بسم الله الرحمن الرحيم

Sudan University of Science &Technology
College of Graduate Studies

Assessment of Antibiotics Susceptibility of Bacterial Isolates from Stethoscopes in Khartoum State Hospitals

تقويم حساسية العزلات الباكتيرية من السماعات الطبية في مستشفيات ولاية الخرطوم للمضادات الحيوية

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

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2014

الآيـــــة

قَالَ تَعَـالَىٰ:

﴿ وَقُلِ ٱعۡمَلُواْ فَسَيَرَى ٱللَّهُ عَمَلَكُمۡ وَرَسُولُهُۥ وَٱلۡمُؤۡمِنُونَۖ وَسَتُرَدُّونَ إِلَىٰ عَٰلِمِ ٱلۡغَيۡبِ وَٱلشَّهَٰدَةِ فَيُنَبِّئُكُم بِمَا كُنتُمۡ تَعۡمَلُونَ ١٠٥ ﴾

صدق الله العظيم

سورة التوبة: الآية (105)

DEDICATION

To my lovely mother, father, grandmother, my sister Leena and brothers suhil and jihad

To the meaning of honest my friends

I dedicate my work

ACKNOWLEDGEMENT

First and all thanks to ALMIGHTY ALLAH who facilitates everything in my life. A great thank to Sudan University of Science and Technology {SUST} College of Medical Laboratory Science for giving me a chance to study in this big edifice.

I would like to thank all teaching staff in this university for being my inspirer in the learning way. Special thanks to my supervisor **Prof. Humodi A. Saeed** for his advice and help to make this research successful, for sharing his valuable time and for giving me fruitful information to finish this project.

Thanks are extended to the technicians Suhair Ramadan and Omer Ibrahim Elzine. I would have not finished this project without the support of my family who has always been there for me whenever I need them and their love to empower me that never fails all the times.

Thanks to my friends: Asmaa Ahmad, Zeinab Mohammed, Doaa Moneer, Sara Tag Alsir and Tagreed Babekir for their help and comments.

ABSTRACT

The stethoscope; a universal tool of medical profession is often used on multiple patients and it's an additional possible vector of infection as they touch many patients. This study was done to assess antibiotics susceptibility of bacteria isolated from stethoscopes in Khartoum hospitals. The study was conducted during the period from April and July 2014.

Bacterial isolates were obtained from the Research Laboratory of Sudan University of Science and Technology. Purity of the isolates was checked by streaking on nutrient agar. Re-identification was carried out by conventional microbiological methods including Gram stain and biochemical tests. Modified Kirby-Bauer disk diffusion method was adopted to assess susceptibility of the isolates against traditionally used antibiotics.

The results revealed that a total of 136 bacterial isolates were re-identified as follows; *S. epidermidis* 37; *S. warneri* 16; *S. aureus* 14; *P. aeruginosa* 12; *S. haemolyticus* 11; *K. pneumonia* 11; *E. coli* 10; *S. hominis* 7; *S. lugdunensis* 6; *Proteus* spp.6; and *S. saprophyticus* 5.

Study on susceptibility of bacterial isolates to different antibiotics revealed that *E. coli* were susceptible (100%) to Ciprofloxacin, Cephalothin and Chloramphenicol; *Klebseilla pneumoniae* were susceptible (91%) to Gentamicin and Ampicillin; *Protus* species were susceptible (100%) to Gentamicin and Ciprofloxacin and *Psedomonas*

aeruginosa were susceptible (100%) to Imipenem, Ciprofloxacin and Gentamicin. Isolates of *Staphylococcus aureus* were susceptible to Ciprofloxacin, Erythromycin and Streptomycin (100%), (71%) and (64%) respectively. On the other hand, Streptomycin was the most potent antibiotic against all *Staphylococcus* species.

The study concluded that susceptibility of Gram-negative rods was high (100%) to Gentamicin, while Gram-positive cocci was high (100%) to Streptomycin. Further studies are required to validate the results of this study.

المستخلص

سماعة الطبيب أداة عالمية لمهنة الطب وكثيرا ما تستخدم للعديد من المرضى وهي أيضا ناقل محتمل للعدوى لأنها تلمس العديد من المرضى, لذلك أجريت هذه الدراسة لتقييم حساسية الجراثيم للمضادات الحيوية للبكتريا المعزولة من السماعات في مستشفيات الخرطوم . أجريت هذه الدراسة في الفترة من أبريل وحتى يوليو لعام 2014.

العزلات الباكتيرية تم الحصول عليها من مختبر الأبحاث بجامعة السودان للعلوم والتكنولوجيا . أستزرعت العزلات على وسط الأجار المغذي للتأكد من نقاوتها و أعيد التعرف عليها بطرق الأحياء الدقيقة التقليدية متضمنة صبغة غرام و الإختبارات البيوكيميائية. أستخدمت طريقة كيربي-باور لنشر القرص لتقييم الحساسية للعزلات ضد المضادات الحيوية التقليدية. وأظهرت النتائج أن من مامجموعه 136 عزلات باكتيرية تم التعرف عليها كالأتي؛ المكورات العنقودية البشروية 37؛ المكورات العنقودية ويرنياي16؛ المكورات العنقودية الذهبية 14؛ الزائفة الزنجارية 12؛ المكورات العنقودية الحالة للدم 11؛ الكليبسيلا الرئوية 11؛ الإسكريشية القولونية 10؛ المكورات العنقودية هومينيس 7؛ المكورات العنقودية لقدونينسيس 6؛ أنواع المتقلبة الرائعة 6 و المكورات العنقودية المترممة 5.

الدراسة على حساسية العزلات الباكتيرية لمختلف المضادات الحيوية أظهرت أن الإسكريشية القولونية كانت حساسة 100% للسبروفلوكساسين, السيفالوتين و الكلورامفينيكول؛ الكليبسيلا الرئوية كانت حساسة 91% للجنتاميسين و الأمبيسيلين؛ أنواع المتقلبة الرائعة كانت حساسة 100% للجنتاميسين و السبروفلوكساسين وعزلات الزائفة الزنجارية كانت حساسة 100% للإيميبينيم، السيبروفلوكساسين والجنتاميسين. عزلات المكورات العنقودية الذهبية كانت حساسية للسيبروفلوكساسين, الإيريثرومايسين و الإستربتومايسين 100%, 71% و 64% بالترتيب. من ناحية أخرى, كان الستربتومايسين المضاد الحيوي الأكثر فعالية ضد كل أنواع المكورات العنقودية.

خلصت هذه الدراسة إلى أن إختبار حساسية المضادات الحيوية للعصيات سلبية الغرام كانت عالية 100% للجنتاميسين, في حين كانت المكورات العنقودية عالية 100% للستربتومايسين. إجراء المزيد من الدراسات مطلوب لدعم نتائج هذه الدراسة.

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LIST OF ABBRIVIATIONS

GEN: Gentamicin

STX: Co-trimoxazole

E: Erythromycin

IMP: Imipenem

MRP: Meropenem

NA: Nalidixic Acid

OF: Ofloxacin

PI: Piperacillin

S: Streptomycin

TE: Tetracycline

VA: Vancomycin

CTR: Ceftriaxone

CEP: Cephalothin

C: Chloramphenicol

CIP: Ciprofloxacin

AK: Amikacin

AMP: Ampicillin

AG: Agumentin

LIN: Lincomycin

CLSI: Clinical Laboratory Standard Institute

ATCC: American Type Culture Collection

HCAI: Health Care-acquired Infection

CDC: Center for Disease Control and Prevention

NNIS: National Nosocomial Infections Surveillance

WHO: World Health Organization

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

CHAPTER ONE

1. INTRODUCTION AND OBJECTIVES

1.1. Introduction

The stethoscope; a universal tool of medical profession is often used on multiple patients except in services such as Intensive Care Units and Neonatal Special Care Units a single stethoscope is often used for all indoor as well as outdoor patients. A routine of disinfection of stethoscope is hardly ever undertaken (Smith *et al*., 1996).

