بسم الله الرحمن الرحيم

Sudan University of Science &Technology
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Detection of Multi-drugs Resistance among Bacteria Isolated from Computers' Keyboards

الكشف عن المقاومة المتعددة للعقاقير عند البكتيريا المعزولة من لوحات مفاتيح الحواسيب

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

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الآيـة

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 (٣٥)

صدق الله العظيم

سورة النور: الآية35

DEDICATION

To my father, mother, husband, brothers and sisters.

To all my colleagues and friends.

AKNOWLEDGEMENT

First of all thanks to ALMIGHTY ALLAH who facilitates uncountable graces.Mere thanks are not enough to explore my thankfulness to my supervisor **Prof. Humodi Ahmed Saeed** for his continuous supervision and guidance.I would like to thank all my colleagues for their collaboration and help. Finally greet thanks to my husband Loui Abdulmonim for his support and encouragement.

ABSTRACT

This is a laboratory-based study, carried out during the period from May to November, 2014. The objective of this study was to detectmulti-drug resistance among bacteria isolated from computers' keyboards.

The isolated bacteria under assessment were obtained from the Research Laboratory, Sudan University of Science and Technology. Gram's stain and biochemical tests were used to confirm the reidentification of the isolates. Multi-drug resistance among the isolates was detected by modified Kirby-Bauer disk diffusion method. The antibiotics assessed were Amikacin, Gentamicin, Amoxi-clav,Cotrimoxazole,Ciprofloxacin, Erythromycin, Ampicillin, Amoxicillin, Tetracycline, Penicillin, Meropenem, Norfloxacin, Cefuroxime, and Cloxacillin.

The results showed that the reidentified isolates were*Pseudomonas* species 38(38%), *S. aureus*22 (22%),*S. epidermis*16(16%),*E. coli*12(12%),*Klebsiella* species8(8%) and *S. haemolytics*4 (4%).

The study concludedhat the result of multi-drug resistance among Gram-negative bacteria was very low. While the multi-drug resistance among Gram-positive bacteria (*Staphylococcus* species) showed that 100% of *S. epidermis*were multi-drug resistant to Gentamicin, Cotrimoxazole,Erythromycin, Tetracycline and Cloxacillin.

المستخلص

هذه دراسة مختبرية ، نفذت خلال الفترة من مايو إلى نوفمبر 2014. وكان الهدف من هذه الدراسة الكشف عن المقاومة لأدوية متعددة بين البكتيريا المعزولة من لوحات مفاتيح الكمبيوتر.

وقد تم الحصول على البكتيريا المعزولة قيد التقييم من مختبر بحوث، جامعة السودان للعلوم والتكنولوجيا. واستخدمت صبغة الجرام والإختبارات البيوكيميائيةلتأكيد هوية من العزلات. تم الكشف عن المقاومة للأدوية المتعددة بين العزلات بواسطة تعديل كيربي باور طريقة نشر القرص. وكانت المضادات الحيوية المقررة الأميكاسين، جنتاميسين، أموكسي كلاف، كوتريموكسازول، سيبروفلوكساسين، الاريثروميسين، الأمبيسلين، أموكسيسيلين، التتراسيكلين، البنسلين، ميروبينيم، نورفلوكساسين، السفيوركسيم، وكلوكساسيلين.

وأظهرت النتائج أن العزلات التي جرى تحديدها الزائفة 38 (38٪)، المكورات العنقودية الذهبية 22 (22٪) المكورات العنقودية البشروية 16 (16٪)، القولونية 12 (12٪)، كليبسيلا 8 (8٪) و العنقودية الدموية 4 (4%).

وخلصت الدراسة إلى أن نتيجة المقاومة لأدوية متعددة بين البكتيريا سالبة الجرام أن جميع الأنواع كانت ليست متعددة المقاومة. في حين أظهرت المقاومة لأدوية متعددة بين البكتيريا إيجابية الغرام (أنواع المكورات العنقودية) أن 100٪ من المكورات العنقودية البشروية متعددة المقاومة لعقار البنسلين، كوتريموكسازول، الاريثروميسين، التتراسيكلين وكلوكزاسيلين.

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LIST OF ABBREVIATIONS

MRO: Meropenem

GEN: Gentamicin

AMK: Amikacin

CIP: Ciprofloxacin

AX: Amoxicillin

CRX: Cefuroxime

NOR: Norfloxacin

AMC: Amoxi-clav

AMP: Ampicillin

PEN: Penicillin

COT: Cotrimoxazcole

ERY: Erythromycin

TET: Tetracycline

CXC: Cloxacillin

S: Staphylococcus

E: Ecoli

Hrs: Hours

MRSA: Methicillin-resistant Staphylococci

MSSA: Methicillin-sensitive Staphylococci

NCCLS: National Committee for Clinical Laboratory Standards

CHAPTER ONE

INTRODUCTION AND OBJECTIVES

1.1. Introduction

Computer is an electronic data processing machine. This machine accepts data from the out-side world.It is inform ofan input and manipulates, calculates, computes on thebasis of set of instructions supplied and stored in thememory.At the end gives the required or desired results in theform of an output to the user (Ravichandran, 2001).

The machines are ubiquitous in everywhere and have been shown to be contaminated with potentially pathogenic microorganisms. There is no economical way to test all the keyboards out there, but there arecommon-sense ways to prevent bacterial contamination or eliminate it if it exists (EltablawyandElhifnawi, 2009).

Bacteria are everywhere; contaminate our body, our houses, workplaces, pets and the whole environment. Fortunately, among many billions of bacteria, only 1,500 can be dangerous for our health. The real problem is that the number of bacterial strains which develop resistances towards disinfectants and especially antibiotics is increasing very fast (ECCMID, 2008).

The prevalence of bacterial infections in humans is increasing and has been shown to result in part from transmission of pathogens from the hospital setting to the community and vice versa (Hidron*et al.*, 2005).

