PREVALENCE AND ANTIBACTERIAL SUSCEPTIBILITY OF SOME NOSOCOMIAL PATHOGENS ISOLATED FROM SOME HOSPITAL ENVIRONMENT IN ZARIA, KADUNA STATE.

BY

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ABSTRACT

This study investigated the prevalence of three nosocomial pathogens on hospital surfaces and to determine the antibiotic susceptibility pattern of the isolates. A total number of 470 samples were collected from the three hospitals; 160 samples were collected from Gambo Sawaba general hospital, 155 samples were collected from Major Ibrahim B. Abdullahi memorial hospital and St Luke Anglican hospital. Sixty three (63) isolates with mean value of 2.133±2.403 were isolated from Gambo Sawaba general hospital out of which 32 (50.8%) were *S. aureus*, 18 (28.6%) were *P. aeruginosa* and *E. coli* was 13 (20.6%). Forty six (46) isolates with mean value of 1.567±1.868 from Major Ibrahim B. Abdullahi memorial hospital; 23 (50.0%), 16 (34.8%) and 7 (15.2%) were *S. aureus*, *P. aeruginosa,* and *E. coli* respectively. From St. Luke Anglican hospital there were 48 isolates with mean value of 1.600±1.813 out of which 27 (56.3%) were *S. aureus*, 13 (27.0%) were *P. aeruginosa,* and 8 (16.7%) were *E. coli*. The difference in the prevalence of the organisms on surfaces in the three hospitals was not statistically significant and there was no significant association between the organisms from the surfaces and the hospital ($P>0.05$) at 95% confidence limit. Only 1 (7.7%) of 13 *E. coli* isolated from Major Ibrahim B. Abdullahi memorial hospital was tested positive for *E. coli* O157:H7 and was not found in the other hospitals. *S. aureus* from Gambo Sawaba general hospital were 100% resistant to ampicillin and 25% resistant to cefoxitin, 95.7% from Major Ibrahim B. Abdullahi memorial hospital were resistant to ampicillin and 13% resistant to cefoxitin and 92.6% and 18.5% from St. Luke hospital were resistant to ampicillin and cefoxitin respectively. *P. aeruginosa* was 100% susceptible to ceftazidime and imipenem, *E. coli* was also 100% susceptible to gentamicin, cefoxitin and ceftazidime. *P. aeruginosa* and *E. coli* were more resistant to tetracycline. *S. aureus* isolates were inhibited by AMP at 15.625 and 31.25µg/ml, the pathogen was inhibited by FOX at 31.25 and 62.5µg/ml. *P. aeruginosa* and *E. coli* isolates were inhibited by tetracyline at 31.25µg/ml. *P. aeruginosa* isolates were inhibited at 31.25µg/ml and the potent MIC of AMP against *E. coli* was 15.625µg/ml. The MBCs of the antibiotics were at 31.25, 62.5 and 125µg/ml. Thus, inanimate surfaces in hospital environment are reservoirs of pathogens including the multidrug-resistant pathogens. The hands of healthcare workers can be a major route of cross-transmission of these pathogens within the hospital environment.

CHAPTER ONE

1.0 INTRODUCTION

Nosocomial pathogens are organisms causing diseases that are obtained from the hospital environment; they are acquired from the hospital environment within few days of admission into the hospital or other health care facilities and are responsible for nosocomial infections (Medubi *et al*., 2006). Nosocomial infections (i.e. infections acquired from the hospital environment secondary to the patients' original condition) have posed a scourge to patients, staff and visitors to the hospitals over the years and 1.4 million people across the world suffered from hospital acquired infection at any given time (Olalekan *et al*., 2011; Anyadoh-Nwadike *et al*., 2011).

According to Singleton (1999), nosocomial infection has been recognized ever since man first gathered patients under the same roof for the diagnosis and treatment of diseases. The hospital exists as a closed community, it is therefore not surprising that certain microorganisms become predominant and may cause disease. It seems obvious that the occurrence of nosocomial infection is related to hospital milieu. These pathogens can be contracted by the contaminated environmental surfaces, and equipment of the hospital through the healthcare workers and even by the patients (Ijioma *et al*., 2010).

According to the US Centre for Disease Control and prevention (CDC, 1993) S*taphylococcus aureus*, P*seudomonas aeruginosa*, and E*scherichia coli*, are the most common nosocomial pathogens. Other organisms that can be isolated from the hospital environment include: *Klebsiella* spp, *Enterococcus* spp, *Acinetobacter*, *Proteus* spp, *Bacillus* spp, *Legionella* spp, *Candida* spp, *Streptococcus* spp, *Serratia* spp and

members of *Enterobacteriaceae*. These pathogens can survive for extended periods and can persist despite attempts in removing them (Wren *et al*., 2008).

The degree of occurrence of one or two of these organisms over others depends on the environment (Chikere *et al*., 2008; Okonko *et al*., 2009). The environment significantly influences multiple factors in the chain of infection (Ekrami *et al*., 2011). Although, contaminated surfaces can serve as reservoirs for pathogens, these surfaces generally may not directly be associated with transmission of infections to either staff or patients. This can be especially troublesome in hospital environments where patients with immunodeficiency are at enhanced risk for contracting nosocomial infections (Jarvis 2007; Chikere *et al*., 2008).

The transmission of microorganisms from environmental surfaces to patients is largely via hand contact with the surfaces. Although, hand hygiene is important to minimize the impact of this transfer, cleaning and disinfecting environmental surfaces appropriately is fundamental in reducing their potential contribution to the incidence of healthcare associated infections (Kampf and Kramer, 2004). Based on US Centres for Disease Control and prevention (CDC) classification, medical and surgical instruments are categorized to ''critical,'' ''semi critical'' and ''non critical'' (Ekrami *et al*., 2011).

Environmental surfaces can further be divided into medical equipment surfaces (e.g. knobs or handles on haemodialysis machines, X-ray machines, instrument carts and dental units) and housekeeping surfaces (e.g. floors, walls, side bed chairs, kitchens, bathrooms, door handles, light switches, counter tops and table tops) (Favero and Bond, 2001). Routine environmental-surface sampling (e.g. surveillance cultures) in healthcare

setting is neither cost effective warranted. Otter *et al*. (2011) reported that surfaces are now playing important role in the epidemic and endemic transmission of the major pathogens linked to healthcare associated infections.

The emergence of antibiotic resistant micro-organisms (e.g., *Staphylococcus aureus* and *P. aeruginosa*) is increasing extremely rapidly around the globe, creating a serious threat to the spread and treatment of infectious diseases (Oli *et al*., 2013). Antibiotic resistance is a worldwide problem. World health leaders have described antibiotic-resistant microorganisms as “nightmare bacteria” that “pose a catastrophic threat” to people in every country in the world (Bockstael and Aerschot, 2009). Many of the pathogens that cause nosocomial infection have a high level of resistance to antibiotic treatment (Jones and Pfaller, 1998). The major nosocomial pathogens increasingly resistant to antimicrobial drugs include *Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa*. Infections from methicillin-resistant staphylococci, vancomycin-resistant enterococci (VRE), and aminoglycoside-resistant *Pseudomonas* spp. are becoming common (Oli *et al*., 2013).

1.1 Statement of problem

Nosocomial infection is a major public health concern these days and a cause of substantial mortality and morbidity for hospitalized patients (Thapa *et al*., 2009). Nosocomial infection caused by the nosocomial pathogens has posed a problem of enormous magnitude globally, hospital localities have proven favourable in transmission of infections due to existing suitable pathogens-host-environment relationship (Samuel *et al*., 2010).

In the past most nosocomial infections were caused by gram positive microbes in which *Staphylococcus aureus* was the primary cause of nosocomial infection. Gram negative bacteria, such as *E. coli* and *Pseudomonas aerugionsa* that has the ability to cause opportunistic skin infections are also the major cause today (Qayyum *et al*., 2010).

The prevalence rate of nosocomial infection as studied by WHO is averagely 11.85% which was reported by Samuel *et al*. (2010) and the risks are increasing in developing nations annually because it has been estimated that between 5 and 10% of patients admitted to acute care hospitals in developing nations acquired one or more infections. This is by far more serious in low-resource countries that do not have the resources either to prevent and control or to manage such situations due to financial constraints (David and Famurewa, 2010).

The economic cost are considerable, the increased length of hospital stay for infected patients is the greatest contributor to the cost (Saka *et al*., 2011). Pittet *et al*. (1994) and Saka *et al*. (2011) reported that the overall increase in the duration of hospitalization for patients with surgical wound infection was about 8 days, ranging 3 days for gynaecology to 9 days for general surgery and 19 days for orthopaedic surgery. Prolong stay not only increases direct cost to patient or payers but also indirect costs due to cost work. The increased use of drugs, the need for isolation and the use of additional laboratory and other diagnostic studies also contribute to the cost (Pittet *et al*., 1994; Samuel *et al*., 2010).

Infections cause by nosocomial pathogens are also one of the leading cause of deaths. More than 70% of these pathogens from the hospital environment are resistant to drugs or multi-drugs which are now the most leading cause of human death worldwide (Anton and David, 2010). The occurrence of multi-drug resistance in hospital-associated

pathogens has resulted in the emergence and re-emergence of difficult - to – treat nosocomial infections in patients.

Therefore, hospital is not only a place where sick people recover from their sickness but also where the illnesses get complicated and healthy people get infected. Whenever clinical procedures are performed, clients are at risk of infection during and after the procedures (Kampf and Kramer, 2004).

1.2 Justification for the study

Many ordinary surfaces in hospitals can adequately be decontaminated with routine disinfection techniques, but many other common hospital surfaces, such as upholstery, side table/bench, floors, carpets and many other areas in the hospital environment cannot and may become reservoirs of pathogens (Byers *et al*., 1998). Commonly used disinfection techniques are sometimes incapable of eradicating fomite reservoir of nosocomial pathogens such as methicillin resistant S*taphylococcus aurues* (MRSA). Intact traditional cleaning techniques even using alkylamines and quaternary ammonium compounds may do more to spread contamination than to reduce it (Exner *et al*., 2004). Surface sampling is used currently for research, as part of an epidemiologic investigation, or as part of a comprehensive approach for specific quality assurance purposes (Olalekan *et al*., 2011).

Environmental surfaces in Healthcare centres act as reservoir for the bacteria gathering and proliferation. Pathogens can be expelled from an infected or colonized patient either through direct contact, aerosol droplets or faeces to the environmental surfaces. Depending on the environmental conditions, these pathogens may remain infectious on the surfaces for weeks after the contamination event (Hassan *et al*., 2004). In humid conditions, some pathogens may actively colonize and transform a passive reservoir

into active one. Furthermore, formation of biofilm by one bacterial agent can affect the survival of other pathogens on the same surface (Rutala and Weber, 2002, Hassan *et al*., 2004).

