Republic of Iraq
Ministry of Higher Education
and Scientific Research
University of Diyala
College of Education for Pure Science

Molecular detection of efflux pumps of Quinolones in the clinical isolates of methicillin -resistance *Staphylococcus aureus* (MRSA)

A Thesis

Submitted to the Council of College of Education for Pure Science, University of Diyala in Partial Fulfillment of the Requirements for the Degree of Doctorate of Philosophy of Science in Biology

By
Iman Abbas Ali AL-Zengena
M.Sc.Biology/Microbiology
University of Diyala 2013

Supervised By

Dr. Abbas Aboud Farhan.
Al-Dulaimi
Prof
1441 A.H.

Dr. Hadi Rahman Rasheed.
Al-Taai
Prof.
2020 A.D

Summary

Atotal of (320) clinical samples were collected from different sources (wounds, burns, urine, ear, nasal carriage and blood) of patients with different infections from Baquba Teaching Hospital in Diyala governorate during the period from February to July 2018) to be isolated, identified and confirmed for the presence of *Staphylococcus aureus* bacteria.

The results demonstrated that 85(26.56%) of the isolates were *S.aureus* distributed according to the sources from the highest to the lowest numbers and percentages as follows: wound, nasal carriage, blood, ear, burn and urine: 25(29.41%), 20(23.52%), 18(21.17%), 10(11.76%), 8(9.41%) and 4(4.70%) respectively depending on the traditional methods of using selective medium (HiCrom agar) and specific differential media, microscopic characteristics, biochemical tests, Vitek-2 system at (98%) probability. All the (85) *S.aureus* isolates showed positive results for the extracellular enzyme coagulase (CoPS), while they showed negative oxidase enzyme results.

Methicillin resistance isolates were identified by using the Cefoxitin disc diffusion method, Oxacillin disc diffusion method and the specialized accurate chromogenic *S. aureus* medium (Chromagar *S. aureus* assay). The results showed that 55(64.70%) of the isolates were methicillin resistant (MRSA), which had modification of penicillin binding proteins, while 30(35.29%) of them were methicillin sensitive *S.aureus* (MSSA).

The susceptibility tests of all *S.aureus* isolates were determined against (16) types of antibiotics (Cefoxtin, Oxacillin, cefepime, Ceftazidime Imipenem, Kanamycin, Gentamicin, Amikacin, Vancomycin, Chloramphenicol,Tetracyclin, Ciprofloxacin, Norfloxacin,

Levofloxacin, Ofloxacin and Nalidixic acid) which were carried out by using the disc diffusion method (Kirby-Bauer). The results revealed that the highest resistance was among the beta lactam group, so there was a complete resistance of (MRSA) isolates (100%) to Cefoxtin (alternative to methicillin), Oxacillin, Cefepime and Ceftazidime, whereas the resistance to Imipenem was (9.09%).

In addition, these isolates showed variable resistance rates to the aminoglycoside group as follow: Kanamycin, Gentamicin, Amikacin (72.7%), (54.5%) and (60%) respectively. However, (58.18%) of the isolates were resistant to Vancomycin (VRSA) and (30.90%) showed intermediate resistance (VISA) and (10.90%) of the isolates were sensitive to Vancomycin (VSSA). The result of the resistance of *S.aureus* isolates to Chloramphenicol and Tetracyclin was (32.72%) and (70.90%) respectively, while it showed moderate resistance (32.72%) to each of Ciprofloxacin, Norfloxacin, Levofloxacin and Ofloxacin and (56.36%) resistance rate to Nalidixic acid.

The study also demonstrated that 18(32.7%) of the (55) (MRSA) isolates showed extensive drug resistance (XDR) and 19(34.54%) of them showed multi drug resistance (MDR), while the rest of the (MRSA) isolates were non multidrug resistant.

