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Sudan University of Science and Technology

College of Graduate Studies

Determination of Antibiogram of Bacteria Isolated from Physicians' White coats in Khartoum State

تحديد مرتسم المضادات الحيوية للبكتيريا المعزولة من معاطف الأطباء البيضاء بولاية الخرطوم

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

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2015

الآية

قال تعالى:

(اللَّهُ نُورُ السَّمَاوَاتِ وَالْأَرْضِ ۚ مَثَلُ نُورِهِ كَمِشْكَاةٍ فِيهَا مِصْبَاحٌ ۖ الْمِصْبَاحُ فِي زُجَاجَةٍ ۖ الزُّجَاجَةُ كَأَنَّهَا كَوْكَبٌ دُرِّيٌّ يُوقَدُ مِنْ شَجَرَةٍ مُبَارَكَةٍ زَيْتُونَةٍ لَا شَرْقِيَّةٍ وَلَا غَرْبِيَّةٍ يَكَادُ زَيْتُهَا يُضِيءُ وَلَوْ لَمْ تَمْسَسْهُ نَارٌ ۚ نُورٌ عَلَىٰ نُورٍ ۗ يَهْدِي اللَّهُ لِنُورِهِ مَنْ يَشَاءُ ۚ وَيَضْرِبُ اللَّهُ الْأَمْثَالَ لِلنَّاسِ ۗ وَاللَّهُ بِكُلِّ شَيْءٍ عَلِيمٌ)

سورة النور: الآية (٣٥)

DEDICATION

To my dear parents

To my sister

To my colleagues and friends

AKNOWLEDGEMENT

All thanks and praise to ALMIGHTY ALLAH the worthy of all praise for all that I am.

With sincere thanks and gratitude, I would like to acknowledge my supervisor **Prof. Humodi Ahmed Saeed** for this outstanding, knowledge encouragement, guidance, patience and constructive advice throughout this study.

All thanks for everyone who helps me somehow in this work.

ABSTRACT

This is a laboratory-based study, carried out during the period from April to June, 2015 to determine antibiogram of bacteria isolated from Physicians' white coats in Khartoum State.

A total of 14 bacterial isolates 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's stain and biochemical tests were used to confirm the identification of the isolates. Modified Kirby–Bauer disk diffusion technique was used to determine antibiogram of isolates against traditionally used antibiotics. The antibiotics used were Gentamicin, Amoxicillin, Ciprofloxacin, Cotrimoxazole, Tetracycline, Azithromycin, Imipenem, Methicillin, and Novobiocin.

The results showed that re-identified isolates were *Staphylococcus epidermidis* (5), *Staphylococcus aureus* (4), *Pseudomonas aeruginosa* (3), *Staphylococcus intermidius* (2). The results of antibiogram of bacterial isolates revealed that all isolates (n=14) were susceptible (100%) to Ciprofloxacin, Gentamicin, Imipenem. Activities of other antibiotics ranged from 0.0% to 80%. Determination of susceptibility of each isolate to same antibiotic was found as follows; *S. aureus* (n=4) were susceptible (100%) to Ciprofloxacin, Tetracycline, Gentamicin, Imipenem, Amoxicillin,

Cotrimoxazole, and Novobiocin. (75%) to Azithromycin, (50%) to Methicillin. *S. intermidius* (n=2) were susceptible (100%) to Ciprofloxacin, Tetracycline, Gentamicin, Imipenem, Azithromycin, Amoxicillin, Cotrimoxazole, Novobiocin.(50%) to Methicillin.

*S. epidermidis (*n=5*)* were susceptible (100%) to Ciprofloxacin, Gentamicin, Imipenem, Novobiocin. (80%) to Amoxicillin and Tetracycline. (60%) to Methicillin and Cotrimoxazole, (40%) to Azithromycin .

*Psedumonas aeurginosa (*n=3*)* were susceptible (100%) to Ciprofloxacin, Gentamicin, Imipenem, and Azithromycin. (0%) to Novobiocin, Cotrimoxazole,

(33.3%) to Amoxicillin, Methicillin and Tetracycline**.**

The study concluded that all isolates recovered from physicians' white coats were completely (100%) susceptible to Ciprofloxacin, Gentamicin, Imipenem. Further studies with more bacterial isolates are required to validate the results of this study.

المستخلص

هذه دراسة قائمه على المختبر ،أجريت خلال الفترة من ابريل إلي يونيو 2015م بغرض تحديد مرتسم المضادات الحيويه للبكتريا المعزولة من معاطف الأطباء.