The latest authoritative review of the role played by contaminated surfaces in the transmission of nosocomial pathogens has recently been published by Otter *et al.* (2011). This categorically dispels the apparent widespread view that contaminated surfaces are not important in transmission. Hardy (2006) reported that some nosocomial pathogens have shown to last for months on hospital floors, for example MRSA can live on plastic laminate surfaces for 2 days and can spread rapidly through contact. Pal *et al*. (2010) also reported that *Pseudomonas aeruginosa* can last for longer period of time in moist surfaces such as suction apparatus, tap, operation theatre wards as well as sink. Nosocomial pathogens shed by patients can contaminate hospital surfaces at concentrations sufficient for transmission. One other key source of transmission explored was the transference to the hands of healthcare providers and subsequently back to the patients. It is imperative to study the distributions of the pathogens on surfaces of hospitals (Dwivedi *et al*., 2009).

1.3. Aim of the study

To determine the prevalence and antibacterial susceptibility of some nosocomial pathogens isolated from some hospital environment in Zaria, Kaduna state.

1.4 Specific objectives

1. To isolate and identify selected bacterial nosocomial pathogens obtained from hospital environment;

2. To determine the antibiogram profile of the bacterial isolates;

3. To determine the MIC and MBC of the antibiotics against the resistant strains of the isolates.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Epidemiology of nosocomial pathogens

All over the world, nosocomial infection is a recognized public health problem. Surveillance Programmes estimate the rate of infection at 5-10 % of hospital admissions (Buttner *et al*., 2001; Harris *et al*., 2010). Nosocomial infections are responsible for about 90,000 deaths in the U.S. per year and approximately 10% of American hospital patients (about 2 million every year) acquired clinically significant nosocomial infections (Harris *et al*., 2010). In Italy in 2000s, about 6.7 % of hospitalized patients were infected; that means, between 450,000 and 700,000 patients had nosocomial infections out of which between 4,500 and 7000 died (Boyce 2007). In Switzerland, extrapolations assume about 70,000 hospitalized patients affected by nosocomial infections between 2 and 14% of hospitalized patients (Harris *et al*., 2010).

In Nigeria, nosocomial infection rate of 2.7 % was reported from Ife, while 3.8 % from Lagos and 4.2 % from Ilorin (Harris *et al*., 2010). The cause of nosocomial infections might be endogenous or exogenous. Endogenous infections are caused by organism present as part of the normal flora of the patient, while exogenous infections are acquired through exposure to the hospital environment, hospital personnel, frequently touched surfaces or medical devices (Medubi *et al*., 2006).

Nosocomial infection rates vary substantially by body site, by type of hospital and by the infection control capabilities of the institution (Ogunsola *et al*., 1998). The proportion of infections at each site is also considerably different in each of the major hospital services and by level of patient risk. This is exemplified by surgical site

infections (SSIs) which are most common in general survey, whereas urinary tract infections and blood stream infections are most frequent in medical services and nurseries. The rates of nosocomial infection vary by surgical subspecialty, low in ophthalmology and high in general surgery. The differences are largely due to variations in exposure to high risk devices or procedures (Taiwo *et al.*, 2004).

2.2 The hospital environment as source of pathogens

Environmental surfaces can be further divided into medical equipment surfaces and housekeeping surfaces. As earlier stated, according to US Centres for Disease Control and Prevention (CDC), medical and surgical instruments are categorized as critical, semi critical, and noncritical (Ekrami *et al.*, 2011). The role of fomites and the inanimate hospital environment in the transmission of infection has been debated for many years, however, there is increasing evidence that contaminated inanimate surfaces, especially those frequently touched by hand, can contribute to the spread of healthcare-associated pathogens (Chopra and Roberts, 2001).

Thorough cleaning of the hospital environment had never been regarded as crucial in the control of multidrug resistant organisms (MDROs) in the hospital (Tsang, 2011). However, hospital environment play an important role in nosocomial infection, because the environment contains diverse population of microorganisms. Microorganisms are present in great numbers in moist and organic environments, but some can also survive under harsh environmental conditions (Fagade *et al.*, 2010). An understanding of how infection occurs after exposure is based on the principles of the chain of infection, which is also important in evaluating the contribution of the environment to nosocomial infection; contaminated surfaces can harbour potential pathogens, these surfaces

generally indirectly associated with transmission of infections to either staff or patients (Otter *et al*., 2011).

Healthcare settings are environment were both infected persons and persons at increased risk of infection congregate. Patients with infections or carriers of pathogenic microorganisms admitted to hospital are potential sources of infection for all patients and staff (Samuel *et al*., 2010). Patients who become infected in the hospital are further source of infection. Over-crowded conditions within the hospital, frequent transfer of patients form one unit to another, and concentration of patients highly susceptible to infection in one area (e.g. newly born infants, burn patients, intensive care) all contribute to the development of nosocomial infections (Ducel *et al*., 2002). In low and middle income countries (LMIC) this challenge is more highlighted because disease prevention and control policies are nonexistent, poorly adapted or insufficiently funded by governments (Raka, 2010). The rates of hospital related infections (HRIs) within a hospital represent the best indicator for the quality of services offered, where a high frequency of HRIs is evidence of a poor quality of health service delivery (Mark *et al*., 2000).

Environmental surfaces in hospital are vectors for drug-resistant bacteria, but the most important sources of these bacteria are the patients coming into the hospital (Boyce *et al*., 1997). Wren *et al*. (2008) reported that healthcare surfaces are often soiled with organic matters that may be left unclean or partially cleaned for variable periods of time. Recent research carried out by Walker *et al*. (2003) highlighted the danger of MRSA lingering on surfaces long after the patient who carried it has been discharged. In one nine-bed intensive care unit (ICU) more than half the patients who picked up methicillin resistant *Staphylococcus aureus* (MRSA) after entering the ICU acquired a

strain of the bacteria not present on other patients in the ICU at the time. In other words, the bacteria had been left behind on floors, bedrails, tables, and other surfaces, by patients already discharged. The hospital environment is contaminated by a variety of pathogenic and non-pathogenic microorganisms that can persist on surfaces for prolonged periods (Wren *et al*., 2008).

The acquisition of nosocomial pathogens by a patient and the resultant development of infection depend on a multifaceted interplay between the environment, a pathogen and a susceptible host (Dancer, 2009). Pathogens that have been linked to transmission via contaminated environmental surfaces and medical equipment include mithiciilin resistant *Staphylococcus aureus* (MRSA), vancomycin resistant *Enterococcus* spp (VRE), *E. coli*, *Pseudomonas* spp, *Clostridium difficile, Acinetobacter spp* and norovirus (CDC, 2003). Microorganisms may be associated to several biological materials in the hospital environment such as floors, walls, ceiling, doors, windows, electro-electronic equipment and specific hospital articles in use for assistance to patients (Dudhagara *et al*., 2011). The environment significantly influences multiple factors in the chain of infection.

2.3 Mode of transmission of pathogens in hospital environment

Scientific evidence suggests that environmental contamination plays an important role in the spread of methicillin-resistant S*taphylococcus aureus* (MRSA), multi-drug resistant P*seudomonas aerugionsa* and vancomycin-resistant *Enterococcus* spp (VRE) (Hota, 2004; Boyce, 2007). Animate and inanimate sources of exogenous infections include hospital staff, other patients, visitors, food, water, fomites, urinary catheter, intravenous devices, respiratory equipment and other prostheses (Boyce, 2007).

The transfer of microorganisms from environmental surfaces to patients is largely through hand contact with the contaminated surfaces (Samuel *et al.*, 2010). It has been estimated 20% to 40% of nosocomial infections have been attributed to cross infection via the hands of health care personnel (Weinstein, 1991). Contamination of the hands of health care workers (HCWs) could in turn result from either direct patient contact or indirectly from touching contaminated environmental surfaces or patients' skin during routine care activities, sometimes even despite glove use (Kramer *et* al., 2006, Allegranzi and Pittet, 2009). Many nosocomial infections are caused by pathogens transmitted from one patient to another, by way of healthcare workers who have not washed their hands, or who don't observe simple hospital hygiene measures, and also between patients (Olalekan *et al*., 2011).

In addition, after admission to hospital, patients become colonized with different, more pathogenic organisms, some acquired in hospital, others brought from the community but increasing in number by stress of illness and antibiotic treatment (Wongworawat and Jones, 2007). These bacteria can become the source of nosocomial infections. After caring for a hospitalized patient, the hands of a HCW become contaminated with these "transient flora," which can then be passed onto the next patient cared for, to a chart, phone, or computer, unless removed by hand hygiene (Kramer *et al*., 2006). For example, admitting a new patient to a room previously occupied by a MRSA- or a VRE-positive patient significantly increases the odds of acquisition for MRSA or VRE (Saha, 2010). Other pathogens that are capable of surviving in hospital reservoirs and for which environmental contamination may play a role in nosocomial acquisition are norovirus, hepatitis B virus, *Acinetobacter* spp, *Clostridium difficile*, and *Candida* spp (Hota, 2004).

Transmission of health-care–associated pathogens from one patient to another via the hands of HCWs require the following sequence of events: Organisms present on the patient's skin, or those that have been shaded on inanimate objects in close proximity to the patient, must be transferred to the hands of HCWs, these organisms must then be capable of surviving for at least several minutes on the hands of personnel, hand washing or hand antisepsis by the worker must be inadequate or omitted entirely, or the agent used for hand hygiene must be inappropriate. Finally, the contaminated hands of the caregiver must come in direct contact with another patient, or with an inanimate object that will come into direct contact with the patient (Bhalla *et al*., 2004).

Recently, other researchers studied contamination of HCWs' hands during activities that involved direct patient-contact wound care, intravascular catheter care, respiratory tract care, and the handling of patient secretions (Ferreira *et al*., 2011). Other studies also have documented that HCWs may contaminate their hands (or gloves) merely by touching inanimate objects in patient rooms (Ferreira *et al*., 2011). The transmission of organisms from artificially contaminated "donor" fabrics to clean "recipient" fabrics via hand contact also has been studied. *Staphylococcus saprophyticus, Pseudomonas aeruginosa,* and *Serratia* spp. were also transferred in greater numbers than was *Escherichia coli* from contaminated fabric to clean fabric after hand contact (Harbath *et al*., 2003). Organisms are transferred to various types of surfaces in much larger numbers (i.e., >104) from wet hands than from hands that are thoroughly dried (Harbath *et al*., 2003).

2.4 The burden of nosocomial pathogens

Hospital related infections (HRIs) impact on the population in many ways. They affect patients directly, causing increased morbidity and mortality; they may lead to disability and may reduce quality of life (Olalekan *et al.*, 2011). They also have impact on the healthcare system by extending hospitalization of affected patients and driving up the costs of diagnosis and treatment. HRIs may be transmitted from healthcare settings into the community, decreasing the reputation of healthcare institutions in the eyes of the public. They also may be a subject of indictment by the treated patients in hospitals (Raka, 2010).

In low and middle income countries (LMIC) the picture of the burden of HRI is unknown due to lack of reliable data and the use of different definitions and methodologies. Many LMIC have not conducted any surveillance studies regarding HRIs and few studies provide information on aetiology and risk factors for HRIs. HRIs represent one of the commonest complications of healthcare, affecting around 2 million persons admitted to acute hospitals annually (Wenzel and Edmond, 2000). They complicate 5-10% of admissions to acute care hospitals in industrialised countries. Average prevalence of HRI in Europe is 7.1 with range 3.5– 14.8% (Stone, 2009). By contrast, HRIs occurred in >40% of hospitalisations in developing countries in Asia, Latin America, and sub-Saharan Africa (Raka, 2010).