The determination of the minimum inhibitory concentration (MIC) value ranged between (8 to up to 1024µg/ ml) for Ciprofloxacin, Levofloxacin and Norfloxacin for *S.aureus* isolates which had resistance to these antibiotics. The minimum bactericidal concentration (MBC) was also determined in this study and their values showed approximately one to two-fold higher than their MIC.

In clinical practice, one of the important inhibitor compounds named Carbonyl cyanide 3-chlorophenylhydrazone (CCCP) which was used in this study as efflux pump inhibitor combined with Ciprofloxacin.

The results appeared that the Ciprofloxacin and CCCP combination displayed a reduction in MIC (1-4) fold ranging between (4-128μg/ml), which might be due to synergistic activity.

The phenotypic activity of efflux pump system was determined in the (18) MRSA Floroquinolone resistant isolates by the Ethidium Bromide cart wheel agar (EtBrCW) based method, and the results showed that (77.7%) of them had positive emotions while (22.22%) were denominated EtBrCW-negative.

In the current study, (18) MRSA Foroquinolone resistant isolates were subjected to polymerase chain reaction technique in a monoplex pattern to amplify two diagnostic genes of *S.aureus* *mec*A and *coa*. The amplicon sizes of these genes were 310 and 595 bp respectively, and they recorded positive results reaching to 100%.

Regarding the prevalence of efflux pump genes, our findings revealed that the 18 MRSA Floroquinolone resistant isolates were subjected to polymerase chain reaction technique in a monoplex pattern to amplify resistance encoding efflux pump genes *norA*, *mde*A, *sdr*M (responsible for hydrophilic fluoroquinolone resistant), *nor*B, *nor*C and *mep*A (responsible for hydrophilic and hydrophobic fluoroquinolone resistant) and *sep*A gene (responsible for inhibitors resistance) they showed to be harboring *nor*A, *nor*B, *nor*C, *sdr*M, *mde*A, *mep*A and *sep*A as (88.8%), (77.7%), (100%), (83.3%), (88.8), (88.8) and (100%) rates respectively.

The (18) MRSA Floroquinolone resistant isolates which were phenotypically coagulase enzyme producers by standard laboratory methods showed positive results for PCR typing of *coa* gene. The amplification of the *coa* gene generated 4 different genotypes: I, II, III, IV based on different sizes of polymorphism ranging between 500-800 bp. The majority of the isolates exhibited *coa* gene size of 600bp. PCR -

RFLP of *coa* gene exhibited four patterns which were obtained with *Alu*I digests of PCR products. The number of fragments varied from two to three, with sizes of the fragments varied from (90 to 500 bp). Class 2 was the most common and accounted for (50%) of the isolates.The isolates of this class were considered epidemic strains and the rest of isolates were considered sporadic strains. The (6) samples of PCR products from *coa* gene related to their variation *coa* genotype and their different sources of isolation were sent for sequencing, then all data obtained from genes sequencing were submitted to gene bank national center for biotechnology information NCBI blastn, under accession number started with (CP012018.1)**.**

The *coa*-based comprehensive tree subdivided the *S. aureus* isolates into two main groups according to their source; group-1 which included S3, S6, S8 and S18, and group-2 which included S7 and S13, indicating the presence of an interesting genetic similarity between the *S. aureus* strains that were isolated from wounds, nasal, blood and ear infections from one side, and the *S. aureus* strains that were isolated from burns and urine samples from the other side.

Finally, investigating gene expression of three efflux pumps genes (*nor*A, *nor*C *and sdr*M) with a reference gene (*gmk*), Tag man qRT- PCR assay by one step was designed to detect and characterize genes. It is the first time to document the detection of *S.aureus* isolates by this method in this region of Iraq. In addition, our study explained was the first one that explained the potentiating effect of Carbonyl Cyanide 3-Chlorophenylhydrazone (CCCP) against expression of efflux pump genes in MRSA isolates that were done by using specfic probe and primer designed. In this part, (6) isolates were chosen according to their source and performed in triplicate.

