Sudan University of Sciences and Technology

College of Graduate Studies

Antimicrobial Resistance of Bacterial Contaminants of Hospital Doorknobs in Selected Hospitals in Khartoum State

مقاومة الملوثات الجرثومية للمضادات الحيوية في مقابض أبواب مستشفيات مختارة في ولاية الخرطوم

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

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الآيـــــة

قال تعالى:

" أَمَّنْ هُوَ قَانِتٌ آنَاءَ اللَّيْلِ سَاجِدًا وَقَائِمًا يَحْذَرُ الْآخِرَةَ وَيَرْجُو رَحْمَةَ رَبِّهِ ۗ قُلْ هَلْ يَسْتَوِي الَّذِينَ يَعْلَمُونَ وَالَّذِينَ لَا يَعْلَمُونَ ۗ إِنَّمَا يَتَذَكَّرُ أُولُو الْأَلْبَابِ"

صدق الله العظيم

سورة الزمر (الاية 9)

Dedication

To my father,

Who taught me the meaning of life

To my mother,

Praying for me to succeed

To my brother and sister,

For their support and kindness

To my friends and colleagues,

The persons, whom I love, respect and appreciate

To everyone from whom I learned

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Alhamdulillah, praise to **ALMIGHTY ALLAH** for blessing and guidance which enabled me to complete this research.

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Abstract

Door handles are documented as breeding grounds for pathogens and presents as focal point of high risk common contact surfacing which facilitates transmission of pathogens within the hospital buildings. Hand hygiene has been singled out as the most important and one of the most effective means of preventing pathogens associated with health care services. This cross sectional study was aimed to investigate the antimicrobial resistance of bacterial contaminant of hospital door knobs in Khartoum state during the period from February to May 2022. Five hospitals of four different wards were included in this study. One hundred (n=100) swabs were taken from (100) door handles from five hospitals of four different locations .These swabs were inoculated on MacConkey and blood agar. Bacterial identification was carried out by different conventional methods and antimicrobial susceptibility testing was done by disc diffusion method. Statistical analysis was done using SPSS version 25. Out of the 100 samples processed, 92 (92%) of them showed bacterial growth. The bacteria isolated were *S. aureus* 34(37%) and nosocomial bacteria, such as *Peudomonas.spp* 24(26%), *Klebsiella.spp 11* (12%), *E coli* 7(8%). *S. epidermidis* 7 and *B.spp* 9 constitutes 7% and 10% respectively. The isolated bacteria showed varying susceptibility pattern to the antibiotics used and were all-resistant to at least two antibiotics. Highest resistance percentage of the isolates was observed against Penicillin (51%) followed by Ceftriaxone (40%) and Clindamycin (39%).

In conclusion findings of this study indicate the presence of bacterial strains resistant to more than two antibiotics in door handles of a hospital which can serve as potential source of diseases.

مستخلص الاطروحة

تم توثيق مقابض الأبواب على أنها مناطق تكاثر لمسببات الأمراض وتقدم كنقطة محورية عالية الخطورة مما يسهل انتقال مسببات الأمراض داخل مباني المستشفى. تم تمييز نظافة اليدين على أنها أهم الوسائل وأكثرها فعالية للوقاية من مسببات الأمراض المرتبطة بخدمات الرعاية الصحية.

هدفت هذه الدراسة للتحقيق في مقاومة مضادات الميكروبات للملوثات البكتيرية لمقابض أبواب المستشفى . كانت هذه دراسة مقطعية أجريت في ولاية الخرطوم خلال الفترة من فبراير إلى مايو ٢٠٢٢. تم تضمين خمسة مستشفيات من أربعة أجنحة مختلفة في هذه الدراسة. تم أخذ مائة (١٠٠) مسحة من (١٠٠) مقابض الأبواب بإستخدام ماسحة قطنية مبللة بمحلول ملحي و تم إستزراعها على أجار ماكونكي و أجار الدم ، تم إجراء التعرف على البكتيريا من خلال اختبارات كيميائية حيوية مختلفة وتم إجراء اختبار الحساسية للمضادات الحيوية بطريقة اختبار انتشار القرص. تم إجراء التحليل الإحصائي باستخدام الإصدار ٢٥ من الحزمة الإحصائية للعلوم الاجتماعية.من أصل ١٠٠ عينة تمت معالجتها، أظهرت ٩٢ (٩٢٪) منهم نمو بكتيري. كانت البكتيريا المعزولة هي المكورات العنقودية الذهبية ٣٤(٣٧%) والزائفة الزحارية٢٤ (٢٦%) والكلبسيلا ١١ (١٢%)، الإشريكية القولونية ٧(٨٪). بينما تشكل المكورات العنقودية البشرية وبكتريا سيريوس العصبية ٧٪ و ١٠٪ على التوالي. أظهرت البكتيريا المعزولة أنماط حساسية متفاوتة للمضادات الحيوية المستخدمة وكانت جميعها مقاومة لمضادات حيوية على الأقل. ولوحظت أعلى نسبة مقاومة للعزلات ضد البنسلين (٥١٪) يليه سيفترياكسون (٤٠٪) والكليندامايسين (٣٩٪). تشير نتائج هذه الدراسة إلى وجود سلالات بكتيرية مقاومة لأكثر من اثنين من المضادات الحيوية في مقابض الابواب بالمستشفى والتي يمكن أن تكون مصدرًا محتملاً للأمراض.

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List of Abbreviations

CDC	-	Centers for Disease Control
HAI	-	Healthcare-associated Infections
ICU	-	Intensive Care Unit
IPA	-	Isopropyl Alcohol
MDR	-	Multi Drug Resistance
MRSA	-	Methicillin Resistant S.aureus
MSSA	-	Methicillin Sensitive S.aureus
MOH	-	Ministry of Health
SPSS	-	Statistical Package for Social Sciences

Chapter I

Introduction

CHAPTER I
INTRODUCTION

1.1. Introduction

Healthcare-associated infections (HAI) are a major public health concern commonly associated with extended length of hospital stay. HAI account for high hospital costs and contribute to increased morbidity and mortality of infected patients (Bonnet *et al*., 2019).

HAI are usually caused by pathogenic bacteria that may emerge from the patient's endogenous microflora during antibiotic therapy in approximately 70% of the cases (Weber *et al*., 2013). HAI may also be acquired from the exogenous environment (30% of the cases) in that the hospital setting plays a significant role in contagion and transmission outbreaks (Weber *et al*., 2013).

