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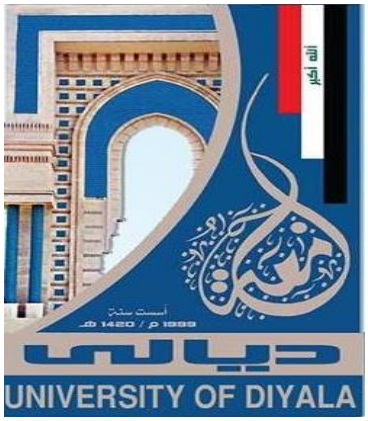
Collage of Science
Department of Biology



Molecular Genotyping of Coagulase gene tandem repeat region in clinical isolates of methicillin resistance *Staphylococcus aureus*

A Thesis

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By

Qutaiba Dhiaa Hashim
B.Sc. Biology / Baghdad university / (2004)

Supervised by

Prof. Dr. Hadi Rahman Rasheed Al-Taai

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Introduction

The *Staphylococcus aureus* bacteria is consider a gram positive cocci which belong to the *Staphylococcus* genus, this bacterium is divided into two major types which are: methicillin resistance *S. aureus* (MRSA) and methicillin sensitive *S. aureus* (MSSA) (Bonar *et al.,* 2018; Torres-Sangiao, 2016). Basically, MRSA developed from MSSA by receiving staphylococcal chromosome cassette *mec* (SCC*mec*) complex which contain the *mec* gene that is accountable for the coding of extra penicillin binding protein which is accountable for the strong resistance toward all Beta lactam antibiotics and other kinds of similar cases (Palavecino, 2014).

The *S. aureus* is a major bacterium that causes nosocomial and community-acquired infections in people, and despite extensive prophylactic efforts, methicillin resistant *S. aureus* (MRSA) infections have risen (Deng *et al.*, 2019).

The *S. aureus* are among the most common infectious agent related factors of morbidity and death globally. This bacterium may lead to a range of illnesses, from mild skin infections to deadly pneumonia and sepsis, antibiotic resistance complicates the treatment of *S. aureus* infections, since there is no viable vaccination. There has been continuous and knowledge accumulated in the extraordinary amount of toxins and other virulence factors produced by *S. aureus*, as well as how they cause illness (Cheung *et al.*, 2021). According to latest studies from 2017, the yearly incidence of fatalities in the United States owing to *S. aureus* bacteremia was found to be 20,000. (Kourtis *et al.*, 2019).

Resistance to various antibiotics is common in *S. aureus*, resistance to classic beta-lactam antibiotics that are beta-lactamase sensitive is nearly universal in *S. aureus* (Lowy, 2003; Cheung *et al.*, 2021). Besides, *S. aureus*

have the capability , frequently in collective system, resistance to the whole presented antibiotics (McGuinness *et al.*, 2017).

Besides the definite antibiotic resistance, non-specific anti-biotic resistance related to formation of biofilm acts a part in various *S. aureus* illnesses that are related to biofilm which consider additional significant manner that *S. aureus* keeps an illness going (Otto, 2018). These might develop on the abiotic surface of catheterization medical devices, and on tissue sites, including heart valves in the scenario of endocarditis. The creation of a biofilm occurs in three stages: adhesion, maturation/proliferation, and separation. Adhesion to human matrix proteins occurs *in vivo* via cell-wall embedded and also other surface proteins (Foster *et al.*, 2019).

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).

The *S. aureus* may be molecularly typed using a variety of approaches. Multiple analytical methodologies have been proposed and utilized in epidemiological research over the last decade to identify and compare *S. aureus* strains (Chadi *et al.*, 2016).

Genomic detection techniques are also employed to identify staphylococcal enterotoxin genes that do not needs providing circumstances for expression of the enterotoxin gene to detect this toxin as in other means of detection such as latex agglutination and ELISA, molecular techniques can identify staphylococci that have synthesized small amounts of the toxin that don't identified by immunological techniques (Hawryluk and Hirshfield, 2002).

Different organizations have used DNA sequencing of the coagulase gene as a reliable diagnostic technique. Due to its varied sequences (81 bp tandem repeats) at its 3' end, the *coa* gene producing coagulase protein is genetically different, allowing distinction of *S. aureus* species. This *coa* gene

polymorphism is used as an epidemiology marker, and typing is done using primers that are homologous to a conserved area of the *coa* gene (Javid *et al.*, 2018). Because the frequency of repeated sequences inside the coa gene varies, the resultant polymerase chain reaction (PCR) outputs of various strains might be of varying lengths (Izadpanah and Asadpour, 2018).

Aims of the study:

Due to disease risk and clinical importance of *S. aureus*, this study was aimed to isolate and diagnose this bacterium from different clinical sources and detect of *mec*A, *nuc*, and *coa* genes and molecular typing by sequencing of the *coa* gene.

الخلاصة

تم جمع 250 عينة سريرية من مصادر مختلفة شملت عينات مهبلية و ادرار ومسحات انف وحروق و جروح. جمعت العينات من مستشفى بعقوبة التعليمي في ديالى خلال الفترة من تشرين الاول 2021 حتى شباط 2022.

اظهرت النتائج ان نسبة النمو الموجب للعينات كان بواقع 198 (79.2%) مثلت بكتيريا العنقودية الذهبية اعلى نسبة عزل من بين المصادر بنسبة 17.6% تليها بكتيريا الاشيريشية القولونية، بكتييا الكليبسيلا، بكتيريا الزائفة، بكتيريا المسبحية، العنقودية البشروية، بكتيريا المتقلبة، و بكتيريا المكورة العنقودية البرازية بنسبة 16.8%، 15.6%، 11.6%، 8.8%، 5.2%، 2% و 1.6% على التوالي.

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 و 12 (24%) من الحروق.

اظهرت نتائج عوامل الضراوة ان 70.5% من المكورات العنقودية الذهبية كانت محللة للدم من نوع بيتا، 56.8% لها القابلية على تحلل الحمض النووي الرايبوزي منقوص الاوكسجين، 45.5% محللة للجيلاتين و 91% منتجة للغشاء الحيوي. 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 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 المقاومة 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.

اظهرت النتائج انتشارا عاليا لعزلات بكتيريا العنقودية الذهبية المتعددة المقاومة و بفرق معنوي (P <0.001)حيث كانت 77.2% منها MDR، بينما كانت عزلات XDR, PDR و الحساسة 14.6%، 2.2% و 6.8% على التوالي.