14 عزله من البكتيريا تم الحصول عليها من مختبر الأبحاث بجامعه السودان للعلوم والتكنولوجيا .
استزرعت العزلات فى وسط الأجار المغذي للتأكد من نقاوتها واستخدمت طريقه جرام والاختبارات البايوكيمائيه للتأكد من هوية هذه العزلات . استخدمت طريقة كيربى-بور لتحديد مرتسم المضادات الحيوية المستخدمة تقليديا تشمل الجينتاميسين، الاموكسيسلين، السيبروفلوكساسين ،الكوتراي مكسازول، الاميبينيم التتراسيكلين ،الازيثروميسين ،الميستيلين ، ، والنوفوبيوسين .
أظهرت نتيجة إعادة التعرف علي هوية البكتريا. المكورات العنقودية البشرويه 5 تليها المكورات العنقودية الذهبية 4 والزائفة الزنجاريه 3 ثم المكورات العنقودية الوسيطة 2.

وأظهرت دراسة تقويم حساسية البكتريا للمضادات الحيوية أن كل البكتريا المعزولة حساسة الايميبنيم للسيبروفلوكساسين والجينتاميسين. والحساسيه للمضادات الحيويه الأخرى فى المدى 0.0%- 80% .

المكورات العنقودية الذهبية حساسة بنسبة100%للسيبروفلوكساسين ،التترسايكلين ،الجنتاميسين ،الايمبينيم الاموكسيسيلين ، الكوتراي موكسازول و النوفوبيوسين 50% للمثيثلين 75% للازيثروميسين .

المكورات العنقودية الوسيطة حساسة بنسبة 100% للسيبروفلوكساسين، التترسايكلين ،الجنتاميسين الايمبينيم، الاموكسيسيلين ، الكوتراي موكسازول ، النوفوبيوسين و للازيثروميسين 50% للمثيثيلين

المكورات العنقودية البشرويه حساسة بنسبة 100% للنوفوبيوسين ،السيبروفلوكساسين ،الجنتاميسين ،الايميبينيم 80% منها حساسة للتتراسيكلين والاموكسيسيلين و60% منها حساسة للمثيثيلين والكوترای موكسازول 40% منها حساسة للازيثروميسين
العزلات من الزائفه الزنجاريه حساسه بنسبه 0%للكوترای موكسازول والنوفوبيوسين. 33.3% منها حساسه للاموكسيسيلين والميثيثلين والتتراسيكلين و100% للازيثروميسين والسيبروفلوكساسين والجنتاميسن والايميبينيم .
خلصت الدراسة إلى أن كل العزلات البكتيرية من معاطف الأطباء بالكامل حساسه للسيبروفلوكساسين والجنتاميسين والايميبينيم . وان إجراء مزيد من الدراسات مع عزلات باكتيريه أكثر مطلوبة للتحقق من هذه النتائج .

TABLE OF CONTENTS

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

CHAPTER TWO

LITERATURE REVIEW

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER FOUR

RESULTS

CHAPTER FIVE

DISCUSSION

LIST OF TABLES

LIST OF ABBREVIATIONS

CoNs	Coagulase Negative staphylococci
DNAse	Deoxyribonuclease
MRSA	Methicillin Resistance *Staphylococcus aureus*
US	United State
PPE	Personal protection equipments
WCC	White coat ceremony
NI	Nosocomial infection
HAIS	Health care associated infections
WHO	World health organization
KIA	Kligler iron agar
MSA	Mannitol salt agar

CHAPTER ONE

INTRODUCTION AND OBJECTIVE

CHAPTER ONE

INTRODUCTION AND OBJECTIVE

1.1. Introduction

The white coat has served as the pre-eminent symbol of physician for 100 years Achilles earliest memory of doctor is the person in the white coat. patients except to be treated in doctors' offices, hospitals and clinics by an individual wearing white. At virtually every medical school, the first symbolic act is the (white coat ceremony). Originated by Arnold P. Gold, MD. This is the ceremonial –cloaking- of doctor –to be as she or he embarks on medical career (Hochberg, 2007).

The white coat is associated with medicine ,science, and the healing ,and it is the most recognized and respected dress of doctor. contamination of skin and clothing by "splashes" or touch is practically unavoidable in hospitals. The white coat worn over personnel clothing is apersonal protection equipments (PPE) from such contamination (Loh *et al*., 2000).

White coats are worn primarily for identification ,but there has always been some concern that white coats, like nurses uniforms and other hospital garments , may play a part in transmitting pathogenic bacteria in a hospital setting, as white coats are known to be potentially contaminated with pathogenic drug resistant bacteria (Wong *et al*., 1991).

Antimicrobial susceptibility tests are used to determine which specific antibiotics a particular bacteria or fungus is sensitive to. Most often ,this testing complements a Gram stain and culture, the results of which are obtained much sooner, Antimicrobial susceptibility tests can guide the physician in drug choice and dosage for difficult- to treat infections (Levinson, 2010).

The performance of antimicrobial susceptibility testing is important to confirm susceptibility to chosen empirical antimicrobial agent, or to detect resistance in individual bacterial isolates, So the susceptibility test of individual isolates is important with species that may possess acquired resistance mechanisms (James and Jane , 2009).

1.2. Rationale

The contamination of physician white coat might be potential vectors of nosocomial infections. 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).

