the resistance of *S. aureus* bacteria to rifampicin, which works to inhibit the synthesis of RNA polymerase, is the occurrence of chromosomal mutations encoded by the *rpoB* gene that prevent the antibiotic from binding to the target site [53]. The current study showed the least resistance to the antibiotics Vancomycin, Linezolid, and Teicoplanin by (5,2, 2%), respectively. [40] found in their study that the sensitivity percentages *of S. aureus* to antibiotics (Linezolid, Tigecycline, and Teicoplanin) were 100%, which was closely consistent with the current study and differed from the [54] study, which showed the percentage of resistance to Linezolid was 16.9%. Vancomycin was an effective antibiotic for staphylococcus resistant to MRSA. The isolates under study showed that the least resistance to vancomycin was 5%. [51] showed that the sensitivity percentage of *S. aureus* bacteria to Vancomycin was 100%, and the result of the current study differed from the study carried out by [55] and [54], who found the percentage of resistance to vancomycin to be 10 and 13.5%, respectively.

Table 5. Shows the percentage of the minimum inhibition values of *S.aureus* bacteria**.**

Antibiotic	Resistance		Resistant Values of MICs
	No. Isolates	Percentage %	
Benzylpenicillin	50	100	0.5 -0.12 ≤
Oxacillin	38	76	1-4 ≤
Gentamicin	10	20	16 ≤
Ciprofloxacin	21	42	2-8 ≤
Moxifloxacin	20	40	2-8 ≤
Erythromycin	39	78	8≤
Clindamycin	37	74	8-16≤
Linezolid	1	2	≤32
Teicoplanin	1	2	≤32
Vancomycin	2	5	32≤
Tetracycline	20	40	2 -16≤
Fusidic Acid	7	14	8- 32≤
Rifampicin	3	6	≤32
Trimethoprim/Sulfamethoxazole	7	14	80- 320 ≤

The *intI1* gene was detected in *S.aureus* isolates and the percentage was 82% and when comparing the multiplied bands with the volumetric index, it was found that the resulting bands with a molecular weight of 280 base pairs as shown in **Figure 2 A and B**. The result showed the detection of the *intI2* gene that the percentage of *S.aureus* possession of the *intI2* gene was 5%

and when comparing the multiplied bands with the volumetric index, it was found that the resulting beams have a molecular weight of 233 as in **Figure 2** and when investigating the *intI3* gene, the result of the current study showed a negative result for all isolates as shown in **Table 6**.

Table 6. Shows the distribution of the integrons between *S.aureus* isolates

No	Source	Number of isolates with integrons gene		
		IntI1	***IntI2***	***IntI3***
		No. (%)	**No. (%)**	**No. (%)**
1	**UTI**	10(20)	0	0
2	**Wounds**	11(22)	0	0
3	**Cervix**	6(12)	0	0
4	**Burns**	10(20)	2(10)	0
5	**Sputum**	4(8)	0	0
	Total No.	41(82)	2(10)	0

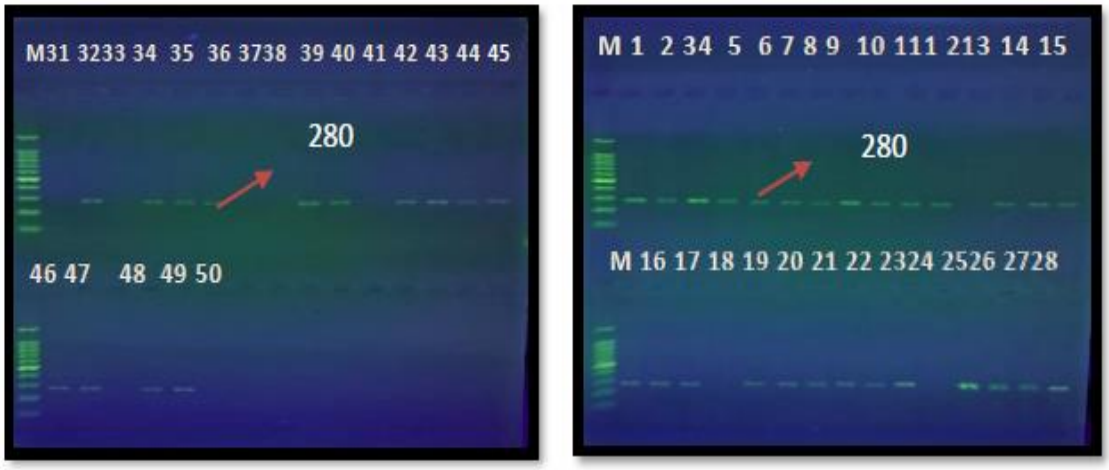

Figure 2. A- Electrophoresis of PCR product for *S.aureus* isolates on agarose gel at a concentration of 2% and a potential difference of 5 V for 60 min for the M path represents the volumetric index (1500-100) ,280 base pairs) A: Pathways (12, 19, 26, 31, 33, 40, 48) of *intI1* for positive isolates

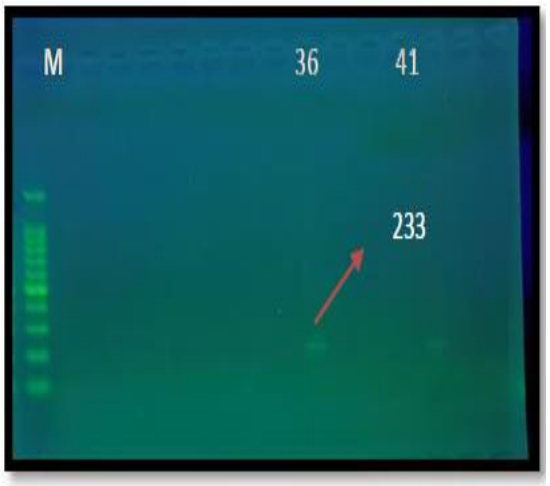

Figure 2. B- Electrophoresis of PCR product for *S.aureus* isolates on agarose gel at a concentration of 2% and a potential difference of 5 V for 60 min for the M pathway represents the volumetric index (1500-100), Pathways (36-40) for *intI2* positive isolates, 233 base pairs.