The results showed that the highest expression of *nor*A gene was in the isolates (SA3,7,15 and18) in presence of Ciprofloxacin and no overexpression of *nor*A was observed in two isolates (SA8 and10). In the presence of CCCP& Cip, the gene expression of the *nor*A was decreased in four isolates (SA3,8,10 and18) and isolate (SA7) remained the same, while (SA15) increased its gene expression. The *nor*C gene was increased and over expression was observed in all isolates treated with Ciprofloxacin except the (SA8) isolate. Expression of the *nor*C gene was decreased in all isolates in the presence of CCCP & Cip and decreased significantly more rapidly and earlier except (SA8) isolates which were minor increased.

The results also showed that qRT-PCR indicated a high expression of *sdr*M gene in four MRSA isolates (SA7,8,15 and18) and no increased expression was seen in two isolates (SA3 and10) after treatment with Ciprofloxacin. Expression of the *sdr*M gene was decreased in all isolates that were treated with CCCP & Cip and decreased significantly more rapidly and earlier. A synergistic effect was observed in this study by CCCP associated with Ciprofloxacin.

The isolates used in this study appeared a predominantly overexpress in the *nor*Cgene and some isolates showed over expression in the *sdr*M gene. One bacterial isolate (SA18) isolated from ear infection was identified as a standard strain that has been deposited in the National Center for Biotechnology Information (NCBI) with the accession number. This isolate harbored 8 genes, 3 diagnosis genes for *S.aureus* (16sRNA ,*mec*A and *coa*) and 5 efflux pump genes (*nor*A, *nor*C, *sdrM*, *sep*A and *med*A).

Introduction

Staphylococcus aureus is a Gram positive coccus, commonly exist on the skin and mucosal membranes and is the main cause of hospital-acquired infections, and Methicillin-resistant *S. aureus* (MRSA) isolates are regarded as a severe health concern (Havae *et al*., 2017). The growth of *S. aureus* infections is usually correlated with immunocompromised and hospitalization conditions (Costa *et al*.,2018). The clinical emphasis of *S. aureus* virulence factors are enzymes, toxins and surface proteins that result in the rapid development of drug resistance (Carrolle *et al*.,2016). *S. aureus* strains often resist to many types of antibiotics. At present, methicillin-resistant *S. aureus* (MRSA) has become a severe problem in hospitals and a main clinical importance a global public health concern worldwide (Safarpoor Dehkordi *et al*.,2018). Documented data showed that nearly 50–70% of the *S. aureus* strains isolated from hospital environment were MRSA .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 (Hasanpour Dehkordi *et al*.,2017).

Quinolones are the most common antibiotics used in clinical practice for treating different bacterial infections, and some of them exhibit excellent *in vivo* and *in vitro* anti-MRSA activitiy (Gao *et al*.,2018). The appearance of methicillin-resistant (MRSA) in hospital-acquired infections as a potential pathogen can deal with these antimicrobial agents (Wang and Ruan,2017). Infections caused by *S. aureus* are difficult to be treated because of it can develop and acquire resistance to multiple antibiotics (Tacconelli *et al.,* 2018). Resistance can be achieved through antibiotic target modification, drug inactivation or drug export by efflux pumps. *S. aureus* encodes different multidrug

resistance efflux pumps (Gupta *et al*.,2019). The ability of several efflux proteins for recognizing different structurally diverse substrates amplifies this problem leading to a phenotype with multidrug resistance (MDR). To date more than ten efflux pumps have been explained for *S. aureus*, most of which belong to the major superfamily (MFS), including NorA, NorB, NorC, MdeA and SdrM (chromosomally encoded) and QacA/B pumps (plasmid-encoded) (Zárate *et al.*,2019). Efflux pump inhibitors (EPIs) may block the action of efflux pumps. Examples of some EPIs that can increase some compound retention within the microbial cells carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (Moazzen *et al* .,2018). Different molecular and phenotypic techniques are available for the differentiation of MRSA isolates, and the most common phenotypic one is the Antimicrobial Susceptibility Testing (AST). Coagulase gene in clinical isolates of methicillin-resistant *S. aureus* with *Alu*I restriction sites by RFLP typing and application is an interesting method since its speed and ease is used for observing the existence of methicillin-resistant *S. aureus* polymorphism as well as DNA-based diagnosis of *S. aureus* (Babu *et al.*,2014).