The main cause of bacterial contamination of keyboards is eating lunch while working so crumbs and spills can wind up on and between the keys; the food

deposits encourage the growth of millions of bacteria. Another cause is thought to be poor personal hygiene such as neglecting to wash hands after going to the bathroom. Dust, also which can trap moisture and enable any bacteria that are already on your keyboard to flourish. One potential cause of a keyboard that can make a person sick, is sharing it among other workers. One of whom may have coughed or sneezed into his hand (ASM, 2005).

Colonization of objects which include computer keyboards, by pathogens and mainly bacteria is reported as one of the important routes for their transmission (Famurewa&David, 2009; Fatma*et al*., 2009).

Nosocomial infections are the main cause of morbidity and mortality as reported by several investigators worldwide (June *et al*., 2000).

Some patient care systems and hospital environment may facilitate the transmission of microorganisms among patients. Recently, computer use is very common in hospitals, and today the computers are considered as a source of nosocomial infection pathogens (Ducel and Fabry, 2001).

Antimicrobial agents represent a main therapeutic tool to control and treat a variety of bacterial infectious diseases. The first antimicrobial compounds used in modern medicine were produced and isolated from living organisms such as the Penicillins from fungi of the genus *Penicillium*, orStreptomycin produced by bacteria of the genus *Streptomyces.* With the advent of organic chemistry many antimicrobial agents are now obtained by chemical synthesis, such as the Sulfa drugs and the Quinolones. At the highest level, antibmicrobial agents can be classified as either bactericidal or bacteriostatic. Bactericidals kill bacteria

directly while bacteriostatics prevent them from dividing. However, in practice, both of these are capable of ending a bacterial infection.

Classification of antimicrobials can also be done according to their mechanism of action. Mechanisms include interference with cell wall synthesis (e.g., β-lactams), inhibition of protein synthesis (Macrolides), interference with nucleic acid synthesis (Quinolones), inhibition of a metabolic pathway (Trimethoprim-Sulfamethoxazole), and disruption of bacterial membrane structure (Polymyxins).

Antibiotic resistance was reported very early in the development of these wonder drugs. Sir Alexander Fleming's original report in 1929 noted that some bacteria, including the microbe now called *Escherichia coli*, were resistant to the effect ofpenicillin. In 1940, Edward Abraham and Ernst Chain reported the presence of anenzyme in *E. coli* that destroyed penicillin, this was several years before the drugbecame widely used to treat patients. In the subsequent decades, bacterial antibioticresistance has become a widespread and well-known phenomenon.

Inappropriate prescription of antibiotics prompted resistance and increased infectiousdisease mortality not only in developing countries but also in developed countries.Aging populations, changes in behavior and a decline in the development of newantibiotics exacerbated a deteriorating situation(Dandekar and Dandekar, 2010). The antibiotic resistance of enteric bacteria has profound clinical implicationsbecause it threats the life and causes

many of serious diseases such as acutegastroenteritis (Georgopapadakou, 2007).

The performance of antimicrobial susceptibility testing by the clinical microbiology laboratory is important to confirm susceptibility to chosen empirical antimicrobial agents, or to detect resistance in individual bacterial isolates. Empirical therapy continues to be effective for some bacterial pathogens because resistance mechanisms have not been observed e.g., continued Penicillin susceptibility of *Streptococcus pyogenes.* Susceptibility testing of individual isolates is important with species that may possess acquired resistance mechanisms (eg, members of theEnterobacteriaceae,*Pseudomonas*species, *Staphylococcus*species,*Enterococcus*species and *Streptococcus pneumoniae*) (Reller*et al*., 2009).

1.2. Rationale

Computer hardware has been implicated as a potentialreservoir for infectious agents. Of increasing concern, however, is the role ofkeyboards in the non-hospital environment as pathogen reservoirs. It follows that theubiquitous sharing of public computers by a broad user base might facilitate increased transmission andprevalence of pathogenic microorganisms throughout the community.

Surveillance and tracking of antibiotic resistant bacteria carried on common-use items will help to elucidate the prevalence of antibiotic resistance within communities. Communication of these data will allow healthcare agencies and

basic researchers to better plan mechanisms for combating the problem of antibiotic resistance.

This study is going to determine the degree of microbial contamination, the efficacy of different disinfectants, and the cosmetic and functional effects of the disinfectants on the computer keyboards.

This certainly has a valuable impact in the selection of suitable disinfectants for routine cleaning of keyboards and mice or transparent plastic covers and of course this will aid the fight against infection. Also, hand washing with selected disinfectants before contact with keyboards will significantly reduce the risk of contamination and cross transmission.

1.3. Objectives

1.3.1. General objective

To detect multi- drug resistance among bacteria isolated from computer keyboards.

1.3.2. Specific objectives

1. To re-identify the isolates.

2. To perform antibiotic susceptibility test.

3. To determine multi- drug resistance isolates.

CHAPTER TWO

LITERATURE REVIEW

Eltablawy and Elhifnawi2009 found that all the tested 24 computer keyboards and mice included in their study, were positive for microbial contamination. The percentage ofpathogenic bacteria, non pathogenic bacteria and mould was 3.0%, 0%; 66.3%; 66.6%; 30.6% and 33.3% forcomputer keyboards and mice, respectively. The isolated pathogens were tested against the 10 different antibiotics. Thedisinfectant dettol wipes were highly effective at removing or inactivating microbial contamination (Eltablawy and Elhifnawi, 2009).

