بسم الله الرحمن الرحيم

Sudan University of Science and Technology

College of Graduate Studies

Detection of Methicillin-resistant *Staphylococcus aureus*in Stethoscopes

الكشف عن المكورات العنقودية الذهبية المقاومة للميثيسلين في السماعات الطبية

A dissertation submitted for partial fulfillment of the requirements of MSc in Medical Laboratory Science (Microbiology)

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الآيـة

بسم الله الرحمن الرحيم

قال تعالى :

يَا أَيُّهَا الَّذِينَ آمَنُوا إِذَا قِيلَ لَكُمْ تَفَسَّحُوا فِي الْمَجَالِسِ فَافْسَحُوا يَفْسَحِ اللَّهُ لَكُمْ ۖ وَإِذَا قِيلَ انْشُزُوا فَانْشُزُوا يَرْفَعِ اللَّهُ الَّذِينَ آمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ ۚ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ ﴿١١﴾

صدق الله العظيم

سورة المجادلة الآية (11)

DEDICATION

To my mother,

brothers, sisters, husband,

and friends

ACKNOWLEDGEMENT

First of all thanks to ALMIGHTY ALLAH for giving me the knowledge and strength to complete this dissertation.

I would like to express my deep gratitude to my supervisor **Prof.HumodiAhmedSaeed** for his keen supervision, encouragement and unlimited support throughout this work.

Thanks are extended to teaching staff and laboratory technicians in the College of Medical Laboratory Science for their endless support.

Finally, all love and thanks, to my extended family, my colleagues and friends for their fruitful comments and discussion.

ABSTRACT

This is a laboratory based study, carried out during the period from June to November,2014. The study objective was to detect Methicillin-resistant *Staphylococcus aureus*(MRSA) in stethoscopes in Khartoum State hospitals.

The bacterial isolates under assessment (n = 14) were obtained from the Research Laboratory, Sudan University of Science and Technology. The isolates were cultured on nutrient agar by streaking to check their purity. Gram's stain and biochemical tests were used to confirm the identification of the isolates. Antimicrobial susceptibility test was carried out by modified Kirby-Bauer disk diffusion method.E-test was done to determine Minimum Inhibitory Concentration (MIC). MRSAweredetected by their resistant Oxacillin.

The results showed that all identified isolates (n = 14) were *Staphylococcus aureus,* study of antimicrobial susceptibility revealedthatall*Staphylococcusaureus* were resistant to Oxacillin.

The study concludedthatMRSA are common in hospitals. Further studies are required with large numbers of isolates to validate the results of this study.

المستخلص

هذه دراسة مختبرية نفذت خلال الفترة من يونيو إلي نوفمبر 2014م ، هدفت الدراسة للكشف عن المكورات العنقودية الذهبية المقاومة للمثيثلين في السماعات الطبية في مستشفيات ولاية الخرطوم.

وقد تم الحصول على البكتريا قيد التقييم من مختبر البحوث بجامعة السودان للعلوم والتكنولوجيا. استزرعت العزلات على وسط الأجار المغذية للتأكد من نقاوتها ثم استخدمت صبغة الغرام والاختبارات البيوكيميائية لتأكد هوية العزلات. أجري اختبار الحساسية للمضادات الحيوية بطريقة) لتحديد التركيز الأدنى من E-testكيربي-باور للانتشار من القرص. وتم إجراء اختبار (الأوكساسيلين المثبط للمكورات العنقودية الذهبية. أظهرت النتائج أن جميع العزلات (عددها 14) هي المكورات العنقودية الذهبية وأظهرت نتائج مضادات الميكروبات للمكورات العنقودية الذهبية المعزولة من السماعات 100% مقاومة للأوكساسيلين.

يوصي باجراء المزيد من الدراسات لعدد اكبر من العزلات للتاكد من صدقية هذه الدراسة .