The results were close to the findings of [7] who found the percentage of integron 1 was 101 isolates (72.6%) out of a total of 139 isolates and the percentage of *intI2* in *S.aureus* isolates was

49 isolates (35.2%). The study carried out by [56] also indicated that the first class integron in *S. aureus* bacteria isolated from different clinical cases in Iran by (56.3%) and the second class integron by (18.7%) and the result of the third class integron was negative for all isolates. [3] who found that the percentage of *S.aureus* had of the *intI1* gene was (46.6%) and the *intI2* gene was (3.4%). [57] were showed in their study at China that the percentage of *S. aureus* bacteria had the *intI1* gene was (85.1%) and the percentage of *intI2* and *intI3* were negative for all isolates. [45] showed that the possession percentage of *S.aureus* class integrin 1 was (24.8%) and all isolates showed negative result of class *IntI* 3 and studies carried out by [7] indicated that the genes of the first class integron carry genes (*aadA*, *aadB*), which are responsible for resistance to aminoglycosides antibiotics such as (Gentamicin, tobramycin, Kanamycin, Streptomycin) and also carry the genes *dfrA1* and *dfrA11* mediate resistance to trimethoprim and also carry *blaoxa2* genes that code resistance to β-lactam and carry genes (*catB3*, *catB8*, *cmlA6*) responsible for resistance to chloramphenicol antibiotic. Studies indicated that the genes of the second class of integron that carry the genes of dihydrofolate reductase (*dfrA1*) acetyltransferase (*sat1*) and aminoglycoside adenyltransferase (*aadA1*) responsible for resistance to trimethoprim, streptothrivin, streptomycin, and the genes of the second class integron also contain the gene *blaoxa2* responsible for resistance to β-lactam antibiotics such as oxacillin and ampicillin, which was found in *S. aureus* bacteria [6]. [58] explained that the genes of the class 1 integron that carry the *sul1/qacEΔ1* gene responsible for resistance to antibiotics, erythromycin, ciprofloxacin trimethoprim/ sulfamethoxazole.

The result of electrophoresis when detecting AGR A genes showed that the percentage of *agr1*, *agr2, and agr3* presence for *S. aureus* bacteria was 88, 6, and 35%, respectively, and by comparing the multiplied bands with the volumetric index, it was found that the resulting bands had a molecular weight of 441, 575, and 323, base pairs, as shown in **Table 7.** and **Figure 3**.

Table 7. Shows the distribution of *agr* in relation to the source of isolates

No	Source	*Agr* group			
		***Agr1* No. (%)**	***Agr2* No. (%)**	***Agr3* No. (%)**	***Agr4***
1	UTI	10	1	8	-
2	Wounds	11	1	10	-
3	Cervix	5	-	7	-
4	Burns	13	1	5	-
5	Sputum	5	-	5	-
	Total No.	44(88)	3(6)	35(70)	-

Figure 3. Electrophoresis of PCR product of genes (*agr1* 441, *agr2* 575, *agr3* 323, *agr4* 659) base pair for *S.aureus* isolates on agarose gel at a concentration of 2% and a potential difference of 5 V for 60 minutes. Pathway M represents volumetric index 1500-100 base pairs, pathways (1-30) for *agr* gene of positive isolates.

The current study agreed with the results of [59] Rasheed and Hussein (2020), who showed that the percentage of the *agr* presence in staphylococcal bacteria MRSA was 51 (76.1%), 4 (5.62%), and 8 (10.52%), respectively, out of a total of 67 isolates, and all isolates showed a negative result for the *agr4* gene.

[13] showed that *S. aureus* had *agr1* of *16* (29.09%), *agr2* of 30 isolates (54.54%), *agr3* of 6 isolates (10.9%), and *agr4* of 3 isolates (5.45%) out of 55 isolates. [60] found that the rate of regulation of the virulence genes *agr1* in the resistance *S. aureus* was 14 (50%), *agr2* was 3 (10.7%), *agr3* was 10 (35.8%), and *agr4* was 1 (3.5%) of a total of 28 isolates. The current result differed from the study carried out by [61], who showed that the percentage of *Agr1* presence in bacteria was 5% and the percentage of *Agr3* presence was 55%. [62] showed that the presence rate of *Agr1* was 131 isolates (78.4%), *Agr2* was 17 isolates (10.2%), and *Agr3* reached 8 isolates (4.8%). [28] showed that the presence rate of *Agr1* reached 82 isolates (55%), *Agr2* was 37 isolates (25%), *Agr3* reached 10 isolates (7%) and *Agr4* was 21 isolates (14%). Studies have indicated that the *AGR* regulator codes for several genes responsible for virulence in *S. aureus* bacteria (such as a and β-hemolysin, leucocidin, lipase, toxic shock syndrome *tsst*, coagulase, and fibronectin-binding protein [63, 64]. Studies have indicated that each group of *agr* regulators mediates several diseases, such as *agr4* encoding exfoliative toxin (*eta etb*) and impetigo, as well as *agr1* and *agr2* encoding many enterotoxin genes (*Seg*, *Sei*, *Sem*, *Seo)*, leukytox-lethal genes *LukF-PV* and *LukS-PV*, and toxic shock syndrome genes (*tst*) regulated by *agr1*, *agr2*, and *agr3* [65, 66]. [67] shows that bacterial resistance to MRSA is regulated by *Agr2*.