Computer terminals in schools were sampled by Boa *et al*2013 for *S. aureus* and methicillin-resistant *Staphylococci*. The overall prevalence of MRSA on computer keyboards was low: 0.68% for a post-secondary institution and 2% and 0% for two secondary institutes. The MRSA isolate from the post-secondary institution did not correspond to the Canadian epidemic clusters, but is related to the USA 700 clusters, which contains strains implicated in outbreaks within the U.S. The isolate from the secondary institute's keyboard was typed as CMRSA7 (USA 400), a strain that has been implicated in both Canadian and U.S. epidemics. Methicillin-resistant *S. haemolyticus* and *S. epidermidis* were also isolated from keyboards, indicating that a mixed community of Methicillin-resistant *Staphylococci* can be present on keyboards. They concluded that although the prevalence was low, the presence of MRSA combined with the high volume of traffic on these student computer terminals

demonstrates the potential for public-access computer terminals and computer rooms at educational institutes to act as reservoirs (Boa *et al*., 2013).

Rutala*et al*2003. assessed the effectiveness of 6 different disinfectants (1 each containing chlorine, alcohol, or phenol and 3 containing quaternary ammonium) against 3 test organisms inoculated onto study computer keyboards. They found that the Potential pathogens cultured from more than 50% of the computers included coagulase-negative *Staphylococci* (100% of keyboards), *Diphtheroids* (80%), *Micrococcus* species (72%), and *Bacillus* species (64%). Other pathogens cultured included ORSA (4% of keyboards), OSSA (4%), Vancomycin-susceptible *Enterococcus* species (12%), and non-fermentative gram-negative rods (36%). All disinfectants, as well as the sterile water control, were effective at removing or inactivating more than 95% of the test bacteria. No functional or cosmetic damage to the computer keyboards was observed after 300 disinfection cycles. They concluded that their data suggest that microbial contamination of keyboards is prevalent and that keyboards may be successfully decontaminated with disinfectants. Keyboards should be disinfected daily or when visibly soiled or if they become contaminated with blood (Rutala*et al*., 2003).

Schultz *et al*. tested 100 keyboards in 29 clinical areas for bacterial contamination. Ninety five were positive for microorganisms. *Streptococcus*, Enterococcus (including one Vancomycin-resistant *Enterococcus*), *S.aureus*, fungi, and gram-negative organisms were isolated. Computer equipment must

be kept clean so it does not become another vehicle for transmission of pathogens to patients (Schultz *et al.*, 2003).

Kassem *et al* 2007. identified putative Methicillin (Oxacillin)-resistant *Staphylococci* isolates from keyboard swabs following a combination of biochemical and genetic analyses. Of 24 keyboards surveyed, 17 were contaminated with *Staphylococci* that grew in the presence of Oxacillin (2 mg l-1). Methicillin (Oxacillin)-resistant*S. aureus* (MRSA),*S.epidermidis* (MRSE) and *S.hominis* (MRSH) were present on two, five and two keyboards, respectively, while all three *Staphylococci* co-contaminated one keyboard. Combined with the broad user base common to public computers, the presence of antibiotic-resistant *Staphylococci* on keyboard surfaces might impact the transmission and prevalence of pathogens throughout the community (Kassem*et al*., 2007).

One of the earliest antimicrobial susceptibility testing methods was the macrobroth or tube-dilution method. This procedure involved preparing two-fold dilutions of antibiotics (eg, 1, 2, 4, 8, and 16 µg/mL) in a liquid growth medium dispensed in test tubes (Ericsson and Sherris, 1971). The antibiotic-containing tubes were inoculated with a standardized bacterial suspension of 1–5×10^5CFU/mL. Following overnight incubation at 35°C, the tubes were examined for visible bacterial growth as evidenced by turbidity. The lowest concentration of antibiotic that prevented growth represented the minimal inhibitory concentration (MIC). The precision of this method was considered to be plus or minus 1 two-fold concentration, due in large part to the practice of

manually preparing serial dilutions of the antibiotics (Balows, 1972). The advantage of this technique was the generation of a quantitative result (ie, the MIC). The principal disadvantages of the macrodilution method were the tedious, manual task of preparing the antibiotic solutions for each test, the possibility of errors in preparation of the antibiotic solutions, and the relatively large amount of reagents and space required for each test (Reller*et al*., 2009).

The antimicrobial gradient diffusion method uses the principle of establishment of an antimicrobial concentration gradient in an agar medium as a means of determining susceptibility. The Etest (bioMérieux AB BIODISK) is a commercial version available in the United States. It employs thin plastic test strips that are impregnated on the underside with a dried antibiotic concentration gradient and are marked on the upper surface with a concentration scale. As many as 5 or 6 strips may be placed in a radial fashion on the surface of an appropriate 150-mm agar plate that has been inoculated with a standardized organism suspension like that used for a disk diffusion test. After overnight incubation, the tests are read by viewing the strips from the top of the plate. The MIC is determined by the intersection of the lower part of the ellipse shaped growth inhibition area with the test strip (Reller*et al.*, 2009).

The gradient diffusion method has intrinsic flexibility by being able to test the drugs the laboratory chooses. Etest strips cost approximately $2-$3 each and can represent an expensive approach if more than a few drugs are tested. This method is best suited to situations in which an MIC for only 1 or 2 drugs is needed or when a fastidious organism requiring enriched medium or special

incubation atmosphere is to be tested (e. g, Penicillin and Ceftriaxone with *pneumococci*). Generally, Etest results have correlated well with MICs generated by broth or agar dilution methods. However, there are some systematic biases toward higher or lower MICs determined by the Etest when testing certain organism-antimicrobial agent combinations (Jorgensen *et al*., 1994). This can represent a potential shortcoming when standard MIC interpretive criteria derived from broth dilution testing are applied to Etest MICs that may not be identical (Prakash*et al*., 2008).

