Sudan University of Science and Technology

College of Graduate Studies

Frequency of Methicillin Resistant *Staphylococcus aureus* Associated with Wound Infections in Patient at some Hospitals –Khartoum State

تردد البكتيريا العنقودية الذهبية المقاومة للميثثلين في عينات الجروح الماخوذة من بعض المسشفيات بولاية الخرطوم

″A dissertation Submitted in Partial Fulfillment for the Requirement of Degree of M.Sc. in the Medical Laboratory Sciences (Microbiology)

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الآيـــــــة

قال تعالى: (قَالُوا سُبْحَانَكَ لا عِلْمَ لَنَا إِلاَّ مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ)(32)

صدق الله العظيم

سورة البقرة الآية "32"

Dedication

To the soul of my lovely father, who worked hardly for

Us.

To my great mother, who taughtme, how I could

Be human.

To my lovely brother and sisters for their support and

Kindness.

To my friends who helped me, believed me and put

A hope on me.

To all of them I dedicate my humble work,

With my best wishes for students to get a success.

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Abstract

The emergence of resistant strains has considerably increased the burden of morbidity associated with wound infections, increased frequency of methicillin resistant *Staphylococcus aureus* (MRSA) and possibility of vancomycin resistance requires rapid and reliable characterization of isolates and control of methicillin resistant *Staphylococcus aureus* spread.

This study had been carried out in Khartoum State during the period between March to July 2018, aimed to determine the frequency of methicillin resistant *Staphylococcus aureus* (MRSA) isolated from wound infections and to detect *mecA* gene among them using phenotypic and genotypic methods.

One hundred wound swabs were collected from patients attending different hospitals including Bahari Teaching Hospital, Omdurman Teaching Hospital and National Public Health Laboratory. All wound swabs were inoculated on blood agar, MacConkey agar and Mannitol Salt agar plates and incubated at 37°C for 24 hours.

The study population ages ranged between 1 up to 70 years old with mean age of 35.7±22 SD, 49 (49%) were males and51 (51%) females.

The types of wounds were as follows, 30% surgical, 50% traumatic and 20% diabetic wounds.

The bacterial growth was observed and identified in 88 swabs (88%), (5%) have mix bacterial growth and 12(12%) showed no obvious growth.

Out of 93 isolates, 26(28%) were *S. aureus,* and the susceptibility test showed that 10 (38.5%) isolated *S.aureus* were methicillin resistant *S.aureus.*

All *S.aureus* isolates which were initially detected as methicillin resistant *S.aureus* by susceptibility test were positive for *mecA* gene by polymerase Chain Reaction(PCR) .

In conclusion *S.aureus* is highly associated with causing wound infections and all MRSA were positive for *mec* A gene, further studies are needed to validate this result.

مستخلص الاطروحة

ظهور السلالات المقاومة ادى الى زيادة معدل الامراضية المصاحبة لالتهابات الجروح ، زيادة معدل العنقودية الذهبية المقاومة للميثلين واحتمالية مقاومتها لمضاد الفانكوميسين يتطلب تصنيف سريع وموثوق به وذلك لمكافحة العنقودية الذهبية المقاومة للميثلين .

اجريت هذة الدراسة في ولاية الخرطوم في السودان في الفترة من مارس 2018 الى يوليو 2018 وكان الهدف من الدراسة معرفة نسبة الكروية العنقودية الذهبية المقاومة الميثيثلين في العينات الماخوذة من الجروح الملتهبة وكشف وجود ال mecA جين في العنقودية الذهبية المقاومة للميثيثلين .

تم جمع 100 عينة من جروح ملتهبة من مستشفى بحري التعليمي ،مستشفى امدرمان التعليمي والمعمل القومي للصحة العامة كل العينات التى جمعت تمت زراعتها في ثلاثة اوساط زراعية تشمل Blood agar MacConkey agar ، Mannitol Salt agar ثم تحضينها في 37 درجة مئوية لمدة 24 ساعة .

العينات تم جمعها من مرضى تتراوح اعمارهم ما بين العام والسبعين عام مع متوسط اعمار 35.7 عام ،49 (49 %)منهم ذكور 51(51%) اناث .

اسباب هذة الجروح مختلفة 30% منها نتيجة لعمليات جراحية ،50% نتيجة لحوادث مختلفة كما ان بعضها جروح سكري 20 % .، ولوحظة ان 88(88 %) من العينات بها نمو واضح كما ان بعضها به اكثر من نوع من البكتريا (5%) ولذلك فان مجموع البكتريا التي تم عزلها 93 بكتريا ، بينما (12%)12 من العينات لم ينمو بها اي نوع من البكتريا .

واظهرت النتائج ان نسبة الكروية العنقودية الذهبية من بين ال 93 بكتريا يساوي(28%) 26 عنقودية ،كما اظهرت نتيجة اختبار الحساسية ان (38.5%) 10 من هذه العنقودية التي تم عزلها عبارة عن العنقودية الذهبية المقاومة للميثيثلين .

استخدمت تقنية الاحياء الجزيئية ال(PCR)للكشف عن وجود ال mec A جين في العنقودية الذهبية المقاومة للميثيثلين وكانت النتيجة ان كل العنقودية الذهبية التى تم مسبقا تحديد انها مقاومة للميثلين بواسطة اختبارات الحساسية تحتوي على ال mecA جين .

الخلاصة تعتبر العنقودية الذهبية مسبب لالتهاب الجروح بنسبة عالية ،كما ان كل العنقوديات الذهبيات المقاومات للمضاد الحيوي الميثلين تحتوي على الجين mec A ،نحتاج الى العديد من الدراسات لمصادقة هذة النتيجة .

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INTRODUCTION

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Introduction

1.1 Introduction

Methicillin-resistant means the bacteria are unaffected by methicillin, a type of antibiotic that used to be able to kill them. Unfortunately some strains of staphylococci have become resistant to methicillin and other similar antibiotics. These strains are known as MRSA, which cannot be cured with traditional penicillin-related drugs (Aly *et al.*, 1977). Instead, MRSA must be treated with alternate antibiotics. MRSA stands for methicillin-resistant *Staphylococcus aureus*, which is a common skin bacterium that is resistant to a range of antibiotics (Aly and Levit, 1987). People can carry it in the nose or on the skin without showing any symptoms of illness. This is called MRSA colonization For *Staphylococcus aureus* the most common body site colonized is the nose. While 25% to 30% of people are colonized in the nose with staphylococci, less than 2% are colonized with MRSA (Kluytmans *et al.*, 1997). MRSA can also causes infections such as boils, wound infections, and pneumonia (Moran *et al.,* 2006). The development of a wound infection depends on the complex interplay of many factors. If the integrity and protective function of the skin is breached, large quantities of different cell types will enter the wound and initiate an inflammatory response. This may be characterized by the classic signs of redness, pain, swelling, and raised temperature (Moran *et al.,* 2006).

MRSA strains are an increasing infection control problem and therapeutic challenge. This strains which are resistant to all β lactam agent, and often to other agents such as the amino glycosides and fluoroquinolones commonly colonize broken skin, but can cause the full range of staphylococcal infection .The resistance gene *mecA* codes for a unique penicillin-binding protein and is transmitted chromosomally, these are predominantly hospital pathogens in debilitated patients (wood *et al*., 2003).

Glycopeptides (eg: vancomycin) are the agents of choice in the treatment of systemic infections, but these agents are expensive and may be toxic (wood *et al*., 2003).

Strains of MRSA with reduced susceptibility to glycopeptides have been found in several countries (wood *et al*., 2003).