In the hospital setting, patients, staff and visitors represent the main reservoir of microorganisms, whereas secondary reservoirs include all environments where nutrients, moisture, and temperature are suitable for microbial survival, such as air humidifiers and nebulizers (Russotto *et al*., 2015). In addition, dry and inanimate surfaces can also serve as a reservoir of pathogens (Russotto *et al*., 2017; Adams *et al.*, 2017), as in mattresses and bed frames (Shams *et al.*, 2016), door knobs (Silva *et al.*, 2012), and even in medical equipment such as stethoscopes and ultrasound devices (Silva *et al.*, 2012). Contamination of these surfaces contributes to pathogen spreading and, as a result, development of horizontal infections (Russotto *et al*., 2017; Adams *et al.*, 2017).

Overall, door knobs may be contaminated by common bacteria of the hand microbiota. More importantly, MDR bacteria have been detected in medical equipment and contact surfaces, especially in critical care units (Costa *et al.*, 2019; Galvin *et al*., 2012). Studies carried out in Brazil and North America have reported contamination of hospital surfaces by bacteria resistant to antibiotics, especially methicillin-resistant *S. aureus* (MRSA) and vancomycin-resistant enterococci . These findings indicate that patients and staff are at risk of contamination by pathogens associated with high mortality rates against which treatment options are restricted. (Weber and Rutala., 2013)

Therefore, Monitoring and evaluation of hospital door handles is necessary for infection control because there is a possibility that contaminated door handles may increase the risk of acquiring infections that often result from contact with door handles contaminated by people who do not practice hand hygiene. Door handles may also get contaminated by gloves and other cross contaminated objects and subjects found within the hospital environment (Ministry of Health , 2015), given the importance of in-hospital transmission, This study therefore aimed at generating data on the level of bacterial contamination, as well as identify bacterial contaminants in door handles of some hospital sections in Khartoum since generally this data is limited.

1.2. Rationale

Door handles of hospitals are taken by many people as vehicles of contamination. On each day of the week, the hospitals receive several hundred of patients; the door handles to these hospitals pose a possible threat to users as microbes could be shared between users. During cleaning up activity of hospitals, handles of door are sometimes not well cleaned. This negligence causes the handles of door to serve as suitable environmental surfaces for bacteria to thrive, resulting in loads of microorganisms on the surfaces which can pose a risk to the health of the staff and patients who touch door knobs. The negligence, ignorance and the risk the microbes may pose to the health of the public therefore needs to be assessed.

1.3. Objectives

1.3.1. General Objective

To investigate the antimicrobial resistance of bacterial contaminants of hospital door knobs.

1.3.2. Specific Objectives

1.To isolate and identify the presence of contaminant bacteria.

2.To enumerate the load of bacterial present on door knobs at selected hospital.

3.To determine the sensitivity of isolated microorganism’s to routine antimicrobials.

Chapter II

LITERATURE REVIEW

CHAPTER II
LITERATURE REVIEW

2.1. Microorganism

A microorganism, or microbe, is an organism of microscopic size, which may exist in its single-celled form or as a colony of cells. They are important in human culture and health in many ways, serving to ferment foods and treat sewage, and to produce fuel, enzymes, and other bioactive compounds. Microbes are essential tools in biology as model organisms and have been put to use in biological warfare and bioterrorism. Microbes are a vital component of fertile soil. In the human body, microorganisms make up the human microbiota, including the essential gut flora. The pathogens responsible for many infectious diseases are microbes and, as such, are the target of hygiene measures (Schopf *et al*., 2017).

2.1.1. History of microorganisms

The microorganisms were first discovered and described by Robert Hooke and Antoni van Leeuwenhoek in 1665 and 1678 respectively, both of whom came from different science backgrounds. Their discovering by using of microscope devises which make change in medical side and life histories. Following historical financial records Leeuwenhoek was frequently defined as the ″first of the microbe hunters″. This cited his renowned letters of the 9 October in 1676 as charitable the first un-mistakable explanations of microbial (bacteria) (Schopf *et al*., 2017).

The Robert Hooke scientist open-minded to microscopy towards recognition of small living things that's way he considered as the backbone of microbiology, moreover he was the first to check explanations of Leeuwenhoek scientist, and he was considered to be dubious by many colleagues. Re-examination of the proceedings and publications of the Royal Culture from 1665 to 1678 expression that Robert Hooke and Antoni van Leeuwenhoek both were the most important discoverers of the microbial in the world (Gest, 2014).

2.1.2. Classification of microorganisms

Microorganisms can be found almost anywhere on Earth. Bacteria and archaea are almost always microscopic, while a number of eukaryotes are also microscopic, including most protists, some fungi, as well as some micro-animals and plants. Viruses are generally

regarded as not living and therefore not considered as microorganisms (Keen *et al.*, 2012).

2.1.3. Bacteria

Are ubiquitous, mostly free-living organisms often consisting of one biological cell. They constitute a large domain of prokaryotic microorganisms. Typically a few micrometres in length, bacteria were among the first life forms to appear on Earth, and are present in most of its habitats. Bacteria inhabit soil, water, acidic hot springs, radioactive waste, and the deep biosphere of Earth's crust. Bacteria are vital in many stages of the nutrient cycle by recycling nutrients such as the fixation of nitrogen from the atmosphere. (Keen *et al.*, 2012).

2.1.3.1. Classification and Identification of Bacteria

Historically, bacteria were considered a part of the Plantae, the Plant kingdom, and were called "Schizomycetes" (fission-fungi). For this reason, collective bacteria and other microorganisms in a host are often called "flora". The term "bacteria" was traditionally applied to all microscopic, single-cell prokaryotes. However, molecular systematics showed prokaryotic life to consist of two separate domains, originally called Eubacteria and Archaebacteria, but now called Bacteria and Archaea that evolved independently from an ancient common ancestor. The archaea and eukaryotes are more closely related to each other than either is to the bacteria. These two domains, along with Eukarya, are the basis of the three-domain system, which is currently the most widely used classification system in microbiology. However, due to the relatively recent introduction of molecular systematics and a rapid increase in the number of genome sequences that are available, bacterial classification remains a changing and expanding field. For example, Cavalier-Smith argued that the Archaea and Eukaryotes evolved from Gram-positive bacteria (Brown and Horswill, 2020).

The identification of bacteria in the laboratory is particularly relevant in medicine, where the correct treatment is determined by the bacterial species causing an infection. Consequently, the need to identify human pathogens was a major impetus for the development of techniques to identify bacteria. (Brown and Horswill, 2020).