The disk diffusion susceptibility method (Jorgensen and Turnidge, 2007) is simple and practical and has been well-standardized. The test is performed by applying a bacterial inoculum of approximately $1–2\times10^{8}$CFU/mL to the surface of a large (150 mm diameter) Mueller-Hinton agar plate. Up to 12 commercially-prepared, fixed concentrations, paper antibiotic disks are placed on the inoculated agar surface. Plates are incubated for 16–24 h at 35°C prior to determination of results. The zones of growth inhibition around each of the antibiotic disks are measured to the nearest millimeter. The diameter of the zone is related to the susceptibility of the isolate and to the diffusion rate of the drug through the agar medium. The zone diameters of each drug are interpreted using the criteria published by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards or NCCLS) (CLSI, 2009) or those included in the US Food and Drug Administration (FDA)-approved product inserts for the disks. The results of the disk diffusion test are “qualitative,” in that a category of susceptibility (ie,

susceptible, intermediate, or resistant) is derived from the test rather than an MIC. However, some commercially-available zone reader systems claim to calculate an approximate MIC with some organisms and antibiotics by comparing zone sizes with standard curves of that species and drug stored in an algorithm (Nijs*et al*., 2000).

Use of instrumentation can standardize the reading of end points and often produce susceptibility test results in a shorter period than manual readings because sensitive optical detection systems allow detection of subtle changes in bacterial growth. There are 4 automated instruments presently cleared by the FDA for use in the United States. Three of these can generate rapid (3.5–16 hrs) susceptibility test results, while the fourth is an overnight system (Richter*et al*., 2007).

Srikanth*et al*2012. undertook study to measure, compare and characterize the aerobic microorganismsincomputer keyboards of hospital and non-hospital settings. Samples were collected from commonly used keys of computers in hospital and non-hospital settings using moistened sterile swabs, inoculated in liquid and solid media, and incubated aerobically at 37°C for 24-48 hrs. Growth was identified as per standard microbiological procedures. Antibiotic susceptibility was determined for pathogenic strains by Kirby-Bauer method. Growth was seen in all 80 samples (40 from each setting). *Staphylococcus aureus* was isolated from both settings (hospital: 6 MRSA, 11 MSSA; non-hospital: 4 MRSA, 9 MSSA). Gram-negative bacilli were isolated morefrequently from hospital (33%). Statistical analysis showed homogeneity

among isolates from computer keyboardsin both settings, except for Pseudomonas. They concluded that isolation of microorganisms from “high-touch” surfaces such as computer keyboards is indicative of the need for awareness on cleaning of such surfaces or disinfection and adequate hand hygiene (Srikanth*et al*., 2012).

CHAPTER THREE

MATERIALS AND METHODS

3.1. Study design

3.1.1. Type of study

This is a laboratory-based study.

3.1.2. Study area

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

3.1.3. Study duration

The study was undertaken in the period from June to December, 2014.

3.1.4. Sample size

A total of one hundred (100) bacterial isolates were used in this study.

3.2. Source of bacterial isolates

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

3.3. Methods

3.3.1. Purification of isolates

The isolates streaked on nutrient agar and incubated overnight at 37°C, descript colony picked up and checked for purity under microscope, and then stored in Bijou bottle for further investigation.

3.3.2. Re-identification of the isolates

3.3.2.1. Gram's stain

The smear prepared from overnight culture on clean and dry slide. The smear left to dry and fixed by rapid pass the slide three times through the flame of a Bunsen burner then allowed to cool before staining. Crystal violet stain added to smear for 30 -60 seconds,and then washed with clean tap water. Lugoĺs iodine added for 30- 60 seconds then washed by clean tap water and decolorized rapidly (few seconds) with acetone- alcohol and washed immediately with clean tap water. Finally, the smear covered with saffranin stain for 2 minutes, and washed by clean tap water. The back of slide wiped clean and placed in a draining rack for smear to air dry. Drop of oil 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 test

3.3.2.2.1. Fermentation of sugars, gas production and H_2S

Triple sugar iron agar (TSI) used to determine whether a Gram-negative rod utilize glucose and lactose and forms hydrogen sulfide (H_2S). TSI contain 10

parts lactose, 10 parts sucrose, 1 part glucose and peptone. Phenol red and ferrous sulfate serve as indicators of acidification and H_2S respectively.

The organism under test inoculated in TSI medium, then incubated at 37℃ for 18 – 24 hrs lactose fermenter organism gave yellow slope and yellow butt, while non lactose fermenter organism gave yellow slope and red butt. The production ofH_2S detected by formation of black colour. The gas production also had been examined (Forbes *et al*., 2002).

3.3.2.2.2. Urease test

The organism under test inoculated in a medium which contains urea and the indicator phenol red then incubated overnight for 37℃. When 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 becomes alkaline as shown by a change in colour of the indicator to pink –red (Forbes *et al.*, 2002).

3.3.2.2.3. Indole production

The conversion of tryptophan to indole by tryptophanase indicated by a colour change following addition of 5% (w/v) P-dimethyl-aminobenz aldehyde (Kovac' s), the indicator of the presence of phenol.

The organism under test inoculated in peptone water,then incubated overnight at 37℃. The detection of Indole done by addition of Kovac`sreagent, which gave red ring within 20 seconds in positive result, while gave yellow or green ring in the negative result (NCCLS, 2002).

3.3.2.2.4. Citrate utilization test

This test used to determine the ability of an organism to utilize sodium citrate as it is only carbon source and inorganic ammonium salts as its only nitrogenous source. Bacteria that can grow on this medium turn the bromothymol blue indicator from green to blue.

The organism under test inoculated in Koser citrate, and then incubated overnight at 37℃. Positive result gave blue colour, while green colour or no change is a negative result (Forbes *et al.*, 2002).

3.3.2.2.5. Catalase test

Some aerobic bacteria produce a catalase enzyme that hydrolyzes hydrogen peroxide into water and oxygen (bubbles).

By a wooden stick, the test organism inoculated into a glass tube containing 3% Hydrogen peroxide, which in positive result gave active oxygen bubbles immediately, while there are no oxygen bubbles in negative result within 10 seconds (NCCLS, 2002).

