

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

(وَمَا أُوتِيتُمْ مِنَ الْعِلْمِ إِلَّا قَلِيلًا)

صدق الله العظيم

سورة الانشاء الآية 85

Dedication

I dedicate this work to

My Parents.....

WHO DID EVERY THINGS FOR ME

My brothers and sisters....

WHO WERE ALWAYS THERE ON MY SIDE

My friends and my colleagues.....

To all who has ever taught me anything

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ABSTRACT

Methicillin resistant *Staphylococcus aureus* (MRSA) is an increasing problem worldwide while in Sudan we still lacking the preliminary data for this pathogen. The objectives of the present study were to provide evidences about the occurrence of MRSA among Sudanese patients as well as to characterize and amplify different genes responsible for this phenomenon. Three hundred and fifty five patients suffering from different clinical diseases ($n= 355$) were included in this study during the period from October 2010 to May 2011. The distribution of the enrolled patients was as follows: general surgery at Wad Medani Teaching Hospital 129/355(36.3%), Gynaecology and Obstetrics Teaching Hospital 107/355 (30.1%), Institute of Nuclear Medicine, Moleculour Biology and Oncology 48/355 (13.5%), Orthopaedic Surgery Hospital 8/355 (2.3%), Dr. Ahmed Abdella Hamadein Hospital for Dermal Diseases 23/355 (6.5%), Gezira Hospital for Renal Diseases and Surgery, National Centre of Paediatrics Surgery 7/355 (2%), Tahily Speciality Hospital 7/355 (2%), Alyaa Speciality Hospital 8/355 (2.3%), and Wad Medani Police Hospital 10/355 (2.8%). All clinical samples were cultured on blood agar and MacConkey agar. Different biochemical tests, Gram's stain and amplification of *arcC* gene were used to identify the causative pathogens. The results confirmed the existence of *Staphylococcus aureus* in seventy two (72);(20.3%) of the enrolled patients among which thirty three (33); (45.8%) methicillin resistant *Staphylococcus aureus* were identified when using modified Kirby Bauer method. The distribution of MRSA among enrolled patients at the hospitals and medical centers were as follows: general surgery at Wad Medani Teaching Hospital

19/72 (26.4%), Gynaecology and Obstetrics Teaching Hospital 5/72 (7%), Orthopaedic Surgery Hospital 1/72 (1.4%), Dr. Ahmed Abdella Hamadein Hospital for Dermal Diseases 2/72 (2.8%) and Institute of Nuclear Medicine, Moleculour Biology and Oncology 6/72 (8.3%). While other medical centers included in the study were appeared free from MRSA.

Confirmation of the results of methicillin disk diffusion Kirby Bauer method was conducted by amplifying *mecA* gene. Furthermore all MRSA isolates were tested against other empirical antibiotics, the results were as follows: 14/33 (42.40%) were resistant to co-trimoxazole, 15/33 (45.50%) were resistant to cephalixin, 22 /33 (66.70%) were resist to tetracycline, 20 /33 (60.60%) were resistant to cefotaxime, 6/33 (18.20%) were resistant to ciprofloxacin and erythromycin, 12 /33 (36.40%) were resistant to pefloxacin and ofloxacin, 33/33 (100%) were resistant to cloxacillin, 7/33 (21.20%) were resistant to clindamycin, 3 /33 (9.10%) were resistant to gentamycin, 30/33 (90.90%) were resistant to ceftriaxone, and 9/33 (27.30%) were resistant to cefuroxime .

All MRSA isolates were examined against vancomycin antibiotic using modified Kirby Bauer disk diffusion method. The results obtained excluded the existent of VRSA among all MRSA isolates. More confirmation was adopted by amplifying *Van A*, *Van B* genes.

The study concluded that MRSA still consider as a great in medical field. Also it confirmed the sensitivity of molecular method in the diagnosis of MRSA as well as VRSA among infected patients. Thus, it can substitute the long conventional methods.