Patient-to-patient transmission of nosocomial pathogens 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 avector for this transmission (Treakle *et al*., 2009). In Sudan there is no previous study conducted to

assess the susceptibility pattern of bacteria isolated from white coat to antimicrobial agents. This study screened selected species of bacteria isolated from white coat against traditionally used antimicrobial agents.

1.3 . Objectives

1.3.1. General Objective

To determine antibiogram of bacteria isolated from white coats.

1.3.2. Specific Objectives

1. To re-identify of the provided bacterial isolates.
2. To perform susceptibility test against the bacterial isolates using different antimicrobial agents .

CHAPTER TWO
LITERATURE REVIEW

CHAPTER TWO
LITERATURE REVIEW

2.1. 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).

The white coat is commonly regarded as the attire that confers a sense of professionalism and authority within the healthcare industry. The history of white coat attire dates back to the late 19th century, when scientists were in the habit of wearing beige-coloured laboratory coats. Wanting to associate themselves with the scientific community in order to gain trust from the public, doctors began to adopt the laboratory coat as a sign of trust worthiness and the ability to provide empirically supported treatments. The white colour was later chosen as a symbol of purity and dedication to 'do no harm'. The white coat eventually became an important symbol of the synergy between the arts and science of Medicine (Jones**,** 1999).

This practice has since spread to many countries and cultures all over the world, with the white coat being strongly associated with the image of western medical practitioners. The significance of and respect towards this attire is reflected by the "white coat ceremony", a ritual which many western medical schools carry out in the beginning of the school years to emphasize professionalism (Huber, 2003).

The white coat is one of the more established symbols of the medical profession and is probably the item of clothing worn by physicians (Kazory,2008).

The symbolism of the white coat is often recognized by formal ceremonies at which medical school graduates are granted the distinction of wearing one to emphasize the humanistic values of medicine (Uneke and Ijeoma, 2010).

2.1.1. The white coat ceremony

The Gold Foundation describes the W.C.C as an experience by which "participating schools alert beginning students to the need to balance excellence in science with compassionate patient care. It "helps to identify the characteristics of a complete doctor. The WCC began in 1993 at Columbia University's College of Physicians and Surgeons, and has since spread across the United States and the world. Because of the short institutional memory of medical schools and its association with the Hippocratic Oath, medical students now perceive the W.C.C as a longstanding tradition. A typical WCC includes the presence of family and friends, a welcome from the school

administration, an inspirational message from a role model, receipt of the white coat from a physician, the swearing of an oath, and a reception with a "party atmosphere. Good will and positive thoughts are meant to welcome and initiate students as novices in the medical profession (Huber, 2003).

2.1.2. Uses of white coat

The physicians white laboratory coat was worn initially for the purpose of protection against cross contamination spills (from reagent) and also because white laboratory coat connotes life , purity , innocence , goodness etc (VanDer, 2001) .

Wearing a white coat is an accepted part of medical practice. The actual use of white coats and how often they are changed varies greatly among individual doctor's and their specialties. There has always been some concern that white coats, like nurse's uniforms and other hospital garments, may actually play apart in transmitting pathogenic bacteria in hospital setting. 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 (Wong *et al*., 1991).

2.1.3. Role of White coat in spreading hospital acquired infection

Health care associated infections are on the rise world wide .Microorganisms are the most commonly transmitted by the hands of health care personnel but materials and articles used in the hospitals could also carry microorganisms (Dharan *et al*., 1999).

It is known that accessories used by physicians can be a potential source of nosocomial infection (Pandy *et al*., 2010).

The World Health Organization (WHO) offers several definitions of a nosocomial infection/ hospital –acquired infection: An infection acquired in a hospital by a patient who was admitted for a reason other than that infection . An infection occurring in a patient in a hospital or other health care facility in whom the infection was not present or incubating at the time of admission. This includes infections acquired in the hospital but appearing after discharge, and also occupational infections among staff of the facility (Bolyard *et al.,*1998).

Health care –associated infections (HAIs) also known as nosocomial infections remain significant hazard for patients and families visiting a hospital or health care facility. The world health organization (WHO) defines an HAI as an infection occurring in a patient in hospital or other Health care facility in whom the infection was not present or incubating at the time of admission (Uneke and Ijeoma, 2010).

Various studies have suggested that health care workers clothing, including white coats, are potential reservoirs for hospital organisms which reinfect the hands of health care workers (HCWs) and may act as vector for transmission of nosocomial pathogens (Sand and Basak, 2015).

Nosocomial infections constitute a major problem globally with major social, economic, moral, and personal effects that increase morbidity and mortality of

hospitalized patients (Sallam *et al*., 2005).

The extended duration of hospital admission and extra drugs or medical management may contribute to additional cost of patient care. These factors increase the emotional stress of the patients and their families and may lead to severe disability and reduce the patients' quality of life (Teng *et al* ., 2009).