Many researchers have confirmed the use of the *agr* method as a method of genotyping [60]. Studies have indicated that the typing method has become important in working to find genetic relationships between bacterial strains, classify bacteria on the basis of their epidemiology, identify methods and sources of infection, distinguish bacterial strains with high virulence and treat them to prevent their spread [68]. The genetic relationship was found and identified by typing *S. aureus* under study by using the *agr* method, where the sequences of this method were found spread in multiple regions of the bacterial genome *agr1*, *agr2*, *agr3*, *agr4*. The results of the current study showed the existence of genetic relationship between bacterial isolates isolated from different clinical sources. The results of the current study showed the presence of four clones, which is clonal A includes 5 (**Figure 4**) bacterial isolates found between them with genetic convergence and in one clonal and isolated these isolates from patients in Baghdad Teaching Hospital and the source from which these isolates were isolated was from cases of wounds and urine and these isolates had the genes *intI1*, *agr3* and resistance to antibiotics and clonal B includes 7 bacterial isolates found between them genetic convergence and isolated from patients in the hospital burns and wounds /Medical City and the source isolates burns and wounds and had the genes (*intI1*, *agr1*) and integron *intI1* and clonal C includes 28 bacterial isolates found between them genetic convergence and isolated from Baghdad Teaching Hospital, Burns and Wounds Hospital and Al-Shaheed Ghazi Al-Hariri Hospital for Specialized Surgery and the source of isolates burns, wounds and sputum characterized by having *agr1*. *agr2*. *agr3*. *intI1*., *int2*. The clonal D includes 7 bacterial isolates found between them genetic convergence and in one clonal isolated from several hospitals in the Medical city and the source of isolates were cervical, sputum and burns and it is characterized by having genes (*agr1*, *agr3*, *int1*). and found three isolates of bacteria that were not related to each other and were isolated from private clinics. it is characterized by having genes (*agr1*, *agr2*, *agr3*, *int1*). The result of the current study as shown in **Table 8.** appeared that isolates have *agr1*, *agr3* genes and they are

characterized by their resistance to many antibiotics The results showed that Integron 1 is characterized by its resistance to antibiotic Tetracycline, Vancomycin, Ciprofloxacin, Moxifloxan).

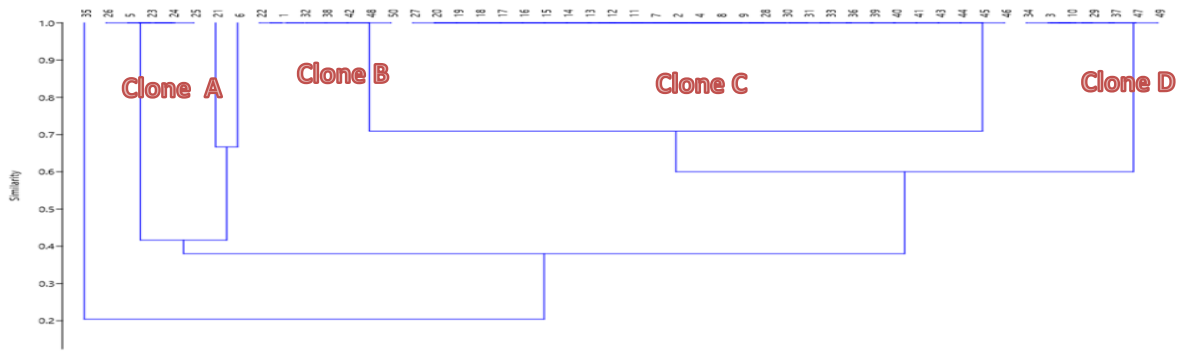

Figure 4. Dendogram of *S.aureus* isolates using Past Jaccard/up GMA.

Table 8. Distribution of integron and agr among *S.aureus* isolates.