Real-time followed by polymerase chain reaction represents a powerful tool and the most suitable method for gene expression quantification via the detection and quantification of mRNA. This assay resulted in a we have introduced data and sample protocols that show the using of the real–time PCR in specific applications such as gene expression analysis, allelic discrimination and detection of genetically modified organism (GMO) (Valihrach and Demnerova, 2012). This protocols faster than the conventional PCR methods and was very reproducible and specific (Lewis and Rice,2014). In Iraq there is no specific study concerned with *nor*C *and sdr*M gene expression, and

prevalence of those genes responsible for constitutive and inducible resistance toward quinolones (Ciprofloxacin) has not been studied yet.

The current study aims to:

detect *Staphylococcus aureus* bacteria with multiple resistance to antibiotics by bacteriological and biochemical methods and to determine the pathways of efflux pumps system by phenotype and genotype methods .Our study also aimed to study coagulase-producing by *S. aureus* genotypically by PCR- RFLP patterns and digested with *Alu*I enzyme .Also our study aimed to determine "Gene Expression" for multiple resistance genes.

These aims established by :

1- Isolation and identification of *Staphylococcus aureus* isolated from different clinical sources by bacteriological and biochemical methods.

2- Studying the sensitivity of *Staphylococcus aureus* against the quinolone beta lactam and aminoglycoside antibiotics as well as determination the Minimal Inhibitory Concentration (MIC) and the Minimal Bactericidal concentration (MBC) for some antibiotics.

3- Phenotypic detection of efflux pumps activity by using Ethidium Bromide agar Cart Wheel (EtBrCW) based method and molecular characterization of some the encoded genes of the efflux pump system.

4- Determination of *S.aureus* that produces coagulase enzyme genotypically by PCR typing of *coa* gene and amplification of *coa* gene by PCR- RFLP patterns and digested with *Alu*I enzyme.

5- Recogination gene expression of multi drug resistance genes by using Taq man RT-PCR by one step technique and using probes designated in this study.

Literature review

1.1 General characteristics of *Staphylococcus aureus*

Staphylococcus aureus, belongs to the *Staphylococcaceae* family was discovered by Sir Alexander Ogston in 1882 (Burda,2015). *S. aureus* is a Gram positive and facultative anaerobic bacteria that grows in clusters of coccoid-shaped cells (Forbes *et., al* 2007). It has been characteristically thought that it is a non-motile bacteria, but a form of dispersion motility has been recently reported involving production of dendrites that enable the bacterial cell to move from their primary colonies (Pollitt *et., al* 2015).

Moreover, they are shown to tolerate a pH 4.2-9.3 with an optimum pH 7.0-7.5 (Brook *et al.*,2007). *S. aureus* can grow most rapidly at 37°C but form pigment best at room temperatures 20–25°C. Their colonies on solid media appear as smooth, round, glistening and raised colonies (Carroll *et al*., 2016). *S. aureus* relatively resists drying, heat (it withstands 50°C for 30 minutes) and 10% sodium chloride but certain chemicals such as (3% hexachlorophene) readily inhibit its growth (Burda,2015). It forms a creamy golden color due to construction staphyloxanthin, a carotenoid pigment that detoxifies the reactive oxygen species (Clauditz *et.,al* 2006). On blood agar, the isolates of *S. aureus* show a characteristic beta-hemolysis, which is related to the production of one of four hemolysin types: alpha, beta, delta or gamma (Vandenesch *et.,al* 2012).