These bacteria have thickened walls compared to vancomycin sensitive strains but are difficult to detect by routine methods as the expression of resistance is heterogeneous (wood, *et al*., 2003).

MRSA produces a penicillin binding protein 2a (mediated through the *mecA* gene) which is carried on the *Staphylococcus* cassette chromosome *mec* (SCC *mec*) of which there are at least six different types recognized, and this results in resistance to all beta─lactam anti biotic (Humphreys, 2012).

Infection may occur following accidental trauma and injection , but post –operative wound infection in hospital are most common , wound infection may be endogenous infection in which infection occurs by patient's own bacterial flora such as *Staphylococcus auerus* from skin and anterior nares or coli forms, exogenous infection many infections are exogenous, skin and anterior nares are important sources of *Staphylococci*. Spread of the organisms from hospital staff and visitors occur by direct airborne routes. More than 60% of hospital acquired infections are due to Gram negative enteric bacilli and only 30% cases Gram positive cocci (Ckakraborty, 2003) .

Wound infection caused by *Staphylococcus auerus* amajor cause of wound infection, the source may be the patient own carrier state, other carriers (eg physicians or nurses) or other infected patients (John *et al.*, 1984).

Microscopic study of smears and culture of specimens from wounds often gives early and important indications of the nature of the infecting organism and thus help in the choice of antimicrobial drugs (Malley and lebowitz, 2007).

1.2 Rationale

MRSA was infecting people who had chronic illness, but now it is becoming a serious problem within health care organizations and community individuals. The emergence of MRSA strains in Sudan may constitute a public health problem with a strong potential for dissemination and high rates of morbidity and mortality. It causes infections ranging from wound infections to endocarditis (Aly *et al*., 1977).MRSA is highly prevalent among populations of *S.auerus* isolated from different clinical specimens (77.6%) in different hospitals in Khartoum State, Sudan, with most of the MRSA isolates being from wound infection (Elimam *et al.,* 2014).The frequency of Methicillin –Resistant *Staphylococcus aureus* in wound infection in Sudan is high as 48.4% of the isolates were resistant to Methicillin (Reza *et al*., 2014).

High level resistance to methicillin is caused by the *mec A* gene, which encodes an alternative penicillin binding protein, PBP2a (Wielders *et al.,* 2002).

So conducting this research will help in identifying MRSA and it is genetic cause for resistance.

1.3 Objective

1.3.1 General objective

To determine the frequency of methicillin resistant *Staphylococcus aureus* associated with wound infections at Khartoum State Sudan.

1.3.2 Specific objectives

1. To isolate and identify *S.aureus* strains from wound swabs using standard microbiological method.

2. To assess antimicrobial susceptibility of *S.aureus* to methicillin by Modified Kirby- Bauer disc diffusion method.

2. To detect *MecA* gene among MRSA using molecular technique.

CHAPTER TWO
LITERATURE REVIEW

CHAPTER TWO

LITERATURE REVIEW

2.1. Staphylococci

2.1.1 Historical Background

Sir Alexander ogston Scottish surgeon first showed in 1880 that number of pyogenic disease in human were associated with cluster forming micro organism (Wood *et al* ., 2003) .

He introduced the name Staphylococcus, and then Rosen Bach in 1884 introduced the term pyogens which detected in 1958to be aureus and described the two pigmented appropriate nomenclature, *Staphylococcus aureus* yellow and *Staphylococcus albus* white (Wood *et al* ., 2003) .

Most Staphylococci are non motile, aerobic or facultative anaerobic, catalase positive; grow in medium containing 10% sodium chloride and temperature ranging from 18C◦-40C◦ (Murry *et al.*, 2002).

2.1.2 Normal habitat

The organism is present on the skin and mucous membrane of human, other mammals and birds, it is widely distributed in environment (Murry, *et al*., 2002).

The species most commonly associated with human disease are *Staphylococcus aureus*, *Staphylococcus epidermids*, *Staphylococcus saprophyt*icus (Murry, *et al*., 200*2).*

2.1.3Morphology and Identification

2.1.3. 1 Morphology

Staphylococc*i are* spherical cells about 1µm in diameter arranged in irregular clusters .single cocci, pairs, and chains are also seen in liquid cultures, strongly gram positive (Malley and lebowitz, 2007).

2.1.3.2 Culture

Staphylococci grow readily on most bacteriologic media under aerobic or micro aerophilic conditions.

The growth most rapidly at 38c◦ but form pigment best at room temperature (20─25 c◦) (Malley and lebowitz, 2007).

Colonies on solid media are round, smooth, raised, and glistening (Malley *et al.*, 2007).

Various degrees of haemolysis are produced by *S.aureus* and occasionally by other species (Malley and lebowitz, 2007)

2.1.3.3 Growth characteristic

Staphylococci produce catalase, which differentiates them from the streptococci (Malley and lebowitz, 2007).

Staphylococci slowly ferment many carbohydrates, producing lactic acid but not gas, proteolytic activity varies greatly from one strain to another, and produce many extra cellular substances (Malley and lebowitz., 2007).

2.1.4 Anti microbial Resistance

2.1.4.1. β─lactamase production

Is common and under plasmid control, andmakes the organisms resistant to many penicillin (Malley and lebowitz, 2007).

The plasmid transmitted by transduction and perhaps also by conjugation (Malley and lebowitz, 2007).

2.1.4.2. Resistance to Nafcillin , Methicillin and Oxacillin

The mec A gene for nafcillin resistance resides in the chromosome, and the gene encodes a low affinity penicillin binding protein (Malley and lebowitz., 2007).

2.1.4.3. Vancomycin Entermediate *S.aureus*

The mechanisms of resistance is associated with increased cell wall synthesis and alteration in the cell wall not due to van gene found in Entero cocci (Malley and lebowitz, 2007).

2.1.4.4. Vancomycin Resistant *S.aureus*

The isolate contained the vancomycin resistance gene van A *(*Malley and lebowitz, *2007).*

2.1.4.5. Plasmid Mediated Resistance

Plasmid mediated resistance to tetracyclines, erythromycins, amino glycosides, and other drugs are frequent in Staphylococci (Malley and lebowitz, 2007).

2.1.4.6. Tolerance

Implies that Staphylococci are inhibited by drugs but not killed by it (Mally and lebowitz., 2007).

2.1.5 Treatment

Serious multiple skin infections ,similar skin infections occur in patients receiving prolonged courses of corticosteroids , Tetracycline s are used for long term treatment (Malley and lebowitz, 2007). Abscesses and other closed suppurating lesions are treated by draining, acute and hematogenous osteomylitis responds well to antimicrobial drugs (Malley and lebowitz., 2007).

In chronic and recurrent osteomylitis surgical draining and removal of dead bone is accompanied by long term administrating of appropriate drugs, hyper baric oxygen and the application of vascularzed myocutaneous flaps have aided healing in chronic osteomyelitis (Malley and lebowitz., 2007).

Bacteremia, endocarditis, pneumonia and other severe infections due to *S.aureus* requires prolong intravenous therapy with β─lactamase resistant penicillin (Malley and lebowitz, 2007).

Vancomycin is often reserved for use with nafcillin resistant *Staphylococci*, if the infection is found due to non─β─lactamase producing *S.aureus* penicillin G is the drugs of choice (Malley and lebowitz, 2007).

Because of the frequency of drug resistant strains, meaningful *Staphylococcal* isolates should be tested for antimicrobial susceptibility to help in the choice of systemic drugs (Malley and lebowitz, 2007).