3.3.2.2.6. Coagulase test

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

3.3.2.2.7.DNase test

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

3.3.2.2.8. Manitol fermentation test

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

3.3.3Assessment of antimicrobial susceptibility test of the isolates (AST)

Modified Kirby-Bauer disc diffusion method was performed to assess the antimicrobialsusceptibility test of the isolates (AST).

3.3.3.1 Antibiotics

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

Ampicillin (AMP) (10µg), Cefuroxime (CRX) (30µg), Cotrimoxazole (COT) (25µg), Tetracycline (TET) (30µg), Amikacin (AMK) (30µg), Gentamicin

(GEN) (10µg), Cloxacillin (CXC) (5µg),Penicillin (PEN) (10µg), Meropenem (MRP) (10µg), Ciprofloxacin (5µg), Norfloxacin (NOR)(5µg),Amoxicillin (AX)(25µg), Amoxi-clav (AMC)(30µg), and Erythromycin (ERY) (15µg).

3.3.3.2 Control strain

Recommended organisms for quality assurance purposes are *S.aureus*ATCC 25923 (BSL 2), *E.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 McFarland standard by adding more organisms if the suspension is too light or diluting with sterile saline if the suspensionis too heavy. Thissuspension was used within 15 minutes of preparation (Cheesbrough, 2006).

3.3.3.4 Seeding of the plates

Asterilenon 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 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 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℃ air incubator for 16 to 18 hrs. The results readafter 18 hrs of incubation (Cheesbrough, 2006).

3.3.3.7 Measuring zone sizes

Following overnight incubation, the zone size was measured to the nearest millimeter using a ruler or caliper. 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 of 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

The assessment of multi- drug resistance among bacteria isolated from computer keyboards was performed between May 2014 and November 2014. The assessment done for 100 isolates (*Staphylococcus* species 42%, *Pseudomonas s*pecies 38%, *E. coli* species 12%, *Klebsiella*species 8%).The most efficient antibiotics used against G-negative bacteria were Amikacin, Meropenem and Gentamicin (100).*E.coli* and *Pseudomonas* species showed 100% sensitivity to Norofloxacin while *Klebsiella*speciesshowed 87.5%.However, sensitivity to Ciprofloxacin showed 75%, 66.66% and 60.5% among *Klebsiella*, *E.coli* and *Pseudomonas*species respectively.*E.coli* and *Klebsiella*speciesshowed 100% sensitivity to Amoxi-clav while *Pseudomonas* was resistant (100%).All isolates showed 100% resistance to Amoxicillin, Cefuroxime, Ampicillin and Penicillin.On the other hand regarding to *Staphylococcus* species the most efficient antibiotics used against *S.aureus* species were Amikacin and Gentamicin which showed 100% sensitivity.*S.aureus*showed 81.80%, 68.2%, 44.55% and 10% sensitivity to Ciprofloxacin, Erythromycin, Tetracycline and Amoxi-clavrespectively.*S.aureus* showed 100% resistance to Cotrimoxazole, Ampicillin, Penicillin and Cloxacillin.*S.epidermidis* showed 100% sensitivity to Ciprofloxacin and Ampicillin, 81.25% to Amikacin, 25% to Penicillin and 50% to

Amoxi-clav.While it showed 100% resistance to Gentamicin, Cotrimoxazole, Erythromycin, Tetracycline and Cloxacillin.*S.haemolyticus* showed 100% sensitivity to Gentamicin, Amoxi-clav, Ciprofloxacin, Erythromycin, Ampicillin, Penicillin and Cloxacillin.It showed 75% sensitivity to Amikacin.While it showed 100% resistance to Cotrimoxazole and Tetracycline.

Table 1. Frequency of bacteria isolates

Isolates	**Frequency (%)**
Staphylococcus species	42%
*Pseudomonas*species	38%
E. coli species	12%
*Klebsiella*species	8%

Table 2.Antibiotic sensitivity & resistance pattern of Gram-negative bacteria

Antibiotic	***E. coli*species(12)**		***Klebsiella* species (8)**		***Pseudomonas* species(38)**	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Amikacin	100% (12)	0.00% (0)	100%(8)	0.00%(0)	100%(38)	0.00%(0)
Gentamicin	100%(12)	0.00%(0)	100%(8)	0.00%(0)	100%(38)	0.00%(0)
Amoxicillin	0.00%(0)	100%(12)	0.00%(0)	100%(8)	0.00%(0)	100%(38)
Ciprofloxacin	66.66%(8)	34.44%(4)	75.0%(6)	25.0%(2)	60.5%(23)	39.5%(15)
Cefuroxime	0.00%(0)	100%(12)	0.00%(0)	100%(8)	0.00%(0)	100%(38)
Norfloxacin	100%(12)	0.00%(0)	87.5.% (7)	12.5%(1)	100%(38)	00.0%(0)
Amoxi-clav	0.00%(0)	100%(12)	0.00%(0)	100%(8)	100%(38)	00.0%(0)
Meropenem	100%(12)	0.00%(0)	100%(8)	0.00%(0)	100%(38)	0.00%(0)
Ampicillin	0.00%(0)	100%(12)	0.00%(0)	100%(8)	0.00%(0)	100%(38)
Penicillin	0.00%(0)	100%(12)	0.00%(0)	100%(8)	0.00%(0)	100%(38)

