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Genetic Bases of Methicillin Susceptible And Methicillin Resistant S*taphylococci*

A Thesis Submitted For The Fulfilment of
Doctor of Philosophy In Clinical Microbiology

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DEDICATION

I dedicate this study.

To my mother, family and colleagues

To all those who offered me their kind assistance & support.

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Abstract

One thousand specimens (wound swab, urine, vaginal swab and prostatic fluid) were collected form patients attending Omdurman military hospital during the period February 2004 to October 2004. The code of each specimen, age and the gender of the patient were recorded. All the specimens were cultured on appropriate media and incubated at 37ºC overnight.

Plates which showed bacterial growth were examined by Gram-stain. All isolates which showed Gram-positive spherical cells arranged in clusters or chains were subjected to subsequent bacteriological tests. The primary selected isolates were examined for catalase, bound coagulase and tube coagulase test.

Organisms showing positive results to the above tests were tested for Mannitol fermentation and sub cultured on nutrient agar for further investigations.

Production of β .lactamase enzyme in the above isolates was studied.

A total of 270 strains were recovered and confirmed to be *S.aureus*. Using eight antibiotics, Kirby-Bauer technique was adopted as the ideal method to study the antimicrobial activity of the isolates. Depending on the susceptibility testing result, forty MRSA isolates were selected in addition to forty MSSA β - lactamase producer and twenty MSSA non β .lactamase producer were randomly selected for genotyping. Wizard R genomic DNA purification kit was used to isolate the DNA. To ensure that all the strains were *S.aureus*. PCR was assayed for the amplification of *S.aureus* specific sequence gene with 30 nucleotide forward and reverse primers and 107 amplicon size. Another PCR program was adopted to isolate mecA gene

with 22 nucleotide forward and reverse primers and 532 amplicon size. All of the 270 *Saureus* isolates showed high degree of sensitivity to vancomycin (100%) and high resistance to penicillin (98.5%). For the other antibiotics the *S.aureus* isolates showed different degree of sensitivity. Methicillin 85.2%, Meropenem 80%. Amoxicillin /clavulanic acid 74% ciprofloxacin 57% gentamicin 62% and cephalexin 62%. The PCR product electrophoresis revealed a clear band of 107 amplicon size which confirmed that all the isolates were *S.aureus*. Another PCR electrophoresis product revealed a gene of 532 base pair (mecA) in 36 strains. When the result was compared with the traditional sensitivity method, it confirmed that all the 36 strains were MRSA. They expressed their resistance with both resistance to methicillin and presence of mecA gene.

The other four strains of *S.aureus* showed resistance to methicillin by the classical method but did not express mecA gene on their PCR product and those are classified as border line methicillin resistant *S.aureus* (BORSA).

Five strains from the non β .lactamase producer expressed mecA gene and resistance to methicillin.

المستخلص

جمعت (1000) عينة من قطيلة الجروح (Wound Swab) والبول (Urine) وربدة المهبل (Vaginal Swab) وسائل البروستاتا (Prostatic Fluid) من المرضى في مستشفى السلاح الطبي بأمدرمان، أبان الفترة فبراير 2004م، إلى أكتوبر 2004م. و قد تم تسجيل ر قم العينة والعمر ونوع المرض؛ كذلك تم استنبات كل عينة في وسيطها الصحيح وتم تفريغها تحت درجة الحرارة 37 سنتيجريد لليلة كاملة.

فُحصت الأطباق التي أظهرت نمواً بائناً من أجل الصبغ الجرامي. و قد أُخضعت كل المعزولات التي أظهرت خلايا مستديرة كروية إيجابية الجرام المرتبة في تلفف (Clusters) أو سلاسل، لاختبارات بكتيريولوجية تالية. و قد فُحصت المعزولات الأولية المختارة، لأجل الكاتالاز (Catalase) والخميرة المجلطة المتحدة (Bound Coaqulase) واختبار هذه الخميرة الأنبوبي (Tube Coaqulase Test).

أجري الاختبار على الكائنات الحية التي أظهرت نتائج إيجابية للاختبارات المذكورة أعلاه لأجل معرفة الاختبار الكحولي المني (Mnnital fermentation) واستنبتت على اغار تغذوي (Nutrient Agar) تمهيداً لدراسات مست قبلية أخرى.

وتمت أيضاً دراسة إنتاج أنزيم اللاكتميز بيتا (Lactamase enzyme β) في المعزولات أعلاه. و قد تم استعادة 270 سلالة ونؤكد أنها عند قودية ذهبية (*S.aureus*)، وباستخدام ثمانية من المضادات الحيوية، ثم إتباع أسلوب كيربي – بور (Kirby - Bauer) على أنه الطريقة المثلى لدراسة النشاط المضاد للمكروب. واعتماداً على حساسية نتيجة الاختبار، تم اختيار 40 MRSA من المعزولات، بالإضافة إلي 40 MSSA من منتج β لاكتاميز و 20 MSSA من لا منتج بيتا لا كتاميز (Lactamase Producer βNon) أختبرت بطريقة عشوائية للنمط الجنسي (Genotype). كذلك استخدمت حقيبة العدد لويزاد آر (Wizard R) لتنقية مجيني الحامض النووي الثنائي (DNA).