Stethoscopes are an additional possible vector of infection as they touch many patients. (Gerken *et al*., 1972). Yet standard sources on infection control still give no advice on cleaning these instruments (Ayliffe *et al*., 1990).

Both the diaphragm and ear-pieces of physician's personal stethoscopes and bedside stethoscopes are frequently colonized with a variety of pathogenic organisms including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) which cause significant morbidity and mortality on the intensive care unit (ICU) (Jones *et al.,* 1995).

Despite remarkable advances in medical research and treatment during the 2othcentury, infectious diseases remain among the leading cause of death worldwide (NIAID, 2010). Of these, nosocomial infections comprise about 5 to 10% (Culver *et al.,* 1985).

It has been estimated that one third of all nosocomial infections may be preventable and are frequently caused by organisms acquired within the hospital environment (Hughe, 1988).

In the United Kingdom, studies have shown that approximately 10% of patients in hospitals are admitted with infection and a further 10% acquire infection while receiving care (Plowman *et al.,* 2000). Recommendations for infection control practices in hospitals are well documented and updated on a regular basis (Garne, 1996). Some studies have evaluated *Staphylococcus aureus* in contamination of various items such as stethoscopes (Cohen and Matalon, 1997).

Hospital acquired infections are frequently caused by microorganisms in the hospital environment and are a significant cause of morbidity and mortality. They also result in increased health care costs. About one third of all nosocomial infection is preventable (Hughe, 1988).

For planning preventive actions, it is essential to identify the reservoirs of microorganisms that cause nosocomial infections. Hands of the hospital staff, medical equipment such as catheters, surgical instruments, implants, ventilators, endoscopes, thermometers, ultrasound probes, otoscopes, etc. may all serve as the reservoir for microorganisms (Verghese and Patel, 1999).

Cleaning stethoscopes with isopropyl alcohol dramatically reduces the number of bacterial colonies on the diaphragm by 94-100% (Bernard *et al.,* 1999).

Examples of such antibiotic-resistant organisms are ceftazidime-resistant *Klebsiella pneumoniae*, vancomycin-resistant enterococci, methicillin-resistant

staphylococci, ciprofloxin-resistant *Pseudomonas aeruginosa*, gentamicin-resistant *Psedomonas aeruginosa*, and penicillin-resistant pneumococci *(*Parmar *et al.,* 2004).

1.2. Rationale

Bacterial contamination of the stethoscope was significant. It can also be noted that the hospital institutions studied are at a particular risk of having microbial population with high antimicrobial resistance. The isolates were potential pathogens and resistant to multiple classes of antibiotics. There are increasing reports of the tremendous risk of transmitting antibiotics resistant bacteria from one patient to another from stethoscopes. Because most of hospital acquired infections are primarily nosocomial and not autoinfection (Hoogkamp *et al.,* 1982*),* their acquisition in the hospital environment adds to morbidity, mortality and economic costs (Parmar *et al.,* 2004).

1.3. Objectives

1.3.1. General objective

To assess antibiotic susceptibility of bacteria isolated from Stethoscopes.

1.3.2. Specific objectives

1. To check purity of bacteria isolates obtained from the Research Laboratory.

2. To confirm identification of the isolates.

3. To perform antibiotic susceptibility test using modified Kirby-Bauer Disk diffusion method.

CHAPTER TWO

LITERATURE REVIEW

CHAPTER TWO

2. LITERATURE REVIEW

2.1. Stethoscope

2.1.1. Definition

The stethoscope is an acoustic medical device for auscultation, or listening to the internal sounds of an animal or human body. It is often used to listen to lung and heart sounds. It is also used to listen to intestines and blood flow in arteries and veins (Ananthi, 2006).

2.1.2. History

The stethoscope was invented in France in 1816 by Laennec at the Necker-Enfants Malades Hospital in Paris. It consisted of a wooden tube and was monaural. The device was similar to the common ear trumpet, a historical form of hearing aid; indeed, his invention was almost indistinguishable in structure and function from the trumpet, which was commonly called a “microphone”. In 1840, Golding Bird described a stethoscope he had been using with a flexible tube. Bird was the first to publish a description of such a stethoscope but he noted in his paper the prior existence of an earlier design (which he thought was of little utility) which he described as the snake ear trumpet. Bird’s stethoscope had a single earpiece (Osler, 1917).

In 1851, Irish physician Arthur Leared invented a binaural stethoscope, and in 1852 George Cammann perfected the design of the instrument for commercial production, which has become the standard ever since. By 1873, there were descriptions of a differential stethoscope that could connect to slightly different locations to create a slight stereo effect, though this did not become a standard tool in clinical practice. The medical historian Jacalyn-Duffin has argued that the invention of the stethoscope marked a major step in the redefinition of disease from being a bundle of symptoms, to the current sense of a disease as a problem with an anatomical system even if there are no noticeable symptoms. This re-conceptualization occurred in part, Duffin argues, because prior to the stethoscopes, there were no non-lethal instruments for exploring internal anatomy (Duffin-Jacalyn, 2012).

Rappaport and Sprague designed a new stethoscope in 1940s, which became the standard by which other stethoscopes are measured, consisting of two sides, one of which is used for the respiratory system, the other for the cardiovascular system. Several other minor refinements were made to stethoscopes, until in the early 1960s Dr. David Littmann, a Harvard Medical School professor, created a new stethoscope that was lighter than previous models and had improved acoustics. In the late 1970s, Littmann introduced the tunable diaphragm: a very hard glass- epoxy resin diaphragm member with an overmolded silicone flexible acoustic

surround which permitted increased excursion of the diaphragm member in a ‘z”-axis with respect to the plane of the sound collecting area (Littmann, 1961).

2.1.3. Types of stethoscopes

2.1.3.1. Acoustic stethoscope

Acoustic stethoscopes are familiar to most people, and operate on the transmission of sound from the chest piece, via air- filled hollow tubes, to the listener’s ears. The chest piece usually consists of two sides that can be placed against the patient for sensing sound; a diaphragm (plastic disc) or bell (hollow cup). This two-sided stethoscope was invented by Rappaport and Sprague in the early part of the 20th century (Finkeistein, 2008).

2.1.3.2. Electronic stethoscope

An electronic stethoscope (or stethophone) overcomes the low sound levels by electronically amplifying body sounds. Electronic stethoscopes require conversion of acoustic sound waves to electrical signals which can then be amplified and processed for optimal listening. Unlike acoustic stethoscopes, which are all based on the same physics, transducers in electronic stethoscopes vary widely. Because the sounds are transmitted electronically, an electronic stethoscope can be a wireless device, can be a

recording device, and can provide noise reduction, signal enhancement, and both visual and audio output.

Electronic stethoscopes are also used with Computer-aided Auscultation programs to analyze the recorded heart sounds pathological or innocent heart murmurs (Palaniappan *et al.,* 2013).

2.2. Hospital acquired infection

2.2.1. Definition

Nosocomial infection is clearly defined by the Center for Disease Control and Prevention (CDC) in the National Nosocomial Infections Surveillance (NNIS) system as a "localized or system condition (WHO, 2009) that results from adverse reaction to the presence of an infectious agent(s) or its toxin(s); that was not present or incubating at the time of admission to the hospital" (Garner *et al*., 1988). Thus, infections that are unrelated to the admitting diagnosis that develop within 48 hours after admission are considered to be nosocomial infections. Nosocomial infections have traditionally referred to infections that develop during hospitalization and so have also been known as hospital-acquired infections. As health care increasingly expands beyond hospitals into outpatient settings, nursing homes, long-term care facilities, and even home care settings, the more appropriate term has become healthcare-acquired infection (HCAI). Nosocomial infections may be considered either as endemic or epidemic. Epidemic infections

occur during outbreaks, when an unusual increase above the baseline of a specific infection or infecting organism occurs (Lynch *et al*., 1997).