The Gram stain, developed in 1884 by Hans Christian Gram, characterises bacteria based on the structural characteristics of their cell walls. The thick layers of peptidoglycan in the "Gram-positive" cell wall stain purple, while the thin "Gram-negative" cell wall appears pink. By combining morphology and Gram-staining, most bacteria can be classified as belonging to one of four groups (Gram-positive cocci, Gram-positive bacilli,

Gram-negative cocci and Gram-negative bacilli). Some organisms are best identified by stains other than the Gram stain, particularly mycobacteria or Nocardia, which show acid-fastness on Ziehl–Neelsen or similar stains. Other organisms may need to be identified by their growth in special media, or by other techniques, such as serology (Tang *et al*., 2019).

Culture techniques are designed to promote the growth and identify particular bacteria, while restricting the growth of the other bacteria in the sample. Often these techniques are designed for specific specimens; for example, a sputum sample will be treated to identify organisms that cause pneumonia, while stool specimens are cultured on selective media to identify organisms that cause diarrhea, while preventing growth of non-pathogenic bacteria. Specimens that are normally sterile, such as blood, urine or spinal fluid, are cultured under conditions designed to grow all possible organisms. Once a pathogenic organism has been isolated, it can be further characterized by its morphology, growth patterns (such as aerobic or anaerobic growth), patterns of hemolysis, and staining (Riley, 2018).

2.2. Bacterial Contamination

Bacterial contamination is a situation which occurs when bacteria end up in a location where they are not supposed to be. It is often used to refer to contamination of food by bacteria which can cause disease, but can also occur in other settings. This situation is not desirable, because it can pose a health threat and cause other problems. As a result, steps are taken to avoid contamination in settings where it can become an issue (Oluduro *et al*., 2012).

Bacterial contamination can also be a problem in medical clinics, operating rooms, and other health care settings. The bacteria can be transferred from patients or health care providers, and they may end up on surgical instruments, medical equipment, door knobs, and numerous other sites. In health care settings, this is an especially big issue because sick people are at risk of becoming sicker if they are exposed to harmful bacteria (Oluduro *et al*., 2012).

Prevention of bacterial contamination can be challenging. Keeping spaces clean and observing proper handling procedure is a big part of prevention. Simple steps like washing hands, dipping shoes in an antibacterial bath after exiting a patient's room, and wearing gloves to handle specimens can cut down a great deal on the risk of passing bacteria from one place to another. It is also important to conduct regular testing to check

for bacterial contamination so that it can be identified before it makes someone sick or causes problems with an experiment or test (Oluduro *et al.*, 2012).

2.3. Door handles pathogens

Bacterial pathogens that have been isolated from door handles in previous studies includes *S. aureus, K.pneumonia, E. coli, Enterobacter spp, Citrobacter spp, P. aeruginosa, Proteus spp, Streptococcus spp, Salmonella spp, Shigella spp, Campylobacter spp* (Nworie *et al*.,2012). These organisms have been known to cause one or more diseases that are mild and could be sometimes serious. The examples of such diseases range from simple skin diseases like pimple, impetigo, scalded skin syndrome to respiratory diseases like, pneumonia to even severe meningitis, osteomyelitis, rhinoscleroma, kidney failure, septicemia and so on (Clauditz *et al.*, 2016).

2.4. Microbial contamination and antibiotic resistance

The discovery of antibiotics turned to more than 70 years, initiated a period of drug innovation and application in human. There are many outbreaks of bacterial infection that are progressively being reported when it is associated with antibiotic resistance, the Centers for Disease Control and Prevention (CDC) tracked a multistate outbreak of *Salmonella enterica*, the enteric serovar Heidelberg infections which related to contaminated ground turkey and disgusted more than 130 people. While these bacteria were resistant to numerous types of antibiotics, the distressed might be preserved with another agent. Also, in Germany, an epidemic of bacteria *Escherichia coli* contaminations caused through vegetables pretentious up to 5,000 people in addition to 50 deaths. Forthcoming large outbreaks is the emergence and universal spread of antibiotic resistance genes. Like the New Delhi metallo βlactamase resistance gene (blaNDM-1), which discusses resistance to penicillin, cephalosporins and a range of their derivatives which has been spread quickly in 2010 (Bush *et al.*, 2012).

From the start of the antibiotic period selective used by antibiotic usage was a soon reflected by resistance improvement in *Staphylococci* and *Micrococcu*s (Gram positive type of bacteria), this was the initiated of immediately with outline of penicillin G in 1941, followed by resistance to additional classes of materials presented one after the other throughout the golden age of antibiotics. The bacterial resistance belongs to Gram-positive in additional to Gram-negative are motionless cumulative. There are numerous drug resistance in pneumococcal infections determination of principal toward extra treatment disappointments fail which so distant consume seen by way of penicillins and pathogens through in height value of resistance, and this condition result in greater

mortality with long term of staying hospital then advanced prices related with methicillin resistant *Staphylococcus aureus* (MRSA) infections, now in contrast through methicillin susceptible *Staphylococcus aureus* (MSSA) infections likewise, vancomycin resistant enterococci. (Bush *et al.*, 2012).

Another significant pathogen is Streptococcus pyogenes that is resistant to macrolides, and the macrolide resistant streptococci of groups B, C, and G, also the coagulase negative staphylococci that are resistant to macrolides, aminoglycosides, B lactam group, glycopeptides and lincosamides. The Gram negative microorganisms characteristically are more resistant to antimicrobials than comparing to Gram-positive bacteria, and this has long been clarified by the presence in the former of the outer membrane penetrability barrier of the cell wall which limits access of the antimicrobial agents to their targets in the bacterial cell. (Bush *et al*., 2012).

The Gram negative bacteria are responsible for a considerable percentage of all bloodstream infections, which lead in patterns of reduced susceptibility to antibiotics were found among Gram-negative bacteria. Despite the high prevalence of antibiotic resistance among Gram-negative bacteria causing bacteremia, the clinical consequences of resistance remain unclear. Important members of the gram-negative bacteria are containing (*Acinetobacter spp, Pseudomonas spp, Stenotrophomonas spp,* and *Burkholderia spp*). Also, these microorganisms are belonging to those function pathogens that principally source of opportunistic infection especially in healthcare associated contaminations who remain disapprovingly ill and/or condition with low immune system. The treatment with multidrug resistance now adays is communal besides to increasing amongst gram negative non-fermenters bacteria, the quantity of straining has currently remained recognized that exhibit resistance to fundamentally altogether generally used antibiotics, as well as anti-pseudomonal penicillins in additional carbapenems, aminoglycosides, sulfamethoxazole, cephalosporins, tetracyclines, trimethoprim- and fluoroquinolones. The polymyxins are outstanding antibiotic medication with justly reliable activity in contradiction of multi drug resistant for *Acinetobacter spp, Stenotrophomonas maltophilia , Pseudomonas aeruginosa* (Bush *et al*., 2012).