Newer antimicrobial agents such as linezolide, daptomycin, and quinupristin are generally reseved for patients with serious Staphylococcal infections that are resistant to more traditional agents who are failing clinically or who are highly allergic (Malley and lebowitz., 2007).

2.1.6 Epidemiology and control

The basic habitat of *Staphylococcus aureus* is the anterior nares; about 30% of the individuals in the community carry the organism in this site at any given time.

Nasal carrier rates among hospital personnel and patients may be much higher when Staphylococcal infections both result from and contribute to the staphylococcal environmental load. Some individuals have extensive colonization of the perineum they and some nasal carriers may disseminate the organism extensively with desquamated epithelial cells and thus constitute a source of infection to others (John *et al.*, 1984).

2.2 *Staphylococcus aureus*

2.2.1 Virulence factors of *Staphylococcus aureus*

2.2.1.1Toxins

2.2.1.1.1 Enterotoxin

Are heat stable exotoxins causing diarrhea and vomiting in human. Entero toxin A and D are resistant to gastric and digestive juices and have been associated with food poising. Entero toxin F causes toxic shock syndrome (Mahon *et al*., 2007).

2.2.1.1.2 Exofoliative toxins

Produced by phage group, it causes epidermal layer of the skin to slough off and known to cause scaled skin syndrome (Mahon *et al*., 2007).

2.2.1.1.3 Cytolytic toxin

It is extra cellular protein that effect red blood cell and leukocytes. (Mahon *et al*., 2007).

S.aureus produce α β and hemolysins, in addition to hemolyzing red blood cell α toxin is capable todestroying platelet and cause severe damage to tissues (Mahon *et al*., 2007).

B hemolycin acts on the sphying, myelin of red cells membrane, hemolycin although it has been described to cause injury in most cells in cultures and to leukocytes, is considerably less lethal than α or β hemolycin (Mahon *et al*., 2007).

2.2.1.1. 4 protein A

One of several cellular components that have been identified in the cell wall of *S.aureus* (Mahon *et al*., 2007).

2.2.1.2 Enzymes

Coagulase: an enzyme that clots plasma and coats Staphylococcal cell which probably prevents bacteria from being phagocytosed, deoxyribonuclase (DNAase) that destroys deoxy ribonucleic acid, lipase which breaks down fat, hyaluronidase that helps *Staphylococcus aureus* to spread in the tissues, staphylococcal kinase which Cause fibrinolysis and beta-lactamase that lead to penicillin resistance (Cheessbrough, 1984).

2.2.2 Pathogenicity

This species causes:

Abscess, boils, styes, and impetigo it may also cause secondary infection of insect bites, ulcer, wound and skin disorders, conjunctivitis especially of newborn,

Septicemia and osteomyelitis, pneumonia and empyema, mastitis antibiotic associated enteritis, food poisoning the food is often contaminated by carrier of *S.aureus*, scalded skin syndrome in young children due to toxin exfoliating, toxic shock syndrome due to colonization of *S.aureus* especially in vagina (Cheessbrough, 1984).

2.2.3 Laboratory diagnosis of infections caused by *Staphylococcus aureus*

2.2.3.1 Specimens

Surface swab pus, blood, tracheal aspirate, or spinal fluid for culture, depending on the localization of the process (Malley and lebowitz, 2007).

2.2.3.2 Smears

Gram positive cocci in cluster in Gram stained smearof pus or sputum (Malley and lebowitz, 2007).

2.2.3.3 Culture of *Staphylococcus aureus*

Specimens planted on blood agar plates give rise to typical colonies in 18 hours at 37c◦ (Malley and lebowitz, 2007).

S.aureus ferment Mannitol, Mannitol salt agar or commercially available chromogenic media are used to screen nasal carriers of *Staphylococcus aureus* and patient with cystic fibrosis (Malley and lebowitz, 2007).

2.2.3.4 Catalase test

This test is used to detect the presence of cytochrome oxidase enzymes (Malley and lebowitz, 2007).

2.2.3.5 Coagulase test

2.2.3.6. SerologicTest

Serologic test for diagnosis of *Staphylococcus aureus* infections have little practical value (Malley and lebowitz, 2007).

2.2.3.7 Typing test

Molecular typing techniques have been used to document the spread of epidemic disease producing clones of *Staphylococcus aureus* (Malley and lebowitz, 2007).

2.2.4. Susceptibility testing

Broth micro dilution or disk diffusion susceptibility testing should be done routinely on staphylococcal isolates from clinically significant infection (Malley *et al.,* 2007).

Resistance to penicillin G can be predicted by a positive test for β─lactamase (Malley *et al*., 2007).

Nafcillin resistance correlated with mecA gene , the gene can be detected by the polymerase chain reaction , most clinical laboratories use a phenotypic method such as oxacillin screening agar plate (Malley *et al* .,2007) .

2.3 Beta-lactams

Beta-lactams contain beta- lactam ring and inhibit cell wall synthesis by binding to penicillin binding proteins. Beta- lactam comprise a very large family of different groups of bactericidal compounds all contain the beta- lactam ring.

The different group within the family is distinguished by the structure of the ring attacked to the beta- lactam ring.

Penicillin binding protein is membrane protein capable of binding to penicillin and is responsible for the final stage cross-linking of the bacterial cell wall structure.

Inhibition of one more of these essential enzymes results in an accumulation of precursor cell wall units, leading to activation of cells, autolytic system and cell lysis (Mims *et al.,* 2006).

2.4. Methicillin

The methicillin being a new synthesized antibiotic was checked against the penicillin-resistant strains with excellent results because it is not a beta-lactamase substrate, although the methicillin target is the same of the penicillin. In fact, both drugs are β-lactamic antibiotics that act by inhibiting the penicillin-binding proteins (PBPs). PBPs are involved in the synthesis of peptidoglycans, essential mesh-like polymers that surround cellular enzymes and are crucial for the bacterium survival (Costantin *et al*., 2009).

2.5. Resistance of MRSA to β lactam antibiotic

MRSA synthesize an additional PBP which has much lower affinity for β-lactam s than the normal PBP, and there for able to continue cell wall synthesis when the other PBP are inhibited. MRSA are resistant to all other β-lactams (Mims *et al*., 2006).

2.6 Screening for MRSA

Comparative evaluations of screening media are limited. Baird Parker medium with ciprofloxacin (BPC) has good overall performance for the recovery of ciprofloxacin-resistant MRSA from screening swabs. The growth of most MRSA on this medium within 24 h offers the advantage of early recognition. The use of this medium, nevertheless, is limited to the detection of ciprofloxacin-resistant isolates. Isolates susceptible to this agent will thus be inhibited and an alternative medium without ciprofloxacin is necessary if ciprofloxacin-susceptible isolates are to be detected. Mannitol Salt Agar (MSA) media may be used and early data on newer chromogenic media appear promising, but there is insufficient evidence to recommend any particular medium among these (Brown *et al*., 2005).

Enrichment of screening swabs is more sensitive than direct plating and may be particularly useful for screening in some high-risk groups of patients and in screening for clearance of MRSA carriage. Comparative evaluations of enrichment media are limited (Brown *et al*., 2005).

Molecular methods for processing screening swabs are a potentially valuable development to reduce test time (Brown *et al*., 2005).

Cefoxitin disc diffusion test is reliable substitute for detection of MRSA in clinical laboratory where MIC detection and molecular methods are not accessible (Reza *et al*., 2014).