By any name, nosocomial infections are a significant problem throughout the world and are increasing. For example, nosocomial infection rates range from as low as 1% in a few countries in Europe and the Americas to more than 40% in parts of Asia, Latin America and sub-Saharan Africa (Lynch *et al*., 1997).

In the US, nosocomial infections affect more than 2 million patients each year (about 5-10% of hospitalized patients) leading to approximately 90,000 deaths per year (Weinstein *et al*., 1998).

The patient is exposed to a variety of microorganisms during hospitalization. Contact between the patient and a microorganism does not by itself necessarily result in the development of clinical disease. A healthy human body has several defences against infection: the skin and mucous membranes form natural barriers to infection, and immune responses (nonspecific and specific) are activated to resist microorganisms that are able to invade. The skin can effectively protect the body from most microorganisms unless there is physical disruption (Beers & Berkow, 1999).

Other disrupters of the natural barrier are lesions or injury or, in the healthcare setting, invasive procedures or devices. In addition to breaks in the skin, other primary entry points for microorganisms are mucosal surfaces, such as the respiratory, gastrointestinal, and genitourinary tracts (Pier *et al.,* 2004).

The membranes lining these tracts comprise a major internal barrier to microorganisms due to the antimicrobial properties of their secretions. The respiratory tract filters inhaled microorganisms, and mucociliary epithelium in the trachea-bronchial tree moves it out of the lung. In the gastrointestinal tract, gastric acid, pancreatic enzymes, bile, and intestinal secretions destroy harmful microorganisms. Commensal bacteria make up the normal flora in the gastrointestinal tract and act as protection against invading pathogenic bacteria (WHO, 2009).

The likelihood of exposure leading to infection depends partly on the characteristics of the microorganisms, including resistance to antimicrobial agents, intrinsic virulence, and amount (inoculum) of infective material. Nosocomial infections are commonly caused by bacteria. They can also be caused by viruses, fungi, and parasites, but these types of infection occur less frequently, especially those caused by parasites (e.g., scabies), and often do not carry the same risks of morbidity and mortality as bacterial infections (Weinstein *et al*., 1998).

Microbes that cause nosocomial infections can be acquired in several ways:

1. The permanent or transient flora of the patient (endogenous infection)

Bacteria present as the normal flora cause infection because of transmission to sites outside the natural habitat (urinary tract), damage to tissue (wound) or inappropriate antibiotic therapy that allows overgrowth (*C. difficile*, *Candida* spp.).

2. Flora from another patient or member of staff (exogenous cross-infection)

Bacteria are transmitted between patients:

1) Through direct contact between patients (hands, saliva droplets or other body fluids).

2) In the air (droplets or dust contaminated with bacteria from a patient).

3) Through staff contaminated through patient care (hands, clothes, nose and throat) who becomes transient or permanent carriers, subsequently transmitting bacteria to other patients by direct contact during care.

4) Through objects contaminated by the patient (including equipment such as stethoscopes and otoscopes), visitors or other environmental sources (e.g. water, other fluids, food).

3. Flora from the health care environment (endemic or epidemic exogenous environmental infections)" (Tietjen *et al*., 2003).

Antibiotic use is an important factor in the development of nosocomial infections. The inappropriate use of antibiotics is a major contributor to the increase in drug-resistant strains of bacteria, and coupled with the natural selection and exchange of genetic resistance elements with microorganisms, drug resistance has emerged as a

worldwide problem, with an increasing number of microorganisms becoming resistant to treatment each year (Knobler *et al*., 2003).

Resistance typically emerges first in the healthcare setting before the community, and drug-resistant bacteria have become the source of approximately 70% of nosocomial infections (Burke, 2003).

In addition, nosocomial infections caused by drug-resistant bacteria are associated with higher rates of morbidity and mortality and other costs (Knobler *et al.*, 2003).

CHAPTER THREE

MATERIALS AND METHODS

CHAPTER THREE

3. MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a laboratory-based experimental study for bacteria isolated from stethoscopes.

3.1.2. Study area

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

3.1.3. Study duration

The study was conducted during the period from April and July2014.

3.1.4. Sample size

This study was done using 136 isolates previously isolated from stethoscopes.

3.2. Source of isolates

This isolates were obtained from the Research Laboratory of Sudan University of Science and Technology, which isolated from stethoscopes in Military Hospital,

Bahry Hospital and Ebraheem Malik Hospital. The isolates were checked for purity and then re-identified by conventional bacteriological methods.

3.3. Experimental work

3.3.1. Purification of isolates

The isolates were streaked on nutrient agar and incubated over-night at 37ºC; a descript colony was picked up and checked for purity under microscope, and then stored in Bijou bottle for further investigations.

3.3.2. Re-identification of the isolates

3.3.2.1. Gram's stain (Appendix 1)

Smear was prepared from overnight culture on clean and dry slide. The smear was left to air dry and fixed was done by rapid pass the slide three times through the flame of a Bunsen burner then allowed to cool before staining. Crystal violet stain was added to smear for 30–60 seconds, and then washed by tap water. Lugol's iodine was added for 30-60minutes then washed by tap water and decolorized rapidly (few seconds) with acetone alcohol and washed immediately by tap water. Finally, the smear was covered with saffranin stain for 2 minutes and washed by tap water. The back of slide was wiped clean and placed in a draining rack for smear to air dry. Drop of oil was added to the dried smear and examined under the light microscope (Carl Zeiss, Germany) by oil lens 100X (Cheesbrough, 2006).

3.3.2.2. Biochemical tests

3.3.2.2.1. Fermentation of sugars, gas and H_2Sproduction

Kligler Iron Agar (KIA) (appendix 2) was used to determine whether Gram-negative rods utilize glucose and lactose fermentatively and form gas and hydrogen sulphide (H_2S). The organism under test was inoculated in KIA medium and incubated at 37ºC for 18-24 hrs. The lactose fermenter organism gave yellow slope and yellow butt, while non lactose fermenter organism gave yellow slope and red butt. The production of H_2S was detected by formation of black colour. The gas production also had been examined (Forbes *et al*., 2002).

3.3.2.2.2. Indole production

This test was used to differentiate Enterobacteriaceae and other genera. The organism under test was inoculated into peptone water (Appendix 3). The tubes were incubated overnight at 37ºC. The detection of indole was done by addition of Kovac's reagent (the component seen in appendix 8) after the incubation period, which gave red ring in the positive result, and yellow or green ring in the negative result (Forbes *et al*., 2002).

3.3.2.2.3. Urease test

This test was used to differentiate bacteria that produce urease enzyme and that not urease producer. The organism under test was inoculated by using of sterile straight wire in a medium that contain urea and phenol red indicator (Appendix 4). The tubes were incubated overnight at 37ºC. If the strain is urease producing, the enzyme will break down the urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium become alkaline as shown by a change in the colour of the indicator to pink (Forbes *et al*., 2002).

3.3.2.2.4. Citrate utilization test

This test was used to determine the ability of an organism to utilize sodium citrate as it is only carbon source and inorganic ammonium salts as it is sole nitrogenous source. By using of sterile wire loop the organism under test was inoculated in Kosser's citrate medium (Appendix 5) and then incubated overnight at 37ºC. Positive result gave blue colour (Forbes *et al*., 2002).