Table 3. Frequency of *Staphylococcus* isolates

Isolates	**Frequency (%)**
S. aureus	22%
S. epidermidis	16%
S. haemolitcus	4.0%

Table 4. Antibiotic sensitivity & resistance of *Staphylococcus* isolates

Antibiotic	*S. aureus*		*S. epidermidis*		*S. haemolitcus*	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Amikacin	100% (22)	0.00% (0)	81.25% (13)	18.75% (3)	75.0% (3)	25.0% (1)
Gentamicin	100% (22)	0.00% (0)	0.00% (0)	100% (16)	100% (4)	0.00 (0)
Cotrimoxazole	0.00% (0)	100% (42)	0.00% (16)	100% (0)	0.00% (0)	100% (4)
Amoxi-clav	10.0% (2)	90.0% (20)	50% (8)	50% (8)	100% (4)	0.00% (0)
Ciprofloxacin	81. 80% (18)	18.2% (4)	100% (16)	0.00% (0)	100% (4)	0.00% (0)
Erythromycin	68.2 % (15)	31.8% (7)	0.00% (0)	100% (16)	100% (4)	0.00% (0)
Ampicillin	0.00% (0)	100% (22)	100% (16)	0.00% (0)	100% (4)	0.00% (0)
Tetracycline	44.55% (12)	55.45% (10)	0.00% (0)	100% (16)	0.00% (0)	100% (4)
Penicillin	0.00% (0)	100% (22)	25% (4)	75% (12)	100% (4)	0.00% (0)
Cloxacillin	0.00% (0)	100% (22)	0.00% (0)	100% (16)	100% (4)	0.00% (0)

Table 5. Biochemical tests adopted for re-identification of bacterial isolates

Isolate code	Biochemical tests																Species
	Indole	Urease	Citrate	Oxidase	KIA				Coagulase	DNase	Mannitol	Sugar fermentation					
					Slope	Butt	Gas	H_2S				Glucose	Maltose	Sucrose	Mannose	Trehalose	
12 Isolates	+	-	-	-	Y	Y	+	-	NT	NT	NT	NT	NT	NT	NT	NT	*E.coli*
8 Isolates	-	+	+	-	Y	Y	+	-	NT	NT	NT	NT	NT	NT	NT	NT	*Klebsiella pneumonae*
38 Isolates	-	D	+	+	R	R	-	-	NT	NT	NT	NT	NT	NT	NT	NT	*Pseudomonas aerginosa*
22 Isolates	NT	NT	NT	NT	NT	NT	NT	NT	+	+	+	+	+	+	+	+	*S. aureus*
16 Isolates	NT	NT	NT	NT	NT	NT	NT	NT	-	-	-	+	+	+	$+^{sl}$	-	*S. epidermidis*
4 Isolates	NT	NT	NT	NT	NT	NT	NT	NT	-	-	V	+	+	+	-	+	*S. haemolyticus*

Key:

NT= Not tested D= different V= Variable reaction

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

CHAPTER FIVE

DISCUSSION

5.1. Discussion

Computers have been commonly used in daily life. This study carried out to search the Multi-drug resistance of bacteria isolated from computer keyboards used in Sudan University.

Keyboards have become reservoirs for pathogens especially in hospitals and schools (Diggs *et al.*, 2008). One should also note here that a reason for the increased percentage of contamination of computers is the difficulty of cleaning and disinfection (Marsden, 2009), as well as the misconception that cleaning keyboards could possibly damage therm.

The result of this study releaved that *E.coli* isolates were highly (100%) susceptible to Amikacin, Gentamicin, Norfloxacin and Meropenem but less susceptible to ciprofloxacin (66.66%). While isolates were highly resistant to Ampicillin (100%). These results were compared to observations of previous studythat found all isolated *E. coli* were susceptible to Gentamicin, 50% were sensitive to Ampicillin and 83.3%were sensitive to ciprofloxacin. (Maryam and Usman, 2014).

Isolates of *Klebsiella* species were highly (100%) susceptible to Amikacin, Gentamicin and meropenem but less susceptible to Norfloxacin (87.5%) and Ciprofloxacin (75%). While isolates were highly resistant to Ampicillin

(100%). In similar study, all*Klebsiella* species isolates were susceptible to Gentamicin but 100% resistant to ciprofloxacin while 100% sensitive to Ampicillin. (Maryam and Usman, 2014).

All the isolated *Pseudomonas* species were highly susceptible (100%) to Amikacin, Gentamicin, Norfloxacin, Meropenem and Amoxi-clav but less susceptible to Ciprofloxacin (60.5%). While isolates were highly resistant to Amoxicillin, Cefuroxime, Penicillin and Ampicillin.While the isolated *Pseudomonas* species in another study were susceptible to Gentamicin and 50% to Ciprofloxacin, While isolates were 100% resistant to Ampicillin. (Maryam and Usman, 2014).

In this study *S. aureus* isolates were highly susceptible (100%) to Amikacin and Gentamicin but less susceptible to Ciprofloxacin (81.80%) and Erythromycin (68.2%). While isolates were highly resistant (100%) to Ampicillin and Cotrimoxazole and 55.45% to Tetracycline. With comparison to another study that showed all *S. aureus* isolates were sensitive to Erythromycin (100%). While the isolates were resistant to Gentamicin.(Maryam and Usman, 2014).

All the isolated *S. epidermidis* were highly susceptible (100%) toCiprofloxacin and Ampicillin. While isolates were highly resistant (100%) to Gentamicin, Cotrimoxazole, Erythromycin and Tetracycline. In similar study, all *S.epidermidis* species were highly sensitive (100%) to Ciprofloxacin and

Erythromycin while less sensitive (50%) to Gentamicin and Cotrimoxazole. (Maryam and Usman, 2014).

5.2. **Conclusion**

The study shows that:

1. The *S. epidrmidis* species were Multi-drug resistant to Gentamicin, Cotrimoxazole, Erythromycin, Tetracycline and Cloxacillin.
2. The antibiotic resistance of G-negative rods was high (100%) to Amoxicillin, Cefuroxime, Ampicillin and Penicillin. While antibiotic susceptibility was high (100%) to Amikacin, Gentamicin and meropenem.
3. The antibiotic resistance of G-positive cocci was high (100%) to Cotrimoxazole.

