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Sudan University of Science and Technology

College of Graduate Studies

Phenotypic Detection of Methicillin-resistant *Staphylococcus aureus* on Physicians' White Coats

الكشف الظاهري عن المكورات العنقودية الذهبية المقاومة للمثيسيلين في معاطف الاطباء البيضاء

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

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قال تعالى:

(وَإِذَا مَرِضْتُ فَهُوَ يَشْفِين)

صَدَقَ الله العَظِيم

سُورَةُ الشُّعَرَاءِ: الآية (80)

DEDICATION

To my mother, father, husband, kids, friends

and all family members.

ACKNOWLEDGEMENT

First of all my thanks to ALMIGHTY ALLAH for helping me to complete this work.

I would like to give my sincere thanks to my supervisor **Prof. Humodi Ahmed Saeed** for his constructive guidance, help and support.

I would like to convey my thanks to Sudan University of Science and Techonlogy and to staff of the Research Laboratory for offering me ideal environment to perform my research project.

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Last but not least, I am greatly indebted to my devoted husband for his love support and encouragement all through the study.

ABSTRACT

This is laboratory-based study, carried out during the period from April to June, 2015 to detect Methicillin-resistant *Staphylococcus aureus* (MRSA) on physicians' white coats.

The bacterial isolates (n=14) were obtained from the Research Laboratory, Sudan University of Science and Technology. Purity of the isolates was checked by streaking on nutrient agar and examined microscopically. Gram stain and biochemical tests were used to confirm the identity of the isolates. Methicillin-resistant *Staphylococcus aureus* (MRSA) was detected by Kirby-Baur disk diffusion technique.

The results showed that re-identified isolates were *Staphylococcus epidermidis* 5 (35.7%), *Staphylococcus aureus* 4(28.6%), *Pseudomonas aeruginosa* 3 (21.4%), *Staphylococcus intermedius* 2 (14.3%). Study on detection of MRSA revealed that only two (50%) out of 4 *Staphylococcus aureus* were MRSA.

It is concluded that physicians' white coats may be contaminated with Methicillin-resistant *Staphylococcus aureus*. Proper handling of white coats by physicians is highly recommended.

المستخلص

هذه الدراسة المعملية أجريت فى الفتره من شهر أبريل الى يونيو 2015 للكشف عن بكتريا المكورات العنقودية الذهبية المقاومة للمثيلين فى معاطف الأطباء البيضاء. تم الحصول على البكتيريا المعزولة من معاطف الأطباء (14عزلة) من مختبر البحوث بجامعة السودان للعلوم والتكنلوجيا. أُختبر نقاء العزلات بزرعهافى الأجار المغذى وفحصها مجهرياً . أُستخدمت صبغة الجرام و الإختبارات البيوكميائية للتاكد من هوية هذه العزلات. تم الكشف عن المكورات العنقودية الذهبية المقاومة للمثيلين بواسطة طريقة الإنتشار من الأقراص.

أظهرت نتيجة اعادة التعرف على هوية البكتيريا أن المكورات العنقودية الجلدية 5 (35.7%) والمكورات العنقودية الذهبية 4 (28.6%) و الزائفة الزنجارية 3 (21.4%) والمكورات العنقودية انترميدياس 2(14.3%). أظهرت الدراسة للكشف عن المكورات العنقودية الذهبية المقاومة للمثيلين أن أثنين (50%) من ضمن أربعة من المكورات العنقودية الذهبية كانت مقاومة للمثيلين. خلصت الدراسة إلى إن معاطف الإطباء قد تكون ملوثة باالمكورات العنقودية الذهبية المقاومة للمثيلين. يوصى بشدة التعامل السليم مع معاطف المختبر بواسطة الأطباء .

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INTRODUCTION AND OBJECTIVES

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

White coat, apron or laboratory coat (abbreviated lab coat) is a knee- length over coat or smock worn by professional in medical field or by those involved in laboratory work to protect their street clothes. The garment is made from white cotton or linen to allow it to be washed at high temperature and make it easy to see if it is clean. There has always been some concern white coat, nurse's uniforms and other hospital garments, may actually play part in transmitting pathogenic bacteria in hospital settings (Priya *et al.,* 2009).

Health care-associated infection is defined as any infection acquired as consequence of person's treatment by health care provider, or which is acquired by health care worker in the course of their duties (Hill, 2011).

Nosocomial infections are a major source of morbidity and mortality in hospital settings. The most important defenses against nosocomial transmission of viral, bacterial and other infections are detailed and continuing of staff and strict adherence to infection control policies (Petroudi, 2009).

