

Sudan University of Science and Technology
College of Graduate Studies and Scientific Research

**Isolation and Molecular Identification of Vancomycin
Resistant and Methicillin Resistant *Staphylococcus aureus*
from Patients with Different Clinical Manifestations in
Khartoum State**

العزل والكشف الجزيئي لجراثومة المكورات العنقودية الذهبية المقاومة
للفانكوميسين والمكورات العنقودية الذهبية المقاومة للميثيسيلين لدى مرضى
مختلفى الأعراض السريرية فى ولاية الخرطوم .

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قال تعالى :

(اقْرَأْ بِاسْمِ رَبِّكَ الَّذِي خَلَقَ)

صدق الله العظيم

سورة العلق الآية (1)

Dedication

I dedicate this work to

My Parents.....

Who gave me the meaning of the life

My husband and my daughter

My brothers and sisters....

For their support and kindness

My friends and my colleagues.....

The persons whom I love, respect and appreciate.....

To all who has ever taught me anything

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ABSTRACT

Emergence of Mecthicillin resistance *Staphylococcus aureus* is of public health concern contributing to increase morbidity and mortality among infected patients. The present study is aimed to determine the prevalence and molecular detection of MRSA, VISA and VRSA among patients in Khartoum state. The enrolled study population (n=426) were patients who attended the main Khartoum Hospitals (Military Hospital, Alrebat University Hospital, Khartoum Teaching Hospital and Khartoum North Teaching Hospital) in Khartoum State, Sudan extending from April 2010 to May 2011.

The study subjects involved 211 males and 215 females with different age categories. Enrolled subjects were suffering from wound infections (105), ear infections (121), Urinary tract infections (UTI) (100), in addition to nasal discharge of medical staff (100).

Clinical samples were cultured on blood agar and MacConkey agar. Cystine lysine electrolyte deficiency (CLED) agar was also used for urine samples. Gram staining and other biochemical tests were also performed for conventional identification. The results confirmed the existence of *Staph. aureus* in 49/426 (11.5%) cases among which MRSA were isolated from 34/49 (69.4%) when modified Kirby-Bauer disk diffusion method was applied. Ten out of these 34 MRSA were confirmed as VRSA by culturing on BHI agar containing 6µg/ml vancomycin according to National Control Culture Standard (NCCLS) criteria.

DNA was extracted from the 34 MRSA isolates (including the 10 VRSA) by phenol-chloroform method. PCR was then performed to amplify of *arcC*, *mecA*, *VanA* and *VanB* genes. Out of the 34 MRSA isolates DNA, 26 were *mecA* positive (76.5%) while 8 (23.5%) were *arcC* positive. But, *vanA* and *B* genes where not amplified

In conclusion, genotype analysis confirmed the existence of MRSA among Sudanese. But the same procedure failed to prove the occurrence of VRSA a mediated resistance indicating that among Sudanese isolates VRSA may be attributed to other factors.

الخلاصة

هدفت هذه الدراسة الى تحديد مدى انتشار جرثومة المكورة العنقودية الذهبية المقاومة للفانكوميسين والمكورة العنقودية الذهبية المقاومة للميثيسيلين وكذلك الكشف الجزيئى لها فى ولايه الخرطوم .

التحق مجتمع الدراسة (ن = 426) وكان من المرضى الذين حضروا مستشفيات الخرطوم الرئيسيه (مستشفى السلاح الطبى – مستشفى الرباط الجامعى – مستشفى الخرطوم التعليمى ومستشفى بحرى). فى الخرطوم , السودان , خلال شهر ابريل 2010 حتى مايو 2011.

شملت الدراسة 211 من الذكور 215 من الأناث من الفئات العمرية المختلفه. والمرضى المسجلين الذين يعانون من التهابات الجروح (105), والتهاب الأذن (121) , أو التهاب المسالك البولية (100), وكذلك مسحات الأنف من الطاقم الطبى ويشمل الممرضات وأطباء المختبرات (100) .

تم زراعة العينات السريرية على أجار الدم وأجار الماكونكى. ثم تم استخدام أجار الكليد لعينات البول. كما أجريت ايضا صبغة الغرام والاختبارات الكيميائية الحيوية الاخرى لتحديد الهوية التقليدية للبكتيريا . وأكدت النتائج وجود المكورات العنقودي الذهبية فى 49\426 (11.5%) شكلت المكورة العنقودية المقاومة للميثيسيلين 49\34 (69.4%) عندما تم أ استخدام طريقة كيربى باور الأنتشارية . كما تأكدت من أن 10 من هذه العزلات تنتمى للمكورات العنقودية الذهبية المقاومة للفانكوميسين (فيرزا) وذلك بزراعتها فى أجار مستخلص القلب والمخ المحتوى على فانكوميسين 6mg/ml وفقا لمعايير NCCL .

تم استخراج الحمض النووى الريبوزي (الدنا) من عزلات المكورات العنقودية المقاومة للميثيسيلين 34 (بما فى ذلك 10 من المكورات العنقودية المقاومة للفانكوميسين) بواسطة طريقه الفينول كلوروفورم و من ثم تم اجراء اختبار البلمره التسلسلى الجزيئى لتضخيم arcC و mec A و van A و vanB وظهرت النتائج أن من مجموع 34 عزله من المكورات العنقودية المقاومة للميثيسيلين (ميرزا) أن 26 macA كانت ايجابية (76.5%) فى حين أن 8 منها كانت ايجابية لـ arcC gene (23.5%). وفى حين انه لم يتم الكشف عن van أو van B .

ختاماً دعمت طرق الكشف الجزيئى لنتائج الكشف التقليدية الميرزا وذلك من خلال وجود جينات mecAgene أو arcCgene فى حين انها فشلت فى التأكيد علي وجود المكورات العنقودية المقاومة للفانكوميسين (الفيرزا) حيث لم يتم الكشف عن vanA أو vanB , وبالتالي قد تعزى الفيرزا الى عوامل أخرى.

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List of Abbreviations

VRSA, Vancomycin Resistant *Staphylococcus. aureus*

VISA, Vancomycin intermediate *Staphylococcus. aureus*

MRSA, Mecthicillin Resistant *Staphylococcus. aureus*

VRE, Vancomycin Resistant *Enterococci*

arcC, *Carbamate Kinase gene*

NCCL, National Committee for Clinical Laboratory Standards

CDC, Control Disease Center

PCR, Polymerase Chain Reaction

SCCmec, *Staphylococcus* Cassette Chromosome *mec*

PBP, Penicillin Binding Protein

BHI, Brain Heart Infusion

MIC, Minimum Inhibition Concentration

DNA, Deoxyribose Nucleic Acid

ELISA, Enzyme linked immune sorbent assay

dNTP, deoxy nucleotide triphosphate

TBE, Tris base ,boric acid, EDTA

SPSS, Statistical package for Social sciences

bp, Base pair(s)

rRNA, ribosomal Ribo Nucleic Acid

mRNA, messenger Ribo Nucleic Acid

SDS, Sodium dodeyle sulphate

PBS, phosphate buffer saline.

E-test, Epsloid test

PVL, panton – valetin leucocidin

TSST, Toxic shock syndrome toxin

CLED, Cystine lysine electrolyte deficiency

CFU, Colony forming unit