The emergence of antimicrobial resistance is an important issue associated with nosocomial infections and most nosocomial infections are often caused by antibiotic resistant organisms (Gashaw *et al*., 2014). Antibiotic resistance increases the morbidity and mortality associated with infections and contributes substantially to rising costs of care resulting from prolonged hospital stays and the need for more expensive drugs (Struelens,1998).

It remains important to have a thorough knowledge of the microflora harboring the white coats of doctors to minimize cross contamination and improve patient safety by reducing the risk of nosocomial infection (Malini *et al* ., 2012).

2.2. Antibiotics

Are substances produced by living organisms. They inhibit the metabolism and /or growth of other microorganism. Antibiotics may be produced naturally or by synthesis (Maarteens *et al* ., 2011).

2.2.1. Mode of actions and mechanisms of bacterial resistance

Antibiotic activity is due to the inhibition of biochemical pathways that are involved in the biosynthesis of essential components of the bacterial cell. The three main bacterial targets of antibiotic agents are cell wall, protein, and nucleic acid biosynthesis. Various mechanisms neutralizing the action of antibiotic agents have developed in bacteria. The most wide spread antibiotic resistance mechanisms are enzymatic drug inactivation, modification or replacement of the drug target, active drug target, active drug efflux, and reduced drug uptake (Peterson and Hayword, 2002).

Bacterial resistance was present before antibiotics were used. this intrinsic innate ability of bacterial species to resist the activity of a particular antibiotic agent is inherent structural or functional characteristic. Acquired bacterial antibiotic resistance is a result of a genetic change. Which occurs in the presence or absence of antibiotic (Guardabassi and Courvalin, 2006).

This genetic change can be the result of mutation horizontal exchange of genetic material via transformation, transduction or conjugation. These genetic events occur in the presence or absence of antimicrobial. however antimicrobial therapy exerts a selective effect and subsequent competitive effect which, when followed by a bacterial genetic transfer, contribute antimicrobial resistance (Fluruya and lowy, 2006)

2.2.2. prosperities of antibiotics

2.2.2.1. Potency

This is the amount of antibacterial active agent in a test substance, determined by mean of a bioassay, usually expressed in microgram per milligram (µg/mg) of the test substance.(EUCAST, 2000).

2.2.2.2. Concentration

This is the amount of antibacterial agent in a defined volume of liquid, preferably expressed as mg/liter (rather than (mg/ml or), or in a defined mass of solid, usually expressed as mg/g or mg/kg.(EUCAST, 2000) .

2.2.2.3. Pharmacokinetics and pharmacodynamics

Pharmacokinetics is the study of drug concentrations over time ,in different body compartments, after a given dose of an antibiotic. Pharmacodynamics is the study of the relationship between pharmacokinetic parameters and the magnitude and time course of the response of the pathogen (EUCAST,2000).

2.3. Previous study

Questionnaire and cross-sectional survey of the bacterial contamination of white coats in two predetermined areas (chest and pocket) on the white coats were done in a rural dental care center in India. Paired sample *t*-test and chi-square test were used for Statistical analysis. The survey was concluded that. 60.8% of the participants reported washing their white coats once a week. Grading by the 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 a more bacteriologically contaminated site as compared to the pocket area. Antibiotic sensitivity testing revealed resistant varieties of microorganisms against Amoxicillin (60%), Erythromycin (42.5%) and Cotrimoxazole (35.2%). And of the 149 participants, the white coats of 23% of participants (34 white coats) were contaminated with *Staphylococcus aureus*,of which ,six(18%)were MRSA (Priya *et al*., 2009).

Another study performed in Nigeria in period between September 2008 to February 2009. to assess the profile of microbial contamination of the white coats used by physicians and to evaluate the relationship between white coat contamination and white coat usage and handling practices by doctors, and to assess the susceptibility of microbial isolates to various antibiotics commonly used in acute practice. Microbiological analysis of swabs taken from the cuffs and pocket mouths of physician's white coats in an acute hospital showed that (91.3%) of the coats had bacterial contamination specifically diphtheroids, *Staphylococcus aureus* and Gram negative bacilli were isolated. The susceptibility of *S. aureus* to ciprofloxacin and gentamicin were 97.8 % ,32.6% respectively. The susceptibility of *Ps.aeruginosa* to ciprofloxacin and cotrimoxazole were 26.1% and 0% respectively (Uneke and Ijeoma, 2010).

Across sectional study was conducted in Department of Microbiology of tertiary care hospital which was attached to Medical College in India. 100 medical students working in various specialties were included in the study. Swabs were taken from 4 different areas of the white coat (collar, pocket, side and lapel) and processed in the Microbiology department according to standard procedures. The study showed that the sides of the coats were the most highly contaminated areas followed closely by the collar and pockets. *Staphylococcus aureus* was the most common isolate followed by coagulase negative *Staphylococci* (CoNs) and Gram negative non fermenters. Most of

the Gram positive cocci were resistant to Penicillin, Erythromycin and Clindamycin (Banu *et al*., 2012).