Lab coat may play part in transmitting pathogenic bacteria in hospital setting, as white coat as known to be potentially contaminated with pathogenic drug resistant bacteria (Banu *et al.*, 2012).

Large proportion of health care workers white coat may be contaminated with *Staphylococcus aureus* including methicillin resistant *S. aureus* (MRSA). White coat may be an important vector for patient-to-patient transmission of *S. aureus* (Treakle *et al*., 2009).

The organism is Gram-positive coccus about 1um in diameter. The cocci are mainly arranged in grape-like cluster, but some especially when examined in pathological specimens, may occur as single cell or pairs or cell. The organisms are non sporing non-motile and usually non-capsulate (Greenwood *et al.,* 2002).

Infections caused by MRSA are being increasingly reported worldwide since 1980. The infection also being increasingly reported now form different hospitals (Chandra, 2009).

MRSA produce penicillin binding protein 2a (mediated through the mecA gene) which carried on the staphylococcal cassette chromosome mec (SCCM) of which there are at least six different types are recognized and this result in resistance to all bete lactam antibiotics (Greenwood *et al.*, 2012).

MRSA usually colonize the broken skin and can cause wide range of local and systemic infections. Hospital staffs harboring MRSA are the chief source of infection for the patients (Chandra, 2009).

1.2. **Rationale**

Infection due to drug resistant bacteria is major health problem. Nosocomial infections are commonly transmitted when health care providers become complacent and do not practice correct hygiene regularly.

Patient-to-patient transmission of nosocomial pathogen has been linked to transient colonization of health care workers, and studies have suggested that contamination of health care workers clothing, including white coats, may be vector for this transmission (Treakle *et al.*, 2009). Therefore, in the absence of authentic data regarding this issue in our home (sudan), this study was conducted to detect Methicillin resistant *S. aureus* in physicians' white coats.

1.3. Objectives

1.3.1. General objective

To detect Methicillin-resistant *Staphylococcus aureus* on physicians' white coats.

1.3.2. Specific objectives

A. To re-identify bacteria isolated from physicians' white coats.

B. To determine Methicillin-resistant *Staphylococcus aureus*.

CHAPTER TWO

LITERATURE REVIEW

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LITERATURE REVIEW

2.1. The White coat

White coat, apron or laboratory coat (abbreviated lab coat) is a knee- length over coat or smock worn by professional in medical field or by those involved in laboratory work to protect their street clothes. The garment is made from white cotton or linen to allow it to be washed at high temperature and make it easy to see if it is clean. There has always been some concern white coat, nurse's uniforms and other hospital garments, may actually play part in transmitting pathogenic bacteria in hospital sitting (Priya *et al.*, 2009).

Many articles of clothing and equipments, such as neckties, stethoscope, pens, lanyards, identify badges along with the doctor's coat have been noted to carry potential pathogens (Kotsanas *et al.*, 2008).

There has also been controversy over whether doctors should be barred from wearing white coats in areas such as staff canteens, tea rooms, and libraries. However wearing white coat is an accepted of medical practice. The actual use of white coats and how often they are changed varies greatly among individual doctors and their specialties. The white coat is associated with medicine, science, and the healing, and it is the most recognized and respected dress of a doctor. Contamination of skin and clothing by "splashes" or touch is practically unavoidable in hospitals. The white coat worn over

personnel clothing, is personal protection equipments (PPE) from such contamination (Loh *et al.,* 2000).

2.1.1. Adopting the lab coat

In the middle of the 19th century, science had damaged the respectability of the medicine by demonstrating that its cures were worthless, thus relegating much of medicine to the realm of quackery and healing cults. While scientists were admired, physicians were distrusted. The medical profession turned to science. After all, it was thought, the laboratories whose inventions could transmit messages instantaneously and had revolutionized transportation could certainly provide breakthrough advances in curing disease. Physicians, seeking to represent themselves as scientists, thus adopted the scientific lab coat as their standard of dress (Jones, 1999).

2.1.2. The evolution of the lab coat

Originally, lab coats were beige, but when adopted by the medical profession in the late 19th century, white was chosen. Early evidence of this change comes from photographs of surgeons wearing short-sleeved white coats over their street clothes at Massachusetts general hospital in 1889. The change to white was appropriate for the times. Earlier in the history of medicine, clerical caretakers in hospitals donned black robes. The sever tone of these robes conveyed a sense of mourning and approaching death, sadly appropriate for the inevitable fate of those brought to the hospital in critical condition. With advances in medical care in 20th century, however, hospitals were no longer regarded as houses for

dying, but institutions of healing. The white uniforms of physicians symbolized this new hope in medicine (Jones, 1999).