3.3.2.2.5. Catalase test

Catalase enzyme causes hydrolysis of hydrogen peroxide solution. Production of an active air bubbles indicated a positive result. Sterile wooden stick was used to remove several colonies of the test organisms and immerse into hydrogen peroxide solution (Appendix 11) (Cheesbrough, 2006).

3.3.2.2.6. Coagulase test

Coagulase enzyme causes plasma to clot by converting fibrinogen to fibrin. A drop of plasma was placed on a clean and dry glass slide. A drop of saline was used as a negative control. By using wooden stick a portion of the isolated colony was emulsified in each drop. Microscopic clumping within 10 seconds considers 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 is the positive result (Forbes *et al*., 2002).

3.3.2.2.7. DNase test

The test organism is cultured on medium which contains DNA (Appendix 6). After overnight incubation, the colonies are tested for DNase production by flooding the plate with a weak hydrochloric acid solution. The acid precipitates un-hydrolyzed DNA. DNase producing colonies are therefore surrounded by clear areas indicating DNA hydrolysis (Cheesbrough, 2006).

3.3.2.2.8. Manitol fermentation test

The organism under test was inoculated on Manitol Salt Agar (MSA) (Appendix 7). Manitol fermented organism indicated by yellow colour, while non manitol fermented organism gave pink colour. Wire loop was used to tough the surface of nutrient agar slope and transfer to culture it in MSA. The media were incubated at 37ºC for 24-48 hours. After incubation period, the plates were examined for significant growth by counting the bacterial colonies. The morphology characters (size, shape, odor and pigment) were observed (Cheesbrough, 2006).

3.3.3. Assessment of antimicrobial sensitivity test of the isolates

Modified Kirby-Bauer disc diffusion method was performed according to the instructions of NCCLS (2012).

3.3.3.1. Antibiotics

The following antibiotics were obtained from Himedia laboratories PVT. Ltd. INDIA:

Ciprofloxacin (CIP) 5 µg, Co-trimoxazole (STX) 25 µg, Tetracycline (TET) 30 µg, Pepracillin (PI) 100µg, Amikacin (AK) 30µg , Gentamicin (GEN) 10 µg, Ampicillin (AMP)10µg, Imipenem (IMP)10µg, Meropenem (MRP) 10µg, Chloramphenicol (C) 30 µg, Ofloxacin (OF) 5µg, Vancomycin (VA) 30µg, Erythromycin (E) 15µg, Agumentin (AG) 30µg, Cephalothin (CEP) 30µg,

Streptomycin (S) 30 µg, Clindamycin (CD) 2µg, Nalidixic acid (NA) 30µg, Lincomycin (LIN) 10µg, Ceftriaxone (CTR) 30µg.

3.3.3.2. Control strain

Recommended organisms for quality assurance purposes are *Staphylococcus aureus* ATCC 25923 (BSL 2), *Escherichia coli* ATCC 25922 (BSL 1), and *Pseudomonas aeruginosa* ATCC 27853 (BSL 2), as the zone of inhibition for these organisms is known.

3.3.3.3. Preparation of inoculums

Sterile inoculating loop was used to touch four to five isolated colonies of the organism to be tested. The organism was suspended in 2 ml of sterile saline. The turbidity of this suspension was adjusted to a 0.5 Mc Farland standard (Appendix 11) by adding more organisms if the suspension is too light or diluting with sterile saline if the suspension are too heavy. This suspension was used within 15 minutes of preparation (Cheesbrough, 2006).

3.3.3.4. Seeding of the plates

A sterile non toxic cotton swab was dipped into the inoculums tube and then the swab was rotated against the side of the tube using firm pressure, to remove excess fluid. The plate of Muller Hinton agar (Appendix 7) was

inoculated by streaking the swab three times over the entire agar surface rotate the plate approximately 60 degrees each time to ensure an even distribution of the inoculums. The swab was discarded into an appropriate container. The plate was allowed to sit at room temperature at least 3 to 5 minutes, but no more than 15 minutes, for the surface of the agar plate to dry before proceeding to the next step (Cheesbrough, 2006).

3.3.3.5. Application of antibiotic discs

An appropriate antimicrobial-impregnated disks was applied on the surface of the agar, using either forceps to dispense each antimicrobial disk one at a time, or a multi-disk dispenser to dispense multiple disks at one time. The lid of the Petri-dish was partially removed. The disk was placed on the plate over one of the dark spots on the template and the disc was gently pressed with the forceps to ensure complete contact with the agar surface (Cheesbrough, 2006).

3.3.3.6. Incubation of the plates

The plate was inverted and placed in a 35°C air incubator for 16 to 18 hours. The results red after 18 hours of incubation unless you are testing *Staphylococcus* against Oxacillin or Vancomycin, or *Enterococcus* against Vancomycin (Cheesbrough, 2006).

3.3.3.7. Measuring zone sizes

Following overnight incubation, the zone size was measured to the nearest millimetre using a ruler or calliper. The plate was placed above a black, non-reflecting surface. The zone size was recorded on the recording sheet (Cheesbrough, 2006).

3.3.3.8. Interpretation of the results

The published Clinical Laboratory Standard Institute (CLSI) guideline was used to determine the susceptibility or resistance of the organism to each drug tested. For each drug, indicate on the recording sheet whether the zone size is susceptible (S), intermediate (I), or resistant (R) based on the interpretation chart. The results of the Kirby-Bauer disk diffusion susceptibility test were reported only as susceptible, intermediate, or resistant (Cheesbrough, 2006).

CHAPTER FOUR

RESULTS

CHAPTER FOUR

4. RESULTS

Bacterial isolates (n=136) were obtained from the Research Laboratory of Sudan University of Science and Technology. The isolates were re-identified by microbiological methods. The results of re-identification were tabulated in table (1). Biochemical tests adopted in re-identification was shown in table (2).

Studies on the susceptibility of bacterial isolates from stethoscopes revealed that all the *E. coli* isolates (n= 10) were susceptible to Ciprofloxacin, Cephalothin and Chloramphenicol (100%), Gentamicin and Co-trimoxazole (90%) and Tetracyclin (60%). Isolates of *Klebseilla pneumoniae* (n= 11) were susceptible to Gentamicin and Ampicillin (91%) and showed less susceptibility to Chloramphinicol (73%), Co-trimoxazole and Ciprofloxacin (55%). The isolates of *Protus* ssp. (n= 6) were susceptible to Gentamicin and Ciprofloxacin (100%) and susceptible to lesser degree to Co-trimoxazole and Tetracyclin (83%).

Isolates of *Psedomonas aeruginosa* (n=12) were susceptible to Imipenem, Ciprofloxacin and Gentamicin (100%), and exhibited less susceptibility pattern to Amikacin (83%), Co-trimoxazole (75%), Tetracyclin (67%) and Pipracilin (58%). *S. aureus* (n= 14) isolates were susceptible to Streptomycin (100%), Ciprofloxacin (71%), and Erythromycin (64%), while other species of staphylococci exhibited different susceptibility patterns to antibiotics (Table 3).

Table 1. Number and percentage of re-identified bacterial isolates obtained from Research Laboratory.