Staphylococci are non spore forming bacteria, and uncommon of them are capsule or pseudo capsule producers making them more critical than the encapsulated strains (Prescott, 2002). Staphylococci produce catalase that converts hydrogen peroxide into oxygen and water. The catalase test distinguishes between staphylococci catalase positive and

streptococci (catalase negative) (Carroll *et al.*,2016). About (40) species of staphylococcus have been reported, (9) of them have two subspecies while (1) has three subspecies (Doškař *et al.*,2010). *S.aureus*, *S.epidermidis* and *S.saprophyticus* are the most commonly species with clinicial importance among others (Forbes *et al*., 2007). *S.aureus* have the ability to produce free and bound coagulase enzyme Coagulase positive (CoPs), which causes blood clotting in the humans. *S. aureus* secretes two forms of coagulase enzyme, bound and free coagulase ,while *S.epidermidis* can not produce this enzyme so they are called coagulase-negative staphylococci (CoNS) (Mahon *et al.*,2011). *Staphylococcus aureus* is often a mannitol fermenting bacteria and possesses protein A, which is an anti-phagocytic virulence factor covalently integrated into bacterial cell walls (MacFaddin, 2000).

1.2 *S. aureus* classification and genetics

The classification of *S. aureus* is related to the similarities in physical properties as shown in table (1-1). They are identified by a marked metabolic diversity of biochemical evolution lines (Towner and Coakayne, 2014).

Table (1-1): Classification of *Staphylococcus aureus* based on Bergey's Manual of General bacteriology (Holt *et al*., 1994)

Kingdam	Bactreriae
Phylum XIII	Firmicutes
Class I	Bacilli
Order I	Bacillales
Family VIII	Staphylococcaceae
Genus I	*Staphylococcus*
Species	*aureus*

S. aureus has a low G+C content genome, which extended from 32.7 to 32.9% in (16) evaluated isolates of *S. aureus* bacteria (Suzuki *et al.,2*012). The genome of *S. aureus* is a circular chromosome that is 2.8–2.9 Mbp in size. The chromosome encodes about 2700 PCDSs (Protein coding sequences) (Plata *et al*., 2009). S. *aureus* genome can be divided into (3) segments: core genome, core variable genes and mobile genetic elements (MGEs) (Stefani *et al.*,2012).

The most variable portion of the *S.aureus* genome is (MGEs), which comprises about (25%) of the total genetic materials of the S. *aureus* isolates (Lindsay *et al*.,2008). The MGEs enable the transfer of DNA fragments between cells or from the surrounding environment by a horizontal gene transference (HGT) (Mell *et al*.,2014). HGT is achieved through (3) main mechanisms including transformation, transduction and conjugation (Munita and Arias, 2016). Mobile genetic elements (MGE) e.g plasmids, transposons and integrons play essential roles in transferring the ARGs in the environment (Kristiansson *et al.*, 2011).

Transformation takes place in naturally competent bacteria possessing the machinery involved up taking the free DNA from the environment (Mell *et al*.,2014). Because of the low natural competency level that requires induction, transformation is the less commonly observed form of HGT in *S. aureus* (Morikawa *et al*., 2012).

In addition, recent studies have demonstrated that bacteriophages also participate significantly in the horizontal spread of resistant genes (Balcazar, 2014). Transduction involves bacteriophage-mediated DNA transferring between cells (Griffiths *et al.*,2000). Bacteriophages are able to encode virulence genes like the immune evasion genes harbored on the haemolysin-converting bacteriophages (van Wamel *et al.*, 2006). It became a coordinator in the bacterial chromosome as a prophage component during the lysogenic cycle (Morikawa *et al*., 2012). It