NO.	*Intl1*	*Intl2*	agrtype	Resistance profile
1	+	-	1	Cip.Oxa.Ben
2	+	-	1.3	Tet.Clind.Eryth.oxa.Ben
3	+	-	1.3	Tet.Clind.Eryth.oxa.Ben
4	+	-	1.3	Tet.Clind.Eryth.oxa.Ben
5	+	-	1	Tet.Clind.Eryth.oxa.Ben.Genta.Vanco.Teico.Linez.Cip.Moxi
6	+	-	1.2.3	Tet.Clind.Eryth.oxa.Ben.Genta.Cip.Moxi
7	+	-	1.3	Tet.Clind.Eryth.oxa.Ben.Genta.Cip.Moxi
8	+	-	1.3	Tet..Eryth.oxa.Ben.Genta.Cip.Moxi
9	+	-	1.3	Tet..Eryth.oxa.Ben.Genta.Cip.Moxi
10	+	-	1.3	Tet..Eryth.oxa.Ben.Genta.Cip.Moxi.Trime
11	+	-	1.3	Clind.Eryth. Ben.
12	-	-	1.3	.Eryth.oxa.Ben.Genta.Cip.Moxi
13	+	-	1.3	Tet..Eryth.oxa.Ben.Genta.Cip.Moxi.Trime.clind
14	+	-	1.3	Tet..Eryth.oxa.Ben.Cip.Clind.Moxi
15	+	-	3	Tet..Eryth.oxa.Ben. Cip.Moxi.
16	+	-	1.3	Clind.Tet.Eryth.oxa.Ben
17	+	-	1.3	Tet..Eryth.oxa.Ben.Genta.Cip.Moxi.
18	+	-	1.3	Tet..Eryth.oxa.Ben..Cip.Moxi.Trime.clind
19	-	-	1.3	Clind,Eryth.Ben
20	+	-	1	Tet..Eryth.oxa.Ben. Cip.Moxi.Trime.clind
21	+	-	1.2	Fusidic.Ben.oxa
22	+	-	1	Clind,Eryth.Ben
23	+	-	3	Oxa.Ben
24	+	-	3	Clind.Eryth.Ben
25	+	-	3	Oxa.Ben
26	-	-	3	Clind.Eryth.Ben
27	+	-	1.3	Clind,Eryth.Ben
28	+	-	1.3	Oxa.Ben
29	+	-	1.3	Clind,Eryth.Ben
30	+	-	1	Oxa.Ben.Clind
31	-	-	1.3	Clind,Eryth.Ben
32	+	-	1	Clind,Eryth.Ben
33	-	-	1.3	Oxa.Ben
34	+	-	1.3	Clind,Eryth.Ben
35	+	-	2	Oxa.Ben

NO.	*Intl1*	*Intl2*	agrtype	Resistance profile
36	+	+	1	Clind,Eryth.Ben
37	-	-	1	Oxa.Ben
38	-	-	1	Clind,Eryth.Ben.oxa
39	+	-	1.3	Tet..Eryth.oxa.Ben. Cip.Moxi..clind
40	+	-	1.3	Tet..Eryth.oxa.Ben. Cip.Moxi..clind.vanco
41	-	+	1.3	Tet..Eryth.oxa.Ben. Cip.Moxi..clind
42	+	-	1	Clind,Eryth.Ben.oxa
43	+	-	1.3	Tet..Eryth.oxa.Ben. Cip.Moxi.Trime.clind.Genta.Fusidic.Rifam
44	+	-	1.3	.Tet..Eryth.oxa.Ben. Cip.Moxi..clind.Genta.Fusidic.
45	+	-	1.3	.Tet..Eryth.oxa.Ben...clind.Genta.Fusidic.
46	+	-	1	Clind,Eryth.Ben
47	+	-	1.3	.Eryth.oxa.Ben..Cip.Moxi.clind
48	-	-	1	Ben.Oxa
49	+	-	1.3	clind..Eryth.oxa.Ben.Cip.Moxi
50	+	-	1	clind..Eryth.oxa.Ben.Cip.Moxi

- **Oxa:Oxacillin, Genta: Gentamicin, Cip:Ciprofloxacin, Moxi: Moxifloxacin, Eryth: Erythromycin, Ben: Benzylpenicillin, Clind: Clindmycin, Linez: Linezolid, Teico Teicoplanin, Vanco: Vancomycin, Tet: Tetracyclin, Tige: Tigecycline, Fusid: Fusidicacid,Rifam: Rifampicin. Trime: Trimethoprim/ Sulfamethoxazle**

4. Conclusions

Staphylococcus aureus isolates were more prevalent in burn samples, according to the research findings. The results of the detection of the genes of IntI1 were major among these isolates and were characterized by their resistance to antibiotics such as Tetracycline, Vancomycin, Ciprofloxacin, and Moxifloxacin. The Agr 1 and Agr 3 methods have shown high efficiency among *S. aureus* isolates

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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Ethical Clearance

The samples were gained according to Scientific Research Ethics Committee approval in College of Education for Pure Sciemces (Ibn Al-Haitham), University of Baghdad No. EC-11 and local research Ethics committee approval in Iraqi Ministry of Health No. 47737 in 13/11/2022

References

1.Taylor, T.A.; Unakal, C.G. *Staphylococcus aureus*. In: *Stat Pearls Treasure Island* (FL): Stat Pearls Publishing **2022**, https://www.ncbi.nlm.nih..gov/books/NBK441868.

2.Deng, Y.; Bao, X.; Ji, L.; Chen, L.; Liu, J; Miao, J.; Chen, D.; Bian, H.; Li, Y., Yu, G. Resistance integrons: class 1, 2 and 3 integrons. *Ann Clin Microbiol Antimicrob*. **2015**, *20(14)*, 45. doi: 10.1186/s12941-015-0100-6. https://doi.org/10.1016/j.envc.2021.100166.

3. El-Baz, A.M; Yahya, G; Mansour ,B; El-Sokkary, M.M.A; Alshaman, R.; Alattar, A; El-Ganiny, A.M. The Link between Occurrence of Class I Integron and Acquired Aminoglycoside Resistance in Clinical MRSA Isolates. *Antibiotics (Basel)*. **2021**, *10(5)*, 488. DOI: 10.3390/antibiotics10050488.

4.Ghaly, T.M.; Gillings, M.R; Penesyan, A.; Qi, Q; Rajabal, V.; Tetu, S.G. The Natural History of Integrons. *Microorganisms* **2021**, *9*(*11*), 22-12. DOI: 10.3390/microorganisms9112212.