Bacterial Isolated	Number	(%)
Staphylococcus epidermidis	38	28
Staphylococcus warneri	16	11.8
Staphylococcus aureus	14	10.3
Pseudomonas aeruginosa	12	8.8
Staphylococcus haemolyticus	11	8
Klebsiella pneumoniae	11	8
Escherichia coli	10	7.4
Staphylococcus hominis	7	5.2
Staphylococcus lugdunensis	6	4.4
Proteus spp.	6	4.4
Staphylococcus saprophyticus	5	3.7
Total	136	100

Table 2. Biochemical tests adopted for re-identification of bacterial isolates

Isolate code	Biochemical tests														
	Indole	Urease	Citrate	KIA				Coagulase	DNase	Manitole	Sugar fermentation				
				Slope	Butt	Gas	H_2S				Glucose	Maltose	Sucrose	Mannose	Trehalose
1stethoscope	+	-	-	Y	Y	+	-	N.T.	N.T	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
2 stethoscope	-	+	+	Y	Y	+	-	N.T	N.T	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
3 stethoscope	+/-	$+^{sl}$	d/+	R	Y	+	+	N.T.	N.T	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
4 stethoscope	-	d	+	R	R	-	-	N.T	N.T	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.
5 stethoscope	N.T.	N.T.	N.T.	N.T	N.T	N.T	N.T	+	+	+	+	+	+	+	+
6 stethoscope	N.T.	N.T.	N.T.	N.T	N.T	N.T	N.T	-	-	-	+	+	+	$+^{sl}$	-
7 stethoscope	N.T.	N.T.	N.T.	N.T	N.T	N.T	N.T	-	-	v	+	+	+	-	+
8 stethoscope	N.T.	N.T.	N.T.	N.T	NT.	N.T	N.T	-	-	-	+	+	$+^{sl}$	-	V
9stethoscope	N.T.	N.T.	N.T.	N.T	N.T	N.T	N.T	-	-	v	+	$+^{sl}$	+	-	+
10 stethoscope	N.T.	N.T.	N.T.	N.T	N.T	N.T	N.T	-	-	-	+	+	+	+	+
11 stethoscope	N.T.	N.T.	N.T.	N.T	N.T	N.T	N.T	-	-	V	+	+	+	-	+

Key: $+^{sl}$= Slow positive reaction+ = positive reaction - = Negative reaction

d= different**N.T**. Not Tested **V**= Variable reaction

Table 3. Susceptibility patterns of bacterial isolates to antibiotics

Bacterial isolates	Susceptibility (%) of bacterial isolates to:																			
	GEN	STX	CTR	OF	CIP	CEP	C	NA	AMP	TE	PI	AK	IMP	MRP	VA	E	AG	LIN	CD	S
S.epidermidis (n=37)	50	39	N.T.	0	74	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	8	47	53	0	0	76
S. warneri (n= 16)	94	12	N.T.	6	88	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	81	100	62	12	0	100
S. aureus (n=14)	36	21	N.T.	0	71	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	21	64	0	29	0	100
P. aeruginosa (n=12)	100	75	N.T.	N.T	100	N.T.	17	N.T.	0	67	58	83	100	0	N.T.	N.T.	N.T.	N.T.	N.T	N.T
S. haemolyticus (n= 11)	90	10	N.T.	0	90	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	100	60	80	0	40	100
K. pneumonia e (n=11)	91	55	0	0	55	0	73	27	91	27	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T	N.T
E. coli (n=10)	90	90	10	0	100	100	100	10	0	60	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T	N.T
S. hominis (n=7)	100	29	N.T.	0	86	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	0	86	86	0	0	100
S. lugdunensis (n= 6)	100	17	N.T.	50	100	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	100	67	83	0	67	100
Proteus spp. (n=6)	100	83	0	0	100	33	50	17	0	83	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T	N.T
S. saprophyticus (n= 5)	100	25	N.T.	0	100	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	N.T.	100	75	75	0	0	100

Key: N.T. = Not Tested

GEN= Gentamicin STX= Co-Trimoxazole MRP= Meropenem CEP= Cephalothin PI= Piperacillin

AMP= Ampicillin C= Chloramphenicol LIN= Lincomycin VA= Vancomycin AK= Amikacin

CTR= Ceftriaxone NA= Nalidixic Acid TE= Tetracycline CD= Clindamicin OF= Ofloxacin

AG= Agumentin CIP= Ciprofloxacin E= Erythromycin

CHAPTER FIVE
DISCUSSION

CHAPTER FIVE

5. DISCUSSION

There are increasing reports of the risk of transmitting antibiotic resistant micro-organisms from one patient to another by stethoscope (Fenelon *et al.,* 2009). These antibiotic-resistant organisms are capable of initiating severe infections in a hospital environment and could require contact isolation and aggressive treatment to prevent the spread of the organisms (Gupta *et al.,* 2004).

The result of this study revealed that *E. coli* isolates were highly susceptible to Ciprofloxacin, Cephalothin, Chloramphenicol, Gentamicin, Co-trimoxazol and Tetracyclin, but less susceptible to Nalidixic acid, Ofloxacin, Ampiciilin and Ceftriaxone. These results were compared to the observations of previous study that found all isolated *E. coli* were susceptible to Gentamicin, Cephalexin and Chloramphenicol (Maryam and Usman, 2014).

Isolates of *Klebsiella pneumoniae* were susceptible to Gentamicin, Ampicillin, Chloramphenicol but it resists Ceftriaxone, Ofloxacin, Cephalothin, Nalidixic acid and Tetracyclin. In similar study, all the *Klebsiella* spp. isolates were susceptible to Gentamicin, Ceftriazone, Chloramphenicol and Ampicillin (Maryam and Usman, 2014).

All the isolated *Pseudomonas aeruginosa* were susceptible to Gentamicin, Ciprofloxacin, Imipenem, Amikacin, Co-trimoxazol and Tetracyclin and showed

high resistance level to Ampiciilin, Meropenem, Chloramphenicol and Pipracillin. While the isolated *Pseudomonas* spp. in another study were susceptible to Gentamicin and Ofloxacin (Maryam and Usman, 2014).

Antibiotic susceptibility testing of isolated Gram negative bacilli of another study showed 100% resistance to Cefuroxime, Lomefloxacin, Ofloxacin and Ceftazidime. However Amikacin, Pefloxacillin were 100% sensitivity (Gurjeet *et al*., 2013). While in this study isolated Gram negative rods showed 100 % resistance to Ceftriaxone, Ofloxacin and Nalidixic acid. However Gentamicin was 100% susceptible.

In this study *Staphylococcus aureus* was susceptible to Streptomycin, Ciprofloxacin and Erythromcin, while *Staphylococcus epidermidis* exhibited susceptibility to Streptomycin Ciprofloxacin, and both bacteria were resistant to Clindamycin, Ofloxacin, Lincomycin, Cotrimoxazole, Vancomycin, Agumentin and Gentamicin. With comparison to another study that showed all the *Staphylococcus aureus* and *Staphylococcus epidermidis* isolates were sensitive to Erythromycin and Streptomycin (100%). The Gram positive isolates were resistant to Gentamicin and Ofloxacin (Maryam and Usman, 2014).

In this study of antibiotic susceptibility of isolated bacteria which is comparable to the observations of previous studies that found *Staphylococcus aureus* is known to have developed resistance to conventional antibiotics (Gentamicin, Lincomycin, Chloramphenicol, Ampicillin, Ofloxacin, Nalidixic acid, Ampicillin, Cefalexin

and Amoxacillin), while the most effective antibiotics were Ciprofloxacin and Erythromycin (Uneke *et al.,* 2008), and this was similar to the case in this study.

Another study of antibiotic susceptibility testing of isolated bacteria, Gram positive cocci were 100% resistant to Roxithromycin and Cefotaxime, whereas Linezolid, Ciprofloxacin and Vancomycin showed 100% sensitivity (Gurjeet *et al.,* 2013).

Conclusion

The study concluded that:

1. Antibiotic susceptibility of Gram negative rods was high (100%) to Gentamicin.

2. Antibiotic susceptibility of Gram positive cocci was high (100%) to Streptomycin.

Recommendations

The study recommended the following:

1. Further studies to assess antibiotics susceptibility of bacterial isolates from stethoscopes with large number of isolates are highly recommended to validate these findings.