الخلاصة

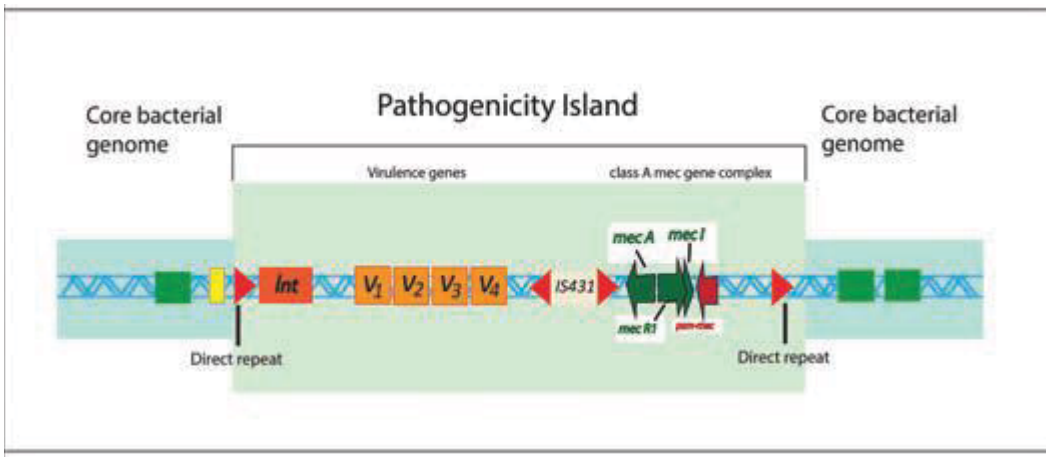
تم في هذه الدراسة جمع (320) عينة سريرية من مصادر مختلفة (كالجروح والحروق والبول والأذن و الأنف اضافة الى الدم) من المرضى الذين يعانون من التهابات مختلفة من مستشفى بعقوبة التعليمي في محافظة ديالى خلال الفترة من شباط إلى تموز 2018) من اجل عزل و تشخيص والتأكد من وجود بكتريا المكورات العنقودية الذهبية فيها.

اظهرت النتائج أن 85(26.56٪) من العزلات كانت لبكتريا المكورات العنقودية الذهبية *S.aureus* توزعت اعدادها ونسبها المئوية وفقًا لمصادر الإصابة من الأعلى إلى الأدنى على النحو التالي: الجروح 25(29.41٪) ثم الانف20(23.52٪) ثم الدم 18(21.17٪) ثم الاذن10(11.76٪) ثم الحروق 8(9.41٪) واخيرا البول4(4.70٪) اعتمادًا على الطرق التقليدية في استخدام الاوساط الانتقائية (HiCrom agar) والاوساط التفريقية الخاصة و الصفات المجهرية و الاختبارات الكيمو حيوية بالاضافة الى نظام Vitek-2 و بنسبة احتمالية بلغت (98٪). أظهرت جميع عزلات المكورات العنقودية الذهبية *S.aureus* نتائج إيجابية لاختبار إنزيم Coagulase خارج الخلية (CoPS) في حين أظهرت نتائج سلبية لاختبار إنزيم Oxidase.

تم تحديد العزلات المقاومة للميثيسيلين باستخدام طريقة انتشار القرص Cefoxitin وطريقة انتشار القرص Oxacillin وطريقة قياس وسط المكورات العنقودية الذهبية الدقيق المتخصص المولد للون (مقايسة Chromagar S. aureus). أظهرت النتائج أن 55(64.70٪) من العزلات كانت مقاومة للميثيسيلين (MRSA) والتي قامت بتحوير البروتينات المرتبطة بالبنسلين, في حين أن 30(35.29٪) من العزلات كانت لبكتريا المكورات العنقودية الذهبية الحساسة للميثيسيلين (MSSA). تم تحديد اختبارات الحساسية لجميع عزلات *S.aureus* ضد (16) نوع من المضادات الحيوية وهي Cefoxtin و Oxacillin و Cefepime و Ceftazidime و Imipenem و Kanamycin و Gentamicin و Amikacin و Vancomycin و Chloramphenicol و Tetracyclin و Ciprofloxacin و Norfloxacin و Levofloxacin و Ofloxacin اضافة الى Nalidixic acid) والتي اجريت باستخدام طريقة انتشار القرص (كيربي-باور). كشفت نتائجنا أن أعلى مقاومة كانت في

conjugates the genetic material exchange from recipient to donor cell via cellular contact, and it is the transcendent tool by which plasmids are exchanged (Griffiths *et al*., 2000).