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CHAPTER ONE

INTRODUCTION AND OBJECTIVES

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

The transmission of infection in the Hospital (Nosocomial infection)from contaminated medical equipment and health-care worker is a major problem. Medical devices, if not sterilized/ disinfected properly may transmit microorganism from one patient to the other. The stethoscope is one of the medical devices which are very commonly used by all health-care workers like doctors, nurses and medical students. Stethoscopes are possible carriers, as this come in contact with many patients following their contact with skin, microorganism can attach and establish themselves on the stethoscopes and subsequently be transferred to other patients if the stethoscopes is notdisinfected before reuse (Sander, 2005, Madar*et al*., 2005, Whittington *et al.,* 2009).

Staphylococcus aureus(*S. aureus*) has been recognized as a challenging organism in human infections since the development of germ theory, because *S.aureus* has ability to develop resistant to the currently available antimicrobial agents. Currently Methicillin- resistant *S.aureus*(MRSA) infections present such a major health care concern (Chini*et al*., 2006), that they may constitute a world-wide health care crisis. MRSA has become endemic in many health care institutions (approximately 50% prevalence in US

and 20% in Europe) and new MRSA strains are developing in the broader community that are affecting people without recognized risk factors for nosocomial MRSA infection (Appelbaum*et al*., 2006).

Methicillin-resistant*S.aureus*is often sub-categorized as Community- acquired MRSA (CA-MRSA) and hospital acquired according to the site where MRSA acquires from, MRSA remain a significant cause of hospital acquired infection (Limp and Stynadka, 2002).

Stethoscopes are known to harbor potentially harmful bacteria. As early as 1972, stethoscopes were identified as a fomite on which bacteria are capable to survive for various amount of time (Gerken*et al*., 1972). *S.aureus*including MRSA survives about 17 days to 7 months (Kramer *et al*., 2006)

1.2. Rationale

Methicillin-resistant *S.aureus*(MRSA) strains have great implication on human health causing mild and severe septicemia. Further complication has been brought about by increased prevalence of MRSA worldwide. Stethoscopes are potential vectors for MRSA which may lead to hospital acquired infections.

1.3. Objectives

1.3.1. General objective

To detect methicillin- resistant *Staphylococcus aureus* in stethoscopes in Khartoum State hospitals.

1.3.2. Specific objectives

a. To confirm the identity of isolates obtained from the research laboratory .

b. To detect Methicillin-resistant *S.aureus* (MRSA).

CHAPTER TWO
LITERATURE REVIEW

CHAPTER TOW

LITERATURE REVIEW

2.1. Epidemiological Background

Multi–drug resistant *Staphylococcus aureus***(***S. aureus***)** has been an international problem since 1950s (Fluit and Schmitz, 2003). Methicillin-resistant *S.aureus* most commonly causes skin infection, but can also cause such more serious, even fatal, infections such as pneumonia(CDC, 2010). The resistance of *S. aureus*to penicillin occurred soon after the drug's development in the era of World War II. This situation required a drug that could be stable in presence of staphylococcal penicillinase, which rendered the drug useless against the organism. In 1960, Methicillin was successful in treating *S. aureus*, however, by 1961 Jevons had reported resistance (Fluit and Schmitz,2003). Later in the 1960s, resistance to erythromycin and 2003 tetracycline were also documented (Flute and Schmitz, 2003). Currently, MRSA is typically resistant to Aminoglycosides, Clindamycin, Flouroquinolones and Macrolides. Resistance to Vancomycin has now also been detected (Fluite and Schmitz, 2003).

2.2. *Staphylococcus aureus*

Staphylococci are very wide spread bacteria their main representative *S. aureus*subspaureus is one of the most important and successful is human pathogens according to current knowledge, the genus *Staphylococcus* has 50

taxons with 39 various types and several sup types (Petras and Jubilejin, 2007).

*Staphylococcus aureus*consist of a single circular from chromosome plusprophages, plasmids, transposons, insertion sequences, and other in completely characterized variable accessory genetic elements which contribute to cell maintenance, growth and adaptation to a variety of environments (Wilkinson, 1997).

2.2.1. Methicillin-resistant *Staphylococcus aureus*

Methicillin-resistant *Staphylococcus aureus* (S.*aureus*)(MRSA) is abacterium resistant to antibiotic methicillin. *S.aureus* is a common MRSA usually infect hospital patient are elderly or very ill (Department of health, 1996).