2. Use of other antibiotic.

REFERENCES

REFERENCES

1. **Ananthi S. (2006).** A Textbook of Medical Instruments. *New Age International*, 290-296.
2. **Ayliffe G.A.J., Collins B.J. and Taylor L.J. (1990).** Hospital Acquired Infections: Principles and Prevention2nd edition. Wright. London.
3. **Beers M. H. And Berkow R. (1999).** Mood disorders in The Merck manual of diagnosis and therapy, 17thed. sec. 15, chap. 189.
4. **Bernard L., Kereveur A., Durand D., Goldstein F., Mainardi J. L., Acar J. And Carlet J.J. (1999).** Bacterial Contamination of Hospital Physician's Stethoscopes. *Infect. Cont. Hosp. Epidemiol.*, **20**(1): 626-628.
5. **Burke J. (2003).** Infection Control: A Problem for Patient Safety. *N. Engl. J.Med.,* **348**: 651-656.
6. **Cheesbrough M. (2006).** District Laboratory Practice in Tropical Countries, Part 2, Cambridge University Press, United Kingdom, PP 60-64.
7. **Cohen H.A. and Matalon J. (1997).** Stethoscope and otoscope- A potential vector of infection. *Fam. Pract*., **14**: 446-448.
8. **Culver D.H., Haley R.W., White J.W. (1985).** The efficacy of infection Surveillance and control programs in preventing nosocomial infection in the hospital. *Am. C. Epidemiol*., **121**: 181-2005.
9. **Duffin-Jacalyn (2012).** Big Ideas: Jacalyn-Duffin on the History of the Stethoscope. TVO.

10. **Finkeistein E. (2008).** The Ultimate Acoustic Stethoscope "Development of laser based heart sound detection system". *JBSE,* **5**:34-37.

11. **Fenelon L., Holcroft L. and Waters N. (2009).** Contamination of stethoscopes with MRSA and current disinfection practices. *J. Hosp. Infect.*, **71**(4): 376-378.

12. **Forbes B. A., Saham D. F. and Weissfeld A. S. (2002).** *Bailey and Scott's Diagnostic Microbiology*, vol. **2**, 12thed. Mosby, Inc. St. Louis., PP. 222-246.

13. **Garne G.S. (1996).** Guidelines for isolation precautions in hospitals. *Infect. Control Hosp. Epidemol.,* **17**: 53-80.

14. **Garner J.S., Jarvis W.R. and Emori T.G. (1988).** CDC definitions for nosocomial infections. *Am. J. Infect. Control,* **16**:128-140.

15. **Gerken A., Cavanagh S. and Winner H.I**. **(1972).** Infection hazard from stethoscopes in hospital. *Lancet* **1**:1214-1215.

16. **Gurjeet S., Urhekar A.D., Anahita V. H., Neha S. and Bhaskar D. (2013).** Bacterial contamination of stethoscope used by health care workers in a tertiary care hospital in Navi Mimba .*Int. J. Pharm. Bio. Sci.,* **3**(1):186-193.

17. **Gupta A., Della-Latta P., Todd B., San G.P., Haas J., Wu F., Rubenstein D. and Saiman L. (2004).** Outbreak of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a neonatal intensive

care unit linked to artificial nails. *Infect. Contrl. Hosp. Epidemiol.,* **25**: 210-215.

18. **Hoogkamp J.A., Cats B., Senders R.C. and Ertbruggen I.V. (1982).** Analysis of Bacterial Infections in a Neonatal Intensive Care Unit.*J. Hosp. Infec.*, **3:**275-284.

19. **Hughe J.M. (1988).** Study on the efficacy of nosocomial infection control. *Infect. Control Epidemol.,* **34**: 553-561.

20. **Jones J.S., Hoerle D. and Riekse R. (1995).** Stethoscopes: a Potential Vector of Infection? *Ann. Emerg. Med.,* **26**(3): 296-299.

21. **Knobler S.L., Lemon S.M., Najafi M. and Burroughs T. (2003).** The Resistance Phenomenon in Microbes and Infectious Disease Vectors: Implications for Human Health and Strategies for Containment-Workshop Summary. Washington, DC: Institute of Medicine. *The National Academies Press*.

22. **Osler, Sir William. (1917).** The Library School in the College. *The Library Association Record*, **19**: 308.

23. **Littmann D. (1961).** An Approach to the Ideal Stethoscope. *JAMA.***178**: 504 - 505.

24. **Lynch J.W., Kaplan G.A. and Salonen J.T. (1997).** Why do poor people behave poorly? Variation in adult health behaviours and psychosocial characteristics by stages of the socio-economic life course. *Social Science and Medicine,* **44**(6): 809–819.

25. **National Institute of Allergy and Infectious Diseases NIAID (2010).** A report on a research agenda for emerging infectious diseases. Clinical Trial .gov. National Committee.

26. **Maryam A. And Usman S. H. (2014).** Characterization and determination of antibiotic susceptibility pattern of bacteria isolated from some fomites in a teaching hospital in northern Nigeri. *Afr. J. Microbiol. Res*., **8**(8): 814-818.

27. **Palaniappan R., Sundaraj K., Ahamed N.U., Arjunan A. And Sundaraj S. (2013).** Computer based Respiratory Sound Analysis: A Systematic Review. *JETE Tech. Rev.,* **30**:248-256.

28. **Parmar R.C., Valvi C.C., Sira P. and Kamat J.R. (2004).** A prospective, randomised, double-blind study of comparative efficacy of immediate versus daily cleaning of stethoscope using 66% ethyl alcohol. *Indian J. Med.Sci*., **58**: 423-430.

29. **Pier G.B., Ramphal R., Mandell G.L., Bennett J.E. and Dolin R. (2004).** *Pseudomonas aeruginosa*. In: Principles and practice of infectious diseases, 6th Ed. New York: Elsevier Churchill Livingstone, 2587–2615.

30. **Plowman R., Grave N. and Griffin N. (2000).** Socio economic burden of hospital acquired infection. London PHLS/CDC Guidelines for Hospital precaution. *Am. J. Infect. Control*, **24**: 24-52.

31. **Smith M.A., Mathewson J.J., Ulert A., Scerpella E.G. and Ericssn C.D. (1996).** Contaminated Stethoscopes Revisited. *Arch. Intern. Med.,* **156**:82-84.

32. **Tietjen L., Bossemeyer D. and McIntosh N. (2003).** Safe Practices in the Operating Room. In Infection Prevention: Guidelines for Health care Facilities with Limited Resources. *JHPIEGO* Corporation, Baltimore.1-18.

33. **Uneke C.J, Ogbonna A., Oyibo P.G. and Ekuma U. (2008).** Bacteriological assessment of stethoscopes used by medical students in Nigeria: implications for nosocomial infection control. *World Health Popul*., **10**: 53-61.

34. **Varghese D. and Patel H. (1999).** Hand washing: stethoscopes and white coats are sources of nosocomial infection. *British Medical Journal,* **319**:519.

35. **Weinstein R.A., Youngster I., Berkovitch M., Heyman E. and Lazarovitch Z. (1998).** Nosocomial infection update. *Emerg. Infect. Dis*., **4**: 416-420.

36. **World Health Organization (2009).** WHO Guidelines for Hand Hygiene in Health Care. First Global Patient Safety Challenge Clean Care is Safer Care. Geneva: WHO 270p.

APPENDICES

APPENDICES

Appendix {1}: Gram's stain

Crystal violate	20.00 gm
Ammonium oxalate	99.00 gm
Ethanol	95.00 ml
D.W	1 L

Lugols iodine

70% alcohol

Saffranine

Appendix {2}: Kligler Iron Agar (KIA) (HiMedia Laboratories Pvt. Ltd. Mumbai, India).