2.1.3. The meaning of whiteness

White was chosen with good reason as the new standard of the medical profession. This color representing purity is visual reminder of the physician's commitment to do no harm. White represents goodness. White also conveys cleanliness and connotes a purging of infection. Further, the white coat symbolizes seriousness of purpose. It communicates the physician's medical intent and serves as symbolic barrier that maintains the professional distance between physician and patient. Perhaps most importantly, the white is a cloak of compassion (Jones, 1999).

2.1.4. Importance of white coats

There are both advantages and disadvantage to wearing the white coat. Objection have been raised about the excessive formality that coat may communicate. For this reason, most Scandinavian physicians, along with many US Pediatricans and psychiatrists, have abandoned its use. However white coat is an important accessory to the image of the physician that should not be carelessly tossed away. Wearing a white coat need not make a physician seem cold or insensitive. His or her attitude matter most. A physician in a white coat may still be worm, friendly, and empathetic. The white coat reminds physician in their professional duties, as prescribed by Hippocrates, to lead their and practice their art in uprightness and honor. In accord with this sentiment, the Arnold P. Gold

foundation of Columbia university college of physicians and surgeons initiated in 1993a (white coat ceremony) that has been adopted by many US medical schools. In 1997, 83 of the 142 accredited medical and osteopathic schools in United States conducted this rite of passage. The ceremony is typically performed for the incoming class at the beginning of each academic year. The white coat as one of medicine's most important symbols signifies that, the word of Dr Gold "a physician's responsibility is not only to take care of patients, but also to care for patients" (Jones, 1999).

Nosocomial infections are a major source of morbidity and mortality in hospital settings. The most important defenses against nosocomial transmission of viral, bacterial and other infections are detailed and continuing of staff and strict adherence to infection control policies (Petroudi, 2009).

2.2. *Staphylococcus aureus* (*S. aureus*)

The organism is Gram-positive cocci about 1um in diameter. The cocci are mainly arranged in grape-like clusters, but some, especially when examined in pathological specimens, may occur in single cell or pairs of cells. The organisms are non-sporing non-motile and usually non-capsulate. When grown on many types of agar for 24 h at 37º C, individual colonies are circular, 2-3 mm in diameter with smooth shiny surface; colonies appear opaque and are often pigmented (golden-yellow, fawn or cream), though a few strain are unpigmented. Staphylococci are salt-tolerant and can be selectively isolated from materials such as faeces and food by use media containing 7-10% sodium chloride (Greenwood *et al.,* 2002).

The main distinctive diagnostic features of *S. aureus* are production of an extracellular, coagulase, which converts plasma fibrenogen into fibrin, aided by an activator present in plasma. This test is done by adding a drop from fresh young broth culture in tube containing .05 ml of citrated human or rabbit plasma diluted 1 in 10. A positive result is seen within a few hours as distinct clot.

Production of thromboblast *nucleases* that break down DNA. This activity is detected by ability of boiled broth culture to degrade DNA in an agar diffusion test. production of a surface-associated protein known as clumping factor or bound coagulase that reacts with fibrinogen. Culming factor is easily detected within few seconds by adding undiluted plasma to saline suspension of the organism on microscope slide (Greenwood *et al.,* 2002).

2.2.1. Pathogenesis

The pathogen *S. aureus* is present in the nose of 30% of healthy people and may be found on the skin. It causes infection most commonly at sites of lowered host resistance, such as damage skin (surgical site infection) or mucous membranes (e.g. ventilator-associated pneumonia) (Greenwood *et al.*, 2012).

2.2.2. Methicillin-resistant *Staphylococcus aureus* (MRSA)

Infections caused by MRSA are increasingly reported, form different hospitals. These strains can cause wide range of infections including bacteremia, indocarditis, and pneumonia. This strains are increasly recognize as important agent of hospital-acquired

infection in hospitalized patients undergoing prosthetic heart valve surgery. MRSA are not resistant only to penicillin, but also to all other β-lactam antibiotics including the third-generation cephalosporin and carbapenems. MRSA strains can be treated with glycopeptide antiboitics such as vancomycin and teicoplanin in serious systemic infections such as pneumonia, bacteremia, and endocarditis. MRSA are senstive to on more of the second line of drugs, which include erythromycin, clindamycin, quinolones, fusidic acid, trimethoprim, chloramphenicol, tetracycline, and rifampicin. However ciprofloxacin, rifampicin,and fusidic acid are not use simply because of possibility of emergence of resistance (Chandra, 2009).