There are variety mechanisms of *P. aeruginosa* towards the resistance including efflux pumps, target-site variations, enzyme creation, porin insufficiencies. Also, there are many genes responsible for resistance regularly cohabit in organism at the same time. Moreover, the many medication resistances in non-fermentative and gram-negative lead to difficult in treatment which lead to both problematic and costly. For the detection of

the resistance of bacteria, a current test should be performed in order to detect different types of bacteria among different types of antibiotics. Moreover, different susceptibility testing methods are necessary when it is suspecting that patients may be infected with these types of microorganisms, for example the developing strains voicing metallo-β-lactamases (Bush *et al*., 2012).

2.5. Microbial contamination and disinfection

The inanimate objects in the environment are known to be contaminated with microorganisms, also mobile phones have become a postponement of the office practice for physicians and others, and it may serve as the perfect substrate for microorganisms, particularly in high temperature and humid conditions. Also, the organisms that cause nosocomial infections are commonly transmitted by hand contacting. Hand hygiene is one of the most important procedures in preventing nosocomial infections. The officials at the CDC mention the hand personal hygiene before and after interaction with patient, also an assessed 1/3 of wholly hospital acquired contaminations are affected by absence of adherence of recognized infection control applies. Moreover, it is very common in health carry surroundings to consume parentages perform first hand and arm scrub upon incoming to the unit. The hand hygiene procedures have been established inspire either by washing hands and/or via antimicrobial lotion or disinfectant beforehand touching patient and after contacting. In spite of this importance on better-quality hand cleanliness, a little emphasis has been prearranged to parent's cell phone usage at the bedside (Brady *et al*., 2012).

Nowadays there are many experimental studies performing regarding to the bacterial pollution of cell phones with microorganisms although the principally attention on health care workers and/or adult in patient locations. Also, there are a little consideration has remained to paid the possible of the transmission rate of bacteria from the cell phone toward the patients and other peoples (Beckstrom. *et al.*, 2013).

The disinfectants are expected to play an even more important role in microbial control in patients and the hospitals in the future. Even though, numerous alcohols have been exposed to be used as antimicrobials, ethyl alcohol, isopropyl alcohol and n-propanol, remain the most commonly used. Alcohol has wide broad spectrum type of the anti-microbial action in contradiction of vary of bacteria, viruses, and fungi but alcohol cannot destroy spore forms (are not sporicidal), conversely, it is recognized to prevent sporulation, moreover, the influence is alterable for the reason the lack ability to sporocidal action, also the alcohols are not suitable options for sterilization but are

extensively used in both solid surface disinfection and skin antisepsis, also the poorer concentrations might be used for the preservers the action of biocides agents (Beckstrom *et al*., 2013).

There are numerous types of alcohol products that contain the little stages biocides than other agents like specific chlorhexidine that preserve on living things skin surface next to be vaporization of alcohol and/or excipients. Emollients that may result in reduction of the vaporization time of the alcohol which are able to significantly increase creation efficacy. Medically, the type of the isopropyl alcohol is deliberated somewhat more efficacious in contradiction of bacteria and ethyl alcohol have more powerful effecting against viruses, this dependent on the amount of the concentrations of both, (i) Active agent. (ii) The test microorganism such as isopropyl alcohol has better lipophilic possessions when comparing with ethyl alcohol and it have fewer activation against hydrophilic viruses. Usually, the ability of the antimicrobial action of alcohols are lesser concentrations under 50%, but optimum the (60 - 90%) variety. There is little knowledge about recent specific method of act toward the alcohols, the idea turned to found the improved efficacy in occurrence of water, because third commonly supposed the reason of the layer destruction in addition to quick lysis of content proteins following interfering by means of metabolism and cell denaturation. This may result in maintained by specific information of analysis of *E. coli* dehydrogenases then the improved the lag bacterial phase development trendy to *Enterobacter aerogenes*, hazarded in line for reserve of metabolic rate necessarily meant for speedy living cell separation (Alwarid *et al*., 2013).

Ethanol needs to have a contact time of at least 10 seconds to kill *Staphylococcus aureus* and *Streptococcus pyogenes*. At a 10 second drying time, ethanol also kills *Pseudomonas aeruginosa, Serratia marcescens, E.coli, Salmonella typhosa, Staphylococcus aureus, Streptococcus pyogenes.* The Isopropyl alcohol mainly in solutions are arranged between the 60% to 90% alcohol in additional to 10 – 40% decontaminated water, it is main and quickly antimicrobial against the (bacteria, fungi, and viruses). Moreover, if the concentration of the alcohol applications drop underneath 50 percentage will be usefulness for disinfection drops sharply, but the higher alcohol concentrations don't prevent additional desirable properties of (bactericidal, virucidal, or fungicidal) (Beckstrom *et al.*, 2013).

Alcohol contain some amount of distilled water; the attendance of water is a crucial influence in an inhibiting the development of pathogenic microorganisms with isopropyl alcohol and destroyed it. The water entertainments as a catalyst and plays an important

role in analysis of the proteins of the cell membranes. Moreover the 70% IPA solutions enter the cell wall of living things more completely which infuses the complete cell, make coagulates to all proteins, and then the microorganism dies. Also, the extra water lead to slows processes of the evaporation, for that reason collective external interaction time and enhancing the efficiency. The IPA concentrations more than 91 percentage will coagulate proteins promptly. Therefore, a defensive coating is created which care for other proteins from further coagulation. Moreover, the substance more than 91% IPA do murder bacteria, however, sometimes need extended interaction of times for disinfection, which enable spores to falsehood in a dormant state without actuality destroyed. In this analysis, moreover the 50% of isopropyl alcohol reagent will murders the Staphylococcus Aureus bacteria within 10 seconds, but the 90% solution with interaction of time over 2 hours is useless. Also, there are many of the disinfectants recognized to kill spores which are categorized as a chemical sterilants compound. In this situation, we know that higher alcohol component harvest less results for bactericidal and antimicrobial results, also there are a product in pharmacy termed a Ethanol Wipes, the 70% Ethanol Wipes for surface and Objects, presaturated ethanol wipes (ethyl alcohol) are a common surface decontamination products for pharmaceuticals, healthcare, and medical device manufacturing. Clean surfaces gloves, notebooks, phones or any compatible material. Use alcohol with care: may degrade some types of plastics, display surfaces, and enamels (Beckstrom *et al.*, 2013).