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE
MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This was a laboratory –based study.

3.1.2. Study area

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

3.1.3. Study duration

The study was carried out during the period from April to June 2015.

3.1.4. Bacterial isolates

Bacterial isolate were obtained from the Research Laboratory, SUST. The isolates were checked for purity and then re-identified by conventional bacteriological methods.

3.2. Culture media

3.2.1. Nutrient Agar

Basic medium used to support the growth of bacteria that do not have special nutritional requirements. It contain peptone, lab-lemco powdered, yeast extract ,sodium chloride and agar (Cheesbrough, 2006).

3.2.2. Macconkey's agar medium

Macconkey's agar is a differential and low selectivity medium use to distinguish lactose fermenting from non lactose fermenting bacteria. It contains peptone, lactose, bile salt, sodium chloride, neutral red and agar (Cheesbrough, 2006).

3.2.3. Manitol salt agar medium

Manitol salt agar is a differential and selective plate medium used to isolate *staphylococcus aureus* from a fecal specimen in the investigation of staphylococcal food-poisoning. It contains peptone, manitol, sodium chloride, phenol red and agar (Cheesbrough, 2006)

3.2.4. Muller Hinton agar medium

This medium is used for sensitivity test. It contains beef infusion casein hydrolysate and starch (Cheesbrough, 2006).

3.3. Methods

3.3.1. Purification of isolates

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

3.3.2. Re –identification of the isolates

3.3.2.1. Grams stain

The Gram stain reaction was used to help identify pathogens in specimens and culture by their Gram reaction (Gram- positive or Gram –negative). and morphology. Gram-positive bacteria stain dark purple with crystal violet and are not decolorized by alcohol and Gram –negative bacteria stain red because after being stained with crystal violet decolorized by alcohol. The smears were fixed by dry heat and then covered with crystal violet for 30-60 seconds the stain was rapidly washed by tap water and tipped off the slide. The stained smear was then covered with iodine for 30-60 seconds. iodine washed off and the smear was decolorized with alcohol and immediately washed with clean water. Safranin was added to the smear for 30-60 seconds the red stain was then washed off with tap water and smear was subsequently

air dried and microscopically examined using high resolution objective power (Cheesbrough, 2006).

3.3.2.2. Biochemical tests

3.2.2.2.1. Catalase test

The differentiation between Staphylococci (which produce catalase) from Streptococci (non catalase production) was made by catalase test. Catalase act as catalyst in the breakdown of hydrogen peroxide to oxygen and water. Using sterile wooden stick, suspected colony was immersed in tube containing 2ml of 3% hydrogen peroxidase. A positive result was indicated by production of air pebbling. A negative indicated by no change on tube (Cheesbrough, 2006).

3.2.2.2.2.DNase test

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

3.2.2.2.3.Coagulase test

Coagulase test 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 37^{o}C up to 4 hours, and then examined for clot **f**ormation (Cheesbrough, 2006).

3.2.2.2.4. Mannitol fermentation test

A useful medium was 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 37^{o}C for 24 hours, and then change in color was observed (Cheesbrough, 2006).

3.2.2.2.5.Indole test

In this test the tested organism produce tryptophanase which breakdown tryptophan and produce indole, which react with kovac's reagent and give pink ring. The tested organism was inoculated into peptone water and inocubatedat 37^{o}C for overnight, the Kovac's reagent was added. If there is pink ring in surface the result was indicated as positive. If there is no pink ring in surface the result was indicated as negative (cheesbrough, 2006).

3.2.2.2.6. Citrate utilization test

In this test organism has ability to use citrate as only sourse of carbon. By straight loop apart of tested colony was emulsified in kosser citrate media and incubate 24 hour in 37^{o} C. Positive result give blue color. Negative result show no change (Cheesbrough, 2006).

3.2.2.2.7. Urease test

In this test organism produce urease enzyme breakdown urea and produce ammonia, which make the pH of media alkaline, in the presence of phenol red Indicator the tested organism inoculated in Christensen's urea agar. Positive : pink color . Negative :No change (Cheesbrough, 2006).

3.2.2.2.8. Kligler Iron Agar (KIA)

Atested organism inoculated by sterile straight loop by stepping on the butt, then blocked the pore and streaked slop media and incubated 24hour in37^{o}C. Glucose fermentation yellow butt, lactose fermentation yellow slop, gas production in the end of the tube and H_2S production blacking in the media(Cheesbrough, 2006).

3.2.2.2.9. Oxidase test

This test depends on the presence in bacteria of certain oxidases that will catalyse the transport of electrons between electron donors in the bacteria and aredox dye

tetramethyle – *P*-phenylene-diamine. The dye is reduced to adeep purple colour. Wet filter paper method. A strip of filter paper is soaked with a little freshly made 1% solution of the reagent and then at once used by rubbing a peck of culture on it with a platinum loop. Positive reaction is indicated by an intense deep –purple hue ,appearing within 5 -10 seconds, and negative reaction by absence of coloration or by coloration later than 60 seconds (Cheesbrough, 2006).