5.Zárate, S.G.; De la Cruz Claure, M.L.; Benito-Arenas, R.; Revuelta J., Santana A.G.; Bastida A. Overcoming aminoglycoside enzymatic resistance: Design of novel antibiotics and inhibitors. *Molecules* **2018**, *23*, 284. DOI: 10.3390/molecules23020284.

6. Ye, C;Hou, F.; Xu, D.; Huang, Q.; Chen, X.; Zeng, Z.; Peng Y.; Fang, R. Prevalence and Characterisation of Class 1 and 2 Integrons in Multi-drug Resistant *Staphylococcus Aureus* Isolates from Pig Farms in Chongqing, China. *J Vet Resp*. **2020**, *64(3)*, 381-386. DOI: 10.2478/jvetres-2020-0061.

7.Mostafa, M.; Siadat, S.D.; Shahcheraghi, F.; Vaziri, F.; Japoni-Nejad, A.; Yousefi, J.V.; Rajaei, B.; Mood, E..H.; Moshiri, A.; Siamdoust, S.A.S. Variability in gene cassette patterns of class 1 and 2 integrons associated with multi drug resistance patterns in *Staphylococcus aureus* clinical isolates in Tehran-Iran. *BMC Microbial*. **2015**, *15*, 1–9. DOI: 10.1186/s12866-015-0488-3.

8.Mohsenzadeh, M.; Ghazvini, K., and Azimian, A. Frequency of specific agr groups and antibiotic resistance in *Staphylococcus aureus* isolated from bovine mastitis in the northeast of Iran. *Vet. Res. Forum* **2015**, *6*, 295–299.

9.Tahmasebi, H.; Sand, D.; Arabestani, M.R. Association between the accessory gene regulator (*agr*) locus and the presence of superantigen genes in clinical isolates of methicillin-resistant *Staphylococcus aureus*. *BMC Res Notes* **2019**, *12(1)*, 130. DOI: 10.1186/s13104-019-4166-7.

10.Khoshnood, S.; Heidary, M.; Asadi, A.; Soleimani, S.; Motahar, M.; Savari, M., Saki, M. and Abdi, M. A review on mechanism of action, resistance, References 88 synergism, and clinical implications of mupirocin against *Staphylococcus aureus*. *Biomedicine and Pharmacotherapy* **2019**, *109*, 1809-1818.DOI: 10.1016/j.biopha.2018.10.131.

11.Khodaverdian, V.; Pesho, M.; Truitt, B.; Bollinger, L.; Patel, P.; Nithianantham, S. Discovery of antivirulence agents against methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother*. **2013**, *57*, 3645–3652. DOI: 10.1128/AAC.00269-13.

12. Saedi, S; Derakhshan, S.; Ghaderi, E. Antibiotic resistance and typing of *agr* locus in *Staphylococcus aureus* isolated from clinical samples in Sanandaj, Western Iran. *Iran J Basic Med Sci*. **2020**, *23(10)*, 1307-1314. DOI: 10.22038/ijbms.2020.46064.10661.

13. Latifpour, M.; Narimani, T.; Sadeghi, A.; Niakan, M. Determination of Virulence Factors and Resistance Profile of Methicillin-Resistant *Staphylococcus aureus* Strains among Different Types of *spa*, *agr*, and SCC*mec*. *Biomed Res Int*. **2022**, *15(2022)*, 5863310. DOI: 10.1155/2022/5863310.

14. Drinka, P.; Bonham, P.; Christopher, J. Swab culture of purulent skin infection to detect infection or colonization with antibiotic-resistant bacteria. *J Am Med Dir Assoc*. **2012**, *13*, 75-79. DOI: 10.1016/j.jamda.2011.04.012.

15. Thille, P.M. *Bailey & Scott's Diagnostic Microbiology*. 14th Edition **2016**, Elsevier

16. Prescott, L.M.; Harley, J.P.; Klein, D.A. *Microbiology: Food and Industrial Microbiology*. 5th Edition, McGraw-Hill, Boston **2002**, 978-981. DOI: 10.14302/issn.2379-7835.ijn-14-479.

17. Karmakar, A.; Dua, P.; Ghosh, C. Biochemical and Molecular Analysis of Biochemical and Molecular Analysis of *Staphylococcus aureus* Clinical Isolates from Hospitalized Patients. *Can. J. Infect. Dis. Med. Microbiol.* **2016**, Article ID 9041636. DOI: 10.1155/2016/9041636.

18. Gilot, P.; Lina, G.; Cochard, T; Poutrel, B. Analysis of the genetic variability of genes encoding the RNA III-activating components Agr and TRAP in a population of *Staphylococcus aureus* strains isolated from cows with mastitis. *J. Clin. Microbiol*. **2002**, *40*(*11*), 4060-4067. DOI: 10.1128/JCM.40.11.4060-4067.2002.