The first worldwide prevalence survey of HRIs conducted under the auspices of World Health Organization (WHO) in 55 hospitals of 14 countries in WHO regions showed an average incidence of 8.7% (23). The majority of HRIs relate to medical devices, e.g. pneumonia which is related to mechanical ventilation, urinary tract infections (UTIs)

related to urinary catheters, surgical site infection (SSI) after trauma or surgery, and bacteraemia derived from intravascular devices. UTIs constitute 30-40% of all HRIs. In intensive care of LMIC 66% of patients admitted to ICU develop HRIs. Ventilator-associated pneumonia (VAP) is a leading cause of death in hospitalised patients (Kollef, 2001).

Many studies have shown the importance of HRIs among neonates in LMIC, where an average of 4384 children die every day of these infections. A major review, reported rates of neonatal infections were 3-20-fold higher than those reported in industrialised countries (Okeke *et al*., 1999). Neonatal infections are estimated to cause 1.6 million deaths e annually, 40% of all neonatal deaths in developing countries (Kollef, 2001). World health organization reported that Nosocomial or hospital acquired infections (HAIs) is a serious global public health issue, causing the suffering of about 1.4 million people across the world at any given time (Olalekan *et al*., 2011).

The costs of HRIs are substantial everywhere, although they varies between countries due to different health care systems. Nosocomial infection can lead to increase in the duration of hospitalization for patients with surgical wound infection was about 8 days, ranging 3 days for gynaecology to 9 days for general surgery and 19 days for orthopaedic surgery (Saka *et al*., 2011). Prolong stay not only increases direct cost to patient or payers but also indirect costs due to cost work. In countries with prospective payment systems based on diagnosis- related groups, hospitals lose from $583 to $4,886 for each HRI.

Annual economic impact of HRIs in Europe is about 7 billion euro per year. The cost to the government of Trinidad and Tobago for HRIs was estimated at $697,000 annually. In Mexico, the annual cost approaches $1.5 billion, and in Thailand 10% of the annual hospital budget is spent on HRIs (Olalekan *et al*., 2011). Some investigators have attempted to measure costs related to hospital outbreaks of HRIs caused by multidrug resistant organisms. In a study of infections caused by MRSA it was estimated that average cost was $4000 per infection, whereas costs of *C. difficile*-associated diarrhoea was approximately $4500 per patient (Olalekan *et al*., 2011).

Resistance to antimicrobial agents is a global challenge in all healthcare facilities. The globalization enabled the rapid spread of multi-resistant microorganisms (CDC, 2003). In law and middle income countries inappropriate and uncontrolled use of antibiotics is very common and antimicrobials are frequently available over the counter in pharmacies. In many low income countries resistance among common pathogens to cheap antimicrobials has already increased drastically, resulting in limited effectiveness. The quality and potency of antibiotics are often suspected, with unregulated import, registration and distribution (Wenzel and Edmond, 2000).

Another factor contributing to resistance is lack of antibiotic policies or basic recommendations at governmental level or within hospitals. Between 20% and 50% of a hospital budget is spent on antimicrobials, which are used to treat more than half of all patients. Even in developed countries >50% of antimicrobials are prescribed incorrectly, either administered in suboptimal doses or for incorrect duration (Raka, 2010). Misuse of antibiotics has been identified as an important factor in the emergence of antimicrobial resistance. In turn, this resistance makes the clinical management of the

patients more difficult. The use of antimicrobials in the veterinary area had an important impact on increase of antimicrobial resistance (Randle *et al*., 2006).

2.5 Staphylococcus aureus

Staphylococcus aureus is a Gram positive coccus that occurs in grape-like clusters. It is a eubacterium that is found on the surface of the human skin and mucous membranes (Prescott *et al*., 2005). It also is found in other areas of human contact such as air, dust and food products (Okeke *et al*., 1999). Cheesborough (2005) describes the *Staphylococcus* species as a group of non motile, non capsulate, Gram positive cocci of uniform size (about 1um in diameter) that occur characteristically groups but also singly and in pairs. Staphylococci are widely distributed in nature. They form part of the normal microbial flora of the skin, upper respiratory tract and intestinal tract (Cheesborough, 2005).

S. aureus is an opportunistic pathogen in man and animals and is the most frequent cause of hospital and community infections (Prescott *et al*., 2005). *S. aureus* can cause a range of illness from minor skin infections such as boils, abscesses to life threatening diseases such as pneumonia, meningitis, toxic shock syndrome and sepsis (Lakshmi and Harasreeramulu, 2011). Most strains become infectious usually when the skin or mucous membrane is punctured by variety of objects such as needles, blades, surgical devices etc. (Cheesborough, 2004; Prescott *et al*., 2005). Little wonder then, that it is a serious threat to hospitalized patients. The bacterium is a short term lived contaminant, short term resident or long term colony forming, non spore forming catalase and coagulase positive organism (Prescott *et al*.,2005).

Generally, the organism presents three broad disease types; a variety of superficial infections such as pimples, boils and toxic epidermal necrolysis (characterized by outer layer of the skin separating from the deeper layers); systemic infections such as endocarditis (inflammation of heart valves), osteomyelitis (inflammation of bone or bone marrow) and toxinoses such as food poisoning or toxic shock syndrome (Landolo, 2000). Anyadoh-Nwadike *et al*. (2011) reported that the pathogenicity of the bacterium depends upon a number of virulence factors: a variety of surface protein on the bacterium's cell membrane which allow attachment and colonization of the bacterium within the cellular and extracellular material of the host, cellular proteins, proteases and toxins that inhibit phagocytises and interfere with the ability of the host to actively hinder bacterial populations in the invasion of host tissues. Cheesborough (2005) confirmed this opinion by suggesting that the versatility of *S. aureus* may be traced to the array of enzymes and toxins it produces.

2.5.1 The antibiotic resistance in Staphylococcus aureus

Drug resistance by the organism is also a major concern (Weinstein, 1998). Both methicillin (oxacillin or cefoxitin) and glycopeptide (vancomycin and teicoplanin) resistance may occur in *Staphylococcus aureus*. A European study demonstrated that 50% of the infectious morbidity in intensive care units (ICUs) can be attributed to Methicillin resistant *S. aureus* (MRSA). MRSA resists desiccation and can survive in hospital dust for up to a year. It is found throughout the hospital environment, particularly around patients known to be colonised or infected with the bacterium (Dancer, 2009). Overall, *S. aureus* is a leading cause of nosocomial infections. It is the most common cause of surgical wound infections and is second only to coagulase negative Staphylococcus (CoNS) as a cause of primary bacteraemia. Increasingly,

nosocomial isolates are resistant to multiple drugs. In the community, *S. aureus* remains an important cause of skin and soft tissue infections, respiratory infections, and (among injection drug users) infective endocarditis (Horst *et al*., 2011).

The increasing prevalence of home infusion therapy is another cause of community-acquired staphylococcal infections (Jones *et al*., 1996). The most prevalent type of resistance among staphylococci is methicillin resistance. The resistance of MRSA against various antimicrobials is globally at alarming rate and treatment of MRSA infections has become more challenging since it is a major concern among healthcare professionals (Dudhagara *et al*., 2011). This resistance is encoded genetically by *MecA* gene, which produces an altered PBP target (Klastersky, 1998). *Staphylococcus aureus* strains continue to be a major problem in many healthcare institutions especially with emergence of MRSA and now account for more than 50% of *S. aureus* recovered from patients in intensive care units and about 40% of *S. aureus* isolated from non intensive care unit (Fagade *et al*., 2010, Lakshmi and Harasreeramulu, 2011). Since MRSA is also resistant to many other antimicrobials, both β-lactams and non-β-lactams, vancomycin is generally relied on for the treatment of MRSA infections.

However, the expanded use of vancomycin has led to the emergence of other resistance problems (Klastersky, 1998). Similarly, when ciprofloxacin was initially introduced to the market, it was highly effective against MRSA. However, almost immediately after release by the US Food and Drug Administration, studies in the United States showed significant increases in the minimum inhibitory concentrations of ciprofloxacin against staphylococci, especially against MRSA (Noble *et al*., 1992). Reports of glycopeptide-resistant *S. aureus* are also alarming. Teicoplanin-resistant coagulase-negative

staphylococci were first reported in 1985; in 1987, vancomycin-resistant coagulase-negative staphylococci were reported (Jones, 1999). Since the initial report from Japan in 1997 (Jones *et al.*, 1998) many other groups from around the world have reported strains of *S. aureus* with intermediate resistance to vancomycin. All of the reported cases occurred in patients who had prolonged (> 1 month) exposure to vancomycin.

2.6 Pseudomonas aeruginosa

Pseudomonas aerugionosa is a non-fermentative, aerobic Gram negative motile rod. It normally lives in moist environments, and uses a wide range of organic compounds for growth. The bacterium is ubiquitous in soil, variety of aqueous solutions, including disinfectants, soaps, eye drops, as well as sinks, respiratory equipment, food, taps toilets, showers and mops. This pathogen needs no sufficient organic matter to grow; it can grow in distilled water. It grows best at about 37°C and even at 42°C. *P. aeruginosa* is capable of multiplying in two days, it can swim from one site to the next as motile cell or it multiplies as an adherent micrcolony biofilm by producing a slimy layer (Harris *et al*., 2010). It is constantly reintroduced into the hospital environment by visitors and patients transferred from other facilities. Spread occurs from patient to patient on the hands of hospital personnel, by direct patient contact with contaminated reservoirs, and by the ingestion of contaminated foods and water. The pathogen has been recognized as an emerging opportunistic pathogen of clinical relevance i.e. it causes infections among immuno-compromised patients (Okonko *et al*., 2009).

This pathogen is commonly implicated in serious nosocomial infections such as pneumonia (lung infection), sepsis, and urinary tract infection (UTI). It also causes infections of the wounds, burns and can lead to death in children and adults with cytis fibrosis (Tavajjohi and Moniri, 2011). *P. aeruginosa* has an abundance of virulence factors, including flagella, pili, lipopolysaccharides, alginate, alkaline protease, elastase, phospholipase C, exotoxin A, quorum sensing mechanisms, type III secretion system, pyocianin, pyoverdin, and produces a number of toxic proteins which not only cause extensive tissue damage, but also interfere with the human immune system's defence mechanisms (Okonko *et al*., 2009).

2.6.1 The antibiotic resistance in Pseudomonas aeruginosa

The worldwide emergence of multidrug- resistant bacterial strains of *Pseudomonas* is of growing concern especially in nosocomial infections caused by *Pseudomonas aeruginosa*. The infections are difficult to eradicate due to resistance to many antimicrobials (Pal *et al.,* 2010). The occurrence of multidrug-resistant *P. aeruginosa* strains is increasing worldwide and limiting our therapeutic options (Bonomo and Szabo, 2006). The spread of this organism is often difficult to control as *P. aeruginosa* exhibit intrinsic resistance to several antimicrobial agents.