2.7 Prevention and Control of MRSA

The control and prevention of MRSA involves the education of all healthcare professionals and the public, fast and reliable detection in the laboratory, active surveillance, prompt patient isolation or cohorting when admitted to hospital, standard precautions and good professional practice by all heath care workers, effective hospital hygiene programmers (Humphreys, 2012).

2.8 Previous studies

Several studies had been conducted regarding MRSA, results of study conducted in Sudan showed that the frequency of mithicillin resistance *Staphylococcus aureus* strains was (26.9%) in all specimens from wound infections in Khartoum Teaching hospital, Sudan (Ahmed, 2016).

Other study at teaching hospitals in Sudan (2012) showed that *Staphylococcus aureus* is major cause of infection in both health care and community setting. it is the one of the most common causes of the health care associated infections reported to the national nosocomial infections surveillance (NNIS) system, it revealed that the *S.aureus* strains were the major causes of hospital acquired infections from postoperative wound infections (55%), and forty-five strains

were identified as MRSA (85.5%) (Ahmed, 2012). Also prevalence of methicillin resistant *Staphylococcus aureus* among Egyptian patients after surgical intervention, 241Staphylococcal species represented the most common isolates(64.8%)among 371 collected isolates from the 208 patients ,out of 241Staphylococcus 127 were *S.aureus* (52%) , the prevalence of *S.aureus* in surgical site infections (SSI), diabetic foot, abscess and burn were (59%), (75%), (56%), and(52%) where as that of MRSA was (16%), (17%), (13%), and (10%) respectively(Ahmed ,*et al.*, 2014) .

Other study in Chitwan Nepal in (2010) showed that the Prevalence of MRSA among skin infection cases at hospital was (68%). MRSA isolation rate was higher from wound (76.9%) followed by purulent exudates (67.7%) and abscess (64.1%) (Khanal, 2010).

In comparisons to study done by Bano *et al* .,(2012) Pakistan in which demonstrates the trends of antibiotic resistance among *S. aureus* of wound isolates and frequency of MRSA from wound infections. The data showed that *S. aureus* was most prevalent among isolates of wound infections. The antibiotic susceptibility data showed that 36.5% (n=19) of the total *S.aureus* isolates were resistant to oxacillin antibiotic and thus were MRSA (Bano *et al.*, 2012*).*

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

3. Materials and methods

3.1 Study type and design

This was descriptive and cross sectional study.

3.2 Study area

This study was done at Khartoum State .The samples were taken from Bahary Teaching Hospital, Omdurman Teaching Hospital, and National Public Health Laboratory.

3.3 Study duration

This study was conducted during the period from March to July 2018.

3.4 Study population

The study populations were patients suffering from wound infections.

3.4.1 Inclusion criteria: All attended patient with wound infection in the previous hospitals were included

3.4.2 Exclusion criteria: Any patients suffering from wound infection with history of antibiotic treatment were excluded.

3.5 Sample size

The sample size was100 patients with wound infections.

3.6 Sampling Technique

This study based on non probability convenience sampling technique

3.7 Data Collection Methods and tools

Data was collected by self interviewing questionnaire (Appendix 1) containing information like age, causes of wound, type of antibiotic if used, and duration of wound.

Exclusion criteria:

Samples were not collected from patients under antibiotic treatment.

3.8 Ethical consideration

Permission to carry out the study was obtained from the Sudan University of Science and Technology Research Ethical Board of collage of medical laboratory science and Permission from hospital was applied from Hospital administration .verbal consent was obtained from participants involved in the study.

3.9 Experimental work

3.9.1 Specimens collection

Wound exudates were collected from deep tissues by sterile cotton wool swab after disinfection with 70% alcohol; delayed samples were preserved in Amis Transport media.

3.9.2 Isolation and preservation of bacteria:

All specimens were cultured onto blood agar, MacConkey agar and Mannitol Salt Agar (Himedia, india) and incubated at 37°C for 24 hours.

Macroscopic examination of culture growth was observed whether it is Mannitol or lactose fermented, hemolytic or not.

3.9.3 Microscopic examination of bacterial growth

Smears were made from purified colonies of a culture and stained by Gram stain method and then examined microscopically for identification of bacteria so as to distinguish between Gram-positive and Gram-negative organisms.

3.9.3.1 Gram Stains (Himedia, India)

Well dried fixed smears were prepared, then they stained by crystal violet for one minute , washed with tap water, then iodine was added for one minute washed with water ,smear was decolorized with alcohol for 10 to 30 seconds , washed with water , finally counter stain safranin was added for two minutes after that washed , dried and examined under microscope (Ckakraborty, 2003).

3.9.4 Biochemical tests for the identification of Gram positive cocci

The following biochemical tests were done:

3.9.4.1 Catalase test

The test demonstrates the presence of catalase in bacteria which release oxygen from hydrogen peroxide.

One ml of 3% hydrogen peroxide was taken in small sterile test tube to which a loop full of bacterial culture was placed, positive catalase reaction showed production of gas bubbles almost immediately (Ckakraborty, 2003).

3.9.4.2 Coagulase test

Coagulase was detected as a clumping factor. A suspension of *S.aureus* in saline was mixed with a loop full of human EDTA plasma on a glass microscope slide after mixing; coagulase causes the formation of fibrin cause the bacteria to clump together (Heritage, *et al.*, 1996).

3.9.5 Antimicrobials test of *S.aureus* isolates by using Standard disc diffusion method (Kirby-Bauer Sensitivity testing technique)

Mueller Hinton sensitivity testing agar (Himedia, india) was prepared and sterilized as instructed by the manufacturer (Appendix III) poured into 90 mm diameter sterile Petri dishes to a depth of 4 mm (about 25 ml per plate). The media was allowed to settle and solidify, and after that, the plates were dried for 5 minutes in an incubator with their lids slightly raised in a 37º C to remove excess moisture from the medium surface and plate cover. The plates were examined for contamination by incubated over night before use.

The inoculums were prepared by emulsifying 3-5 colonies of the test organism in about 3-5 ml of sterile normal saline and compared to0.5 Macfarland standared. In order to prevent further growth the diluted and standardized inoculums were not be allowed to stand longer than 15-20 minutes before inoculation on the plates. A sterile cotton swab is dipped into the suitably diluted suspension and rotated; the swab was pressed against the side of the tube to remove excess fluid, and streaked across the medium in three directions and rotating the plate approximately 60º to ensure even distribution.

Using sterile forceps methicillin 1µl antibiotic discs was carefully placed onto the inoculated plates; each disc was lightly pressed down to ensure its contact with the agar.

After overnight incubation at 37º C aerobically the culture was examined for zones of inhibition of bacterial growth around the respective discs, zones were measured in mm by ruler.

Using the interpretative chart, interpret the zones sizes of methicillin 1µl antibiotic reporting the organisms as Resistant, Intermediate/moderately sensitive, Sensitive (susceptible).

3.9.6 Molecular detection of MecA gene by using PCR method

3.9.6.1 DNA extraction by boiling method

About 3-5 *S.aureus* colonies were collected from blood agar plate and resuspended in 300µl D.W in 1.5 ml eppendorf tube using micropipettes. The samples were boiled at 100ºC for 15 minutes, and then centrifuged at 13000(rpm) for 10 minutes the supernatant was used as a template (Abdalla *et al*., 2014).

3.9.6.2. Primer Sequences and Target Gene

The oligonucleotide primers used in this study were synthesized and purchased from Genekam Biotechnology AG-Germany.