2.6. Previous Studies

In study conducted by Wojgani *et al*.,(2012) in United Kingdom , to determine whether microbial contamination of door handles in two busy intensive care units and one high dependency unit was related to their design, location, and usage. They found a significant correlation between the frequency of movements through a door and the degree to which it was contaminated (p=0.01)(Wojgani *et al*.,2012)

Further study by Bashir, *et al* (2016) in Jigawa State, north-western Nigeria to isolate, identify and evaluate the presence or absence of bacterial contaminants on the door handles of public toilet in the Federal University, in order to take the necessary remedial measures. Frequency distribution of the isolates showed that *Staphylococcus aureus* were 44(38.3%), *Bacillus species* 26(22.6%), *Escherichia coli* 16(13.9%), *Micrococcus spp* 13(11.3%), *Salmonella spp* 10(8.7%) and *Klebsiella spp* 6(5.2%). The level of contamination varies depending on the traffic exposure and the environment. This means that it is necessary to practice good personal hygiene through hand washing and use of

hand sanitizer as well as daily washing and cleaning of toilets to reduce the incidence of microbial transmission (Bashir *et al* .,2016).

Also in study by Dharm Bhatta, *et al*., (2018) in Nepal, to determine the bacterial contamination of common hospital objects frequently touched by patients, visitors and healthcare workers. A total of 232 samples were collected and 219 bacterial isolates were recovered from 181 samples. *Staphylococcus aureus* was the most common bacterial isolate (44/219). Majority of *S. aureus* isolates were recovered from elevator buttons, biometric attendance devices and door handles. Among the *S. aureus* isolates, 36.3% (16/44) were methicillin resistant *Staphylococcus aureus* (MRSA) while remaining were methicillin sensitive *Staphylococcus aureus* (MSSA). Out of 44 *S. aureus* isolates, 12 (29.5%) were multidrug resistant and 14 (31.8%) were biofilm producers. The majority of MRSA isolates 62.5% (10/16) were biofilm producers. *Acinetobacter* was the most common Gram negative isolate followed by *E. coli* and *Pseudomonas species*(Dharm Bhatta *et al.,*2018)

Another study by Dayane Rodrigues *et al*., (2019) in Northern Brazil to analyze the epidemiology of bacterial contamination (contaminated sites, pathogen species and their antimicrobial susceptibility, and tracking of multidrug-resistant microorganisms - MDR) of inert hospital surfaces and medical equipment in two public hospitals. They found that most inert surfaces and equipment analyzed presented bacterial contamination (95.5%). *Staphylococcus aureus* was the main pathogen of clinical significance detected both in 40 Hospital A (61.8%) and B (68.6%). Hospital A showed higher rates of isolated MDR bacteria than Hospital B, especially in the Adult Intensive Care Unit, which included methicillin-resistant *Staphylococcus aureus* (MRSA) (52.7%), Enterobacteria resistant to 4th generation cephalosporins (19.4%), and multidrug-resistant *Pseudomonas aeruginosa* (2.78%)(Dayane R *et al* .,2019)

Further study by Charles Maina at 2020 in Kenya to determine the type of bacterial contaminants on door handles within Murang'a District Hospital. The findings showed that 68 doors did not indicate disease causing bacteria. The highest frequencies of disease causing bacteria were *E. coli* and *Citrobacter ssp* at a frequency of 11 each. The lowest disease causing bacteria was *P. aeruginosa* at a frequency of 6. (Charles M *et al* 2020)

CHAPTER III

MATERIAL AND METHODS

CHAPTER III

MATERIALS AND METHODS

3.1. Study Design

This is descriptive cross sectional study.

3.2. Study area and Duration

The study was conducted in selected hospitals (Al fouad hospital, Fedail hospital, Health care hospital, Ibrahim Malik hospital and Jabra hospital for emergency and injuries) at Khartoum State during the period from February 2022 to May 2022.

3.3. Study case

Swabs were collected from doorknobs of different sections in selected hospitals.

3.4. Ethical Considerations

Ethical approval to conduct this study was obtained from Scientific Research Committee , College of Medical Laboratory Science, Sudan University of Science and Technology, and from hospitals authorities.

3.5. Sampling technique

Non probability convenience sampling technique.

3.6. Sample size

Hundred swabs were taken from the doorknobs(20 samples from each hospital. In each hospital 5 samples were taken from each section ,and the sections were as following : ICU, operation room , lab and reception.

3.7. Laboratory processing

3.7.1. Collection of specimens

Samples were collected from hospital door handle from inner and outer side by using sterile cotton swab moisted in normal saline. Then the swabs were transported immediately to laboratory within 30 minutes.

3.7.2. Bacterial Isolation

The samples were cultured onto Blood and MacConkey Agar plates and incubated at 37°C for 24 hrs.

3.7.3. Identification of isolates

3.7.3.1. Colonial morphology

A comment on colonial morphology was carried out based on lactose fermentation and size of the colony.

3.7.3.2. Gram's stain

A Primary stains "Crystal violet" was applied to the dry–heat–fixed smear of microorganism for 1 minute. Then the stain was washed with tap water and cover with Lugol's iodine for 1 minute, then washed with tap water. And decolorized by acid alcohol and washed with tap water. Then the stain was covered with safarnin for 2 minutes. The slide was placed in a rack to dry, examined at (100 X) (Oil-immersion lens) (Cheesbrough, 2006).

3.7.3.3. Biochemical tests

The following tests have been done according to standard laboratory procedures. Using sterile straight wire loop, the colonies were touched and inoculated, and then incubated at 37°C in an incubator, then interpreted according to their reactions (Cheesbrough, 2006).

3.7.3.3.1. Biochemical tests of Gram positive cocci

3.7.3.3.1.1. Catalase test

A drop of the catalase reagent 3% Hydrogen peroxide was placed on the glass slide. Using wooden stick, a small amount of bacteria from 24-hour pure culture was placed onto the reagent drops of the microscopic slide. An immediate bubbles formation indicated a positive result and no bubbles formation indicated catalase negative result (Cheesbrough, 2006).

3.7.3.3.1.2. DNAase test

The tested organism was cultured on a medium which contain DNA, after overnight incubation, the colonies are tested for DNA-ase production by flooding the plate with a weak hydrochloric acid solution. The acid precipitates un hydrolyzed DNA. DNA-ase producing colonies are therefore surrounded by clear areas due to DNA hydrolysis (Cheesbrough, 2006).