مستخلص الأطروحة

تعتبر المكورات العنقودية الذهبية المقاومة للميثيسيلين مشكلة متفاقمة في جميع أنحاء العالم بينما في السودان لا نزال نفتقر إلى البيانات الأولية لهذا الكائن الممرض.

هدفت هذه الدراسة إلى تقديم أدلة عن تواجد المكورات العنقودية الذهبية المقاومة للميثيسيلين بين المرضى السودانيين وكذلك معرفة خصائصه وتضخيم الجينات المختلفة المسؤولة عن هذه الظاهرة .

ثلاثمائة وخمسة وخمسون مريضاً يعانون من أمراض سريرية مختلفة (عدد = 355) تم تضمينها في هذه الدراسة خلال الفترة من أكتوبر 2010 إلى مايو 2011. وتوزيع المرضى المسجلين على النحو التالي: الجراحة العامة بمستشفى ود مدني التعليمي 355/ 129 (36.3٪)، المستشفى التعليمي لأمراض النساء والتوليد 355/107 (30.1٪) ومعهد الطب النووي والأحياء الجزيئية والأورام 355/48 (13.5٪)، مستشفى جراحة العظام 355/8 (2.3٪)، ومستشفى الدكتور أحمد عبد الله حمدين للأمراض الجلدية 355/23 (6.5٪)، مستشفى الجزيرة لأمراض وجراحة الكلى ، المركز القومي لجراحة الأطفال 355/7 (2٪)، المستشفى التأهيلي التخصصي 355/7 (2٪)، مستشفى علياء التخصصي 355/8 (2.3٪)، ومستشفى الشرطة 355/10 (2.8٪). تم زراعة جميع العينات السريرية على آجار الدم وآجار الماكونكي. وعمل الاختبارات البيوكيميائية المختلفة، كما استخدمت صبغة جرام وتم الكشف عن الجين *arcC* بالتضخيم والبلورة لتحديد مسببات المرض. أكدت النتائج وجود المكورات العنقودية الذهبية في 72 (72، 20.3٪) من المرضى المسجلين، ثم تم الكشف عن وجود المكورات العنقودية الذهبية المقاومة للميثيسيلين باستخدام طريقة الانتشار القرصي باور كيربي (لمعرفة تحسس الجراثيم للمضادات الحيوية). عددها ثلاثة والثلاثين (33، 45.8٪). بينما كان توزيع المكورات العنقودية الذهبية المقاومة للميثيسيلين بين المرضى المسجلين في المستشفيات والمراكز الطبية على النحو التالي: جراحة العامة بمستشفى ود مدني التعليمي 72/19 (26.4٪)، المستشفى التعليمي لأمراض النساء والتوليد 72 / 5 (7٪)، مستشفى جراحة العظام 72/1 (1.4٪)، ومستشفى الدكتور أحمد عبد الله حمدين للأمراض الجلدية 72/2 (2.8٪)، ومعهد الطب النووي الأحياء الجزيئية والأورام 72/6 (8.3٪) . والمراكز الخالية من المكورات العنقودية الذهبية المقاومة للميثيسيلين كانت: المركز القومي لجراحة الأطفال، مستشفى علياء التخصصي، مستشفى الشرطة، مستشفى التأهيلي التخصصي ومستشفى الجزيرة لأمراض وجراحة الكلى.

نتائج طريقة الإنتشار القرصي باور كيربي (لمعرفة تحسس الجراثيم للمضادات الحيوية) للميثيسيلين تم تأكيدها بتضخيم الجين *mecA*. ثم تم اختبار جميع العزلات (المكورات العنقودية الذهبية المقاومة للميثيسيلين) لمعرفة مقاومتها للمضادات الحيوية المستخدمة الأخرى، وقد كانت النتائج على النحو التالي: 33/14 (42.40%) كانت مقاومة للكوتريموكسازول، و 33/15 (45.50%) مقاومة للسيفاليكسين، و 33/22 (66.70%) مقاومة لالنتراسيكلين، كانت 33/20 (60.60%) مقاومة للسيفوتاكسيم، 33/6 (18.20%) كانت مقاومة للسيبروفلوكساسين والاريثرومايسين، 33/12 (36.40%) كانت مقاومة للبفلوكساسين وأوفلوكساسين، و 33/33 (100%) كانت مقاومة للكلوكساسيلين، 33/7 (21.20%) كانت مقاومة للكلينداميسين، 33/3 (9.10%) كانت مقاومة للجنتاميسين، و 33/30 (90.90%) كانت مقاومة للسيفترياكسون، و 33/9 (27.30%) كانت مقاومة للسيفروكسيم.