Ingredients:	Gms/litre
Peptic digest of animal tissue	15.00
Beef extract	3.00
Yeast extrac	3.00

Proteose peptone	5.00
Lactose	10.00
Dextrose	1.00
Ferrous sulphate	0.20
Sodium chloride	5.00
Sodium thiosulphate	0.30
Phenol red	0.024
Agar	15.00

Final pH (at 25 ºC) 7.4

Preparation:

A mount of 42.524 grams was suspended in 1000 ml distilled water, sterilized by autoclaving at 15 Ibs pressure (121ºC) for 15 minutes and Cooled to about 50ºC. Then mixed well and poured into sterile tubes. The medium was allowed to solidify in a slope position to give a butt and slope.

Appendix {3}: Peptone water (HiMedia Laboratories Pvt. Ltd. Mumbai, India).

Ingredients:	Gms/litre
Peptic digest of animal tissue	10.00
Sodium chloride	5.00

Final pH (at 25ºC) 7.2 ± 0.2

Preparation:

A mount of 15.0 grams was suspended in 1000 ml distilled water, mixed well and dispensed into tubes. Then sterilized by autoclaving at 15 Ibs pressure (121ºC) for 15 minutes.

Appendix {4}: Urea agar base (Christensen) (HiMedia Laboratories Pvt. Ltd. Mumbai, India).

Ingredients:	Gms/litre
Peptic digest of animal tissue	1.00
Dextrose	1.00
Sodium chloride	5.00
Di sodium phosphate	1.20

Monopotassium phosphate	0.80
Phenol red	0.012
Agar	15.00

Final pH (at 25ºC) 6.8 ± 0.2

Preparation:

A mount of 240.0 grams was suspended in 950 ml distilled water, heated to boiling to dissolve the medium completely and sterilized by autoclaving at 10 Ibs pressure (115ºC) for 20 minutes. Then Cooled to about 50ºC and aseptically 50 ml of sterile 40% urea was added. Mixed well and poured into sterile tubes.

Appendix {5}: Kosser's citrate (Mast Group Ltd., Merseyside, U.K.).

Ingredients:	Gms/litre
Magnesium sulphate	0.20
Potassium disulphate phosphate	1.00
Sodium ammonium phosphate	1.50
Tri sodium citrate	2.50
Bromothymole blue	0.016

Preparation:

A mount of 5.2 grams was suspended in 1000 ml distilled water, mixed well and dispensed into tubes. Then sterilized by autoclaving at 15 Ibs pressure (121ºC) for 15 minutes.

Appendix {6}: DNase agar

Appendix {7}: Manitol salt agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India).

Ingredients:	Gms/litre
Proteose peptone	10.00
Beef extract	1.00
Sodium chloride	75.00
D-Mannitol	10.00
Phenol red	0.025
Agar	15.00

Final pH (at 25ºC) 7.4 ± 0.2

Preparation:

A mount of 111.02 grams was suspended in 1000 ml distilled water, heated to boiling to dissolve the medium completely and sterilized by autoclaving at 15 Ibs pressure (121ºC) for 15 minutes. Then mixed well before pouring.

Appendix {8}: Muller Hinton agar (HiMedia Laboratories Pvt. Ltd. Mumbai, India).

Ingredients:	Gms/litre
Beef infusion	300.00
Casein acid hydrolysate	17.50
Starch	1.50
Agar	17.00

Final pH (at 25ºC) 7.3± 0.1

Preparation:

A mount of 38.0 grams was suspended in 1000 ml distilled water, heated to boiling to dissolve the medium completely and sterilized by

autoclavingat 15 Ibs pressure (121ºC) for 15 minutes. Then mixed well before pouring.

Appendix {9}: Kovac's reagent

(p)- di methyl aminobenzaldehyde	2 gm

Appendix {10}: Physiological saline (0.85%)

NaCl	0.85 gm
Distilled water	100 ml

Appendix {11}: McFarland standard NO.3

1.0% H_2SO_4 (1.0 ml H_2SO_4 + 99 mldistilled water)	0.3 ml
1.0% $BaCl_2$ (1.0 gm$BaCl_2$ + 100 ml distilled water)	9.7 ml

Appendix {12}: Catalase reagent

3% H_2O_2	2 ml

Appendix {13}: The module

Incubator (GALL Enk AMP GE, U.K.).

Autoclave (Graffin and Italy George Ltd.).

Hot air oven (Leader Engineering, U.K.).

Appendix {14}: Raw data

E. coli

No.	GEN	COT	CE	OF	CIP	CEP	C	NA	AMP	T
121	20	29	20	10	45	20	33	8	0	15
58	27	30	24	15	37	25	37	0	0	20
69	29	23	21	12	40	30	35	23	0	18
114	20	28	29	8	33	18	30	5	0	11
35	14	30	20	12	45	17	27	5	0	17
71	28	28	20	15	42	28	34	7	0	15
80	17	35	20	17	38	22	38	8	5	25
139	22	15	0	10	33	28	30	10	0	22
79	26	30	20	9	40	30	33	6	0	18
140	24	29	20	10	37	30	30	4	0	20

Klebsiella pneumoniae

No.	GEN	COT	CE	OF	CIP	CEP	C	NA	AMP	T
7	19	22	10	0	15	17	22	12	22	18
133	16	15	0	8	0	12	17	15	17	0
132	18	25	0	10	25	11	27	13	18	0
94	27	10	0	5	30	15	25	22	24	20
67	17	15	0	11	0	12	15	18	20	0
38	20	20	0	4	35	11	18	14	22	15
88	25	25	8	0	25	10	20	17	17	11
157	28	45	15	7	37	15	35	23	18	20
158	30	40	0	7	45	16	20	15	15	28
175	0	12	0	8	11	13	20	20	19	0
178	17	10	0	0	0	8	0	18	20	0

Psedomonas aeruginosa

No.	P	AK	IMP	MER	COT	CIP	AM	T	GEN	C
8	27	20	33	0	22	26	2	24	18	12
44	25	28	40	0	25	33	0	28	22	15
66	25	33	52	14	30	38	0	21	25	20
77	22	17	50	8	35	34	0	14	26	15
78	28	17	36	7	32	25	4	35	33	13
92	26	23	25	0	21	30	0	29	26	10
137	19	20	25	0	30	30	10	28	30	12
146	20	27	33	0	34	42	0	18	24	18
148	18	28	44	4	22	28	5	35	25	15
156	25	35	39	0	30	33	0	13	28	17
159	20	30	43	10	35	45	2	35	24	13
180	30	32	54	11	26	40	0	18	26	14

***Proteus* spp.**

No.	COT	CE	NA	CIP	OF	CE	AM	TET	GEN	C
63	31	10	18	42	12	14	0	24	31	16
55	44	0	17	38	10	20	0	27	32	13
64	27	0	19	31	11	18	0	20	28	17
89	13	0	18	30	4	13	0	22	26	22
181	33	0	13	40	9	17	0	20	32	25
182	27	0	13	28	11	13	0	17	27	18

S. aureus

No.	GEN	CIP	VA	COT	E	AG	LINCO	OF	CD	S
1	24	33	22	24	22	13	21	11	15	14
3	16	25	4	20	27	11	25	13	13	20
27	18	28	0	21	23	20	20	10	11	16
39	30	20	35	18	13	13	22	14	14	22
42	23	30	22	19	20	10	17	11	8	21
47	14	19	12	17	23	11	27	8	10	18
56	12	24	11	24	30	14	22	14	13	20
57	17	18	0	20	28	16	21	13	14	14
96	18	18	8	19	22	15	18	10	10	22
104	16	25	5	20	20	13	25	5	12	21
107	20	27	15	22	15	19	22	9	11	20
108	13	31	0	25	25	14	19	13	9	15
149	22	30	11	18	21	10	25	10	0	14
169	15	22	10	20	30	14	21	12	11	20