3.7.3.3.1.3. MSA

Mannitol Salt Agar (MSA) is used as a selective and differential medium for the isolation and identification of *staphylococcus aureus*. Mannitol is the fermentable carbohydrate, fermentation of which leads to acid production, detected by phenol red indicator, coagulase positive staphylococci *(S.aureus*) produce yellow colonies and coagulase

negative staphylococci produce red colonies and no color change of phenol red indicator(Cheesbrough, 2006).

3.7.3.3.2. Biochemical tests of Gram negative bacilli

3.7.3.3.2.1. Oxidase test:

A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. Alternatively an oxidase reagent strip can be used. When the organism is oxidase-producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour (Cheesbrough, 2006).

3.7.3.3.2.2. Indole test

The tested organism was cultured in peptone water, a medium which contains tryptophan. Indole production was detected by Kovac,s reagent . In sterile peptone water, the test organism was inoculated and incubated at 37℃ for overnight. The production of indole was detected by adding 0.5 ml of Kovac,s reagent .Then the test tube was mixed gently and examined for a red color in the surface layer within 10 minutes (Cheesbrough, 2006).

3.7.3.3.2.3. Citrate utilization test

A dense bacterial suspension was prepared in 0.25 ml sterile normal saline in small tube, citrate tablet was added and the tube was incubated overnight at 35-37°C, positive citrate test is indicated by red color while yellow – orange color, indicate negative citrate test (Cheesbrough, 2006).

3.7.3.3.2.4. Urease test

The tested organism was cultured in a medium which contains urea and the indicator phenol red . When the strain is urease-producing, the enzyme will break down the urea (by hydroly-sis) to give ammonia and carbon dioxide. With the release of ammonia, the medium becomes alkaline by a change in colour of the indicator to pink-red (Cheesbrough, 2006).

3.7.3.3.2.5. Kligler Iron Agar (KIA)

The tested organisms were inoculated into KIA medium, using a straight wire loop, the agar butt was stabbed, and then the slope was streaked in a zigzag pattern, after inoculation (make sure the tube tops are left loose). KIA reactions are based on the fermentation of lactose and glucose and the production of hydrogen sulphide, yellow butt (acid production) and red-pink slope indicates fermentation of glucose only, cracks and bubbles in the medium indicate gas production from glucose fermentation and blackening

along the stab line or throughout the medium indicates hydrogen sulphide production (Cheesbrough, 2006)

3.7.3.3.2.6. Motility test

The tested organisms were inoculated into semisolid media, using straight wire loop, touch to the colony, stab once to a depth of only 1/3 to 1/2 inch in the middle of the tube . Motile organisms will spread out into the medium from the site of inoculation, nonmotile organisms remain at the site of inoculation(Betty *et al*.,2007)

3.8. Antimicrobial susceptibility testing

The disk diffusion susceptibility testing was performed according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotic discs used in this study included Penicillin (P, 30 µ) Amoxicillin/Clavulinic acid (AMC, 20/10 µg), Clindamycin (CM, 30 µg), Cefoxitin (FOX, 30µg), Erythromycin (E, 20µg), Gentamicin (GEN, 20 µg), Ciprofloxacin (CIP, 5 µg), Cotrimoxazole (SXT, 30 µg), Tetracycline (TE, 30 µg), Cefotaxime (CTX, 30 µg), Ceftazidime (CAZ, 30 µg) and Ceftriaxone (CRO, 30 µg). The antibiotic discs were stored at -20°C and placed at room temperature prior to use. A single colony of organism was sub cultured overnight on Mueller Hinton agar plates (Oxoid) at 37°C. After an overnight incubation at 37°C, zones of inhibition or clear zones were measured and compared to the CLSI guidelines. Multidrug resistance (MDR) is defined as resistance to at least three drugs.

3.9. Statistical Analysis

Data was computed and analyzed by Statistical Package for Social Science (SPSS) software version 25.0 .Categorical variables were described by number and percent (N,%). Chi-square test was used to compare between categorical variables. A two-tail $P < 0.05$ was considered statistically significant. All analyses was performed with the SPSS.

Chapter IV

RESULTS AND DISSCUSION

CHAPTER IV

RESULTS AND DISSCUSION

4.1. Prevalence of isolated bacteria from door handles

A total of 100 swab samples were collected from different hospital sections showed bacterial contamination which was detected in 92 of 100 sampled surfaces (92%), with total of 92 bacterial isolates were obtained and no microbial growth in only 8 surfaces and there was mixed infection in some swab samples.

The biochemical tests of isolated bacteria showed predominance of G+ve bacteria such as S.aureus, S.epidermidis and Bacillus.species (Table 4.1), also G-ve bacteria such as E.coli, Klebsiella.species and Pseudomonas species (Table 4.2).

Table 4.1: Biochemical tests results of G+ve bacteria.

	Biochemical tests for G +ve bacteria		
Isolated bacteria	Catalase test	DNAase test	MSA
S.aureus	Positive	Positive	Yellow colonies (lactose ferment)
S.epidermidis	Negative	Negative	Pink colonies (non lactose ferment)
B.species	Positive	_	Pink colonies (non lactose ferment)

Table 4.2: Biochemical tests results of G-ve bacteria.

	Biochemical tests for G-ve bacteria					
Isolated bacteria	Oxidase test	Indole test	Citrate utilization test	Urease test	KIA	Motility test
E.coli	Negative	Positive	Negative	Negative	Yellow butt yellow slop cracking and no H2s	Motile
K. species	Negative	Negative	Positive	Positive	Yellow butt yellow slop cracking and no H2s	Non motile
P. species	Positive	Negative	Positive	Negative	Pink butt pink slop no cracking and no H2s	Motile

The microbiological analysis of the doorknobs in hospitals showed a predominance of common bacteria of the human flora, such as *S. aureus* (37%) and nosocomial bacteria, such as *Pseudomonas. species* (26%), *Klebsiella.species*(12%), *E. coli* (8%). *S. epidermidis* and *Bacills .species* constitutes 7% and 10% respectively (Table 4.3).

Table 4.3: Distribution of isolated bacteria from hospitals doorknobs .

isolated Bacteria	Frequency	Percentage
S.aureus	34	37%
S.epidermidis	7	7%
P.species	24	26%
E.coli	7	8%
K.species	11	12%
B.species	9	10%
Total	92	100%

.

Both Gram positive and Gram negative organisms were found among the identified isolates. The number and the percentage of the identified Gram positive and Gram negative bacteria are shown in Figure 4.1.