Drug resistance in P. *aeruginosa* may be mediated via several mechanisms. The resistance mechanisms include; production of β-lactamases, efflux pumps and target-site or outer membrane modification (Tavajjohi and Moniri, 2011). The enzymes, β-lactamases are bacterial enzymes that are encoded by chromosomal or by plasmid-born genes and protecting the microorganisms against the lethal effects of β-lactams drugs by rendering them effectless. Extended-spectrum β-lactamases (ESBLs) are enzymes that

mediate resistance to extended spectrum cephalosporin (ESCs) such as cefotaxime, ceftriaxone and ceftazidime and the monobactam aztreonam. ESBLs are inactivated by the β-lactamases inhibitor, calvulanate (Yu *et al*., 2006; Jazani and Zahedi, 2012).

Resistance to multiple drugs is usually the result of the combination of different mechanisms in a single isolate or the action of a single potent resistance mechanism (Zavascki *et al*., 2010). Antimicrobial resistance to clinical isolates of P. *aeruginosa* may complicate treatment of infections and can adversely affect clinical outcomes and treatment cost for patients (Hota, 2004).

2.7 Escherichia coli

Escherichia coli is the predominant, facultative anaerobe of the human intestine. The pathogen is aerobic Gram negative, short rods in shape. Beneficial strains of *E. coli* typically colonize the infant gastrointestinal tract within a few hours after birth. The presence of this bacterial population in the intestine suppresses the growth of harmful bacteria and is important for synthesizing appreciable amounts of B vitamins. *E. coli* usually remains harmless when confined to the intestinal lumen (Johnson *et al*., 1996). However, in debilitated or immuno-suppressed humans, or when gastrointestinal barriers are violated, even normal, "non-pathogenic" strains of *E. coli* can cause infection *(*Paton and Paton, 1998). It is also known that some *E. coli* strains have developed the ability to cause disease of the gastrointestinal, urinary, or central nervous system in even very healthy people.

Strains of *E. coli* that cause diarrhoea include strains that cause traveller's diarrhoea which is caused by enterotoxigenic *E. coli* (*E. coli* O157:H7), persistent diarrhoea (enteroaggregative *E. coli)*, watery diarrhoea of infants (enteropathogenic *E. coli),* hemorrhagic colitis (bloody diarrhoea), and haemolytic uremic syndrome (enterohaemorrhagic *E. coli*) (CDC, 1993).

E. coli O157:H7 infections occur worldwide; infections have been reported on every continent and are transmitted by the faecal-oral route. They can be spread between animals by direct contact (Alam and Zurek, 2006). *Escherichia coli* is an important cause of urinary tract infections (UTIs). The systemic infections include bacteraemia, nosocomial pneumonia, cholecystitis, cholangitis, peritonitis, cellulitis, osteomyelitis, and infectious arthritis. *E. coli* is also leading cause of neonatal meningitis (Buchanan and Doyle, 1997). The presence of this microbe on surfaces of hospital could be as a result of faecal contamination.

2.7.1 The antibiotic resistance in Escherichia coli

The antibiotic sensitivities of different strains of *E. coli* vary widely. As Gram-negative organisms, *E. coli* are resistant to many antibiotics that are effective against Gram-positive organisms. Antibiotics which may be used to treat *E. coli* infection include amoxicillin, as well as other semi synthetic penicillins, many cephalosporins, carbapenems, aztreonam, trimethoprim-sulfamethoxazole, ciprofloxacin, nitrofurantoin and the aminoglycosides.

Antibiotic resistance is a growing problem. Some of this is due to overuse of antibiotics in humans, but some of it is probably due to the use of antibiotics as growth promoters in animal feeds (Johnson *et al*., 2006). A study published in the journal Science in August 2007 found the rate of adaptative mutations in *E. coli* is "on the order of 10^{-5} per genome per generation, which is 1,000 times as high as previous estimates," a finding which may have significance for the study and management of bacterial antibiotic resistance (Perfeito *et al*., 2007).

Antibiotic-resistant *E. coli* may also pass on the genes responsible for antibiotic resistance to other species of bacteria, such as *Staphylococcus aureus*, through a process called horizontal gene transfer. *E. coli* bacteria often carry multiple drug-resistance plasmids, and under stress, readily transfer those plasmids to other species. Indeed, *E. coli* is a frequent member of biofilms, where many species of bacteria exist in close proximity to each other. This mixing of species allows *E. coli* strains that are piliated to accept and transfer plasmids from and to other bacteria. Thus, *E. coli* and the other enterobacteria e.g. enterococci are important reservoirs of transferable antibiotic resistance genes (Salyers *et al*., 2004).

CHAPTER THREE

3.0 MATRERIALS AND METHODS

3.1 Study area

The study area of this work encompass some hospitals in Zaria including Gambo Sawaba general hospital, Kofan Gaya which is situated at 11° 02' N and 007° 41' E, St Luke Anglican hospital, Wusasa located at 11° 04' N and 007° 40' E, then Major Ibrahim B. Abdullahi memorial hospital which is the third hospital selected for this study is situated at 11° 06' N and 007° 41' E all at Greenwich meridian. These areas were located using Taiwan made Etrex® high-sensitive geographic positioning system (GPS) receiver.

3.2 Ethical approval

The ethical approval was obtained from ethical committee of Kaduna state Ministry of Health and was used for sampling (Apendix 1).

3.3.0 Sampling designing and techniques

3.3.1 Sample size

The sample size was determined using an average of 11.85% WHO prevalence rate of nosocomial infection as reported by Samuel *et al*. (2010). The sample size was determined as described by Naing *et al*. (2006) using the formula:

$$n = \frac{Z^2 P(1-P)}{d^2}, \quad n = \text{number of sample}$$

P = prevalence rate of contamination of previous study = 11.85% = 0.1185

Z = standard normal distribution at 95% confidence limit = 1.96

d = absolute desired precision of 5% = 0.05

Therefore,

$$n = \frac{1.96^2 \times 0.1185\,(1 - 0.1185)}{0.05^2} = 160$$

The sample size for this study was increased to four hundred and seventy (470) samples; 160 samples from Gambo Sawaba general hospital, 155 each from St. Luke's Anglican hospital Wusasa and Major Ibrahim B. Abdullahi memorial hospital.

3.3.2 Sample collection

The samples were collected in the morning before commencement of work but hand swab of the staff were collected during working hours. Samples for the studies were collected from hands of some of the hospital staff and nurses (46), floors (96), toilets seats (28), operation tables (15), door knobs/door handles (68), Nurses' table tops (36), bedrails (58), stretchers (35), cupboards (52) and sinks (36) using sterile swab sticks using wetted sterile cotton swabs.

3.4.0 Laboratory analysis

Each sample swab was inoculated into prepared sterile bacteriological peptone water and incubated at 37°C for 24 hours for enrichment after which the turbid broth was subcultured on differential media such as Mannitol salt agar, Eosin methylene blue agar (EMB), Pseudomonas cetrimide selective agar and MacConkey agar plates and incubated again at 37°C for 24 hours. Discrete colonies were further subcultured onto fresh prepared plates of the selective media and nutrient agar plates to obtain pure cultures. The purified cultures were gram stained and stored on nutrient agar slants for biochemical tests and identification.

3.4.1 Observation of colonial morphology and characteristics

Presumptive morphological identification of the colonies was done by observing their individual appearances on the selective media that were used for the isolation.

3.4.2 Gram staining

Gram staining reaction is a basic staining technique in bacteriology used to help identify pathogens in specimens and cultures by their Gram reaction (Gram positive or Gram negative) and morphology. The following method was used as reported by Cheesbrough (2005).

With the aid of sterile wire loop, a loopful of normal saline was placed on a grease free slide by passing it over flame of Bunsen burner. A distinct colony of each of the organism was smeared evenly covering an area of about 15-20mm on the slide containing the normal saline. The smear was allowed to air-dry completely and the smear was heat fixed by rapidly passing the slide opposite of the smear three times through the flame of Bunsen burner. The fixed smear was covered with crystal violet stain for 60 seconds. It was washed with distilled water, the excess water was tipped off and the smear was covered with Gram's iodine for another 60 seconds and was washed off with distilled water.

Acetone was used to rapidly decolourize the smear in few seconds and was washed immediately with distilled water. The smear was counter stained with safaranin for 2 minutes; the stain was washed off with distilled water. The water at the back of the slide was wiped with a clean tissue and air-dried on a draining rack. The smear was examined microscopically with oil immersion objective lens (100×) and the bacteria were observed and recorded (Cheesbrough, 2005).

3.4.3 Biochemical tests:

Catalase test

Three (3) ml of hydrogen peroxide solution was poured into a test tube. With the aid of sterile glass rod several colonies of the test organism were carefully removed and immersed into 3ml solution of hydrogen peroxide. Immediate bubbling within few seconds was recorded to be positive test of *Staphylococcus* species (Cheesbrough, 2005).

Coagulase test

A drop of distilled water was added on each end of a slide. A colony of a suspected organism of 24 hours culture from blood agar (previously checked by gram staining) was emulsified in each of the drops of the distilled water. A loopful of the plasma was then added to one of the suspensions and mixed gently. Clumping or agglutinations of the organisms with the plasma within ten (10) seconds indicated a positive result of *Staphylococcus aureus*; negative result indicates *Staphylococcus epidermidis* (Cheesbrough, 2005).

IMViC test for identification of *E. coli*

IMViC reactions are a set of four useful reactions that are commonly employed in the identification of members of family enterobacteriaceae. The four reactions are: Indole test, Methyl Red test, Voges Proskauer test and Citrate utilization test. The letter “i” is only for rhyming purpose (MacFaddin, 2000).

Indole test

Testing for indole production is important in the identification of enterobacteria, for example most strain of *Escherichia coli***.**

The colony of the 24 hours culture of the test organism (after being checked by Gram staining) was inoculated in a bijou bottle containing 3ml of sterile tryptone water. It was incubated at 35-37°C for up to 48 hours, then it was tested for production of indole by dispensing 0.5ml of Kovac's reagent. After it was gently shaked, red colour in the surface layer within ten (10) minutes indicated positive result for *E. coli* (Cheesbrough, 2005).

Methyl red (MR) test

A 24 hours culture of the bacterium was inoculated into a test tube of 3ml of glucose phosphate broth, which contained glucose and a phosphate buffer and was incubated at 37°C for 48 hours. The pH of the medium was tested for production of acid as a result of fermentation by adding 5 drops of methyl red solution (0.04%). Development of red colour indicated positive result for *E. coli* (MacFaddin, 2000).

Voges-Proskauer (VP) test

A 24 hours culture of the bacterium was inoculated into a test tube of 3ml of glucose phosphate broth, which contain glucose and a phosphate buffer and was incubated at 37°C for 48 hours. The culture was treated with 0.6ml of α- naphthol and shake. Then 0.2ml of 40% KOH was added to the broth and shaked. The tube was allowed to stand for 15 minutes. There was no colour appearance even after it was allowed to stand for 1

hour; since maximum colour development occurs within one hour after addition of reagents the negative result indicated *E. coli* (MacFaddin, 2000).