Primer pair	**sequence(5…→3)**	**Ampilicon size bp**	**Target gene**
Mec A Forward	F □ AAA ATC GAT GGT AAA GGT TGGC	532	*mec A*
MecA Revers	R□ AGT TCT GCA GTA CCG GAT TTGC		

3.9.6.3 PCR amplification

Amplification was carried out using specific primer target gene *mecA* ampilicon size of 532pb. The total volume of reaction mix was 20µl.

3.9.6.3.1Master mix

The premix (INtRON Biotechnology, Seongnam , Korea) was used, it contain all the reagent required for PCR(except water, template and primer)and additional compound needed for direct loading into agarose gel tracking blue dye that allow the monitor progress during the electrophoresis. It store at ─20◦c can be used up to one year.

3.8.6.3.2 Preparation of reaction mixture

For one sample PCR pre mix tube 11 µl from DNase RNase free sterile water was transferred , lµ forward primer ,1 µl reveres primer were added, then 7 µl from DNA sample was added to the mix .

3.9.6.4 The thermo cycler (PTC-100 programmable Thermal Controller, USA) program as follow:

	Temperature	Time	Cycling	Reference
Initial denaturation	94	4min		Strommenger *et al*, 2003
Denaturation	94	30sec	35 cycles	
Annealing	59	30 sec		
Extension	72	60 sec		
Final extension	72	4min		
Hold	4	∞		

3.9.6.5 Detection of PCR products

Preparation of 1.5% agarose gel:

Weight 1.5gram from agarose powder ,transfer it to sterile flask ,then add 100ml of 1X Tris bas Boric acid EDTA(TBE) buffer ,mix well to dissolve, complete solving by heating in microwave ,let it to cool before 10 µl of ethidium bromide add because it is highly carcinogenic so take care when use it ,poured into tray supplied with combs ,let to solidify then remove the combs .

Preparation of 250ml of 10X Tris bas Boric acid EDTA (TBE) buffer:

Tris base 27.0g

Boric acid 13.75g

Distell water 250ml

Preparation of 1X Tris bas Boric acid EDTA (TBE) buffer:

Add 10ml of 10X Tris bas Boric acid EDTA (TBE) buffer to 90ml of sterile D.W and mix.

3.8.6.5. Gel electrophoresis

The PCR products were analyzed on a 1.5% agarose gel. 6µl of a DNA molecular weight marker 50 base pair (INtRON,Biotechnology ,Korea) was transferred into the first well, In the following wells , 6µl PCR products samples were transferred followed by NTC(Non Template Control) which is the master mix without addition of any template , Gel electrophoresis was performed at 85 volt for 45 minutes, and the analysis was done by using ultra violet trans illuminator.

3.10 Data analysis

Data was analyzed by statistical package for social sciences (SPSS) soft ware program version 16 by using chi-square test. P.value equal or less than .05 consider significant.

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

A total of 100 wound swabs were collected from patients suffering of wound infections .Their age ranged between 1 up to70 years old with mean 35.7 ±22 SD, 49 (49%) were males, 51 (51%) females.

The wounds were surgical (30%), traumatic (50%) and diabetics (20%) wound. The total number of swabs that showed growth were 88(88%), (5%) from 88% showed mix growth, while the remaining 12(12%) showed no obvious growth as show in (Figure 4.1).

A total of 93 isolates were obtained, 41(44%) were Gram-positive isolates and 52(56%) were Gram-negative isolates (Figure 4. 2).

Among 41 Gram positive *S.aureus* represented as 26(63.4%) as show in (Figure 4.3), Regarding Antibiotic sensitivity testing the result showed that 16(61.5%) out of 26 *S.aureus* were sensitive to methicillin while 10(38.5%) were resistant (Figure 4. 4).

The frequency of MRSA according to gender as follow, 6 (6%) were from males and 4(4 %) were from females. There was no significant association between gender and MRSA wound infections (table 4.1). The results also showed that there was no significant association between age group and frequency of MRSA wound infections (Table 4.2).

Molecular analysis for detection of *mec* A gene among MRSA isolates showed that all MRSA isolates (10) were positive for the presence of mecA gene which was detected by PCR assay (Figure 4.5).