The control and prevention of MRSA involves the education of all health care professionals and the public, fast and reliable detection in the laboratory (including perhaps the use of molecular methods), active surveillance (even universal surveillance), prompt patient isolation or cohorting when admitted to hospital, standard precautions and good professionals practice by all health-care workers (including compliance with hand hygiene guidelines), effective hospital hygiene programs and antibiotic stewardship programs, e.g. avoidance of the excess use of cephalosporines and fluoroquinolones (Greenwood *et al.*, 2012).

2.2.3. Resistance to antimicrobial drugs

Bacterial resistance to drugs is condition in which the bacteria which were earlier susceptible to the antibiotics develop resistance against antibiotics and are not susceptible to the action of the same antibiotics. Antibiotics resistance among bacteria is major

concern in the treatment of patient. Emergence of antibiotic resistance to old as well as new antibiotics by bacteria is posing a major challenge in treatment of infection caused by bacteria. The antibiotic susceptibility is seen more commonly in hospital-acquired infections than in community-acquired infections. The antibiotic-resistant bacteria are more commonly seen in hospital environment due to wide spread use of antibiotics in hospital that select for these bacteria. These hospital strains of bacteria are characterized by developing resistance to multiple antibiotics at the same time. Common examples of such strains of bacteria showing drug resistance include hospital strains of *S. aureus* and Gram-negative enteric bacteria such as *Escherichia coli* and *Pseudomonas aeruginosa*. The resistance to multiple antibiotics is mediated by plasmid-carrying several genes that encode enzymes responsible for the resistance (Chandra, 2009).

2.2.4. Epidemiology and prevention

The organism *S. aureus* is frequent colonizer of skin and mucosa. High carrier rate (up to 80%) are rules among hospital patients and staff. The principle localization of colonization in these persons is the anterior nasal mucosa area, from where bacteria can spread to hand or with dust into air and be transmitted to susceptible person. *S. aureus* is frequently the causal pathogen in nosocomial infections. Certain strains are known to cause hospital epidemics. Identification of the epidemic strain requires differentiation of relevant infection isolates from other ubiquitous strains. Lysotyping can be used for this purpose, although use of molecular methods to identify genomic DNA (fingerprints) is now becoming more common. The most important preventive measure in hospitals is

washing the hands thoroughly before medical and nursing procedures; intranasal application of antibiotics (mupirocin) is the method of reducing bacterial count in carriers (Kayser *et al.*, 2001).

2.3. Previous studies

Microbiological analysis of swabs taken from the cuff and pocket mouths of physicians' white coats in acute care hospital showed that 91.3% of coats had bacterial contamination. Specifically dihptheroids, *S. aureus,* and Gram-negative bacilli. In contrast comparatively lower rates of bacterial contamination were observed in white coats of visiting physicians, of the medical unit compared with the rest of the hospital, that were less 1 year old, and that were laundered daily. Further, the white coat of physicians who wore them only when seeing patients had significantly lower bacterial contamination than white coat of physician who was theirs during clinical and non clinical duties. The bacterial isolates were resistance to nearly all antibiotics tested; the most effective, however, was ciproflox. Results suggest that physicians' white coat may increase nosocomial infection transmission. Proper handling of white coats by physician and other health care workers could minimize cross-contamination and improve patient safety by potentially reducing nosocomial infections (Uneke and Ijeoma, 2010).

Banu and his colleagues (2012) conducted cross-sectional survey of the bacterial contamination of white coats in tertiary care hospital. 100 medical students working in variouse specialties were included in the study. Swabs were taken from 4 different area of white – collar, pocket, side and lapel and processed in microbiology department

according to standard procedures. Although most of the white coats had been washed within the past 2 weeks, the sides of coats were the most highly contaminated areas followed closely by the collar and the pockets. *S. aureus* was the most common isolate followed by coagulase negative staphylococci and Gram-negative none fermenters. Most of Gram-positive cocci were resistant to penicilln Erythromycin and Clindamycin. White coats have been shown to harbor potential contaminants and may have role in nosocomial transmission of pathogenic microorganisms. Thus, a yearly purchase of white coats and the possession of two or more white coats at any point in time should be made compulsory. There is pressing need to promote scrupulous hand washing before and after attending patients and alternatives to white coats, including universal use of protective gowns, should be considered.