Citrate test

Two sets of slants of Simmons citrate agar were prepared in bijou bottles, one of the sets were used to identify *Escherichia coli* and the other sets were used to identify *Pseudomonas aeruginosa*. Then with the aid of sterile straight wire normal saline suspension of the test organisms were streaked on the slants and were incubated at 37°C for 48 hours. A bright blue colour on the agar slants in the tubes inoculated with *Escherichia coli* and *Pseudomonas aeruginosa* indicated positive result (Cheesbrough, 2005).

Oxidase test

A clean piece of filter paper was placed on a clean Petri dish; three (3) drops of freshly prepared oxidase reagent were added on the filter paper. With the aid of glass rod a colony of 24 hours culture of the suspected organism was removed and smear on the filter paper containing the oxidase reagent**.** Development of a blue-purple colour within ten (10) seconds indicated the positive result of *P. aeruginosa* (Cheesbrough, 2005).

3.4.4 Confirmation test

The isolates for the study were confirmed using microgen Gram negative (GNA or GNA + GNB) and microgen STAPH identification kits from Microgen Bioproducts Ltd (www.microgenbioproducts.com) UK.

3.4.4.1 Microgen staphylococci identification kit

The Microgen Staph-ID system comprises of a single microwell test strip containing 12 standardized biochemical substrates which have been selected on the basis of extensive computer analysis i.e. each well contains dehydrated substrates. A colour change occurs if the individual substrates are metabolized by the organism during incubation, or after addition of specific reagents.

Procedure

Inoculation and incubation

A single colony from 24 hours culture was emulsified in a suspending medium supplied with the kit and mixed thoroughly. The adhesive tape sealing the microwell test strip was carefully peeled. With aid of a sterile Pasteur pipette 3 drops of the bacterial suspension was added to each of the microwells of the test strips. After inoculation, well 10 and 11 were overlayed with 3 drops of mineral oil. The top of the microwell test strips were sealed with adhesive tape removed earlier and incubated at 37°C for 24 hours. The microwells test strips were read after 24 hours of incubation.

Reading and addition of reagents

The adhesive tape was removed and all the positive reactions were recorded with the aid of colour chart and substrate reference table. The results were recorded on the forms provided.

1. Appropriate reagents were added to the following microwells:

a) To well 12 a drop of L- pyrrolidonyl-α-naphthylamide (PYR) reagent was added and read after ten (10) minutes. Formation of a very deep pink/red colour indicated a positive result.

b) Nitrate reduction test on well 9 was done after reading and recording the β-Glucuronidase reaction. 1 drop of Nitrate A reagent and 1 drop of Nitrate B reagent was added to the well and the reaction was read after 60 seconds. The development of a red colour indicated Nitrate reduction. All the results were recorded on the provided report form appropriately.

Identification

On the Microgen staphylococci identification report form which contained the substrates used for the biochemical tests organised into triplets (sets of 3 reaction) with each substrate assigned a numerical value (1,2,4). The sum of the positive reactions for each triplet formed a single digit of the Octal Code (profile number) that was used to determine the identity of the isolate. The Octal Code of each isolate was entered into the Microgen Identification System Software (MID-60), which generated a report of the 5 most likely organisms in the selected database. The software provides the identification base on probability (%) with analysis of quality of identification (www.microgenbioproducts.com).

3.4.4.2 Microgen GN-identification system

The Microgen GN-ID system comprises two separate microwell test strips; GN A and GN B. each strips contains 12 standardized biochemical dehydrated substrates. Each well contains dehydrated substrates. If the individual substrates are metabolized by the organism, a colour change occurs during incubation, or after addition of specific reagents. The GN A microwell kit is for the identification of oxidase negative, nitrate positive glucose fermenters example the family of *Enterobacteriaceace*. GN A and GN B (GNA+B) kit is used together to produce a 24 substrate system for the identification of oxidase positive, non-fastidious gram negative (www.microgenbioproducts.com).

Procedure:

Inoculation and incubation

A single colony from 24 hours culture was emulsified in 5ml of sterile 0.85% normal saline and mixed thoroughly. The adhesive tape sealing the microwell test strips was carefully peeled backward. Then with the aid of sterile Pasteur pipette 3 drops of the bacterial suspension (*E. coli*) was added to each of the microwell tests trips.

After the inoculation of the isolates, wells 1, 2, 3 and 9 (GNA microwell test strip) and well 24 (GNB microwell test strip) which was used to test for oxidase positive (*P. aerugionsa*) were overlayed with 3 drops of mineral oil. The top of the microwell test strip was resealed with the adhesive tape and was incubated at 37°C for 24 hours. The results of the biochemical reactions were read recorded after 24 hours. For oxidase positive like the *P. aerugionsa*, microwell 13 and 24 were read after 48 hours of culture.

Reading and addition of reagents

1. Appropriate reagents were added to the following microwells:

a) To well 8 two drops of Kovac's reagent were added. The results of the reaction were read and recorded after 60 seconds.

b) To well 10 one drop of VP I reagent and VP II reagent were added. The result of the reaction were read and recorded after 30 minutes.

c) To well 12 one drop of Tryptophan deaminase (TDA) reagent was added. The result was read and recorded

2. For oxidase positive isolates nitrate reduction was performed on well 7 by adding 1 drop of Nitrate A and Nitrate B after reading and recording Ortho-Nitrophenol-β-galactoside (ONPG) result.

The identification of *E. coli* and *P. aerugionsa* using GNA and GNA+B kits respectively

On the Microgen GN-ID A+B report form which contained the substrates used for the biochemical tests organised into triplets (sets of 3 reaction) with each substrate assigned a numerical value (1,2,4). The sum of the positive reactions for each triplet formed a single digit of the Octal Code (profile number) that was used to determine the identity of the isolate. The Octal Code of each isolate was entered into the Microgen Identification System Software (MID-60), which generated a report of the 5 most likely organisms in the selected database. The software provides the identification base on probability (%) with analysis of quality of identification.

3.4.4.3 The serology test for identification of *E. coli* O157:H7

With the aid of micropipette 1 drop (30µL) of isotonic saline (M40) was dispensed on to two wells of a clean, dry Microgen® agglutination slide. Several 24 hours culture colourless colonies of *E. coli* where picked using wire loop several from Sorbitol MacConkey agar plate(the morphology of *E. coli* on this medium is colourless). The colonies were emulsified in the two drops of saline on the test slide to produce a heavy, smooth suspension. The suspension was spread evenly over the surface of the wells and the slide was rocked well for 30 seconds and the suspension remained smooth. Then 1 drop of Microgen® *E. coli* O157:H7 test latex was added to one of the bacterial suspensions, and one drop of Microgen *E. coli* O157:H7 control latex to the other. The suspensions were mixed with a fresh mixing stick for each combination. The slide was

gently rocked for two minutes and agglutination was observed. An agglutination reaction was indicated by visible aggregation of the latex particles (www.microgenbioproducts.com).

The interpretation of the serology test:

Test Latex	Control Latex	Interpretation
+	-	*E. coli* O157:H7 present
-	-	*E. coli* O157:H7 not present
+	+	Non-specific agglutination
-	+	Inconclusive result

3.5.0 McFarland turbidity standard

The turbidity standard of the organisms used was 0.5. One percent (1%) v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of distilled water and mixed well. One percent (1%) w/v of barium chloride solution was also prepared by placing 1g of the dehydrated salt ($BaCl_2.2H_20$) in a 100ml measuring cylinder distilled water and dissolved it to using distilled water to the 100ml mark of the measuring cylinder. Then 0.6ml of the barium chloride was added to 99.4ml of the sulphuric acid solution and was mixed well. The small portion of the turbid solution was transferred into a test tube which was used to compare with the inoculated organisms in Mueller-Hinton broth (Cheesbrough, 2004).

3.6.0 Antibiotic susceptibility test

The antimicrobial susceptibility pattern was determined using Kirby-Bauer-NCCLS modified single disc diffusion technique (Cheesbrough, 2004). Disc diffusion technique

was performed according Kirby-Bauer method, as described in the guidelines of Clinical and Laboratory Standards Institute, CLSI (2008). Single antibiotic disc such as Ampicillin (10µg), Vancomycin (30µg), Tetracycline (30µg), Cefoxitin (30µg), Chloramphenicol (30µg), Imepenem (10µg), Ceftazidime (30µg), Linezolid (10µg) and Gentamicin (10µg), all the discs were obtained from Oxoid England.

3.7.0 The standardization of inoculums

The dilution of each of the suspension of the test organisms and the standard isolates were prepared by picking a 24 hours colony of the organism using sterile wire loop into sterile test tube containing sterile normal saline to form turbidity that match with 0.5 scale of MacFarland's standard (1.5×10^8 cells/ml) (Coyle, 2005). *Staphylococcus aureus*, ATCC 25923 and *Escherichia coli*, ATCC 25922 were obtained from National Institute of Pharmaceutical Research and Development (NIPRD), Abuja. *Pseudomonas aeruginosa* ATCC 9027 was obtained from National Veterinary Research Institute (NVRI), Vom, Jos. These standard strains were used as the antibiotics susceptible control. The cell suspensions was inoculated by streaking on prepared Mueller-Hinton agar using sterile swab stick, then the antibiotic disc was placed on the inoculated medium aseptically with help of sterile forceps and incubate at 37^oC for 24 hours. The zones of inhibition created by each of the antibiotics against the test organisms and the standard strains as positive control were measured and the result was interpreted using guideline from CLSI (2008). The results were recorded as sensitive, intermediate and resistant.

3.8.0. Determination of multiple antibiotics resistance (MAR) index

The multiple antibiotics resistance index was determined for each of the selected bacterial isolate using a formula MAR = x/y, where x is the number of antibiotics to which test isolate displayed resistance and y is the total number of antibiotics to which the test organism has been evaluated for sensitivity (Olayinka *et al*., 2004, Tula *et al*., 2013).

3.9.0 Minimum inhibitory concentrations

This was determined by broth microdilution method. Standard antibiotics powders were obtained from Glaxosmithkline and Evans pharmaceutical companies. Two-fold dilutions of the antibiotics were prepared in Muller Hinton broth (Wayne, 2002) about 10 dilutions were made with concentration gradient concentration covering from 0.244-125µg/ml of each of the antibiotic using a stock concentration of 250µg/ml. For each series of the dilution all the first tubes of the Mueller Hinton broth were double strengthened (10ml) the remaining tubes were single strength (10ml). Then10ml of the antibiotic from the stock concentration was used for dilution. The concentration of each of the suspension of the test organisms were prepared by picking a 24 hours colony of the organism using sterile wire loop into sterile test tube containing sterile normal saline to form turbidity that match with 0.5 scale of MacFarland's standard (1.5×10^8 cells/ml) (Coyle, 2005). The tubes containing 10ml of the sterile Mueller-Hinton broth were used to carry out two-fold dilution using 10ml of the antibiotic (125µg/ml as the highest concentration). Three controls were set up: positive control (containing Mueller Hinton broth and standard strain), negative control (containing Mueller Hinton broth and antibiotic), and sterility control (containing only Mueller Hinton broth) (Terry-Alli *et al*., 2011). All the tubes were inoculated with 0.1ml of the isolates using micro pipette except negative control and sterility control and were incubated at 35°C for 24 hours. The lowest concentration that inhibited the growth or showed no growth of the bacterial

isolate in the tube was taken as the MIC. Readings were taken and recorded according the guidelines of CLSI (2008).