These hospital–associated MRSA infections were associated with a small number of *S.aureus* clones strains; with defined genetic identifiers, and were frequently multi- drug resistant (Oliveira *et al.,* 2002, Ribeiro*et al.,*2005).

β- Lactamases are enzymes that selectively destroy β-lactam molecule, which constitute the large and most diverse group of antimicrobial drugs. Development of these agents differs principally with respect to spectrum, susceptibility to enzymatic activation and pharmacology (WHO, 1997).

Resistance to Methicillin and other β-lactamantibiotic is caused by the*mec*A gene which is situated on the staphylococcal cassette chromosome *mec*. The *mec*A gene encodes the 78-k Da penicillin – binding protein (PBP) 2a or (I –V) have been distinguished, and several varian of these Scc*mec* types have been

described – All SCC*mec* element carry genes for resistance to β- lactam antibiotics. Additionally, SCC*mec* types II and III carry non – β - Lactam antibiotic–resistance genes an integrated plasmids and a transposon (Deurenberg*et al.,* 2007).

2.2.2. DetectionofMRSA

2.2.2.1.Dilution methods

Agar dilution test on Muller-Hinton or Columbia agar with 2% NaCl and aninoculum of 10^4cfu/ml will distinguish most resistant strains from susceptible strains (NCCLs,2003). With NCCLs method only Muller-Hinton with 2% NaCl incubation for24^h 33-35C^ooxacillin MIC of>2mgl indicates that strain is susceptible and>2 mg\l resistant. Broth with 2% NaCl an inoculum of 5×10^5cfu ml and incubation at 33-35C^o for Broth microdilutionuse of MH 24^h (NCCLs, 2003).

2.2.2.2. Dilution and diffusion method

E-test also known as epsilometer test is a quantitative method for antimicrobial susceptibility testing applies both the dilution of antibiotic and diffusion of antibiotic into the medium.

2.2.2.3. Break point methods

Include both agar and broth methods and similar to dilution MIC methods but test only the breakpoint concentration (2mg/l Oxacillin, 4 mg/l Methicillin)(NCCLs,2003).

2.2.1.1.5. Agar screening method

Requires suspending the test organism to the density of a 0.5 McFarland standard and inoculating MH agar containing 4%NaCl and 6mg/l oxacillin with spot or a streak of the organism plates are incubated at 35C^{o} or les for 24 h and any growth other than a single colony is indicative of resistance (NCCLs,2003)

2.2.1.1.6. Disc diffusion methods

Direct colony inoculum preparation MH agar 1µg oxacillin disc incubate for 24^{hrs} at 35C^{o} or cefoxitin(30µg).latexagglutinationtest, rapid slid latex agglutination test based on detection of PBP2a is commercially available as kit from several suppliers. The method involves extraction of PBP2a from suspension of colonies and detection by agglutination with latex particles with monoclonal antibodies to PBP2a (Griethuysen*et al.,*1999).

2.2.1.1.7.Polymerase Chain Reaction(PCR)

To detect the *mec*A gene.Other methods are automated methods and Quenchingfluorescencemethods.

2.3. Stethoscope as fomites

Data have supported the idea that stethoscopes can act as fomites for over thirty years (Gerken*et al.*, 1972). The majority studies have focused broadly on the stethoscopes of nurses physician in hospital setting in one of the first studies , the stethoscopes of medical interns, residents faculty and nurses (N: 50) were cultured. Thirteen stethoscopes (26%) were reported as contaminated with potential pathogen, meaning bacterial colonies that were

not common skin flora (Mangi and Andriole, 1972) . the same year bacterial contamination of stethoscopes was reported again (Gerken*et al.,* 1972).This finding resound throughout each decade physican stethoscopes (N=29) were cultured and 26 (89%) yielded potentially pathogenic bacteria (Breathnach*et al.,* 1992). In study limited to one ICU, ear buds and the diaphragms of stethoscopes were examined. Out of 24stethoscopes tested to digrams (8.3%) Contained pathogens. The result shows that bacterial colonization with potential pathogens is a common finding (Whittington *et al*., 2009).