وللتأكد من أن كل السلالات كانت عند قودية ذهبية (*S.aureus*) فقد قيست PCR لتوسع العند قودية الذهبية لتوالي الجين المعين، مع 30 نكليوتيد (Nucleolide) أمامي وتمهيدي عكسي و 107 أمبليكون (Amplicon). و قد تم تبني برنامج (PCR) آخر لعزل جين (mecA) مع 22 نكليوتيد أمامي وتمهيدي عكسي و 532 حجم أمبلكون. وكانت السلالات العند قودية الذهبية قد أظهرت درجة عالية من الحساسية للفانكوميسين (100%) (Vancomycin) ومقاومة عالية للبنسلين (98.5%). وبالنسبة للمضادات الحيوية الأخرى، فإن سلالة العند قودية الذهبية تظهر درجات مختلفة من الحساسية – ميثيسلين (85.2%) (Methicillin، ميروبنيم (80%) (Meropenem، حامض موكيسيلين/كالفين بولانك (74%)(Amoxicillin/Calvulanic acid، سيبروفلوكساسين (57%) (Ceprofloxacin، جينتاميسين (62%) (gentamicin وسيفالبكسين 62%. و قد كشف منتج PCR للرحلان الكهربائي – (PCR Product electrophoresis) شريطاً واضحاً 107 أمبلكون (amplicon size) الذي أكد أن كل

المعزولات كانت عند قودية ذهبية. و قد كشف منتج PCR للرحلان الكهربائي الآخر جين لـ 532 قاعدة زوجية (mecA) في 36 سلالة وحينما قورنت النتيجة مع طريقة الحساسية التقليدية، أكدت أن كل الـ 36 سلالة، كانت (Methicillin Resistant *staphylococcus aureus* (MRSA التي عبرت عن مـ قاومتها مع كلاً من مـ قاومة الميثيلين (Methicillin) ووجد جين (mecA).

و قد أظهرت السلالات الأربع للعنقودية البرتقالية (أو الذهبية) مـ قاومة للميثيسلين بالطريقة التقليدية (الكلاسيكية، لكنها لم تعبر عن جين mecA في منتج التفاعل التسلسلي المحفز للبلمرة (PCR)، وتلك تصنف على أنها مـ قاومة عند قودية ذهبية ميسيسيلنية في خط الحد الفاصل (Borderline methicillin resistant *S.aurous* "BORSA"). و قد عبرت 5 سلالات لمنتج لاكتمايس غير بيتا (Non βLactamase Producer) عن جين mecA ومـ قاومة الميثيسيلين.

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Introduction

This research was planned and carried out about *Staphylococci* for several reasons.

First, for more than a century *Staphylococci* remain of major concern and the most common cause of nosocomial infections. The increasing frequency of multiresistant *Staphylococci* as a cause of infections in both hospitalized and, increasingly, non hospitalized individuals have drawn the attention of physicians to understand the pathophysiology, disease spectrum, and management of *Staphylococcal* infections.

Second, the large amount of information about *Staphylococci* has become available in recent years.

The molecular biology revolution has led to a new understanding of the molecular basis of the pathogenesis of *Staphylococcal* infections. These new methods also provide techniques to aid in the study of the epidemiology of infections and provide a diagnostic method, and management of *Staphylo coccal* infection.

Beta lactam antibiotics are the preferred drugs for serious *S.aureus* infections. However since the introduction of methicillin into clinical use, the occurrence of methicillin resistant. *S.aureus* (MRSA) strains have increased steadily, and nosocomial infections caused by methicillin Resistant *S.aureus* became a significant epidemiological problem world wide.

In addition bacterial genes are constantly mutating. Some strains genetic make up will give them a slight advantage when it comes to fighting off antibiotics. So when weaker strains encounter antibiotics, they die while these naturally resistant strains may prove harder to kill.

The advice from doctors who prescribe antibiotics is always to complete the entire course an advice which many of us ignore. When patient don't complete the course, there is a chance that most of the organisms will be killed but not all of them and the one that survive are of course likely to be those that are most resistant to antibiotics. Over time, the bulk of the *staphylococci* Strains will carry resistance gene and further mutations may only add to their survival ability.

Strains that carry two or three resistance genes will have extraordinary powers of resistance to antibiotics.

Patients are at higher risk than normal of picking up a *staph.* Infection on the wards. Because the population in hospital tend to be older, more sicker and weaker than the general population making them more vulnerable to the infection. MRSA infections can prove difficult to treat because they are resistant to treatment, making them more dangerous than a simple case of *staph.*

Doctors are very worried about what the future holds for MRSA. The number of reports of MRSA infections rises year by year and the spectrum of the organism resistant to all antibiotic is approaching. Vancomycin resistant *Staphylococcus aureus* has acquired resistance to a drug considered the last line of defense, when all other antibiotics have failed. Therefore rapid and accurate identification of *S.aureus* and its methicillin susceptibility pattern has important implications for therapy and management of both colonized and infected patients.