3.10.0 Minimum bactericidal concentrations

The MBC was determined by subculturing the tube next to the MIC tube and all the tubes in which there was no growth that showed no visible growth of the bacterial isolates on freshly prepared plates of Mueller Hinton agar containing 5% of tween 80 (help to inactivate the antibiotic). The plates of the inoculated medium were incubated at 37°C for 24 hours. The lowest concentration on the plate that showed no growth of the bacterial isolate was the MBC (Cheesbrough, 2005).

3.11.0 Statistical analysis

Data analysis was performed using Statistical Package for Social Science (SPSS) version 17.0. The statistical methods employed were Chi-Square and Kruskal-wallis test and p-values greater than or equal to 0.05 ($P \geq 0.05$) were considered not significant or no associations.

CHAPTER FOUR

4.0 RESULTS

Of the total number of 470 samples collected from the three hospitals for this study, 157 (33.40%) showed positive result for *Staphylococcus aureus*, *Pseudomonas aeruginosa* and *Escherichia coli*.

Table 4.1 indicates the total number of 63 isolates with mean value of 2.133±2.403 isolated from Gambo Sawaba general hospital out of which 32 (50.8%) were tested positive for *S. aureus*, 18 (23.6%) for *P. aeruginosa* and 13 (20.6%) were tested positive for E. coli. The total number of isolates from Major Ibrahim B. Abdullahi memorial hospital was 46 with mean value of 1.567±1.813 out of which 23 (50.0%), 16 (34.8%) and 7 (15.2%) were *S. aureus*, *P. aeruginosa* and *E. coli* respectively. The total number of isolates isolated from St Luke Anglican hospital was 48 with mean value of 1.567±1.813, the number of *S. aureus*, *P. aeruginosa* and *E. coli* were 27 (56.3%), 13 (27.0%) and 8 (16.7%) respectively. The statistical analysis showed that there was no significant difference in the prevalence rate of the organisms on the surfaces of the three hospitals ($P > 0.05$) at 95% confidence limit using Kruskal-Wallis test.

Table 4.2 shows the distribution of the pathogens in relation to the surfaces of the three hospital environment. *S. aureus* was the only pathogen isolated from the hand swab of the nurses in the three hospitals. This pathogen was isolated from door knob, nurses' table top/staff table, stretcher, bedrail, floor and cupboard from all the hospitals. It was

isolated from toilet seat, sink and floor of the three hospitals. *S. aureus* was isolated from operation table of Gambo Sawaba general hospital.

Table 4.1: Occurrence of bacterial isolates in the three hospital environment in Zaria

Hospital	No. of sample screened	Total isolates	Total % isolates	*P. aeruginosa*	% prevalence of *P. aeruginosa*	*S. aureus*	% prevalence of *S. aureus*	*E. coli*	% prevalence of *E. coli*	Mean ±SD	P value
GSGH	160	63	39.4	18	28.6	32	50.8	13	20.6	2.13±2.403	0.941
MIBAMH	155	46	29.7	16	34.8	23	50.0	7	15.2	1.60±1.868	0.631
SLAH	155	48	30.9	13	27.1	27	56.3	8	16.7	1.57±1.813	0.935
Total isolates	470	157	33.4	47	29.9	82	52.2	28	17.8		

P value > 0.05

KEY: GSGH = Gambo Sawaba General Hospital Zaria, MIBAMH = Major Ibrahim B. Abdullahi Memorial Hospital Zaria, SLAH = St Luke's Anglican Hospital Zaria

Pseudomonas aeruginosa was found predominantly contaminating toilet seat, sink, stretcher and floors. This pathogen was also isolated from operation table of St. Luke's Anglican hospital and Major Ibrahim B. Abdullahi memorial hospital and some cupboard in Gambo Sawaba general hospital. *Escherichia coli* in this work was isolated from door knob/door handle of toilets of the three hospitals. Some were isolated from bedrail of St Luke's Anglican hospital. The surfaces of the three hospitals were more contaminated by *S. aureus*. Even though the statistical analysis showed that there was no significant association between the organisms from the surfaces and the hospitals ($P > 0.05$) at 95% confidence limit using Chi-Square test.

Table 4.3 shows the rate of prevalence of the pathogens in Gambo Sawaba hospital. The prevalence of the three pathogens on the surfaces of Gambo Sawaba general hospital showed that 100, 83.3, 53.8, 66.7, 75.0, 100, and 57.1% of *Staphylococcus aureus* was isolated from hand swab, nurses' table top, door knob/handle, operation table, stretcher, bedrail and cupboard respectively and the total percentage distribution of this pathogen from this hospital was 50.8%. The prevalence rate of *S. aureus* was high compared to the other two pathogens. The prevalence rate of *Pseudomonas aeruginosa* isolated from toilet seat, sink, stretcher, floor and cupboard was 44.4, 100, 25.0, 100, and 42.9% respectively and the total percentage distribution of this pathogen was 28.6%. *Escherichia coli* was isolated from nurses' table top (16.7%), Door knob/handle (46.2%), toilet seat (55.5%) and operation table (33.3%) and the total percentage distribution of this organism was 20.6%.

Table 4.2: Bacterial isolates in relation to the surfaces in the three hospital environment

Surface	Organism	Hospital			Chi-Square value	P value
		GSGH	MIBAMH	SLAH		
NHS	*S. aureus*	+	+	+	2.400	0.663
	P. aeruginosa	-	-	-		
	E. coli	-	-	-		
DK(DH)	*S. aureus*	+	+	+	3.000	0.809
	P. aeruginosa	-	-	-		
	E. coli	+	+	+		
NTT/ST	*S. aureus*	+	+	+	6.000	0.423
	P. aeruginosa	-	-	-		
	E. coli	+	+	-		
OT	*S. aureus*	+	-	-	4.500	0.609
	P. aeruginosa	-	+	+		
	E. coli	+	-	-		
Sink	*S. aureus*	-	-	-	1.286	0.5260
	P. aeruginosa	+	+	+		
	E. coli	-	-	-		
Stretcher	*S. aureus*	+	+	+	4.500	0.609
	P. aeruginosa	+	+	+		
	E. coli	-	-	-		
TS	*S. aureus*	-	-	-	3.600	0.463
	P. aeruginosa	+	+	-		
	E. coli	+	+	+		
Floor	*S. aureus*	-	+	+	2.400	0.663
	P. aeruginosa	+	+	+		
	E. coli	-	-	-		
BR	*S. aureus*	+	+	+	6.000	0.423
	P. aeruginosa	-	-	+		
	E. coli	-	-	+		
CB	*S. aureus*	+	+	+	2.250	0.325
	P. aeruginosa	+	-	-		
	E. coli	-	-	-		

The P value > 0.05

KEY: + = Presence of pathogen, - = Absence of pathogen, NHS = Nurses' hand swab, NTT/ST = Nurses table top/staff table, DK/DH = Door knob/Door handle, TS= Toilet seat, OT= Operation table, BR = Bedrail, CB = Cup board, GSGH = Gambo Sawaba General Hospital, MIBAMH = Major Ibrahim B. Abdullahi Memorial Hospital, SLAH = St Luke's Anglican Hospital.

Table 4.3: Prevalence (%) of the isolates in Gambo Sawaba General Hospital

Sample source	Sample size	Total positive isolates	Total % of isolates	*S. aureus*	(%)	*P. aeruginosa*	(%)	*E. coli*	(%)
NHS	20	5	25.0	5	100	-	-	-	-
NTT/ST	11	6	63.6	5	83.3	-	-	1	16.7
DK/DH	23	13	56.5	7	53.8	-	-	6	46.2
TS	13	9	69.0	-	-	4	44.4	5	55.6
OT	5	3	60	2	66.7	-	-	1	33.3
Sink	12	5	41.7	-	-	5	100	-	-
Stretcher	11	4	36.3	3	75.0	1	25.0	-	-
Floor	31	5	16.1	-	-	5	100	-	-
BR	17	6	35.2	6	100	-		-	-
CB	17	7	41.1	4	57.1	3	42.9	-	-
Total	160	63	39.4	32	50.8	18	28.6	13	20.6

KEY: SHS = Nurses’ hand swab, NTT/ST = Nurses table top/staff table, DK/DH = Door knob/Door handle, TS= Toilet seat, OT= Operation table, BR = Bedrail, CB = Cup board.

As it is presented in table 4.4 the prevalence of the three pathogens isolated from surfaces at Major Ibrahim B. Abdullahi memorial hospital showed that *S. aureus* was isolated from hand swab (100%), nurses' table top (60.0%), door knob/handle (66.7%), stretcher (50.0%), floor (14.3%), bedrail (100%) and cupboard (66.7%). The total percentage distribution of this pathogen on the surfaces was 50.0%. *P. aeruginosa* was isolated from toilet seat (33.3%), sink (100%), stretcher (50.0%) and floor (85.7%) and the total percentage distribution of the pathogen on the surfaces. *E. coli* was isolated from some nurses' table top/staff table (40.0%), door knob (22.2%), and toilet seat (66.7%) and the total percentage distribution of *E. coli* on the surfaces of this hospitalwas15.2%.

Table 4.5 shows the distribution of the three pathogens on surfaces at St. Luke's Anglican hospital. *Staphylococcus aureus* was isolated from hand swab (100%), nurses' table tops (100%), door knob/handle (57.1%), stretcher (66.7%), bedrail (60.0%) and cupboard (100%) and total percentage distribution of the pathogen was 56.3%.

Pseudomonas aeruginosa was isolated in this hospital from operation table (100%), sink (100%), stretcher (33.3%), floor (83.3%) and bedrail (30.0%). The total percentage distribution of *P. aerugionsa* was 27.0%. *Escherichia coli* was isolated from some door knob (42.9%), toilet seat (100%), floor (16.7%), bedrail (10.0%) and total prevalence of this pathogen isolated from this hospital was 16.7%.

The result of serotyping of *E. coli* as shown in table 4.6 showed that all the eight (8) isolates of the *Escherichia coli* from St. Luke's Anglican hospital were negative for *E. coli* O157:H7 and all the isolate of *E. coli* from Major Ibrahim B. Abdullahi memorial hospital, none was positive for *E. coli* O157:H7. Only 1 (7.7%) of the 13 isolates of *E. coli* showed positive result for *E. coli* O157:H7.