Plasmids are commonly found in *S. aureus* and associated with harboring antimicrobial resistance genes, genes convoluted in metabolic pathways and virulence genes including toxins (Malachowa *et al*., 2010). Assembly of MGEs are found in *S. aureus*, and comprise plasmids, insertion sequences, transposons, pathogenicity islands, genomic islands, chromosomal cassettes as well as bacteriophages (vanWamel *et al.*,2006). These elements usually encode virulence genes or antimicrobial resistance genes that participate in the accomplishment of S. *aureus* in specific environments as shown in fig (1-1) (Malachowa *et al*., 2010).



Figure(1-1): Staphylococcal genome (Plata *et al*.,2009)

1.3 Colonization and carrier

Staphylococcus aureus is aopportunistic bactrria . Humans seem to have little resistance to the surface colonization of *S. aureus*, so these bacteria can easily colonize the skin and nose (Miller *et al*., 2014). In healthy persons, these surface bacteria almost never invade the body to cause serious infections. The damaged tissues predispose patients to develop more severe staphylococcal infections (Peacock and Paterson, 2015). Up to (30%) of human-beings are continuously and

الخلاصة

تم في هذه الدراسة جمع (320) عينة سريرية من مصادر مختلفة (كالجروح والحروق والبول والأذن و الأنف اضافة الى الدم) من المرضى الذين يعانون من التهابات مختلفة من مستشفى بعقوبة التعليمي في محافظة ديالى خلال الفترة من شباط إلى تموز 2018) من اجل عزل و تشخيص والتأكد من وجود بكتريا المكورات العنقودية الذهبية فيها.

اظهرت النتائج أن 85(26.56٪) من العزلات كانت لبكتريا المكورات العنقودية الذهبية *S.aureus* توزعت اعدادها ونسبها المئوية وفقًا لمصادر الإصابة من الأعلى إلى الأدنى على النحو التالي: الجروح 25(29.41٪) ثم الانف20(23.52٪) ثم الدم 18(21.17٪) ثم الاذن10(11.76٪) ثم الحروق 8(9.41٪) واخيرا البول4(4.70٪) اعتمادًا على الطرق التقليدية في استخدام الاوساط الانتقائية (HiCrom agar) والاوساط التفريقية الخاصة و الصفات المجهرية و الاختبارات الكيمو حيوية بالاضافة الى نظام Vitek-2 و بنسبة احتمالية بلغت (98٪). أظهرت جميع عزلات المكورات العنقودية الذهبية *S.aureus* نتائج إيجابية لاختبار إنزيم Coagulase خارج الخلية (CoPS) في حين أظهرت نتائج سلبية لاختبار إنزيم Oxidase.

تم تحديد العزلات المقاومة للميثيسيلين باستخدام طريقة انتشار القرص Cefoxitin وطريقة انتشار القرص Oxacillin وطريقة قياس وسط المكورات العنقودية الذهبية الدقيق المتخصص المولد للون (مقايسة Chromagar S. aureus). أظهرت النتائج أن 55(64.70٪) من العزلات كانت مقاومة للميثيسيلين (MRSA) والتي قامت بتحوير البروتينات المرتبطة بالبنسلين, في حين أن 30(35.29٪) من العزلات كانت لبكتريا المكورات العنقودية الذهبية الحساسة للميثيسيلين (MSSA). تم تحديد اختبارات الحساسية لجميع عزلات *S.aureus* ضد (16) نوع من المضادات الحيوية وهي Cefoxtin و Oxacillin و Cefepime و Ceftazidime و Imipenem و Kanamycin و Gentamicin و Amikacin و Vancomycin و Chloramphenicol و Tetracyclin و Ciprofloxacin و Norfloxacin و Levofloxacin و Ofloxacin اضافة الى Nalidixic acid) والتي اجريت باستخدام طريقة انتشار القرص (كيربي-باور). كشفت نتائجنا أن أعلى مقاومة كانت في