19. Cerasela, P.; Lazar, A.; Irina, P.; Maria, I.; Giancarla, V., Banu, C. Somaclonal variation at the nucleotide sequence level revealed by RAPD and ISSR markers. *J. Hortic., Fores. and Biotech*. **2011**, *15(4),* 119-123. DOI: 10.1007/BF03195229.
20. Delost, G.R.; Delost, M.E.; Armile, J.; Lloyd, J. *Staphylococcus aureus* carriage rates and antibiotic resistance patterns in patients with acne vulgaris. *J Am Acad Dermatol*. **2016**, *74(4),* 673-8. DOI: 10.1016/j.jaad.2015.11.025.
21. Harley, J.P.; Prescott, L.M. *Laboratory Exercises in Microbiology*. 7th ed. McGraw-Hill Higher Education. New York **2007**.
22. Levinson, W. *Review of medical microbiology and immunology*. 14th ed., McGraw-Hill Education, USA **2016**.
23. Al-Ameen, N.T.T.A. *Effect of aqueous and alcoholic extracts of garlic and ginger plants on some types of bacteria isolated from tonsillitis*. MSc. Thesis, College of Education for Girls, University of Tikrit **2017**.
24. Lateef, K.R.; Aziz, I.H.; Faik, A.J. Molecular Typing of *Staphylococcus aureus* by DNA Restriction Fragment Length Polymorphism of *coa* Gene. *Iraqi J. Biotechnol.* **2015**, *14(1),* 23-28.
25. Chen, K.; Lin, Sh.; Li, P.; Song, Q.; Luo, D.; Liu, T.; Zeng, L. and Zhang, W. Characterization of *Staphylococcus aureus* isolated from patients with burns in a regional burn center, Southeastern China. *BMC Infect. Dis.* **2018**, *18*, 5. DOI: 10.1186/s12879-018-2955-6.
26. Shamkhi, G.J; Saadedin, S.M.K.; Jassim, K.A. Detection the Prevalence of Some Chromosomal Efflux Pump Genes in Methicillin Resistant *Staphylococcus aureus* Isolated from Iraqi Patients. *Iraqi J. Biotechnol.* **2019**, *18(3),* 33-42.
27. Mohammed, S.W.; Radif, H.M. Detection of *icaA* Gene Expression in Clinical Biofilm-Producing *Staphylococcus aureus* Isolates. *Iraqi J. Sci.* **2020**, *61(12),* 3154-3163. DOI: https://doi.org/10.24996/ijs.2020.61.12.2.
28. Ahmed, Z.F.; Al-Daraghi, W.A.H. Molecular Detection of *medA* Virulence Gene in *Staphylococcus aureus* Isolated from Iraqi Patients. *Iraqi J. Biotechnol.* **2022**, *21(1),* 8-18.
29. Al-Hayali, R.T.G.H. *Phenotypic and genetic relationship of Staphylococcus aureus isolated from different infections and the inhibitory effect of antibiotics, nanoparticles and vitamin C on them*. MSc. thesis. College of Science, University of Mosul **2020**.
30. Javdan, S..L; Narimani T., Abadi, M.S,S; Gholipour, A. Agr typing of *Staphylococcus aureus* species isolated from clinical samples in training hospitals of Isfahan and Shahrekord. *BMC Res Notes.* **2019**, *12*, 363–368. DOI: 10.1186/s13104-019-4396-8.
31. Guo, Y; Song, G.; Sun, M; Wang, J.; Wang, Y. Prevalence and Therapies of Antibiotic-Resistance in *Staphylococcus aureus*. *Front. Cell. Infect. Microbiol*. **2020**, *10*, 107. DOI: 10.3389/fcimb.2020.00107.
32. Okorie-Kanu, O.J.; Anyanwu, M.U.; Ezenduka, E.V.; Mgbeahuruike, A.C; Thapaliya, D.; Gerbig, G.; Ugwuijem; E.E.; Okorie-Kanu, C.O.; Agbowo, P.; Olorunleke; S.; Nwanta, J. A.; Chah, K.F. and Smith, T.C. Molecular epidemiology, genetic diversity and antimicrobial resistance of *Staphylococcus aureus* isolated from chicken and pig carcasses, and carcass handlers. *PloS one* **2020**, *15(5),* e0232913. https://doi.org/10.1371/journal.pone.0232913.
33. Mohammadi, M; Bahrami's**,** N.; Mona Khajavian's, M. The Occurrence of Type I, II, and III Integrons in Multi-drug Resistance and Methicillin-Resistant *Staphylococcus aureus* Isolates in Iran. *Curr. Microbiol*. **2020,** *77*, 1653–1659. https://doi.org/10.1007/s00284-020-01956-x .
34. Ahmed, M.E.; Mousa,I.S.; Al-Halbosiy, M.F.; Saheb, E.J. The anti-Leishmaniasis activity of Purified Bacteriocin Staphylococcin and Pyocin isolated from *Staphylococcus aureus* and *Pseudomonas aeruginosa*. *Iraqi J. Sci.* **2018**, *59*, 2A, 645-653. https://ijs.uobaghdad.edu.iq/index.php/eijs/article/view/289.