Treakle and his colleagues (2009) performed across-sectional study involving attendees of medical and surgical grand rounds at a large teaching hospital to investigate the prevalence of contamination of white coats with important nosocomial pathogens, such as methicillin-sensitive *S. aureus*, methicillin-resistant *S. aureus* (MRSA), and vancomycin-resistant enterococci (VRE). Each participant completed a brief survey and cultured his or her white coat using a moistened culture swab on lapels, pockets, and cuffs. Among 149 groud rounds attendees' white coats, 34 (23%) were contaminated with *S. aureus*, of which 6(18%) were MRSA. *S.aureus* contamination was more prevalent in residents, those working in patient setting, and those who saw inpatient that day. This study suggests that a large proportion of health care workers white coats may be contaminated

with *S. aureus* including MRSA. White coat may be an important vector for patient-to-patient transmission of *S. aureus*.

In study carried out by loh and his colleagues has demonstrating that the white coats of medical students are more likely to be bacteriologically contaminated at points of frequent contact, such as the sleeve and pocket. The organisms identified were principally skin commensls including *S. aureus*. The cleanliness of the coat as perceived by the student was correlated with bacteriological contamination, yet despite this; a significant proportion of student only laundered their coats occasionally. This study supports the view that the students' white coat is potential source of cross infection on the ward and its design should be modified in order to facilitate hand washing. Hospital training medical students should consider taking on burden of providing freshly laundered white coats for the students.

In another study, swabs were taken from the white coats of undergraduate students posted in various clinical departments, interns, and postgraduate students. The microbial contamination was studied by observing and recording the colony morphology on the culture plates, Gram's staining with light microscopic screening of slides, and biochemical characterization of the isolates using standard microbiology protocols. Microbiological analysis of swabs taken from white coats in dental operatory showed that 100% coats had bacterial contamination. Out of 30 swabs collected, 46 isolates were obtained. 50% of the isolates showed Gram-positive cocci, making is major microbial group contaminating the white coats in dental operatory. The presented study highlights

the fact that the white coats are a potential source of cross infection. The result of this study mandate a strict audit process and protocols to be set in place for preventing cross-contamination from the white coats in a dental operatory (Malini *et al.,* 2012).

Another study carried to determine the level and type of microbial contamination present of the white coats of dental interns, graduate students and faculty in a dental clinic. Questionnaire and cross-sectional survey of the bacterial contamination of white coats in tow predetermined areas (chest and pocket) on the white coats were done in rural dental care center. Paired sample t-test and chi-squire test were used for statistical analysis. 60.8% of the participants reported washing their white coats once a week. Grading by examiner revealed 15.7% dirty white coats. Also, 82.5%of the interns showed bacterial contamination of their white coats compared to 74.7% graduate students and 75% faculty members irrespective of the area examined. However, chest area was consistently more bacteriologically contaminated site as compared to the pocket area. Antibiotic sensitivity testing revealed resistant varieties of micro-organisms agaienst Amoxicillin (60%), Erythromycin (42.5%) Cotrimoxazole (35.2%). The white coats seem to be potential source of cross-infection in the dental seting. The bacterial contamination carried by white coats, as demonstrated in this study, supports the ban on white coats from non-clinical areas (Priya *et al.*, 2009).

CHAPTER THREE

MATERIALS AND MEHTODS

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3.1. Study design

3.1.1. Type of study

This was retrospective study conducted to detect Methicillin-resistant *Staphylococcus aureus* on physicians' white coat.

3.1.2. Study area

This study was done in Research Laboratory, College of Medical Laboratory Science, Sudan University of Science and Technology (SUST).

3.1.3. Study duration

The study was carried out in the period from April to June, 2015

3.2. Source of bacterial isolates

Bacterial isolates were obtained from the Research Laboratory, SUST. The isolates were previously recovered from physicians' white coats.

3.3. Sample size

Total of 14 (n=14) bacterial isolates were used in this study.

3.4. Laboratory work

3.4.1. Checking purity of the isolates

The isolates were streaked on nutrient agar and incubated over-night at 37ºC. At the end of incubation period, a discrete colony was picked up and checked for purity under microscope; then stored in Bijou bottle containing nutrient agar slant for further investigations.

3.4.2. Re-identification of the isolates

3.4.2.1. Gram stain

Bacterial smear was prepared by transferring portion of discrete colony to a drop of normal saline. The smear was covered with crystal violet stain for 30-60 seconds, rapidly washed the stain with clear water, then smear was covered with lougou's iodine for 30-60 seconds, washed of the iodine with clear water, decolorized rapidly (few seconds) with acetone-alcohol, washed immediately with clean water, then the smear was covered with sufranine for minutes, washed off the stain with clean water, wiped back of the slide clean and placed in draining rack for the smear to air dry, the smear was examined microscopically with oil immersion objective to report bacterial gram reaction and cell shape. Gram positive bacteria; stain dark purple, Gram negative bacteria; stain red (Cheesbrough, 2000).