3.2.3. Susceptibility of bacterial isolates to antibiotic

Bacterial Isolates were subjected to antibiotic sensitivity analysis using the kirby Bauer disc diffusion method.The organisms' suspension was prepared from pure culture for each isolates and the turbidity of suspension was compared to McFarland turbidity standard. Mueller Hinton medium was used for disk diffusion test. The plate surface was inoculated using a swab that was impregnated in bacterial suspension standardized to match turbidity of the 0.5 McFarland turbidity standard, the plate was swabbed in three directions to insure complete distribution of the inoculums over entire plate, within 15 minute over inoculation the antimicrobial disks are applied then incubate at 37^{o}C aerobically. The disc used was commercially available and contained several antibiotic: Ciprofloxacin (5mcg), Amoxicillin (30mcg), Gentamicin (10mcg), Methicillin (5mcg), Imipenem (10mcg), CO-trimoxazole (25mcg), Tetracycline (30mcg) and Novobiocin (30mcg).

3.2.3.1. Application of antibiotic discs

Using sterile forceps, antibiotic discs were applied and evenly distributed on the inoculated plate. The plate was inverted and incubated aerobically at 37^{o}C, for 18-24 hours.

3.2.3.2. Reading of zones of inhibition

Using a ruler on the underside of the plate, the diameter of each zone of inhibition was measured in mm. The end point of inhibition was where the growth started.

3.2.3.3. Interpretation of the results

The zone size of each antibiotic was compared to their standard inhibiton zone on the chart provided by manufacture's.(Appendix 3).

CHAPTER FOUR

RESULTS

CHAPTER FOUR

RESULTS

Bacterial isolates (n=14) were obtained from the Research Laboratory (SUST). Biochemical test adopted for re-identification and their results were tabulated in table (1 and 2). These were *S. aureus* (4), S. *epidermidis(* 5), *S. intermidius(* 2), and *Ps*. *aeruginosa* (3).

Studies on the assessment of susceptibility of the isolates to antibiotics revealed that all isolate were susceptible (100%) to Ciprofloxacin, Gentamicin, Imipenem. Susceptibility to other antibiotic ranged from 0.0% to 80% Assessment susceptibility of each isolate to same antibiotic was found as follows, all 4/4 *S*. *aureus* isolate were susceptible to Ciprofloxacin and Tetracycline and Gentamicin, Imipenem, Amoxicillin, Cotrimoxazole, and Novobiocin. and 3/4 to Azithromycin, 2/4 to Methicillin.

The 2 isolate of *S*. *intermidius* were susceptible to ciprofloxacin, Tetracycline, Gentamicin, Imipenem, Azithromycin, Amoxicillin, Cotrimoxazole, Novobiocin, one was susceptible to Methicillin. The 5 isolates of *S*. *epidermidis* were susceptible to Ciprofloxacin, Gentamicin, Imipenem, Novobiocin and 4/5 were susceptible to Amoxicillin and Tetracycline.3/5 were susceptible to Methicillin and cotrimoxazole , 2/5 were susceptible to Azithromycin .

The 3 isolate of *Ps*. *aeurginosa* were susceptible to Ciprofloxacin, Gentamicin, Imipenem, Azithromycin, and 3 isolate were resistant to Novobiocin, Cotrimoxazole, and one isolate was susceptible to Amoxicillin, Methicillin, Tetracycline Table (3).

Antibiotic susceptibility (%) showed in (Table 4).

Table 1. Re-identification of Gram- negative bacterial isolates

Isolate code	Biochemical test								Identified organism
	KIA				Urease	Indole	Citrate	oxidase	
	S	B	G	H_2S					
C1	R	R	_	_	Negative	negative	positive	positive	*Pseudomonas aeruginosa*
C2	R	R	_	_	Negative	negative	positive	positive	*Pseudomonas aeruginosa*
C3	R	R	_	_	Negative	negative	positive	positive	*Pseudomonas aeruginosa*

Table 2. Re-identification of Gram- positive bacterial isolates

Isolate code	Biochemical test				Identified organism
	Catalase	Coagulase	Mannitol fermentation	DNase	
C4	Positive	positive	positive	positive	*S. aureus*
C5	Positive	negative	negative	positive	*S. intermidius*
C6	Positive	negative	negative	negative	*S. epidermidis*
C7	Positive	positive	positive	positive	*S. aureus*
C8	Positive	negative	negative	negative	*S. epidermidis*
C9	Positive	positive	positive	positive	*S. aureus*
C10	Positive	positive	positive	positive	*S. aureus*
C11	Positive	negative	negative	negative	*S. epidermidis*
C12	Positive	negative	negative	positive	*S. intermidius*
C13	Positive	negative	negative	negative	*S. epidermidis*
C14	Positive	negative	negative	negative	*S. epidermidis*