Stethoscopes were first identified as potential vectors for bacterial infection over 30 years ago (Petras and Jubilejin, 2007). Both the diaphragm and earpieces of physician's personal stethoscopes and bedside stethoscopes are frequently colonized with a variety of pathogenic organisms including Methicillin-Resistant S. *aureus* (MRSA) and Vancomycin-Resistant Enterococci (VRE) which cause significant morbidity and mortality on the intensive care unit (ICU) (John*et al.,* 1995).

2.3.1. Pathogen transmission from stethoscope

Transfer of pathogen from stethoscope to human skin is necessary for infection to be possible. Transmission of *Micrococcus lutens* on stethoscope diaphragm to human skin was reported on an intentionally contaminated the diaphragm (Marinella*et al.,* 1997). Because of the favorable conditions for MRSA growth on skin, it believed MRSA would follow the same pattern of transmission. *Staphylococcus aurous* with all other contaminated surfaces,

contact with stethoscope harboring MRSA can allow the spread of bacteria to patients skin (CDC, 2010).

Although it had long been held that microorganisms in the inanimate hospital environment do not play a significant role in the acquisition of nosocomial infection, it has become evident in recent years that surfaces in hospitals touched by patients or health care workers readily become contaminated by "environmental pathogens," such as MRSA, VRE, *Acinetobacterbaumanii*, *Clostridium difficile*, Respiratory Syncytial virus, and *Norovirus*, which collectively have a unique capacity to survive desiccation in a viable, transmissible form for days to months. Compelling epidemiologic data indicate that contamination of inanimate surfaces in hospitals is an important reservoir of these pathogens and has driven a move toward more comprehensive surface decontamination with bleach solutions, ultraviolet light, or aerosolization of hydrogen peroxide or peroxacetic acid (Weber *et al.,*2010) Auscultation of the heart, lungs, abdomen, and major arteries with a stethoscope has long been considered an integral part of the physical examination, and most health care providers prefer to use their own stethoscope. It has long been known that the diaphragms and bells of stethoscopes randomly sampled in a health care setting, such as a hospital, are almost universally contaminated by potential nosocomial pathogens, most often staphylococci MRSA up to 32% of the time but also *Clostridium difficile*, resistant Gram-negative bacilli, and even viruses, and studies have shown that

stethoscope contamination by these microorganisms is commonly acquired from colonized or infected patients (Bernard, 2010; Zachary *et al.,* 2001).

Expected bacterial growth on stethoscopes include common skin flora organism Staphylococcus (non – pathogenic form) and Corynebacterium. There is little concern for the transmission of normal skin flora between individuals. However, stethoscopes may become contaminated with pathogenic bacteria, Although MRSA is commonly cited organism on stethoscopes, other pathogenic bacteria have been reported, such as *Escherichiacoli* ,Enterobocter, *Klebsiella* (Mangi and Andriole, 1972) and *Micrococcusluteus* (Marinella*et al.,* 1997).

2.3.2. MRSA isolated from stethoscopes

Multiple studies have reported MRSA colonization on stethoscopes, in one 200 stethoscopes of physicians, nurses and hospital personnel were tested among four hospitals and out patient clinics of those cultured (N=200), *S.aureus* was noted on 17 (8.5), with four (2%) of those being resistant to methicillin (Smith *et al.,* 1996). Similarly, MRSA was isolated in a study conducted at single community- based hospital and satellite family health center (Schroeder *et al.*, 2009). Three stethoscopes (3.2%) of the 93cultured (N=93) reported by carried MRSA of stethoscopes (N=50) of emergency medical service providers (EMS) in one emergency department in a large hospital, 16(3.2% had MRSA colonization (Merlin *et al.,* 2009).

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This study is a laboratory-based study for detection of MRSA in stethoscope.

3.1.2. Study area

The experimental work of the present study was carried out in the Research Laboratory, College of Medical Laboratory Science, Sudan University of Science and Technology (SUST).

3.1.3. Study duration

The study was conducted during period from June to November, 2014.

3.2. Bacteriological methods

3.2.1. Source of isolates

The isolates were obtained from the Research Laboratory of Sudan University of Science and Technology. The isolates were checked for purity and then re-identified by conventional bacteriological methods.

3.2.2. Purification of isolates

The isolates were streaked on nutrient agar and incubated over night at 37ºC; a descripte colony was picked up and checked for purity under microscope.