Table 4.4: Prevalence (%) of the isolates in Major Ibrahim B. Abdullahi Memorial Hospital

Sample source	Sample size	Total positive isolates	Total % of isolates	*S. aureus*	(%)	*P. aeruginosa*	(%)	*E. coli*	(%)
NHS	11	4	36.4	4	100	-	-	-	-
NTT/ST	9	5	55.6	3	60.0	-	-	2	40.0
DK/DH	25	8	32.0	6	75.0	-	-	2	25.0
TS	8	3	37.5	-	-	1	33.3	2	66.7
OT	4	2	50.0	-	-	2	100	-	-
Sink	14	5	35.7	-	-	5	100	-	-
Stretcher	14	4	28.6	2	50.0	2	50.0	-	-
Floor	35	7	20.0	1	14.3	6	85.7	-	-
BR	19	5	26.3	5	100	-		-	
CB	16	3	18.8	2	66.7	-	-	1	33.3
Total	155	46	29.7	23	50.0	16	34.8	7	15.2

KEY: SHS = Nurses' hand swab, NTT/ST = Nurses table top/staff table, DK/DH = Door knob/Door handle, TS= Toilet seat, OT= Operation table, BR = Bedrail, CB = Cup board.

Table 4.7 shows the antibiotic susceptibility profile of the pathogens from Gambo Sawaba general hospital. The antibiotic profile of *S. aureus* showed that 100% were resistant to ampicillin, 25.0% were resistant to cefoxitin and 6.3% were resistant to both ceftazidime and tetracycline. The susceptibility pattern of the pathogen showed that 100% were susceptible to vancomycin and linezolid, 68.7, 56.2 and 53.1% were susceptible to tetracycline, ceftazidime and gentamicin respectively. Then, 25.0, 37.5 and 46.9% of the *S. aureus* were of intermediate resistance to tetracycline, ceftazidime and gentamicin respectively.

Some of the isolates of *P. aeruginosa* were resistant to tetracycline (27.8%), chloramphenicol (11.1%) and gentamicin (5.6%). The susceptibility of the isolates showed that 72.2, 88.9 and 94.4% were susceptible to tetracycline, chloramphenicol and gentamicin respectively. All the isolates (100%) of *P. aeruginosa* were susceptible to imipenem and ceftazidime. Also, some isolates of *E. coli* were resistant to ampicillin (7.7%) and tetracycline (46.2%). Some were susceptible to ampicillin (61.5%), tetracycline (23.0%), then 61.5% and 53.8% were susceptible to both ceftazidime and gentamicin and 100% were susceptible to cefoxitin.

The antibiotic susceptibility profile of isolates from Major Ibrahim B. Abdullahi memorial hospital as in table 4.8 showed that *S. aureus* isolates were highly resistant to ampicillin (95.7%), and 30.0% were resistant to both tetracycline and cefoxitin, very few were resistant to ceftazidime (8.7%). All the isolates of *S. aureus* were susceptible to vancomycin and linezolid, some were susceptible to tetracycline (52.2%), ceftazidime (43.5%), cefoxitin (87.0%) and gentamicin (60.9%).

Table 4.5: Prevalence (%) of the isolates in St. Luke Anglican Hospital

Sample source	Sample size	Total positive isolates	Total % of isolates	*S. aureus*	(%)	*P. aeruginosa*	(%)	*E. coli*	(%)
NHS	15	6	40.0	6	100	-	-	-	-
NTT/ST	16	5	31.2	5	100	-	-	-	-
DK/DH	20	7	35.0	4	57.1	-	-	3	42.9
TS	7	3	42.9	-	-	-	-	3	100
OT	6	1	16.7	-	-	1	100	-	-
Sink	10	3	30.0	-	-	3	100	-	-
Stretcher	10	3	30.0	2	66.7	1	33.3	-	-
Floor	30	6	20.0	-	-	5	83.3	1	16.7
BR	22	10	45.5	6	60.0	3	30.0	1	10.0
CB	19	4	21.0	4	100	-	-	-	-
Total	155	48	31.0	27	56.3	13	27.0	8	16.7

KEY: SHS = Nurses' hand swab, NTT/ST = Nurses table top/staff table, DK/DH = Door knob/Door handle, TS= Toilet seat, OT= Operation table, BR = Bedrail, CB = Cup board

Some the isolates *P. aeruginosa* were resistant to tetracycline (43.8%) and chloramphenicol (12.5%), some were also susceptible to gentamicin (75.0%), imipenem (56.2%), chloramphenicol (50.0%), ceftazidime (68.7%) and tetracycline (56.2%).

Of the isolates of *E. coli* 71.4 and 14.3% were resistant to tetracycline and ampicillin respectively. All the isolates of *E. coli* were 100% susceptible to gentamicin, cefoxitin ceftazidime and chloramphenicol, others were susceptible to ampicillin (57.1%) and tetracycline (28.6%).

The antibiotic susceptibility profile of pathogens from St. Luke's Anglican hospital as presented in table 4.9 showed that all isolates *S. aureus* susceptible to vancomycin and linezolid. This pathogen was also highly resistant to ampicillin (92.6%). Some were resistant to tetracycline (3.7%) and cefoxitin (18.5%). Of the total number of *S. aureus* isolates from this hospital, 7.4, 51.9, 81.5, 51.9 and 59.3% were susceptible to ampicillin, tetracycline, cefoxitin cetazidime and gentamicin respectively. Of the total number of isolates of *P. aeruginosa* from this hospital a high percentage of 61.5% were resistant to tetracycline and 30.8% were resistant to chloramphenicol. All the isolates of *P. aeruginosa* were 100% susceptible to ceftazidime and imipenem. Some of them were susceptible to tetracycline (38.5%), chloramphenicol (30.8%) and gentamicin (61.5%).

Table 4.10 shows the multiple antibiotic resistance patterns of the isolates from the hospital environments. One of the isolates of *P. aeruginosa* was resistant to three antibiotics; seven isolates were resistant to two antibiotics. Some isolates of *S. aureus* were resistant to three different antibiotics while some were resistant to only two antibiotics and two isolates of *E. coli* were resistant to two antibiotics.

Table 4.6: The serotype of *Escherichia coli* for *E. coli* O157:H7

Hospital	Number of isolates	Test latex	Control text	Interpretation	Total number of *E. coli* O157:H7 identified
SLAH	8	-	-	*E. coli* O157:H7 not present	0
MIBAMH	7	-	-	*E. coli* O157 not present	0
GSGH	13	+	-	*E. coli* O157 present	1 (7.7%)

KEY: GSGH = Gambo Sawaba General Hospital, MIBAMH = Major Ibrahim B. Abdullahi Memorial Hospital, SLAH = St Luke's Anglican Hospital.

Figure 4.1 shows the percentage distribution of the total multidrug resistant strains of the three pathogens from the three hospitals. The isolates from Gambo Sawaba hospital showed that 31.3% of *S. aureus*, 11.1% of *P. aeruginosa* and 7.7% of *E. coli* were multidrug resistant. The multidrug resistant of the isolates from Major Ibrahim B. Abdullahi memorial hospital showed that 21.7% of *S. aureus*, 12.5% of *P. aeruginosa* and 14.3% of *E. coli* were also multidrug resistant. There was no multidrug resistant isolates among *E. coli* from St. Luke's Anglican hospital but 18.5% of *S. aureus* and 30.8% of *P. aeruginosa* were multidrug resistant isolates.

The multiple antibiotic resistance (MAR) index of the isolates as presented in table 4.11 showed that 16 (80%) and 4 (20%) of the 20 multidrug resistant *S. aureus* from the three hospitals had multiple antibiotic resistant index of 0.285 and 0.43 respectively. One of the multidrug resistant isolates of *P. aeruginosa* from the three hospitals had MAR index of 0.40 and 7 isolates had MAR index at 0.40. The multidrug resistant

Table 4.7: Antibiotic profile of the three isolates from Gambo Sawaba General Hospital

	Pseudomonas aeruginosa (N = 18)			*Staphylococcus aureus* (N = 32)			*Escherichia coli* (N = 13)		
ANTIBIOTIC	R	I	S	R	I	S	R	I	S
VA (30µg)		NT		0 (0%)	-	32(100%)		NT	
AMP (10µg)		NT		32(100%)	-	0(0.0%)	1 (7.7%)	4(30.8%)	8(61.5%)
TE (30µg)	5(27.8%)	0(0%)	13(72.2%)	2(6.3%)	8(25.0%)	22(68.7%)	6(46.2%)	4(30.8%)	3(23.0%)
LZD (10µg)		NT		0(0.0%)	-	32(100%)		NT	
CAZ (30µg)	0(0.0%)	0(0.0%)	18(100%)	2(6.3%)	12(37.5%)	18(56.2%)	0(0.0%)	6(41.2%)	7(53.8%)
C (30µg)	2(11.1%)	0(0.0%)	16(88.9%)		NT		0(0.0%)	5(38.5%)	8(61.5%)
IMP (10µg)	0(0.0%)	0(0.0%)	18(100%)		NT			NT	
FOX (30µg)		NT		8(25.0%)	-	24(75.0%)	0(0.0%)	(0.0%)	13(100%)
CN (30µg)	1(5.6%)	0(0.0%)	17(94.4%)	0(0.0%)	15(46.9%)	17(53.1%)	0(0.0%)	6(41.2%)	7(53.8%)

Where:

VA = Vancomycin

AMP = Ampicillin

TE = Tetracycline

NT = Not Tested

R = Resistant

I = Intermediate

S = Sensitive

FOX = Cefoxitin

CN = Gentamicin

LZD = Linezolid

CAZ = Ceftazidim

C = Chlorampehnicol

MP = Imipenem

isolates of *E. coli* from Gambo Sawaba general hospital and Major Ibrahim B. Abdullahi Memorial hospital had multiple antibiotic resistance index of 0.33.

Table 4.12 shows the minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the antibiotics against the resistant strains of the isolates from Gambo Sawaba hospital. The MICs of ampicillin against *S. aureus* were at 7.8125, 15.625 and 31.25µg/ml but more at 15.625 and 31.25µg/ml. Cefoxitin inhibited more isolates of *S. aureus* 31.25µg/ml and MIC of tetracycline and ceftazidime against this pathogen was at 62.5 and 31.25µg/ml respectively. The MBCs of ampicillin that killed *S. aureus* were at 31.25 and 62.5µg/ml. Cefoxitin and ceftazidime were able to kill some of the *S. aureus* at 62.5 and tetracycline at 125µg/ml. *P. aeruginosa* was inhibited by gentamicin at 31.25µg/ml with MBC at 62.5µg/ml, and MICs of tetracycline and chloramphenicol at 15.625 and 31.25µg/ml with MBCs at 31.25 and 62.5µg/ml. The MICs of tetracycline against *E. coli* were at 15.625 and more of the isolates were inhibited at 31.25µg/ml with MBCs at 62.5µg/ml. The MIC of ampicillin against this pathogen was at 15.625µg/ml with MBC at 31.25µg/ml.