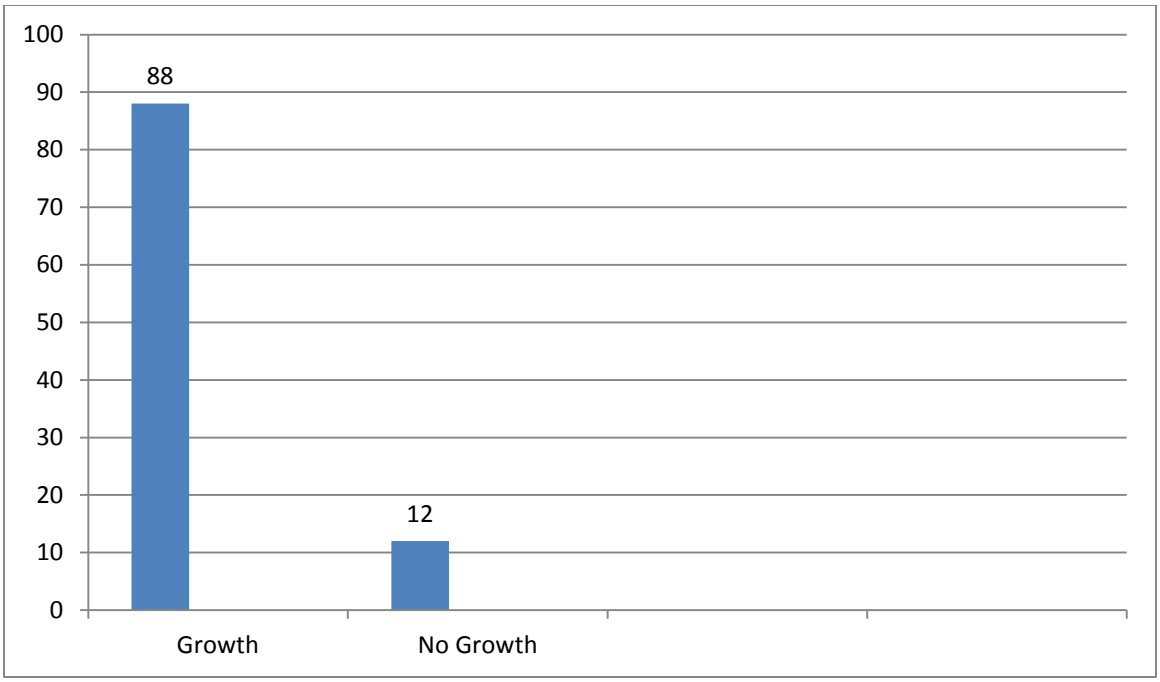

Figure (4.1) Frequency of bacterial growth among patients with wound infections

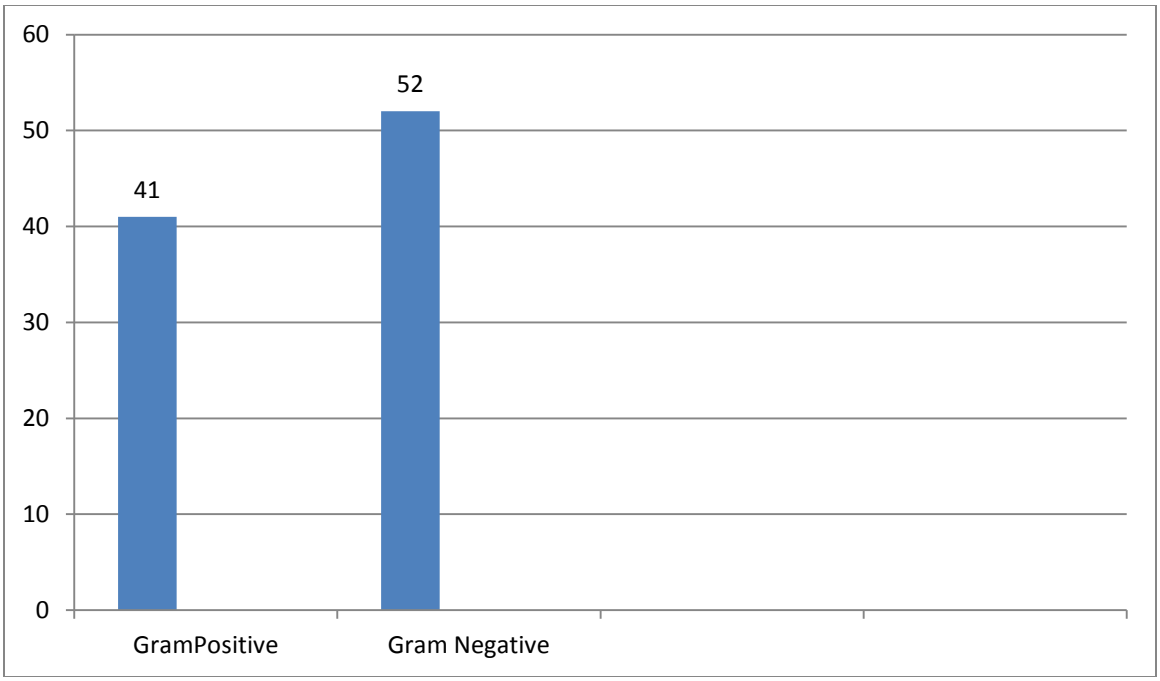

Figure (4.2) Distribution of isolates (n=93) according to Gram stain technique.

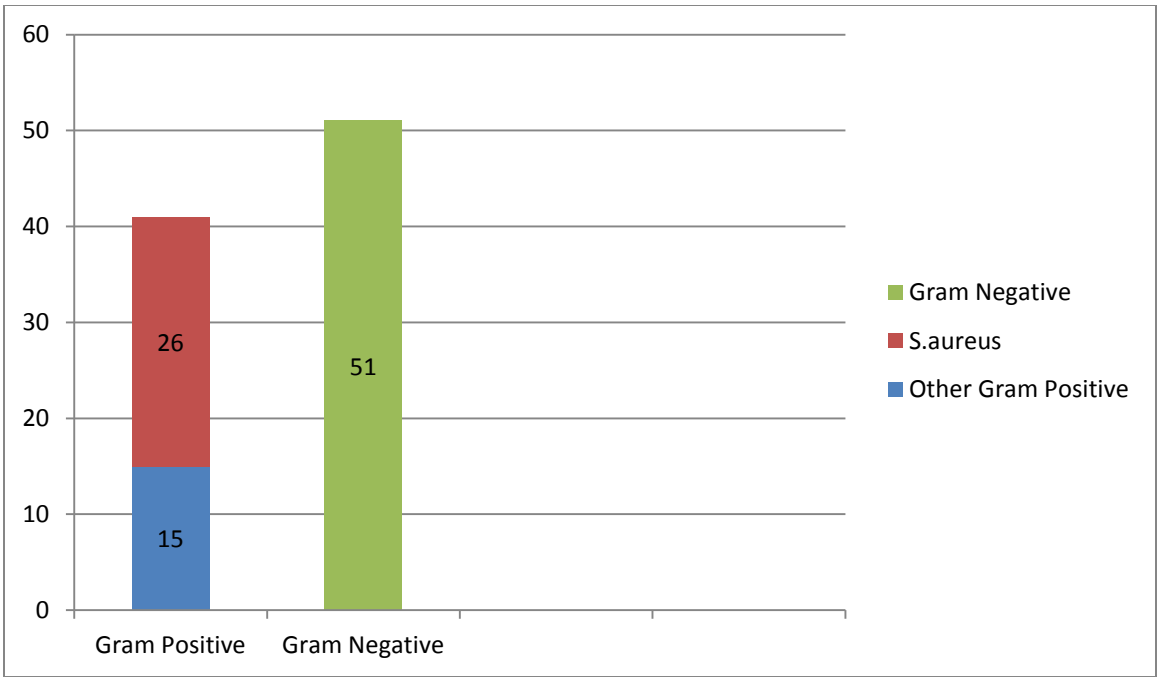

Figure (4.3) Distribution of *S.aureus* among isolates (n=93)

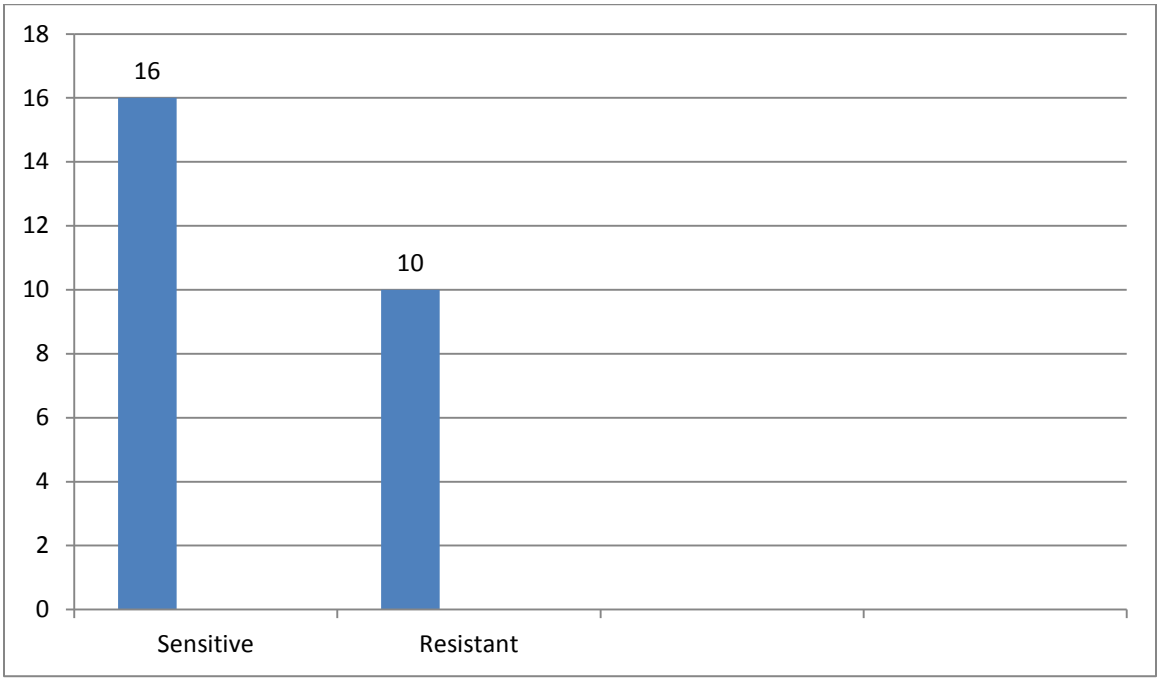

Figure (4.4) Susceptibility to Methicillin among *Staphylococcus aureus* wound isolates (n=26)