Table 3. Susceptibility of bacterial isolates to antibiotics

Antibiotics	Susceptibility of			
	S. aureus **n=4**	***S. epidermidis*** **n=5**	***S. intermidius*** **n=2**	***Ps. aeurginosa*** **n=3**
CIP (5mcg)	4/4	5/5	2/2	3/3
TE (30mcg)	4/4	4/5	2/2	1/3
GEN (10mcg)	4/4	5/5	2/2	3/3
MET (5mcg)	2/4	3/5	1/2	1/3
IPM (10mcg)	4/4	5/5	2/2	3/3
AZM (15mcg)	¾	2/5	2/2	3/3
AMX (30mcg)	4/4	4/5	2/2	3/3
COT (25mcg)	4/4	3/5	2/2	0/3
NV (30mcg)	4/4	5/5	2/2	0/3

Key:

CIP=Ciprofloxacin, MET=Methicillin**,** TE=Tetracycline, IPM=Imipenem**,** GEN=Gentamicin, AZM=Azithromycin**,** AMX=Amoxicillin, COT=Cotrimoxazole**,** NV=Novobiocin

Table 4. Antibiotic susceptibility (%)of bacterial isolates

Antibiotic	**Abbreviation**	**Susceptibility (%)of bacterial isolates**			
		S. aureus **n=4**	***S. epidermidis*** **n=5**	***S. intermidius*** **n=2**	***Ps. aeruginosa*** **n=3**
Ciprofloxacin	CIP	100	100	100	100
Amoxicillin	AMX	100	80	100	33.3
Gentamicin	GEN	100	100	100	100
Azithromycin	AZM	75	40	100	100
Methicillin	MET	50	60	50	33.3
Imipenem	IPM	100	100	100	100
CO-tri moxazole	COT	100	60	100	0
Tetracycline	TE	100	80	100	33.3
Novobiocin	NV	100	100	100	0

CHAPTER FIVE

DISCUSSION

CHAPTER FIVE
DISCUSSION

Traditionally, the white coat is thought to bring credibility and dignity to the medical profession (Muhadi *et al*., 2007). However, white coats have been shown to harbor potential contaminants and so these may have a role in the nosocomial transmission of pathogenic microorganisms. The high rates of the bacterial contamination of white coats may be associated with the following two facts: Firstly, patients continuously shed infectious microorganisms in the hospital environment, and the health care providers are in constant contact with these patients. Secondly, it has been demonstrated that bacteria can survive between 10 and 98 days on fabrics which are used to make white coats, which include cotton, cotton and polyester, or polyester materials (Banu *et al*., 2012)

Various studies have found contamination of HCWs, clothing during patient care activities and transmission of bacteria through uniforms and white coats. these contaminated clothing act as a reservoir hence even after proper hand hygiene procedures, the hands of HCWs may get re-contaminated allowing transmission of pathogens to patients or the environment .

This study was conducted to assess the susceptibility of potentially pathogenic bacteria isolated from physician's white coats to selected antibiotics .

These isolates were *S. aureus*, *S. epidermidis*, *S. intermidius*, and *Ps. aeruginosa*. Study on antibiogram of *S.aureus* revealed that high susceptibility to ciprofloxacin (100%) and gentamicin (100%). These results are high than the results of Uneke and Ijeoma (2010) in Nigeria who reported susceptibility of *S.aureus* as ciprofloxacin (97.8%) and Gentamicin (32.6%). Moreover *S.aureus* isolates were (50%) resistant to Methicillin (MRSA). This result was higher than that obtained by Priya *et al* (2009) in India who reported that *S.aureus* were 18% resistant to Methicillin. This variation may be attributed to number of *S.aureus* under test in the two studies. In the present study the susceptibility of *Ps. aeuroginosa* to cotrimoxazole was 0%. This result is similar to Uneke and Ijeoma (2010) in Nigeria who reported that that *Ps. aeruginosa* isolate were susceptible to cotrimoxazole as 0% . On other hand, susceptibility of *Ps. aeruginosa* to ciprofloxacin (100%) was higher than result of Uneke and Ijeoma (2010) in who reported susceptibility to *Ps.aeruginosa* to ciprofloxacin as 26.1%.

Conclusion

The study concluded that all isolates were susceptible 100% to Ciprofloxacin, Gentamicin and Imipenem.

Recommendation

1. Modified Kirby–Baurer method must be adopted for all microbiological laboratories to assess susceptibility of clinical isolates to different antibiotics.

2. The wearers of the white coats should washed their white coats and exclude it from the non clinical areas of the hospital such as the libraries and the dining room.