3.4.2.2. Biochemical tests

Catalase test

The differentiation between Staphylococci (which produce catalase) from Streptococci (non catalase production) was made by catalase test. Catalase acts as catalyst in the breakdown of hydrogen peroxide to oxygen and water (Cheesbrough, 2006).

Using sterile wooden stick, suspected colony was immersed in tube containing 2ml of 3% hydrogen peroxide. A positive result was indicated by production of air pebbling. A negative indicated by no change on tube.

DNase test

Using sterile loop suspected colonies were inoculated under a septic condition into DNA medium. After overnight aerobic incubation at 37ºC hydrochloric acid (1% HCL) was added to the spots of an organism. Clearing round colonies mean positive result (Cheesbrough, 2006).

Coagulase test

Coagulase is an enzyme that causes plasma to clot. The test use to differentiate *S. aureus,* which produce coagulase enzyme from other staphylococci. 0.5 ml of diluted plasma was placed in small test tube. 5 drops from bacterial suspension was added and mix gently, incubated at 37ºC up to 4 hours, and then examined for clot formation (Cheesbrough, 2000).

Mannitol fermentation test

Mannitol salt agar medium was used for identifying staphylococci species, which are able to grow on agar containing 70-100g/L sodium chloride. Some species of Staphylococci are able to ferment mannitol and other cannot ferment.

The test done by inoculating organisms under test in MSA medium which contain phenol red as indicator, and then incubated the plate at 37ºC for 24 hours, and then change in color was observed (Cheesbrough, 2000).

Oxidase test

A strip of filter paper is soaked with little freshly made 1%solution of the reagent (tetramethyl-*p*-phenylene-diamine oxalate) and then at once used by rubbing a speck of culture on it with glass loop. A positive reaction is indicated by an intense deep-purple color, appearing within 5-10 seconds, delayed positive within 10-60seconds and negative reaction by absence of coloration or by coloration later than 60 seconds (Collee *et al.*, 1996).

Indole test

In this test the tested organisms produce tryptophanase which break tryptophan and produce indole, which react with kovac's reagent and give pink ring. The tested organisms was inoculated into peptone water and incubated at 37ºC for overnight, the kovac's reagent was added. If there is pink ring the result was indicated as positive. If there is no pink ring in surface the result was indicated as negative (Cheesbrough, 2006).

Citrate utilization test

In this test the organisms has ability to use citrate as only source of carbon. By straight loop a part of tested colony were emulsified in Kosser citrate media and incubate 24 hour in 37ºC. Positive: blue color. Negative: no change (Cheesbrough, 2006).

Urease test

In these test organisms produce urease enzyme break down urea and produce ammonia, which make PH media alkaline, in the presence of phenol red indicator the tested organism inoculated in the Christensen's urea agar. Positive: pink color. Negative: on change (Cheesbrough, 2006).

Fermentation of sugar and production of gas and H_2S

Tested organisms inoculated by sterile straight loop by stepping on the butt, then blocked the pore and streaked slop media and incubated 24 hour in 37ºC. Glucose fermentation yellow butt, lactose fermentation `yellow slop, gas production in the end of tube and H²s production blacking in the media (Cheesbrough, 2006).

3.4.2.3 Antimicrobial susceptibility test

This was done by Kirby-Bauer disk diffusion technique according to NCCLS (1997).

Culture media

Sterilized Mueller-Hinton agar was prepared. Cooled to 45-50Cº, and poured in sterile dry Petri dish on level surface, to depth of 4mm. the presence of any excess surface moister on the medium was removed by keeping the plates inverted in an incubator at (35-37Cº)

Preparation of inoculums

The inoculums were prepared by direct colony suspension method. Five well selected colony (similar appearance) of the organisms to be tested were bucked with sterile loop and the growth was transferred to sterile saline, then the inoculums turbidity was adjusted by using McFarland standard turbidity (Cheesbrough, 2002).

Inoculation of Muller Hinton agar

The plate was inoculated by dipping a sterile cotton swabs into the inoculums. The excess inoculums was removed by pressing rotating the swab firmly against the side of the tube above level of the liquid. The swab was seeded evenly over the surface of the medium in three directions, the inoculums was left to dry, with lid closed for a few minutes at room temperature.

Antimicrobial disc application

By using a pair of sterile forceps, antibiotic disc were applied, and evenly distributed on the inoculated plate. The plate was inverted and incubated aerobically at 37Cº for 18-24 hours.