35. Abd Zaid, A.M.; Kandala, N.J. Rapid discrimination among methicillin resistant *Staphylococcus aureus* isolates using variable number tandem repeat analysis in a sample of Iraqi patients and. *Iraqi J. Agric. Sci.* **2021**, *52(5)*, 1185–1193. DOI: https://doi.org/10.36103/ijas.v52i5.1456.
36. Omar, N.N.; Mohammed, R.K. A molecular study of toxic shock syndrome toxin gene (tsst-1) in β-lactam Resistant *Staphylococcus aureus* Clinical Isolates. *Iraqi J. Sci.* **2021**, *62(3)*, 825-837. DOI: https://doi.org/10.24996/ijs.2021.62.3.13.
37. Fitranda, M; Salasia, S.I.O; Sianipar, O; Dewananda, D.A; Arjana, A.Z; Aziz, F; Wasissa, M; Lestari, F.B.; Christin, C.M. Methicillin-resistant *Staphylococcus aureus* isolates derived from humans and animals in Yogyakarta, Indonesia. *Vet. World* **2023**, *16*(*1*), 239-245. DOI: 10.14202/vetworld.2023.239-245.
38. Al-Hayanni, H.S.A. Antimicrobial resistance in staphylococci and detection of *meca* and *blaz* genes in *Staphylococcus aureus*, *Staphylococcus sciuri* and *Staphylococcus epidermidis* isolated from fresh beef. *Biochem. Cell. Arch*. **2021**, *21(1)*, 1571-1577.
39. AbdEl-Mongy, M.; Awad, E.T.; Mosaed, F. Vancomycin resistance among methicillin resistant *Staphylococcus aureus* Isolates from neonatal sepsis attending intensive care unit in Shibin El-Kom teaching hospital Egypt. *J. Pure Appl. Microbiol* **2018**, *12*, 1093-1100. DOI: https://doi.org/10.22207/JPAM.12.3.07.
40. Budzyńska, A.; Skowron, K.; Kaczmarek, A.; Wietlicka-Piszcz, M.; Gospodarek-Komkowska, E. Virulence Factor Genes and Antimicrobial Susceptibility of *Staphylococcus aureus* Strains Isolated from Blood and Chronic Wounds. *Toxins* **2021**, *13*, 491. DOI: 10.3390/toxins13070491.
41. Khodabandeh, M; Mohammadi, M.; Abdolsalehi, M.R. Analysis of resistance to macrolide–lincosamide–Streptogramin B among mecA-positive *Staphylococcus aureus* isolates. *Osong Public Health Res Perspect*. **2019**, *10(1)*, 25. DOI: 10.24171/j.phrp.2019.10.1.06.
42. Al-Daoudi, H.F. *Study of the effect of some plant extracts and antibiotics on some species of Staphylococcus spp. Isolated from the upper respiratory tract*. MSc. Thesis, College of Education for Girls, Tikrit University **2018**.
43. Cavalcanti, V.P.; Camargo, L.A.; de Moura, F.S.; Fernandes, E.J.; de, M.; Lamaro Cardoso, J.; Braga, C.A. da, S.B.; André, M.C.P. *Staphylococcus aureus* in tonsils of patients with recurrent tonsillitis: prevalence, susceptibility profile, and Genotypic characterization. *Braz. J. Infect. Dis.* **2019**, *23(1)*, 8–14. DOI: 10.1016/j.bjid.2018.12.003.
44. Jaddoa, N.T.M; Al-Mathkhury, H.J.F. Gentamicin Modulates the Gene Expression of HLA in Methicillin Resistance *Staphylococcus aureus* Biofilm. *J. Global Pharma Technol.* **2018**, *10(7)*, 61-70.
45. Mahmoud, S.A; Einas, H; El-Shatoury, S. T.M; Abdallah, N.A. Detection of *mecA* and class1 integron in *Staphylococcus aureus* isolated from Egyptian hospitals. *African J. Biol. Sci*. **2021**, *17(1)*, 251-259. DOI: 10.21608/AJBS.2021.201677.
46. Emaneini, M.; Bigverdi, R.; Kalantar, D.; Soroush, S.; Jabalameli, F., Noorazar Khoshgnab, B.; Asadollahi, P. and Taherikalani, M. Distribution of genes encoding tetracycline resistance and aminoglycoside modifying enzymes in *Staphylococcus aureus* strains isolated from a burn center. *Ann. Burns Fire Disast.* **2013**, *26(2)*, 76–80.
47. Hamad, S.L. *The Association of Nasal and Oral Presence of Bacteria in Obese Type Two Diabetes Patients*. M. Sc. Thesis. College of Science, University of Baghdad **2017**.
48. Kwoji, I.D.; Tambuwal, F.M.; Abubakar, M.B.; Yakubu, Y.; Bitrus, A.A.; Jauro, S. Occurrence of methicillin resistant *Staphylococcus aureus* in chickens and farm personnel in Sokoto, North-western Nigeria. *J Adv. Vet. Anim Res.* **2017**, *4(3)*, 255–260. DOI: https://doi.org/10.5455/javar.2017.d220.
49. Jead, M.R.; Mohammed, R.K. Detection of lmrS, mepA, norC genes among *Staphylococcus aureus* Isolates. *Int. J. Pharmac. Res.* **2020**, *12(1)*, 1471-1476. DOI: 10.31838/ijpr/2020.12.01.243.
50. Bessa, G.R.; Quinto, V.P.; Machado, D.C.; Lipnharski, C.; Weber, M.B., Bonamigo, R.R.; D'Azevedo, P.A. *Staphylococcus aureus* resistance to topical antimicrobials in atopic dermatitis. *An Bras Dermatol.* **2016**, *91(5)*, 604–610. DOI: 10.1590/abd1806-4841.20164860.