3.3. Recommendations

1. Implementation measures of hygiene by providing disinfectants at entry and at several critical contamination points in computer offices to minimize hand contamination.
2. Hygienic standards education for community to take care when using computer keyboards
3. Methods of decontamination and disinfection of computers keyboards should be elaborated to consumers

4. Frequent hand cleansing, especially with instant hand sanitizers is the most significant step to help prevent faeco-oral and droplet transmissions.
5. The computer keyboards should be in a manner that does not get contaminated with dirt and/or disease-causing agents.
6. The computer keyboards should be regularly cleaned with relevant disinfectants.
7. Covering the mouth or nose when coughing or sneezing decreases droplet spread and makes hand cleansing even more important.

REFERENCES

1. **ASM,(2005).** Women better at hand hygiene habits, hand down. ASM Press Releases: Washington, DC. Citeseerx. Ist. psu. edu.
2. **Balows A, (1972).** Current techniques for antibiotic susceptibility testing. Springfield, IL:Charles C. Thomas.
3. **Boa TT, Rahube TO, Fremaux B, Levett PN and Yost CK. (2013).** Prevalence of methicillin-resistant *staphylococci* species isolated from computer keyboards located in secondary and postsecondary schools. *J EnvironHealht*; **75**(6):50-8.
4. **Cheesbrough M, (2006).** District Laboratory Practice in Tropical Countries, Part2, Cambridge University Press, United Kingdom, PP 60-64.
5. **Clinical and Laboratory Standards Institute, (2009).** Performance standards for antimicrobial susceptibility testing. Nineteenth informational supplement M100–S19. Wayne, PA: Clinical and Laboratory Standards Institute.
6. **Dandekar T and Dandekar G,. (2010)**.Pharmacogenomic strategies against microbial resistance: from bright to bleak to innovative. *Fut. Med. Ltd*; **11**(9):1193-1196.
7. **Diggs R,Diallo A, Kan** H, **Glymph C, Furness BW and ChaiSJ, (2008).** Norovirus outbreak in an elementary school—Districtof Columbia,February 2007. Centers for Disease Control and Prevention: Morb.Mortal. Wkly. Rep; **51**: 1340-1343.

8. **Ducel G and Fabry J, (2001).** Epidemiology of nosoccmial infections. 2nd ed. Newyork: WHO, 4-9.

9. **Eltablawy, S.Y. and Elhifnawi, H.N. (2009)**. Microbial Contamination of Some Computer Keyboards and Mice in National Center for Radiation Research and Technology (NCRRT). *World Appl. Sci. J;***6** (2): 162-167.

10. **Ericsson JM and Sherris JC, (1971).** Antibiotic sensitivity testing: report of an international collaborative study. *ActaPatholMicrobiolScand*; **217**(Suppl):1-90.

11. **European Congress Clinical Microbiology and Infectious Diseases (ECCMID), (2008).** Infections control and contamination of daily use objects. The 18th ECCMID, Barcelona, 19-22 April.*www.eccmid.org*.

12. **Famurewa O and David OM**, **(2009).** Cell phones: A medium of transmission of bacterial pathogens. World Rural Observations;**1**(2):69-72.

13. **Fatma U, Saban E, Ahmet D, Yanik K, Murat G and Hakan L, (2009).** Are we aware how contaminated is our mobile phones with nosocomial pathogens? *Ann Clin Microbiol Antimicrob*;**8**(7):1-4.

14. **Forbes BA., Saham DF, and Weissfeld AS, (2002).** Bailey and Scott's diagnostic microbiology, vol**2**, 12th ed. Mosby, Inc., St. Louis.Chapter 7, P.111. Chapter 13, P. 222, 225, 243, 245, 246.

15. **Georgopapadakou, N. (2007).** Antibiotic Resistance in *Enterobacteria*.Wax R.,Bacterial Resistance to Antimicrobials, 2nded., Taylor & Francis Group, Boca Raton London New York, p:343.

16. **Hidron AL, E.V. Kourbatova, J. Halvosa, B.J. Terrell, L.K. McDougal and F.C. Tenover et al., (2005).** Risk factors for colonization with methicillin- resistant *Staphylococcus areus* (MRSA) in patients admitted to on urban hospital; emergence of community-associated MRSA nasal carriage. *Clin. Infect. Dis*; **41**: 159-166.

17. **Jorgensen JH and Turnidge JD, (2007).**Antibacterial susceptibility tests: dilution disk diffusion methods. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, eds. Manual of clinical microbiology. 9th ed. Washington, DC: American Society for Microbiology, 1152–1172.

18. **Jorgensen J.H, Ferraro M.J,McElmeel M.L,Spargo J,Swenson J.M and Tenover, F.C. (1994**). Detection of penicillin and extended-spectrum cephalosporin resistance among *Streptococcus pneumoniae* clinical isolates by use of the E test. *J ClinMicrobiol*; **32**:159-63.

19. **June K**, **Eun P and Jae J (2000).** Multicenter surveillance study for nosecomial infection in major hospitals in Korea. *American Journal of InfectionControl*; **28:454**-457.

20. **Kassem I.k, Sigler V and Esseili M .A, (2007) .** Public computer surfaces are reservoirs for methicillin-resistant *staphylococci*. *The ISMEJournal*; **1**: 265–268.

21. **Marsden R (2009). ASolid-Surfaced Infection Control Computer Keyboard.** www.cleankeys.nl/whitepaper.pdf, pp. 1-5.

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

23. **NCCLS. Performance Standards forAntimicrobial Susceptibility Testing; Twelfth Informational Supplement.(2002).**NCCLS document M100-S12 (ISBN 1-56238-454-6). NCCLS, 940 West Valley Road, Suite 1400, Wayne, Pennsylvania 19087-1898, USA (General NCCLS website: http://www.nccls.org).

24. **Nijs A, Cartuyvels R, Mewis A, Peeters V, Rummens JL and Magerman K, (2000).** Comparison and evaluation of Osiris and Sirscan antimicrobial susceptibility systems in the clinical microbiology laboratory. *J ClinMicrobiol; 41:3627*–30.