Reading and interpretation

The diameter of each zone of inhibition (including the diameter of the disc) was measured to nearest millimeter by using ruler. The susceptibility of isolates was reported according to manufacture standard zone size interpretative manual. Sensitive organisms were when the zone of inhibition was equal to or greater than the standard.

CHAPTER FOUR

RESULTS

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RESULTS

Bacterial isolates were obtained from Research Laboratory (SUST). These were previously recovered from physicians' white coats. Total 14 isolates were re-identified. These were *Staphylococcus epidermidis* 5 (35.7%), *Staphylococcus aureus* 4 (28.6%), *Pseudomonas aeruginosa* 3 (21.4%) and *Staphylococcus intermedius* 2(14.3%) (Table1).

The percentages of MRSA among isolated Staphylococcus aureus were 2(50%) out of four *Staphylococcus aureus*.

Table 1. Isolated bacteria from physicians' white coats

Re-identified bacteria	Frequency
Pseudomonas aeruginosa	3
Staphylococcus aureus	4
Staphylococcus epidermidis	5
Staphylococcus intermmidius	2

Table 2. Biochemical test of isolated Gram negative bacteria

Isolate code	Biochemical tests								Identified bacteria
	Oxidase test	Indole test	Urease test	Citrate test	KIA				
					Slope	Butt	Gas	H2s	
C1	+ve	-ve	-ve	+ve	R	R	_	_	*Pseudomonas aeruginosa*
C4	+ve	-ve	-ve	+ve	R	R	_	_	*Pseudomonas aeruginosa*
C10	+ve	-ve	-ve	+ve	R	R	_	_	*Pseudomonas aeruginosa*

Key: R=Red; +ve=positive ; -ve=negative

Table 3. Biochemical test of isolated Gram-positive bacteria

Isolate code	Biochemical tests				Identified bacteria
	Manitol fermentation	Catalase test	DNase test	Coagulase test	
C2	+ve	+ve	+ve	+ve	*S. aureus*
C3	-ve	+ve	-ve	-ve	*S. epidermidis*
C5	-ve	+ve	+ve	+ve	*S.intermmidius*
C6	+ve	+ve	+ve	+ve	*S. aureus*
C7	+ve	+ve	+ve	+ve	*S. aureus*
C8	-ve	+ve	+ve	+ve	*S.intermmidius*
C9	+ve	+ve	+ve	+ve	*S. aureus*
C11	-ve	+ve	-ve	-ve	*S. epidermidis*
C12	-ve	+ve	-ve	-ve	*S. epidermidis*
C13	-ve	+ve	-ve	-ve	*S. epidermidis*
C14	-ve	+ve	-ve	-ve	*S. epidermidis*

Key: +ve=positive ; -ve=negative

CHAPTER FIVE

DISCUSSION

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5.1. Discussion

Traditionally the white coats are thought to bring credibility and dignity to medical profession (Muhadi *et al.*, 2007). However, white coats have been shown to harbor potential contaminant and these may have a role in nosocomial transmission of pathogenic microorganisms. The high rates of bacterial contamination of white coats may be associated with the following 2 facts: firstly, patient continuously shed infectious microorganisms in the hospital environment, and health care providers are in constant contact with these patients. Secondly it has been demonstrated that microorganisms can survive between 10 and 98 days on fabrics which are use to make white coats, which include cotton, cotton and polyester, or polyester materials (Uneke and Ijeoma, 2010; Chacko *et al.,* 2003).

This study was done to detect the methicillin-resistant *S. aureus* in physicians' white coats.

S. aureus including susceptible and resistance isolates, were found in this working. Our data suggest that physicians' white coats were contaminated with *S. aureus* and some of isolates were methicillin-resistant. in this working found that *S. aureus* contamination in 4 (28.6%) tow of them was MRSA (14.3%). Previous studies have found variable rates of *S. aureus* contamination, wong *et al.*, (1991) evaluated white coats of 100 physician by

pressing contact plates into 3 area of each coat and found *S. aureus* contamination in 29 of the coats (none of which was MRSA). Loh *et al.,* (2000) evaluated white coats of 100 medical students at 3 sites in blood agar plates and found bacterial contamination in all coats but *S. aureus* in only 5 of these. Treakle *et al.*, (2009) evaluated 149 lab coats 34 (23%) were contaminated with *S. aureus* 6(18%) were MRSA.

Coagulase negative staphylococci were also isolated (35.7%) which are skin commensales and these can be potentially infectious to the patients who are admitted to hospital. This finding is higher than reported by (Banu *et al.,* 2012*)* 10.3%.