S. epidermidis

No.	GEN	CIP	VA	COT	E	AG	LINCO	OF	CD	S
2	15	33	18	12	30	15	14	16	11	14
10	20	20	14	10	20	14	10	13	14	20
12	16	22	13	23	25	11	12	14	0	15
16	14	26	11	9	22	11	13	18	13	14
18	13	30	12	12	30	16	14	10	12	18
19	12	24	10	18	23	13	8	13	10	13
21	23	20	14	12	20	10	20	14	12	22
23	15	20	15	13	20	14	14	9	0	16
24	18	22	13	18	24	10	14	15	11	20
25	16	30	14	20	34	15	11	14	13	20
31	10	26	10	19	30	18	10	19	10	14
34	14	24	0	13	23	12	15	13	12	14
36	11	25	13	19	21	14	14	15	8	14
41	25	22	14	12	22	15	12	16	15	22
45	15	21	11	12	22	10	13	12	5	16
52	13	30	5	20	19	11	10	5	10	21
59	24	32	12	21	23	13	5	11	13	17
61	14	18	11	17	17	14	11	13	12	20

62	20	22	14	10	22	20	12	18	0	23
81	13	25	9	15	22	15	15	14	11	22
82	16	34	12	10	26	13	10	12	11	16
83	12	20	13	11	30	0	14	13	14	19
85	10	19	10	17	21	14	12	19	8	22
97	20	30	16	4	22	17	0	14	0	21
100	13	25	12	15	26	0	14	20	13	20
101	15	33	10	22	30	8	13	15	4	14
111	14	24	12	10	20	14	10	13	13	17
126	11	20	14	13	32	11	12	18	11	19
134	14	30	14	16	22	15	9	20	2	20
142	12	28	12	0	18	5	15	16	10	14
145	9	24	13	21	15	10	14	10	12	20
147	16	20	12	9	28	14	12	8	9	19
164	13	22	0	18	30	19	11	17	9	22
165	25	30	9	13	16	18	14	13	14	22
167	14	29	13	10	22	20	18	10	20	18
172	23	20	12	13	27	10	15	16	13	14
173	15	22	10	19	33	14	10	16	10	20
189	20	18	14	12	22	15	12	13	5	22

S. saprophyticus

No.	GEN	CIP	VA	COT	E	AG	LINCO	OF	CD	S
95	19	29	21	10	30	18	13	12	10	23
117	26	22	15	15	24	20	14	8	5	28
122	22	30	19	20	22	24	20	10	13	22
127	21	23	19	16	26	21	20	11	2	28
162	20	27	17	13	25	26	17	15	9	18

S. haemolyticus

No.	GEN	CIP	VA	COT	E	AG	LINC	OF	CD	S
5	21	30	21	15	15	26	11	8	18	25
15	28	28	19	14	22	27	13	20	24	19
46	13	39	21	11	23	24	18	11	14	23
51	21	18	20	12	24	18	10	13	19	25
87	27	40	20	15	21	20	13	5	22	20
93	22	34	17	13	25	22	11	14	19	19
110	24	29	18	17	23	24	9	15	21	26
112	15	31	16	11	30	19	10	13	20	27
113	19	25	19	12	23	26	12	0	22	23
129	22	22	17	15	23	25	13	10	16	24
198	20	20	22	10	22	23	11	10	20	22

S. hominis

No.	GEN	CIP	VA	COT	E	AG	LINCO	OF	CD	S
4	19	28	8	10	30	20	11	12	0	30
43	25	34	0	15	35	26	8	16	12	22
99	27	25	2	16	22	22	17	10	0	28
109	20	30	13	14	27	27	10	13	5	25
144	24	22	5	18	28	19	11	13	10	33
161	22	33	0	11	24	23	15	8	8	30
188	18	20	10	11	30	26	13	14	2	27

S. warneri

No.	GEN	CIP	VA	COT	E	AG	LINC	OF	CD	S
26	18	33	20	10	24	29	10	16	8	27
30	19	34	17	13	27	27	11	12	12	23
33	20	22	16	11	30	17	18	15	12	23
37	20	19	23	10	33	23	22	0	2	30
40	22	23	20	8	35	20	19	8	0	18
50	18	30	13	16	24	14	21	12	8	27
74	15	23	21	11	30	16	17	13	13	28
115	13	33	11	14	29	29	15	20	13	27
119	27	21	18	16	27	21	20	14	11	31
168	24	17	14	10	25	23	14	10	0	19
174	23	25	16	12	24	18	12	14	14	25
177	18	35	17	15	35	18	10	17	13	26
186	22	21	17	12	30	21	13	11	5	22
187	27	36	25	0	32	22	12	14	10	30
193	29	29	27	12	34	26	17	5	14	20
194	19	27	21	8	25	15	16	13	9	19

S. lugdunensis

No.	GEN	CIP	VA	COT	E	AG	LINCO	OF	CD	S
49	28	35	20	11	22	26	15	20	22	25
75	33	29	19	16	21	24	15	18	24	22
90	29	40	22	10	24	22	13	17	22	28
91	28	30	24	15	25	18	20	11	29	28
124	25	33	18	11	25	22	15	18	20	26
153	21	43	22	10	23	20	18	15	19	30

Bacteria	GEN		COT		CTR		OF		CIP	
	S	R	S	R	S	R	S	R	S	R
E.coli	9	1	9	1	1	9	0	10	10	0
Klebsiella pneumoniae	10	1	6	5	0	11	0	11	6	5
*Protus*spp.	6	0	5	1	0	6	0	6	6	0

Cont.

Bacteria	CEP		C		NA		AMP		TE		Total
	S	R	S	R	S	R	S	R	S	R	
E.coli	10	0	10	0	1	9	0	1	6	4	10
Klebsiella pneumoniae	0	11	8	3	3	8	10	1	3	8	11
Protus spp.	2	4	3	3	1	5	0	6	5	1	6

Bacteria	PI		AK		IMP		MEM		COT	
Psedomonas aeruginosa	S	R	S	R	S	R	S	R	S	R
	7	5	10	2	12	0	0	12	9	3

Cont.

Bacteria	CIP		AM		TET		GEN		C		Total
Psedomonas aeruginosa	S	R	S	R	S	R	S	R	S	R	
	12	0	0	12	8	4	12	0	2	10	12

Bacteria	GEN		CIP		VA		COT		E	
	S	R	S	R	S	R	S	R	S	R
Staphylococcus aureus	5	9	10	4	3	11	3	11	9	5
Staphylococcus epidermidis	19	19	28	10	3	35	15	23	18	20
Staphylococcus saprophyticus	5	0	5	0	5	0	2	3	4	1
Staphylococcus haemolyticus	10	1	9	2	11	0	1	10	8	3
Staphylococcus hominis	7	0	6	1	0	7	2	5	6	1
Staphylococcus warneri	15	1	14	2	13	2	2	14	16	0
Staphylococcus lugdunensis	6	0	6	0	6	0	1	5	4	2

Cont.

Bacteria	AG		LIN		OF		CD		S		Total
	S	R	S	R	S	R	S	R	S	R	
Staphylococcus aureus	0	14	4	10	0	14	0	14	14	0	14
Staphylococcus epidermidis	20	18	0	38	6	32	0	38	29	9	38
Staphylococcus saprophyticus	4	1	0	5	0	5	0	5	5	0	5
Staphylococcus haemolyticus	9	2	0	11	1	10	5	6	11	0	11
Staphylococcus hominis	6	1	0	7	0	7	0	7	7	0	7
Staphylococcus warneri	10	6	2	14	1	15	0	16	16	0	16
Staphylococcus lugdunensis	5	1	0	6	3	3	4	2	6	0	6