3.2.3.Re-identification of *S. aureus*

3.2.3.1. Gram stain

A smear was prepared by emulsifying a small portion of the bacterial colony in a drop of normal saline and spread evenly on a clean slide. The smear was allowed to air dry on a safe place- protected from dust and sun light. Then the smear was fixed by passing the slide, the smear upper most, three times through the flame of a Bunsen burner and was allowed to cool before staining.

The fixed smear was covered with crystal violet stain for 30 -60 seconds. The stain was washed off rapidly with clean tap water. All the water was tipped off and the smear was covered with Lugol's iodine for 30- 60 seconds. The iodine was washed off with clean tap water and decolorized rapidly (few seconds) with acetone-alcohol and washed immediately with clean water. Then the smear was covered with neutral red stain for 2 minutes, and washed off with clean water. The back of slide was wiped clean and placed in a draining rack for the smear to air dry. The dried smear was examined microscopically, first with the 40X objective to check the staining and then with oil immersion objective to observe the bacteria (Cheesbruogh, 2000).

3.2.3.2. Biochemical tests

3.2.3.2.1. Catalase test

This test is used to differentiate those bacteria that produce catalase enzyme from non catalase producing bacteria. Catalase catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism was tested for catalase

production by bringing into contact with hydrogen peroxide. Bubbles of oxygen were released if the organisms are catalase producer. The culture should not be more than 24 hours old (Cheesbrough, 2000).**3.2.3.2.2. Coagulase Test**

Coagulase causes plasma to clot by converting fibrinogen to fibrin. A drop of human or rabbit plasma was placed on a clean, dry glass slide. A drop of saline was used as a negative control. With a wooden stick a portion of the isolated colony was emulsified in each drop. Microscopic clumping within 10 second was a positive result. In the tube method, several colonies were emulsified in 0.5 ml of diluted plasma, and then incubated for 4 hours. Clot formation was the positive result (Forbes *et al*., 2002).

3.2.3.2.3. DNAse Test

This test was used to identify *S. aureus* which produce deoxyribonclease enzyme. DNase hydrolyses deoxyribonucleic acid (DNA). The tested organism was cultured on a medium which contain DNA, after overnight incubation the colonies were tested for DNase production by flooding plate with a week (1 mole) hydrochloric acid solution. DNase producing colonies were surrounded by clear area due to DNA hydrolysis (Cheesbrough, 2000).

3.3. Susceptibility of bacterial isolates to antibiotics

Modified Kirby-Bauer disc diffusion method was performed according to the instructions of (NCCLS, 2003) as follow;

3.3.1. Culture media

Sterilized molten Muller-Hinton agar (pH 7.4±2) was prepared, cooled to 45-50°C, and poured in sterile, dry Petri plates on a level surface, to a depth of 4mm. Some representative plates after solidification were incubated at 35°C, for 24-72 hours to check sterility. The presence of any excess surface moister on the medium was removed by keeping the plates inverted in an incubator at (35-37°C).

3.3.2. Antibiotic

Disc of Oxacillin (OX) 1µg and other antibiotics including; Gentamicin (GEN) 10µg, Ciprofloxacin (CIP) 5µg, Erythromycin (ERY) 15µg, Clindamycin 2µg, Vancomycin 30µg, and Ciprofloxacin (CIP) 5µg were obtained from (Bioanalyse-Turkey).

3.3.3. Preparation of inoculums

The inoculums were prepared by transferring 4-5 colonies from pure cultures, (confirmed by Gram's staining) with wire loop to 4 ml of sterile normal saline. The inoculums turbidity was adjusted to that of McFarland standard (prepared by mixing 0.6 ml of 1% w/v barium chloride and 99.4 ml of 1% v/v sulfuric acid).

3.3.4. Seeding of the plates

A sterile non toxic cotton swab was dipped into each standardized inoculum. The swab was rotated firmly against the upper inside wall of the tube to get rid of excess fluid. The entire agar surface of the plate was streaked with the

swab three times while turning the plate at 60° angle between each streaking. The plate was allowed to dry for 3-5 minutes with lid in place.