Gram-negative bacilli were isolated from the physician lab coats (21.4%), these were potentially infectious.

5.2. Conclusion

In conclusion, this study suggests that physicians' white coats may be contaminated with *Staphylococcus aureus* including Methicillin-resistant *Staphylococcus aureus*. The presented study highlights the fact that the white coats are a potential source of cross infection.

5.3. Recommendations

1. Proper handling of white coats by physicians could minimize cross contamination and improve patient safety by potentially reduces nosocomial infections.
2. More frequent change of white coats.
3. Scrupulous hand washing should be observed before and after examining patients.
4. Exclusion of white coats from non clinical area on hospital.

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APPENDICES

7. APPENDICES

A) Culture media

Nutrient Agar

Approximate formula per Liter

Beef extract …….....3.0g

Peptone……..5.0g

Agar…….15.0g

Manitol salt agar

Ingredient

Meet extract…….1.0g

Casein peptone ……...5.0g

Sodium chloride ……...75.0g

D.mannitol ……..10.0g

Phenol red…….0.025g

Agar ……..15.0g

Preparation

111g of powder dissolve in 1L of D.W and sterilize by autoclave at 121c for 15 minutes then cool and pour in petridishes.

DNase agar

Ingredient

Casein enzymatic hydrolysate…….15.0g

Papic digest of soya bean meal ……..5.0g

Deoxy ribonucleic acid ……..2.0g

Sodium chloride …….5.0g

Agar ……..…15.0g

Preparation

42g of powder dissolve in 1 L of D.W and sterilize by autoclave at 121c for 15 minute then cool and pour in petridishes.

Urea agar base (Christensen)

Ingredient

Peptide digest of animal tissue ……..1.0g

Dextrose …….....1.0g

Sodium chloride …….....5.0g

Monopotassium phosphate ……..0.80g

Phenol red …….0.012g

Agar …….15.0g

Preparation

24g of powder dissolve in 1L of D.W then sterilize by autoclaving at 15 Ibs pressure at 121c for 15 minutes then cool and add aseptically 50ml of 40%urea, mix and pour tube in vertical position.

Kosser citrate medium

Ingredient

Magnesium sulfate …….0.2g

Potassium dihydrogen sulfate ……1.0g

Sodium ammonium sulfate ……1.5g

Trisodium citrate…….....2.5g

Bromothymole blue ……..0.016g

Prepration

5.2g dissolve in 1 L of D.W sterilize by autoclaving at 15 Ibs pressure at 121c for 15 minute and pour in tube.

Kilger iron agar (KIA)

Ingredient

Peptic digest of animal tissue ……..15.0g

Beef extract ……...3.0g

Yeast extract……...3.0g

Protease peptone ……..10g

Lactose ……10g

Dextrose …….1.0g

Ferrous sulfate ……0.20g

Sodium chloride ……..…5.0g

Sodium thiosulfate …….0.3g

Phenol red ……..0.024g

Agar ……..15.0g

Preparation

57.5g dissolve in 1L D.W and sterilize by autoclave at 121c for 15 minute then cool and pour in tube in slop slant position.

Peptone water

Ingredient

Peptic digest of animal tissue ……..10.0g

Sodium chloride ……5.0g

Preparation

15g of powder dissolve in 1Lof D.W then sterilize by autoclaving at 15 Ibs pressure at 121c for 15 minutes

B) Preparation of reagents

1. Gram stain reagent

Crystal violet

Approximate formula per Liter

Crystal violet …….20.0g

Ammonium oxalate ……9.0g

Ethanol absolute ……95ml

Distilled water ……..to 1 litter

Lugols iodine

Approximate formula per Liter

Potassium iodine ……20.0g

Iodine …….10.0g

Distilled water…….up to litter

Acetone-alcohol decolorizer

Approximate formula per Liter

Acetone ……..…….500ml

Ethanol, absolute ……495ml

Distilled water ……25ml

Suffranin

Approximate formula per Liter

Suffranin ……..2.5g

95% ethanol ……10ml

Distilled water …….up to 1 litter

2. **Physiological saline (8.5g\L)**

Sodium chloride …….8.5g

Distilled water …….up to litter

3. **Hydrochloric acid**

Hydrochloric acid, concentrated ……8.6ml

4. **Kovac's reagent**

Approximate formula per Liter

Amyle or isoamyle alcohol ……..…….15ml

p-dimethyle –aminobenzaldehyde ……....10g

hydrochloric acid concentrated ……50ml

5. **Hydrogen peroxide**

H_2O_2 solution ……10 volume