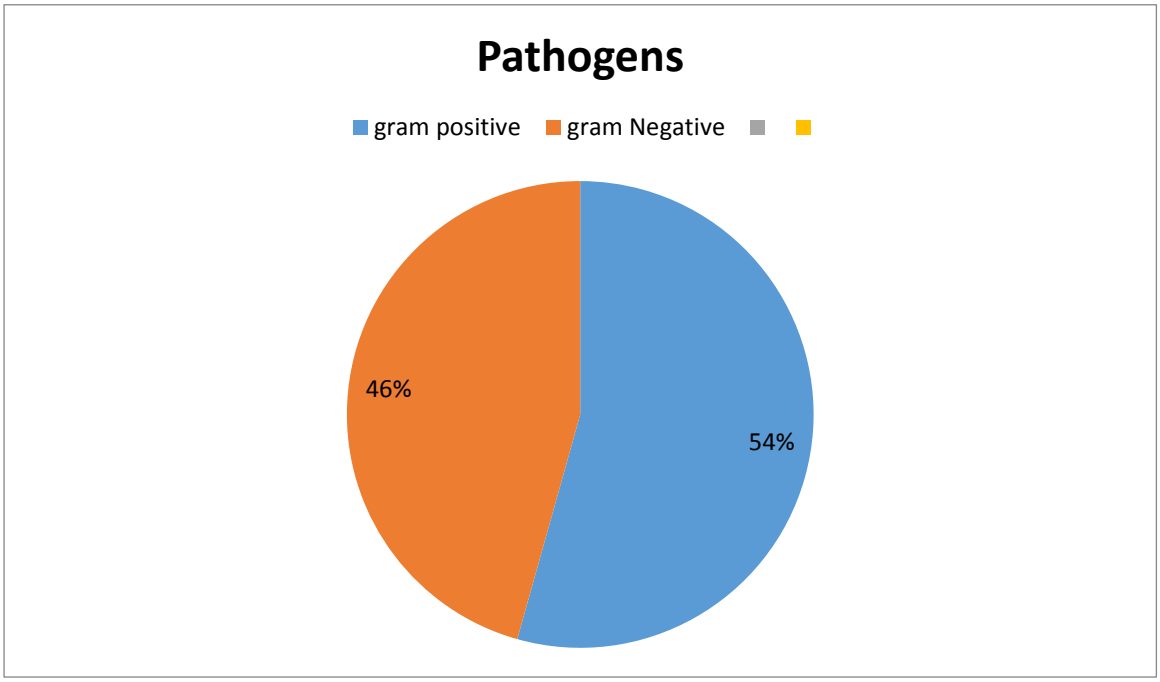

Figure 4.1: Distribution of the isolates according to Gram's Reaction

Also the results of study showed that the doorknobs of operational room was most contaminated place (35%), followed by ICU (26%), then laboratory and reception (19.5% for each one) (Table 4.4).

Table 4.4: Frequency of clinically important microorganisms isolated from doorknobs of hospitals sections

Organisms	ICU	Operation room	LAB	Reception
S. aureus	13	9	7	5
S. epidermidis	-	-	2	5
P.species	8	11	3	2
K.species	2	7	2	-
E. coli	1	2	3	1
B.species	-	3	1	5
Total	24 (26%)	32 (35%)	18 (19.5%)	18 (19.5%)

The study demonstrated no significant association between types of organisms and sections of different hospitals (Table 4.5_ 4.9).

Table 4.5: Association of types of organism with sections of Al fouad hospital

Isolated bacteria	Hospitals sections				P value
	ICU	OR	Lab	Reception	0.16
S. aureus	4	1	1	1	
S. epidermidis	_	_	_	1	
B.spp	_	_	_	_	
E. Coli	_	1	_	_	
K.spp	_	1	_	_	
Pseudomonas.spp	1	2	_	1	

Table 4.6: Association of types of organism with sections of Fedail hospital

Isolated bacteria	Hospital sections				P value
	ICU	OR	Lab	Reception	0.82
S. aureus	2	1	1	1	
S. epidermidis	_		_		
B.spp	_	1	_	1	
E. Coli	1	1	_	_	
K.spp	_	1	_	_	
Pseudomonas.spp	1	3	_	_	

Table 4.7: Association of types of organism with sections of Health care hospital

Isolated bacteria	Hospital sections				P value
	ICU	OR	Lab	Reception	0.07
S. aureus	3	2	1	1	
S. epidermidis	_	_	_	1	
B.spp	_	1	_	1	
E. Coli	_	_	1	_	
K.spp		1	_	_	
Pseudomonas.spp	1	1	_	1	

Table 4.8: Association of types of organism with sections of Ibrahim malik hospital

Isolated bacteria	Hospital sections				P value
	ICU	OR	Lab	Reception	
S. aureus	2	3	2	1	0.24
S. epidermidis	_	_	1	3	
B.spp	_	_	1	2	
E. Coli	_	_	2	1	
K.spp	1	3	2	_	
Pseudomonas.spp	3	4	2	_	

Table 4.9: Association of types of organism with sections of Jabra hospital

Isolated bacteria	Hospital sections				P value
	ICU	OR	Lab	Reception	0.62
S. aureus	2	2	2	1	
S. epidermidis	_	_	1		
B.spp	_	1	_	1	
E. Coli	_	_	_	_	
K.spp	1	1	_	_	
Pseudomonas.spp	2	1	1	_	

4.1.1 Antibiotic Susceptibility testing of isolates

Table 4.10 showed resistance and sensitivity pattern of isolates to different antibiotics. Some bacteria were resistant to more than two antibiotics and some were resistant to at least two antibiotics. The interpretation of each bacterium either resistant or susceptible to antibiotic is shown in Table 4.10.

Table 4.10: Antimicrobial susceptibility profiles of bacterial isolates from hospitals door knobs