تم اختبار جميع العزلات للمضادات الحيوية المكورات العنقودية الذهبية المقاومة للميثيسيلين ضد الفانكوميسين باستخدام طريقة الإنتشار القرصي باور كيربي (لمعرفة تحسس الجراثيم للمضادات الحيوية). النتائج التي تم الحصول عليها دلت على خلو جميع عزلات المكورات العنقودية الذهبية المقاومة للميثيسيلين من وجود المكورات العنقودية الذهبية المقاومة للفانكوميسين. و للتأكيد تم الإعتماد على طريقة التضخيم والبلورة للجينات *Van A*, *Van B*.

الدراسة أكدت ان المكورات العنقودية الذهبية المقاومة للميثيسيلين لاتزال تحدي كبير في الحقل الطبي. كما أكدت الدراسة ايضا حساسية الطريقة الجزيئية في الكشف عن المكورات العنقودية الذهبية المقاومة للميثيسيلين وايضا المكورات العنقودية الذهبية المقاومة للفانكوميسين على حد سواء بين المرضى المصابين. عليه، يمكن استبدالها عن الطريقة التقليدية الطويلة.

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List of Abbreviations

<i>arcC</i>	Carbamate kinase
BMC	Bio Medical Center
bp	Base pairs
CA-MRSA	Community-acquired methicillin resistant <i>Staphylococcus aureus</i>
CDC	The Centers for Disease Control and Prevention
CNS	Coagulase-negative staphylococci
DM	Diabetes Mellitus
DNA	Deoxyribonucleic acid
DNase	Deoxyribonuclease
dNTP	Deoxynucleotide triphosphate
(ds)DNA	Double-stranded
DST	Drug Susceptibility Testing
EDTA	Ethylene diaminetetracetic acid
ET	Exfoliative toxins
FAME	fatty acid modifying enzyme
G	gram
HA-MRSA	Hospital acquired methicillin resistant <i>Staphylococcus</i>

	<i>aureus</i>
ICU	Intensive care unit
IgG	Immunoglobulin G
IV	Intravascular
Mb	Mega base pair
McF	McFarland
MIC	Minimum inhibitory concentration
min.	minute
ml	milliliter
mm	millimeter
MRSA	Methicillin resistant <i>Staphylococcus aureus</i>
MSA	Mannitol salt agar
MSSA	Methicillin susceptible <i>Staphylococcus aureus</i>
PBP2	Penicillin binding protein 2
PCR	Polymerase chain reaction
pH	Power of Hydrogen
PRSA	Penicillin-resistant <i>Staphylococcus aureus</i>
PTSGs	Pyrogenic toxin super antigens

Psi	Pounds per square inch
PVL	Panton Valentine Leukocidin
<i>Staph. aureus</i>	<i>Staphylococcus aureus</i>
SCCmec	Staphylococcal Cassette Chromosome mec
<i>spp.</i>	Species
<i>Staph</i>	<i>Staphylococcus</i>
SSSS	staphylococcal scalded-skin syndrome
SSTI	Skin and soft tissue infection
TSS	Toxic shock syndrome
U	unit
USA	United States of America
UTI	Urinary tract infection
UV	Ultraviolet
VISA	Vancomycin intermediate <i>Staphylococcus aureus</i>
VRE	Vancomycin-resistant <i>enterococci</i>
VRSA	Vancomycin-resistant <i>Staph. aureus</i>
v/v	volume per volume