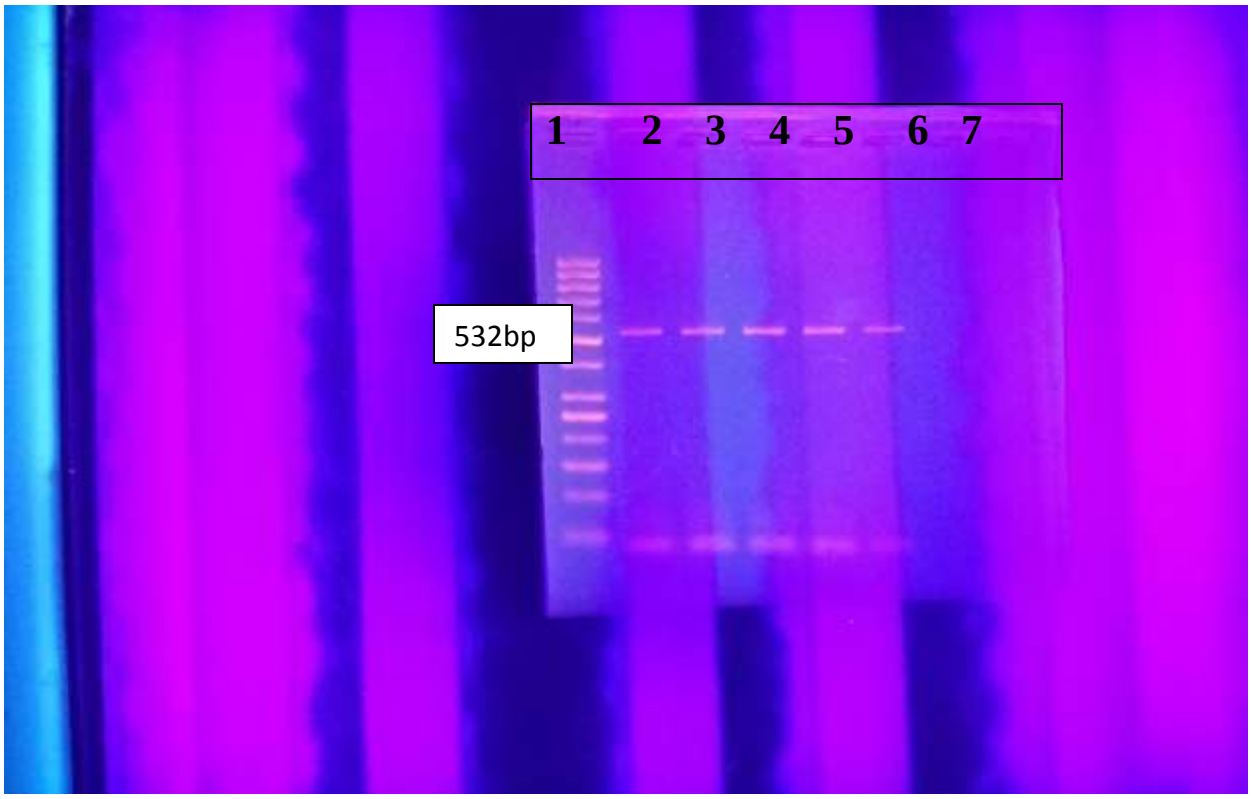

Figure (4.5) Gel electrophoreses show MRSA *mecA* gene 532 bp(Ladder 50 bp)

Lane "1" ladder, lane 2positive Control, lanes 3, 4, 5, 6, *mec A* positive samples, lane 7 negative test control .

Table (4.1): Frequency of MRSA according to gender

Gender	positive		negative		Total	P.Value
	Frequency	percentage	Frequency	Percentage		
Males	6	6%	43	43%	49	0.46
Females	4	4%	47	47%	51	
Total	10	10%	90	90%	100	

Table (4.2): Frequency of MRSA according to Age Group

Age Group	positive		negative		Total		p.value
	Frequency	percentage	Frequency	percentage	Frequency	%	
1-15	2	2	22	22	24	24	0.7
16-30	2	2	10	10	12	12	
31-45	4	4	24	24	28	28	
46-60	1	1	19	19	20	20	
Over 60	1	1	15	15	16	16	
Total	10	10	90	90	100	100	

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION, CONCLUSION AND RECOMINDATIONS

5.1 Discussion

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a type of staph bacteria that is resistant to certain antibiotics called beta-lactams. These antibiotics include methicillin and other more common antibiotics such as oxacillin, penicillin, and amoxicillin. In the community, most MRSA infections are skin infections (Aly and Levit, 1987). Methicillin-resistant *Staphylococcus aureus* (MRSA) have emerged as an important cause of nosocomial and community-acquired infections ranging from mild to severe life-threatening infections. Therefore, a reliable detection of such strains is required for effective treatment (Osman *et al.,* 2018).

This study aimed to detect the frequency of MRSA isolates from wound infections in Khartoum state in Sudan, and to detect the present of *mec A* gene among MRSA isolates.

The result obtained revealed that 88% of wound swabs specimens showed growth of different bacterial pathogens, and *Staphylococcus aureus* (MRSA, MSSA) were predominant 26 (28%) from all isolates, and 10 (38.5%) from all *Staphylococcus aureus* isolates were MRSA so that MRSA is less than MSSA . This result same as to the result of Osman *et al.*, (2018). in Khartoum state, Sudan who said that MRSA strains are less frequent than MSSA 38.1%, In this study the frequency of MRSA was higher than results of Ahmed (2016), who showed 26.9% in all specimens from wound infections in Khartoum Teaching Hospital, Sudan, and to that obtained by El Amin in (2016) , the percentage rate of MRSA isolates was 28%.

The present study revealed that all MRSA isolates 10(100%) posses *mec A* gene which was similar to that obtained by Haroun and Eidha ,(2011) in Khartoum Teaching Hospital in Sudan in which 9 *Staphylococcus aureus* isolates initially classified as Methicillin-resistant *Staphylococcus aureus* based on diffusion method , and PCR confirmed the presence of *mec A* gene in these isolates .

Our finding agreed with results obtained by Helal and his collags(2015) in Egypt all Methicillin-resistant *Staphylococcus aureus* isolates were *mec A* gene positive by PCR, however it was different to Elhassan *et al*.,(2015) , when amplified a 310 base pair fragment of the mec A gene by PCR, 12 out of the 123 MRSA isolates were mec A gene negative .

In this study there was no association between gender and MRSA wound infections.

Different results was observed by Kupfer *et al*, (2010) who found that males' gender is significantly correlated with increased risk of MRSA acquisition ($P< 0.001$).

In my study the age group of 31-45 years considered as a risk group for MRSA this results were disagree by Kupfer *et al*, (2010) who found that75% of MRSA positive patients are over 50 years of age.

5.2 Conclusion

S.aureus is highly associated with causing wound infections and all MRSA were positive for *Mec A* gene. This study also showed no association between MRSA, age group and gender.

5.3 Recommendations

Nasal screening to the medical and paramedical staff to detect carriers of MRSA, also sensitivity test must be done to select suitable drugs ,PCR technique can be used to detect MRSA, recommended to do more research to validate these results ,carry out study in large number of samples ,detection of MRSA from different sites of infections ,determine other resistant genes like *mec c and pvl* .

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Appendix I

Sudan University of Science and Technology

Collage of Graduate studies

Questionnaire

Frequency of Methicillin Resistant *Staphylococcus aureus* associated with wound infections

Hospital Name ……..

Date ……\.....\.....

Specimen number:……

Age:……

Gender ……

Causes of wound

Laboratory investigation:……..