25. **Prakash V, Lewis JSII and Jorgensen JH, (2008).** Vancomycin MICs with methicillin-resistant *Staphylococcus. aureus* (MRSA) isolates differ based upon the susceptibility test method used. *Antimicrob Agents Chemother*; **52**:4528.

26. **Ravichandran, D, (2001).** Introduction to Computers and Communication. 1st Ed., Tata McGraw-Hill Education, New Delhi, ISBN-10: 0070435650. pp: 584.

27. **Reller LB, Weinstein M, Jorgensen J H and Ferraro MJ, (2009).** Antimicrobial Susceptibility Testing: A Review of General Principles and Contemporary Practices. *Clin Infect Dis*; **49** (11):1749-1755.

28. **Richter SS and Ferraro MJ, (2007).** Susceptibility testing instrumentation and computerized expert systems for data analysis and interpretation. In: Murray PR, Baron EJ, Jorgensen JH, Landry ML, Pfaller MA, eds. Manual of clinical microbiology. 9th ed. Washington, DC: American Society for Microbiology: 245–56.

29. **Rutala WA, White MS, Gergen MF, Weber DJ. (2003).**Bacterial contamination of keyboards: efficacy and functional impact of disinfectants. *Infect Control Hosp Epidemiol.*; 27(4):372-373.

30. **Schultz M, Gill J, Zubairi S, Huber R and Gordin F, (2003)**. Bacterial contamination of computer keyboards in a teaching hospital.*Infect. ContHospEpidemiol*; **24**(4):302-3.

31. **Srikanth P, Sivasubramanian S, Sudharsanam S, Thangavel G and Jagannathan K, (2012).** Assessment of Aerobic Bacterial Contamination of Computer Keyboards in a Tropical Setting. *J Associate Physicians India*; VOL. 6.

Appendices

Appendix (1): culture media

1.1. Difco™Nutrient Agar

Approximate formula * per Liter

Beef Extract .....……...….3.0g

Peptone ……...…...…. 5.0g

Agar …….....15.0g

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

1.2. Difco™MacConkey Agar

Approximate formula * per Liter

Peptone…….....….20.0g

Lactose ……...…….....10.0g

Bile Salts…….…...5.0g

Sodium Chloride ……...…..….5.0g

Agar…….....…...12.0g

Nutrient Red…….…...….....0.05g

1.3. Difco™Manitol Salt Agar

Approximate formula * per Liter

Proteose Peptone No.3…….…….....10.0g

Beef Extract…….……...…...….1.0g

D-Mannitol…….…….....10.0g

Sodium Chloride…….....….....….…75.0g

Agar…….....…….15.0g

Phenol Red……...25.0g

Final PH (at 25℃) 7.4 ± 0.2

1.4. Mueller Hinton Agar

Approximate formula * per Liter

Beef Extract 2g

Acid Hydrolysate of Casein 17.5g

Starch 1.5g

Agar 17g

Final pH 7.3 ± 0.1 at 25°C

Formula may be adjusted and/or supplemented as required to meet performance specifications.

1.4. Kligler Iron Agar

Approximate formula * per Lite

Peptic digest of animal tissue……..15.00g

Beef extract……..……3.00g

Yeast extract……...……..3.00g

Proteose peptone……...…….5.0g

Lactose……...…..10.0g

Dextrose…….……..1.00g

Ferrous sulphate……...0.20g

Sodium chloride……...5.00g

Sodium thiosulphate……...…….0.30g

Phenol red……...…...0.024g

Agar……..….15.00g

Final PH (at 25℃) 7.4℃

1.5. Urea agar base

Approximate formula * per Lite

Peptic digest of animal tissue……..1.00g

Dextrose…….……..1.00g

Sodium chloride……...5.00g

Di sodium thiosulphate……1.20g

Monopotassium phosphate……..……..0.80g

Phenol red……...0.012g

Agar……..…..….15.00g

Final PH (at 25℃) 6.8 ± 0.2

1.6. Peptone water

Approximate formula * per Lite

Peptic digest of animal tissue……...……..10.00g

Sodium chloride…….…...5.00g

Final PH (at 25℃) 7.2 ± 0.2

1.7. Kosser ’s citrate

Approximate formula * per Lite

Magnesium sulphate…….…0.20g

Potassium disulphate phosphate……..…….……...1.00g

Sodium ammonium phosphate……..……...1.50g

Tri sodium citrate……..2.50g

Bromothymole blue……...…….0.016g

Appendix (2) reagents

2.1. Kovac' s reagent

(P)- di methyl aminobenzaldehyde……..…….2gm

2.2. Physiological saline (0.85%)

NaCl……..…….0.85gm

Distilled water…….….100ml

2.3. McFarland standard NO.3

1.0% H_2SO_4 (1.0 ml H_2SO_4 + 99 ml distilled water)…….....…..….0.3ml

1.0% $BaCl_2$ (1.0 gm $BaCl_2$ + 100 ml distilled water)……...…….9.7ml

2.4. Catalase reagent

3% H_2O_2……...…..…..…2ml

Appendix (3) Gram Stain

Crystal violate……...…20.00 gm

Ammonium oxalate……...……99.00 gm

Ethanol…….…..…95.00 ml

Distilled water……..…….….1ml

Logols iodine

70% alcohol

Saffranine

Appendix (4) Instruments

2.1 Safety cabinet Daihan lab tech co LTD, made in UK .

2.2 IncubatorGALL ENK AMPMade in UK

2.3 Freezer-20 Made in EUROP

2.4 Water bath Model: LWB-111D, made in UK

2.5 Microscope Model A15120-4, made in Germany

2.6 Sensitive balances

2.7 Ultra low temperature freezer-70

Model MDF-392, made in Japan

2.8. Refrigerator with glass door

Made in Saudi Arabia

2.9 Autoclave

Dixons, surgical instrument LTP, made in UK