3. Further studies with a large number of bacterial isolates are highly recommended to validate these finding .

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APPENDICES

Appendix (1):

A) Culture media

Difco™ Nutrient Agar

Approximate formula*per Liter

Beef Extract …….....…3.0g

Peptone……...….5.0g

Agar ……...15.0g

Difo ™ MacConkey Agar

Approximate formula*per Liter

Peptone…….....20.0g

Lactose……..…....10.0g

Bile Salts……..5.0g

Sodium Choloride…….....12.0g

Agar…….....12.0g

Nutrient Red ……..….0.05g

Difco™ Manitol Salt Agar

Approximate formula*per Liter

Proteose Peptone No.3……..……10.0g

Beef Extract……...1.0g

D-Manitol ……10.0g

Sodium Choloride……75.0g

Agar……..15.0g

Phenol Red……25.0g

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 -+ o.1 at 25C

Kliglar iron agar (KIA)

Lab-Lemco powder……..…..3.0g /l

Yeast extract …….……3.0g/l

Peptone……....20.0g/l

Sodium chloride ……...5.0g/l

Lactose ……...10.0g/l

Dextrose (glucose)……....1.0g/l

Ferric citrate……..….0.3g/l

Sodium thiosulphate ……...…..0.3g/l

Phenol red ……...0.05g/l

Agar …….12.og/l

DNAse agar

Tryptose……20g/l

Deoxyribonuclic acid…….2g/l

Sodium chloride ……5g/l

Agar……12g/l

Christensen's urea agar

Glucose ……..5g

Sodium chloride……..…5g

Potassium dihydrogen phosphate ……..2g

Peptone ……...…1g

Agar……20g

Distilled water ……1 liter

Simmons' citrate medium

Koser's medium ……....1liter

Agar ……..…20g

Bromothymole blue, 0.2%.....40ml

B) Preparation of reagents

1.Gram's Stain reagent

Crystal violet

Approximate formula *per liter

Crystal violet…….……20.0g

Ammonium oxalate……..……..9.0g

Ethanol,obsolute ……..……..95ml

Distilled water …….……to 1 liter

Lugols Iodine

Approximate formula *per liter

Potassium iodine ……..……...…20.0g

Iodine ……..10.0g

Distilled water…….….to 1 Liter

Acetone- alcohol decolorizer

Approximate formula* per liter

Acetone…….….500ml

Ethanol, absolute……475ml

Distilled water……..…..25 ml

Saffranin

Approximate formula*per liter

Saffranin ……....2.5g

95% ethanol……..10 ml

Distilled water……...…to 100ml

2.Physiological saline (8.5g/l)

Sodium chloride ……...…...8.5g

Distilled water ……..……..to 1 liter

3.Hydrochloric acid

Hydrochloric acid,concentrated ……...8.6ml

Distilled water …….……to 100ml

4.Kovac's reagent

Approximate formula*per liter

Amyle or isoamyle alcohol ……..15ml

P-Dimethyl- aminobenzaldehyde……..…..10g

Hydrochloric acid concentrated ……..…….50ml

5.Hydrogen peroxide

H_2O_2 solution ……10 vol

Appendix 2: Diameter of inhibition zones of various discs of antibiotics

Code of Isolate	CIP	AMX	GEN	AZM	MET	IPM	COT	TE	NV
S 1	28	21	20	25	7 R	42	25	27	28
S 2	32	16	24	11 R	17	45	25	28	24
S 3	32	16	21	25	6 R	45	25	30	30
S 4	35	33	25	34	17	50	35	30	27
S.e 1	28	34	19	38	15	42	35	32	30
S.e 2	28	11 R	25	14	18	28	9	15	33
S.e 3	34	17	26	16	16	50	29	25	32
S.e 4	35	23	28	10 R	5 R	55	27	35	27
S.e 5	40	22	15	9 R	6 R	55	8	40	30
S.i 1	37	22	24	38	7 R	45	30	30	29
S.i 2	30	35	25	19	10	45	23	25	30
Ps.1	43	12 R	20	25	14	34	9 R	15	12 R
Ps. 2	38	18	21	32	5 R	30	8 R	12 R	15 R
Ps.3	32	11 R	21	34	6 R	40	7 R	11R	13 R

against bacteria

Key:

S = *Staphylococcus aureus* *R=resistant*

S.e =*Staphylococcus epidermidis*

S.i = *Staphylococcus intermidius*

Ps =*pseudomonas aeruginosa*

Appendix 3: Himedia zone size Interpretation

Antimicrobial Agent	Symbol	Disc Content	Interpretation Criteria		
			Sensitive (mm or more)	Intermediate (mm)	Resistant (mm or less)
Ciprofloxacin	CIP	5 mcg	21	16-20	15
Tetracycline	TE	30mcg	19	15-18	14
Cotrimoxazole	COT	25mcg	16	11-15	10
Amoxicillin	AMX	30mcg	18	14-17	13
Azithromycin	AZM	15mcg	28	14-17	13
Novobiocin	NV	30mcg	22	18-21	17
Imipenem	IPM	10mcg	16	14-15	13
Methicillin	MET	5mcg	14	10-13	9
Gentamicin	GEN	10mcg	15	13-14	12