Wound swab culture

Growth ( ) no growth ( )

S.aureus( ) others ( )

Methicillin susceptibility test

Sensitive ( )

Resistant ( )

Moderate ( )

Molecular detection of mec Agene:

Positive ( ) Negative ( )

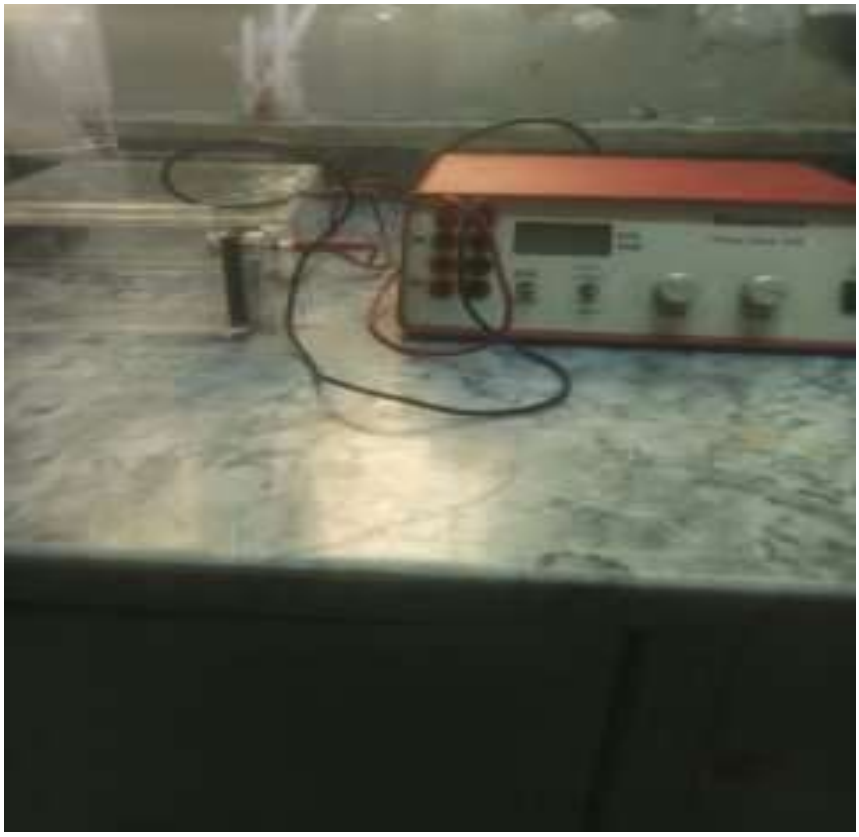
Appendices II

Color plat (1) *Staphylococcus aureus* in Mannitol Salt agar media

Color plat (2) Coagulase negative staphylococcus in Mannitol Salt agar

Color plat (3) *Staphylococcus aureus* resist to methicillin(the disc in the middle)

Color plat (4) Thermo cycler (PCR machine)



Color plat (5) Gel electrophoreses

Color plat (6) Ultra violet Trans illuminator

Appendix III

Reagent and culture media

Blood agar media:

To make about 35 blood agar plates

Nutrient agar 500ml

Sterile defibrinated blood 25ml

Preparation:

1 prepares the agar medium as instructed by the manufacture. Sterilize by autoclaving at 121◦c for 15minutes.

2. When the agar has cooled to50◦c, add aseptically the sterile blood and mix gently, avoid forming air bubbles.

3. Dispense aseptically in 15ml amounts in sterile Petri dishes

4. Date the medium and give it a batch number

5. Stor the plates at 2─8◦c, in sealed plastic bags to prevent loss of moisture

Mac Conkey agar media (himedia):

Ingredients	gram
Peptic digests of animal tissue	20.00
Lactose	10.00
Bile salt	5.00
Neutral red	.075
Agar	12.00

Final PH 7.4 ±.2 at 25◦ c

to prepare 1 liter

Suspend 47.07 g in 1 liter of distill water heat until completely dissolved. autoclave at 121◦c for 15minutes. Cool to 45-50 and power in to sterile Petri plates

Mannitol Salt Agar media: (Himedia)

Ingredients	gram\liter
Proteose peptone	10.00
Beef extract	1.00
Sodium chloride	75.00

D ─mannitol	10.00
Phenol red	0.025
Agar	15.00

Final PH at 25◦c is 7.4 ±02

Dissolve 111.02 gram in 100ml distill water heat until completely dissolved, sterile by autoclaving at 15 I bs pressure 121◦c for 15 minutes.

Note: this product contains 7.5% sodium chloride as one of its ingredients, on repeated exposure to air and absorption moisture sodium chloride has tendency to form lumps, there for we strongly recommended storage in tightly closed container in dry place away from bright light.

Gram stain:

Crystal violet (Himedia laboratories Pvt. Ltd. Mumbai, India)

Ingredients

Crystal violet	20g
Ammonium oxalate	9g
Ethanol or methanol, absolute	95ml

Preparation

Weight the crystal violet using sensitive balance ,transfer to a brown bottle pre marked to hold 1 liter ,add absolute ethanol or methanol and mix until the dye is completely dissolved ,weight the ammonium oxalate and dissolve in 200ml of D.w add to the stain ,make up to 1liter mark with D.W and mix well ,label the bottle and store at room temperature ,the stain is stable for several month .

Lug's iodine (Himedia laboratories Pvt.ltd. Mumbai, India)

Ingredients	g\L
Potassium iodates	20g
Iodine	10g

Preparation

Weight the potassium iodate transfer to brown bottle, add about quarter of the volume of water ,mix until completely dissolved ,weight the iodine and add it to the potassium iodate solution ,mix until the iodine dissolved and make up to 1 liter with D.W ,store at room temperature in dark place .

Acetone alcohol decolorizer (Himedia laboratories Pvt.ltd. Mumbai, India)

Ingredients L\L

Acetone 500ml

Methanol or ethanol absolute 475ml

Preparation:

Mix the D.W with the ethanol or methanol ,transfer the solution to screw cap bottle ,technical grade is adequate ,measure the acetone ,add it immediately to the alcohol solution mix well(caution: acetone is a highly flammable chemical that vaporizes rapidly ,there for use it well away from an open flame),label the bottle and mark it as highly flammable .store in safe place at room temperature .

Safranin (Himedia laboratories Pvt.ltd. Mumbai, India)

Ingredients	g\L
Safranin O	.5g
Ethyl alcohol 95%	100ml

Catalase reagent:

Ingredient

Hydrogen peroxide (3%)

Store at 4─8◦c and protect from light, and has shelf life of 26 weeks.

Muller Hinton agar (Himedia laboratories Pvt.ltd. Mumbai, India)

Ingredients	g\L
Casein acid hydrolysate	17g
Meat infusion solids	2g
Starch, soluble	1.5g
Agar	17g

Final PH 7.3±.1 at 25c

Preparation

Dissolve 38 gram in 1000ml D.W heat until boiling to dissolve the medium completely and sterilize by autoclaving at 121c and 15 Ibs pressure for 15 minutes .cool to 45◦c, mix well and pour into sterile Petri dish plates.

Appendices (7): .5% Mac Farland standard:

Prepare a1% v\v solution of sulfuric acid by adding 1ml of concentrated sulfuric acid to 99ml of water. Mix well, prepare 1%v\v solution of barium chloride by dissolving .5gram of dehydrate barium chloride in 50ml of D.W .add 0.6ml of barium chloride solution to 99.4ml of the sulfuric acid, and mix .transfer a small volume of the turbid solution to a capped tube ,when stored in well sealed container in the dark at room temperature ,can be use for up to 6month.