51. Abbas, N.J. *Bacteriological and molecular study of Staphylococcus aureus bacteria isolated from different clinical sources*. MSc. thesis, College of Education for Pure Sciences, Ibn Haitham, University of Baghdad **2019**.
52. Ahmed, K.D.; Salim, R.J. Isolation and Diagnosis of Aerobic Bacteria from Patients with Respiratory Tract Infection and Partial Characterization of Their Plasmid Profile. *Ibn Haitham J. Pure Appl. Sci.* **2016**, *29*(*3*), 260-270. DOI: https://jih.uobaghdad.edu.iq/index.php/j/article/view/716.
53. Zhou, W.; Shan, W.; Ma, X; Chang, W; Zhou, X; Lu, H. Molecular characterization of rifampicin-resistant *Staphylococcus aureus* isolates in a Chinese teaching hospital from Anhui, China. *BMC Microbiol.*. **2012**, *12*, 240. DOI: 10.1186/1471-2180-12-240.
54. Rasmi, A.H; Ahmed, E.F; Abdou; M.A.; Gamal, F.M.F.G. Virulence genes distributed among Staphylococcus aureus causing wound infections and their correlation to antibiotic resistance. *BMC Infect. Dis.* **2022**, *22(1)*, 652. DOI: 10.1186/s12879-022-07624-8.
55. Zaidan, I.A.; Al-Kazaz, A.K.A.; Mohammed, A.S. Effect the combination of antibiotics on clinical isolates of *Staphylococcus aureus*. *Baghdad Sci. J.* **2009**, *6*(*4*), 683-692. DOI: https://doi.org/10.21123/bsj.2009.6.4.683-692.
56. Goudarzi, M.; Goudarzi, H.; SáFigueiredo, A.M.; Udo, E.E., Fazeli, M.; Asadzadeh, M. Molecular Characterization of Methicillin Resistant *Staphylococcus aureus* Strains Isolated from Intensive Care Units in Iran: ST22-SCCmec IV/t790 Emerges as the Major Clone. *PLoS ONE* **2016**, *11(5)*, e0155529. DOI: 10.1371/journal.pone.0155529.
57. Li, L.; Zhao, X. Characterization of the resistance class 1 integrons in *Staphylococcus aureus* isolates from milk of lactating dairy cattle in Northwestern China. *BMC Vet Res* **2018**, *14*, 59. DOI: https://doi.org/10.1186/s12917-018-1376-5.
58. Marathe, N.P.; Nagarkar, S.S.; Vaishampayan, A.A.; Rasane, M.H.; Samant, S.A.; Dohe, V.; Kagal, A.; Shouche, Y.S.; Deshpande, N. High prevalence of class 1 integrons in clinical isolates of methicillin-resistant *Staphylococcus aureus* from India. *Indian J Med Microbiol*. **2015**, *33*(*2*), 231-236. DOI: 10.4103/0255-0857.154905.
59. Rasheed, N.A.; Hussein, N.R. Characterization of different virulent factors in methicillin-resistant *Staphylococcus aureus* isolates recovered from Iraqis and Syrian refugees in Duhok city, Iraq. *PLoS ONE* **2020**, *15(8)*, e0237714. DOI: https://doi.org/10.1371/journal.pone.02377.
60. Olya, Z.N.; Najar-Peerayeh, S.; Yadegar, A.; Bakhshi, B. Clonal diversity and genomic characterization of Panton-valentine Leukocidin (PVL)-positive Staphylococcus aureus in Tehran, Iran. *BMC Infect Dis*. **2021**, *21(1)*, 372. DOI: https://doi.org/10.1186/s12879-021-06060-4.
61. Bibalan, M.H.; Shakeri, F.; Javid, N.; Ghaemi, A.; Ghaemi, E.A. Accessory Gene Regulator Types of *Staphylococcus aureus* Isolated in Gorgan, North of Iran. *J Clin Diagn Res.* **2014**, *8(4)*, DC07- DC09. DOI: 10.7860/JCDR/2014/6971.4219.
62. Abbasian, S., Farahani; N.N.; Mir, Z.; Alinejad, F.; Haeili, M.; Dahmardehei, M.; Mirzaii, M.; Khoramrooz, S.S.; Nasiri, M.J.; Darban-Sarokhalil, D. Genotypic characterization of *Staphylococcus aureus* isolated from a burn centre by using *agr*, *spa* and SCC*mec* typing methods. *New Microbes New Infect*. **2018**, *26*, 15-19. DOI: https://doi.org/10.1016/j.nmni.2018.08.001.
63. Singh, R.; Ray, P. Quorum sensing-mediated regulation of staphylococcal virulence and antibiotic resistance. *Future Microbiol*.; **2014**, *9*, 5, 669-81. DOI: 10.2217/fmb.14.31.
64. Bronner, S.; Monteil, H.; Prévost, G. Regulation of virulence determinants in Staphylococcus aureus: complexity and applications. *FEMS Microbiol Rev*. **2004**, *28(2)*, 183-200. DOI: 10.1016/j.femsre.2003.09.003.
65. Gomes, A. R.; Vinga, S.; Zavolan, M.; de Lencastre, H. Analysis of the genetic variability of virulence-related loci in epidemic clones of methicillin-resistant *Staphylococcus aureus*. *Antimicrob. Agents Chemother*. **2005**, *49*, 366–379. DOI: 10.1128/AAC.49.1.366-379.2005.

66. Tan, L.; Li, S.R.; Jiang, B.; Hu, X.M.; Li, S. Therapeutic targeting of the *Staphylococcus aureus* accessory gene regulator (*agr*) system. *Front. Microbiol.* **2018**, *9*, 55. DOI: 10.3389/fmicb.2018.00055.
67. Sakoulas, G., Eliopoulos, G.M.; Moellering, R. C.; Jr. Novick, R.P; Venkataraman, L.; Wennersten, C. *Staphylococcus aureus* accessory gene regulator (*agr*) group II: is there a relationship to the development of intermediate-level glycopeptide resistance? *J. Infect. Dis*. **2003**, *187*, 929–938. DOI: 10.1086/368128.
68. Al-Zahraa, F.; Gomaa, M.; Helal, Z.H. Phenotypic Characterization and ERIC-PCR Typing of MDR *Acinetobacter* species in Egypt. *Egypt. J. Med. Microbiol.* **2011**, *20*, 1.