3.3.5. Application of antibiotic discs

Using sterile forceps, 5 antibiotic discs were applied, and evenly distributed on the inoculated plate. The plate was inverted and incubated aerobically at 35°C, for 24 hours.

3.3.6. Reading of zones of inhibition

After overnight incubation the control strain and the test plates were examined to ensure that growth is confluent or near confluent. Using a ruler on the underside of the plate, the diameter of each zone of inhibition was measured in mm.

3.3.7. Interpretation of the results

The zone size of each antibiotic was measured. The susceptibility of isolates was reported according to the manufacture's standard zone size interpretative manual.Resistant MRSA ≤ 10 mm zone size of inhibition.

3.4. Determination of MIC

E-test method was performed to determine MIC ofOxacillin.

3.4.1. Materials

3.4.1.1. Antibiotic powder

The antibiotic powder ofOxacillin was obtained from Spansules, Venpetrochemical, India.

3.4.1.2. Paper

Art paper type, normally, China clay (kaolin) coated on both sides was selected for preparing the discs. The selection was based on its ability to uniformly absorb sufficient volumes of antibiotic solutions.

3.4.1.3. Test organisms for quality control

Quality control was performed to measure the effectiveness of antimicrobial agents by using a control *Staphylococcus aureus* ATCC 25923 obtained from the Central Public Health Laboratory.

3.4.2. Manual E-test method

3.4.2.1. Preparation of paper discs

By using an ordinary office two-hole puncher, paper discs with approximate diameter of 6.3mm were punched out one by one from a sheet of the paper. Precautions were taken to avoid overlapping of holes, and since the paper discs had a tendency to curl after punching, they were flattened by spreading them in a single layer on a clean smooth surface then pressed by rolling a bottle repeatedly. The discs were placed in a Petri dish then autoclaved for 15 minutes at 15Ibs pressure and allowed to cool.

3.4.2.2. Preparation of antibiotic solutions

Powder was accurately weighed and dissolved in the appropriate diluents (distilled water) to yield the required concentration. Stocks solution was prepared using the formula according to National Committee for Clinical Laboratory Standerds:

1000 xVxC=W

P

Where P=Potency given by the manufacturer in relation to the base.

V=Volume in ml required.

C=final concentration of solution (multiplies of 1000).

W=weight of antibiotic to be dissolved in the volume.

The concentration of the antibiotic solution was expressed in µg/ml and was based on the potency per disk prescribed by WHO Expert Committee on Biological standardizations.

3.4.2.3. Preparation of serial dilutions

Ten different concentrations were obtained for Oxacillinsolution 2-folddilution was prepared for Oxacillin.Sixty four µg/ml were prepared from the stock of each antibiotic solution, and then serial double dilutions were prepared in conventional dilution method.

3.4.2.4. Impregnation of discs

Blank sterile prepared discs were soaked in the following concentration ofOxacillin;(64,32,16,8,4,2,1,0.5,0.25,0.13) µg/ml. Then the impregnated discs were transferred into sterile Petri-dishes and labeled with their defined concentrations.

3.4.2.5. Drying and storage

Without covering the Petri dishes, the discs were allowed to dry in a hot air oven at 50°C for 20 minutes. After drying each 50 to 100 discs were placed in small dark sterile air tight labeled containers, with a desiccant at the bottom,

and a layer of sterile cotton or foam over the desiccant to avoid contact with the disks. The disks were stored in a freezer at -14°C .Unopened containers were removed from the freezer 1 or 2 hours before use to equilibrate to room temperature before they were opened to minimize the amount of condensation that mightoccur when warm room air reached the cold containers.

3.4.3. The procedures

The discs with gradient concentrations that had been impregnated with Oxacillin. Muller-Hinton agar supplemented with 2% NaCL was used. An inoculum of *S. aureus*was inoculated on the surface of the agar plate.With sterile forceps, beginning from the minimum concentration and upwards, the discs were applied to the inoculated agar surface. Discs were in complete contact with the agar surface, and were in one line beginning with the low concentration to the high, so that the disc with maximum concentration was nearest the rim of the plate. The antibiotics diffused out into the agar, producing an expotentional gradient of the Oxacillin. After 24 hours of incubation at 35°C, the point at which the ellipse met the defined disc concentration gave a reading for the Minimum Inhibitory Concentration (MIC) of the antibiotic.