		Gram positive			Gram negative		
Antibiotic		***S aureus [34]***	***S.epidermidis [7]***	***B.spp [9]***	***E.coli [7]***	***Pseudomonas spp [24]***	***Klebsiella spp.[11]***
P	S	2 (6%)	1 (14%)	0	-	-	-
	R	32 (94%)	6 (86%)	9 (100%)	-	-	-
AMC	S	34 (100%)	2 (28%)	0	4 (56%)	24 (100 %)	3 (27%)
	R	0 (0 %)	5 72%)	9 (100%)	3 (42%)	0 (0%)	8 (73%)
CM	S	29 (85%)	0	7 (78%)	-	-	-
	R	5 (15%)	7 (100%)	2(22%)	-	-	-
FOX	S	27 (79%)	3 (42%)	1 (11%)	-	-	-
	R	7 (21%)	4 (56%)	8 (89%)	-	-	-
E	S	17 (50%)	1 (14%)	8 (89%)	-	-	-
	R	17(50 %)	6 (86%)	1 (11%)	-	-	-
GEN	S	29 (85%)	4 (56%)	8	0 (0%)	18 (75%)	11 (100 %)
	R	5 (15%)	3 (42%)	1 (11%)	7(100%)	6 (15%)	0 (0%)
CIP	S	34 (100%)	0	8 (89%)	7 (100%)	8 (33%)	11 (100 %)
	R	0	7 (100%)	1 (11%)	0 (0 %)	16 (67%)	0
SXT	S	-	-	1 (11%)	7 (100%)	18 (75 %)	11(100%)
	R	-	-	8 (89%)	0 (0 %)	6 (25 %)	
TE	S	32(94%)	1 (14%)	-	0	-	0 (0 %)
	R	2 (6%)	6 (86%)	-	7	-	11 (100%)
CTX	S	-	-	-	7(100%)	-	11 100
	R	-	-	-	0 (0 %)	-	0
CAZ	S	34 (100 %)	4 (56%)	0	7 (100 %)	4 (17%)	8 (73%)
	R	0 (0 %)	3 (42%)	9 (100%)	0 (0 %)	20 (83%)	3 (27%)
CRO	S	34(100 %)	5 (72%)	3 (34%)	0 (0%)	6 (25%)	7 (64%)
	R	0 (0 %)	2 (28%)	6 (66%)	7 (100 %)	18 (75%)	4 (36%)

P= Penicillin **AMC** = Amoxicillin/Clavulanic acid **CM**=ClindamSycin **FOX=** Cefoxitin **E =** Erythromycin **GEN** = Gentamicin **CIP** =Ciprofloxacin **SXT** = Cotrimoxazole **TE** = Tetracycline **CTX** = Cefotaxime **CAZ** = Ceftazidime CRO = Ceftriaxone **S** = Sensitive **R**= Resistant

4.1.2. Resistance pattern of the organisms to the tested antibiotics

The most resistance was seen against penicillin (47 (51%)) isolates being resistant against it. Next to penicillin, 37 isolates were resistant to Ceftriaxone, giving a percentage of 40. The third highest resistance was seen against, Clindamycin where (36 (39%)) isolates were resistant to it.

Table 4.11: Percentage of resistant bacteria among isolates

Antimicrobial agents	Frequency	Percentage
Penicillin	47	51%
Amoxicillin/Clavulinic acid	25	27%
Clindamycin	36	39%
Cefoxitin	14	15%
Erythromycin	19	21%
Gentamicin	22	24%
Ciprofloxacin	24	26%
Cotrimoxazole	14	15%
Tetracycline	26	28%
Cefotaxime	0	0
Ceftazidime	35	38%
Ceftriaxone	37	40%

4.2 Discussion

Inert hospital surfaces and medical equipment's can be a reservoir for multidrug resistant pathogens. Understanding the epidemiology of bacterial contamination in this setting is essential to prevent in-patient contamination and health care associated infections development (Johani *et al.,* 2018).

Bacterial pathogens can survive and remain viable on inert surfaces and equipment due to their ability to form biofilms and to environmental factors (such as surface porosity and humidity) (Russotto *et al.,* 2017, Esteves *et al*., 2016), which works as a major factor driving pathogen thriving and dissemination.

The results of study showed that *S. aureus* (37%) was the main microorganism recovered from doorknobs. This was anticipated as it is a major component of the normal flora of the skin and nostrils. The findings of other researchers (Nworie *et al.,* 2012, Boone and *Gerba* ,2007), was in accordance with this finding.

Similar in selected hospitals in Akoko, Ondo State Southwest Nigeria (Alabi *et al.,* 2013) showed that the frequency of Gram positive bacteria was higher than the Gram negative bacteria, this also corroborates the findings of this study and agrees with the statement that Gram-positive bacteria have overtaken the Gram-negative as the major bacteria isolated from fomites (Inweregbu *et al*., 2005). In contrast, the result of this study did not agree with the work of Orji *et al*., (2005) in Nigeria which showed that *Staphylococcus aureus* was the least isolated bacteria, this different between these two studies may be due to environment or the number of sample. Isolation of more Gram positive bacteria than Gram negative can be explained, as they are members of the body flora of both asymptomatic carriers and sick persons. These organisms can be spread by the hand, expelled from the respiratory tract or transmitted by animate or inanimate objects (Chikere *et al.,* 2008).

Number of *Bacillus spp* was isolated from hospital door handles, this is also in agreement with the research carried out by Boone *et al*., (2007) in Arizona who reported that *Bacillus spp* was found to be the predominant organism among all the organisms that were isolated from door handles.

The results showed that 92% of the doorknobs analyzed (n = 100) were contaminated. Consistent with this, international studies (Weber and Rutala,2013, Johani *et al.,* 2018)in Jeddah, Saudi Arabia, have shown that only less than 50% of hospital surfaces are properly cleaned and disinfected with germicides(Weber and Rutala.,2017). These

alarming findings strongly suggest that the hospital environment can act as a reservoir of pathogens and enable their cross-transmission to the patient.

From the findings in this study, it was observed that most of the isolates obtained were resistant to most commonly used antibiotics. These antibiotics were Penicillin, Ceftriaxone and Clindamycin. The resistance to these antibiotics which is in accordance with the research carried out by Adewoyin *et al*.,(2013), who reported that antibiotic resistant microorganism contaminates environmental surfaces such as doorknobs. The result of susceptibility of antibiotics presented different degree of resistance to the different drugs used against different organisms. From the result of antibiotic susceptibility test, all isolates were resistant to at least one of the 10 antibiotics tested.

CHAPTER V

CONCLUSION AND RECOMMENDATIONS

CHAPTER V

CONCLUSION AND RECOMMENDATIONS

5.1. Conclusion

This study was concluded that; there was a high level of bacterial contamination on door handles of hospitals. The most frequent potentially pathogenic bacteria were *S.aureus* and *Pseudomonas aeruginosa*. Also found that; doorknobs of operational room was most contaminated place, followed by ICU, laboratory and reception. Moreover, the antibiotic susceptibility of isolates showed high resistance to penicillin, ceftriaxone and clindamycin.

5.2. Recommendations

_ Regular disinfection of door handles as well as frequent washing of hands could also go long way.

_Hand-washing practice after using toilet should be adopted by everyone to prevent the spread of microorganisms.

_The hospital management should give more attention to the distribution of hand sanitizers to the users.

_More trained cleaner should be employed for maintaining proper cleaning of hospital washrooms.

_The patients, visitors, employees, nurses, doctors, even cleaners should maintain personal hygiene.

_ further study can be done to find out any correlation between multidrug resistance of bacteria and presence of plasmids.

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