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

A total of 14 *Staphylococcus aureus*were obtained from the Research Laboratory, Sudan University of Science and Technology (SUST). Biochemical tests adopted for re-identification and their results were tabulated in (Table 1).

Antibiotic Susceptibility Tests was done for isolated bacteria by modified Kirby-Bauer Disc Diffusion Technique. E-test was used to determine Minimum Inhibitory Concentration (MIC) for antibiotic.

Results of the present study revealed that all *Staphylococcus aureus* isolated were (100%)resistant to Oxacillin (Table 2).

E-test was done using concentration ranged from 64 to 0.13 µg\ml to determine Minimum Inhibitory Concentration (MIC) of oxacillin against *S.aureus*isolates. The results revealed that all strains by disc diffusion method were resistant when confirmed by E-test with MIC>64 µg/ml (Table3).

The antibiogram of MRSA isolates in this study showed resistant to Clindamycin (92%) , Erythromycin , Gentamicin ,Vancomycin (71%) Tetracyclin (64%) and Ciprofloxacin (57%) (Table 4).

Table 1. Biochemical tests adopted for re-identification of isolates

Isolate code	Biochemical tests				
	Catalase	Coagulase	Manitol	DNAse	Suggested organisms
1	+	+	+	+	*S. aureus*
3	+	+	+	+	*S. aureus*
27	+	+	+	+	*S. aureus*
39	+	+	+	+	*S. aureus*
42	+	+	+	+	*S. aureus*
47	+	+	+	+	*S. aureus*
56	+	+	+	+	*S. aureus*
57	+	+	+	+	*S. aureus*
96	+	+	+	+	*S. aureus*
104	+	+	+	+	*S. aureus*
107	+	+	+	+	*S. aureus*
108	+	+	+	+	*S. aureus*
149	+	+	+	+	*S. aureus*
169	+	+	+	+	*S. aureus*

Key

+ =Positive reaction

Table 2. Activity of Oxacillin against *S. aureus*

Isolate code	Activity
S. aureus 1	R
3 *S. aureus*	R
S. aureus 27	R
S. aureus 39	R
S. aureus 42	R
S. aureus 47	R
S. aureus 56	R
S. aureus 57	R
S. aureus 96	R
S. aureus 104	R
S. aureus 107	R
108*S. aureus*	R
149*S. aureus*	R
S. aureus 316	R

Table 3. Minimum Inhibitory Concentration (MIC) of the Oxacillin of MRSA strain

Isolate code	MIC Range(64-0.13 mg/ml)
S. aureus 1	> 64
S. aureus 3	> 64
S. aureus 27	> 64
S. aureus 39	> 64
S. aureus 42	> 64
S. aureus 47	> 64
S. aureus 56	> 64
S. aureus 57	> 64
S. aureus 96	> 64
S. aureus 104	> 64
S. aureus 107	> 64
S. aureus 108	> 64
S. aureus 149	> 64
S. aureus 169	> 64

Table 4.Antibiogram of MRSA isolates

Antibiotic	Percentage of Resistant %
Clindamycin	92%
Vancomycin	71%
Erythromycin	71%
Gentamicin	71%
Tetracycline	64%
Ciprofloxacin	57%

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE

DISCUSSION

Stethoscopes potential vectors of Methicillin –resistant *Staphylococcus aureus*(MRSA). Methicillin-resistant *Staphylococcus aureus*(MRSA) is a major challenge in health-care institutions worldwide. For very severe infections,the risk of death is about two times higher with MRSA than with Methicillin-Susceptible *Staphylococcus aureus*. Stethoscopes have been shown to have a high rate of bacterial contamination, with 0-20% of those tested being colonized with MRSA (Sanders, 2005).

This study was done to detection of Methicillin-resistant *Staphylococcus aureus* in stethoscopes it found that 14 of physician's stethoscopes were contaminated with MRSA. This similar to results obtained by (Nathania,2011) who found the stethoscopes, diaphragm are colonized with micro-organism on average 87.3% of the time. An average,14% of stethoscopes carry MRSA.(Smith *et al.,* 1996) who found that bacteria on 80% of 200 stethoscopes and MRSA on 34%. Antimicrobial Susceptibility Test of the isolated organisms were done by disc diffusion test and E-test to detect MRSA .The oxacillin disc diffusion test showed 14(100%) were Methicillin–resistant *S.aureus* and similar result obtained by E-test all *S.aureus*isolated showed resistant to Oxacillin,had MIC>64µg/ml.The two methods showed similar results.As already reported,the Oxacillin disc diffusion test was the least reliable test for detection of MRSA .Both methods are easy to perform and they are affected by test conditions.Regarding disc diffusion tests for MRSA detection the sensitivity of Oxacillin and Cefoxitin disc diffusion test amounted to 100%(Venkatakrishna*et al*., 2011).

Theantibiogram of MRSA isolates in this study showed resistant toVancomycin,Gentamicin, Erythromycin(71%), andCiprofloxacin (57%), Clindamycin (92%),Tetracycline(64%).

5.2. Conclusion

The study concluded that:

1. The prevelance of MRSA in stethoscopes was very high.

2. Both methods gave accurate results for detection of MRSA, with Minimum Inhibitory Concentration (MIC) >64µg/ml.

3. Some MRSA are multidrug resistant.

5.3. Recommendations

The following points are highly recommended:

1. Modified Kirby-Bauer method must be adopted for all microbiological laboratories
2. MIC test is very important tool to evaluate pathogenic microorganisms, degree of susceptibilities and to detect specific resistant mechanisms, so reference laboratories must determine MIC as reference points in the evaluation and comparison of new and existing of new antimicrobial agents.
3. Further studies are required for better detection of MRSA. PCR for mecA gene is considered the gold standard method for detection MRSA.

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APPENDECIS

Appendices

Appendix (1)

Culture media

2.2. Difco™NutrientAgar

Approximate formula * per Liter

Beef Extract.....3.0g

Peptone..... 5.0g

Agar 15.0g

*Adjusted and \or supplemented as required to meet performance criteria.

2.3. Difco™MacConkeyAgar

Approximate formula * per Liter

Peptone.....20.0g

Lactose 10.0g

Bile Salts.....5.0g

Sodium Chloride 5.0g

Agar.....12.0g

Nutrient Red.....0.05g

2.4. Difco™Manitol Salt Agar

Approximate formula * per Liter

Proteose Peptone No.3.....10.0g

Beef Extract.....1.0g

D-Mannitol.....10.0g

Sodium Chloride.....75.0g

Agar.....15.0g

Phenol Red.....25.0g

2.5. Difco™Muller Hinton Agar

Approximate formula * per Liter

Beef Extract 2g

Acid Hydrolysate of Casein.....17.5g

Starch.....1.5g

Agar.....17g

Final PH: 7.3 ±0.1 at $25C^0$

Appendix (2) Instruments

1.1 Safety cabinet

Daihan lab tech CO.LTD.

Made in UK.

1.2 Incubator

GALLENKAMP

Made in UK.

1.3 Freezer-20

Made in EUROP.

1.4 Water bath

Model: LWB-111D.

Made in UK.

1.5 Microscope

Model A15120-4.

Made in Germany.

3.6 Sensitive balances

3.7 Ultra low temperature freezer-70

Model MDF-392.

Made in Japan.

3.8. Refrigerator with glass door

Made in Saudi Arabia.

3.9 Autoclave

Dixons, surgical instrument LTP.

Made in UK.

Appendix 3

McFarland standard (0.5%)

Solution of 1% v/v sulphuric acid prepared by adding 1ml of concentrated sulphuric acid to distilled water mixed well and prepared 1.17%w/v solution of barium chloride 100ml distilled water also was prepared. The turbidity standard 0.5ml of 1.17%w/v barium chloride solution was added to 99.5ml of 1% sulphuric acid solution and mixed 0.5ml McFarland standard. Transferred a small volume of the turbid solution to a screw-cap bottle of the same types as used for preparing the test and control inoculum .Stored in a well seal scaled bottle in a dark temperature (20-28°c) standard. This standard has the turbidity of a suspension of approximately 1.5×10^{8} bacterial /ml.