Table 4.8: Antibiotic profile of the three isolates from Major Ibrahim B. Abdullahi Memorial Hospital

	Pseudomonas aeruginosa (N = 16)			*Staphylococcus aureus* (N = 23)			*Escherichia coli* (N = 7)		
ANTIBIOTIC	R	I	S	R	I	S	R	I	S
VA (30µg)		NT		0(0.0%)	-	23(100%)		NT	
AMP (10µg)		NT		22(95.7%)	-	1(4.3%)	1(14.3%)	2(28.6%)	4(57.1%)
TE (30µg)	7(43.8%)	0(0.0%)	9(56.2%)	3(13.0%)	8(34.8%)	12(52.2%)	5(71.4%)	0(0.0%)	2(28.6%)
LZD (10µg)		NT		0(0.0%)	-	23(100%)		NT	
CAZ (30µg)	0(0.0%)	5(31.3%)	11(68.7%)	2(8.7%)	11(47.8%)	10(43.5%)	0(0.0%)	0(0.0%)	7(100%)
C (30µg)	2(12.5%)	6(37.5%)	8(50.0%)		NT		0(0.0%)	0(0.0%)	7(100%)
IMP (10µg)	0(0.0%)	7(43.8%)	9(56.2%)		NT			NT	
FOX (30µg)		NT		3(13.0%)	-	20(87.0%)	0(0.0%)	0(0.0%)	7(100%)
CN (30µg)	0(0.0%)	4(25.0%)	12(75.0%)	0(0.0%)	9(39.1%)	14(60.9%)	0(0.0%)	0(0.0%)	7(100%)

Where:

VA = Vancomycin

AMP = Ampicillin

TE = Tetracycline

NT = Not Tested

R = Resistant

I = Intermediate

S = Sensitiv

FOX = Cefoxitin

CN = Gentamicin

LZD = Linezolid

CAZ = Ceftazidime

C = Chloramphenicol

IMP = imipenem

Table 4.9: Antibiotic profile of the three isolates from St. Lukes' Anglican Hospital

	Pseudomonas aeruginosa (N = 13)			*Staphylococcus aureus* (N = 27)			*Escherichia coli* (N = 8)		
ANTIBIOTIC	R	I	S	R	I	S	R	I	S
VA (30µg)		NT		0(0.0%)	-	27(100%)		NT	
AMP (10µg)		NT		25(92.6%)	-	2(7.4%)	1(12.5%)	2(25.0%)	5(62.5%)
TE (30µg)	8(61.5%)	0(0.0%)	5(38.5%)	1(3.7%)	12(44.4%)	14(51.9%)	2(25.0%)	2(25.0%)	4(50.0%)
LZD (10µg)		NT		0(0.0%)	-	27(100%)		NT	
CAZ (30µg)	0(0.0%)	0(0.0%)	13(100%)	0(0.0%)	13(48.1%)	14(51.9%)	0(0.0%)	0(0.0%)	8(100%)
C (30µg)	4(30.8%)	5(38.4%)	4(30.8%)		NT		0(0.0%)	0(0.0%)	8(100%)
IMP (10µg)	0(0.0%)	0(0.0%)	13(100%)		NT			NT	
FOX (30µg)		NT		5(18.5%)	-	22(81.5%)	0(0.0%)	2(25.0%)	6(75.0%)
CN (30µg)	0(0.0%)	5(38.5%)	8(61.5%)	0(0.0%)	11(40.7%)	16(59.3%)	0(0.0%)	0(0.0%)	8(100%)

Where:

VA = Vancomycin

AMP = Ampicillin

TE = Tetracycline

NT = Not Tested

R = Resistant

I = Intermediate

S = Sensitive

FOX = Cefoxitin

CN = Gentamicin

LZD = Linezolid

CAZ = Ceftazidime

C = Chloramphenicol

IMP = imipenem

Table 4.10: Multiple antibiotic resistant patterns of the isolates from the three hospitals

P. aeruginosa	Combination of antibiotics	*S. aureus*	Combination of antibiotics	*E. coli*	Combination of antibiotics
1	CN, TE, C	12	AMP, FOX	2	AMP, TE
7	TE, C	2	AMP, CAZ		
		1	AMP,CAZ, FOX		
		2	AMP, TE, FOX		
		2	AMP, TE		
		1	AMP, CAZ, TE		

Table 4.13 shows the minimum inhibitory concentration (MICs) and minimum bactericidal concentration (MBCs) from Major Ibrahim B. Abdullahi memorial hospital. The MICs of ampicillin against *S. aureus* isolates was from 7.8125 – 31.25µg/ml with MBCs ranged from 31.25 – 62.5µg/ml. more were inhibited and killed at 15.625 and 31.25µg/ml respectively. The MICs cefoxitin against some *S. aureus* was 31.25µg/ml with MBC at 62.5µg/ml. The MIC of tetracycline was at 31.25µg/ml and started killing at 62.5µg/ml also; the MIC of ceftazidime against one of the *S. aureus* isolates was at 31.25µg/ml.

The MICs of chloramphenicol against *P. aeruginosa* was at 31.25µg/ml with MBCs at 62.5µg/ml respectively. Tetracycline started inhibiting more of the *P. aeruginosa* and *E. coli* at 31.25µg/ml; these pathogens were killed more at 62.5µg/ml by the antibiotic. The MIC of ampicillin against *E. coli* was at 15.625µg/ml with MBC at 31.25µg/ml.

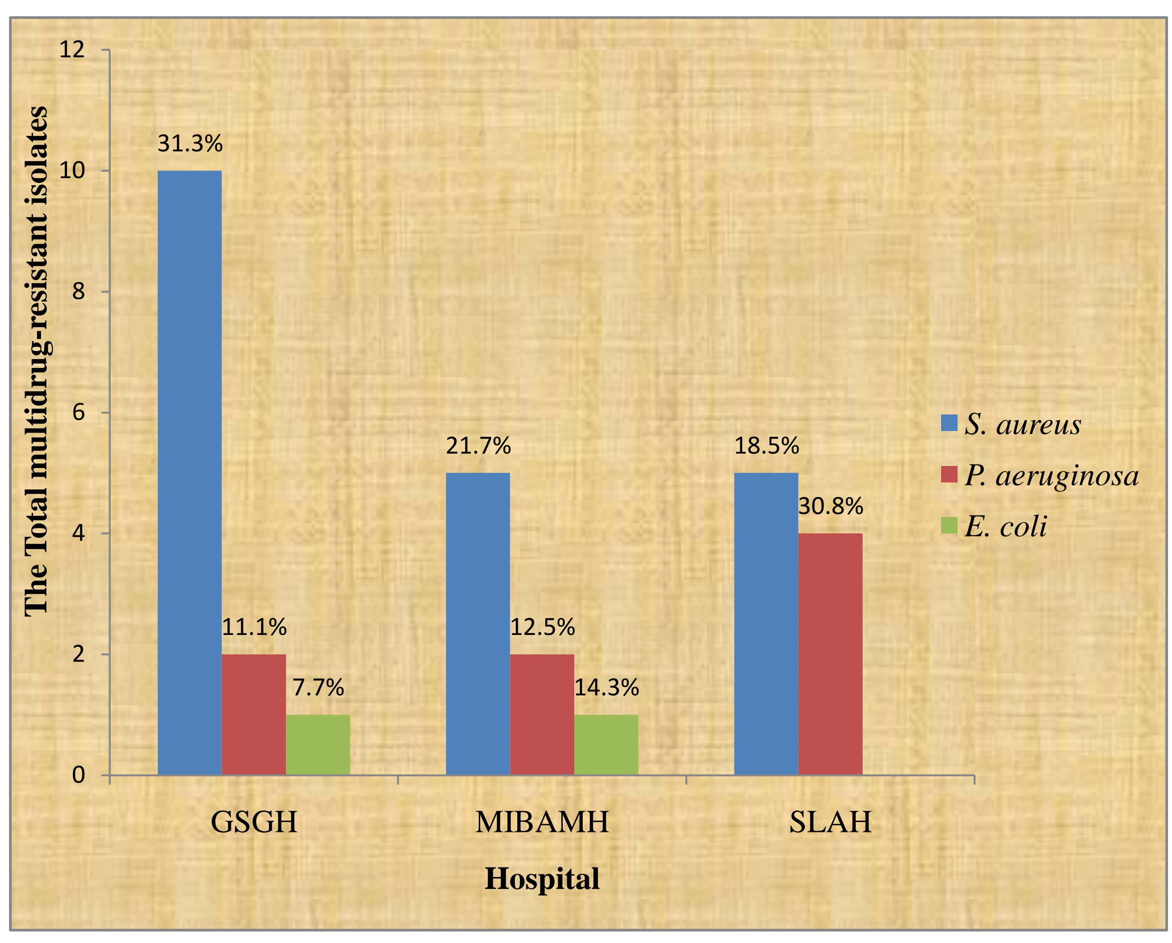

Fig.4.1: Prevalence of the total multidrug-resistant isolates from the three hospital environment.

KEY: GSGH = Gambo Sawaba General Hospital, MIBAMH = Major Ibrahim B. Abdullahi Memorial Hospital, SLAH = St Luke's Anglican Hospital

Table 4.11: Multiple antibiotic resistance (MAR) indices of the pathogens isolated from the three hospitals

Organism	No. of resistance isolates	MAR Index	Percentage (%)
S. aureus	16	0.29	80
	4	0.43	20
P. aeruginosa	1	0.60	12.5
	7	0.40	87.5
E. coli	2	0.33	100

Table 4.14 shows the MICs and MBCs of the tested antibiotics against the resistant strains of isolates from St. Luke's Anglican hospital. *Staphylococcus aureus* was inhibited by the minimum concentration of ampicillin from 15.625 – 62.5µg/ml with MBCs ranged from 31.25 - 125µg/ml it was more inhibited at 15.625 and 31.25µg/ml. The MICs of cefoxitin against *S. aurues* was from 31.25 – 62.5µg/ml with killing activity at 62.5 and 125µg/ml. *S. aureus*, *P. aeruginosa* and *E. coli* were inhibited by minimum concentration of tetraclycline at 31.25 µg/ml and MBC at 62.5µg/ml. The MICs of chloramphenicol against *P. aeruginosa* was at 31.25µg/ml with MBCs at 62.5µg/ml respectively. The MIC of ampicillin against *E. coli* was 7.8125 µg/ml and MBC at 31.25µg/ml.

Table 4.12: The MIC and MBC of the tested antibiotics against the resistant strains of the isolates from Gambo Sawaba Hospital

Organism	Antibiotic	MIC (µg/ml)	No. of Isolates	MBC (µg/ml)	No. of isolates
S. aureus	AMP	7.8125	3	31.25	3
		15.625	15	31.25	15
		31.25	14	62.5	14
	FOX	15.625	2	62.5	6
		31.25	6	62.5	2
	TE	62.5	2	125	2
	CAZ	31.25	1	62.5	1
P. aeruginosa	CN	31.25	1	62.5	1
	TE	15.625	2	62.5	2
		31.25	3	62.5	3
	C	15.625	1	62.5	1
		31.25	1	62.5	1
E. coli	TE	15.625	1	62.5	1
		31.25	5	62.5	5
	AMP	15.625	1	31.25	1

Table 4.13: The MIC and MBC of the tested antibiotics against the resistant strains of the isolates from Major Ibrahim B. Abdullahi Memorial Hospital

Organism	Antibiotic	MIC(µg/ml)	No. of Isolates	MBC(µg/ml)	No. of isolates
S. aureus	AMP	7.8125	3	31.25	3
		15.625	10	31.25	10
		31.25	7	62.5	7
	FOX	31.25	2	62.5	1
	TE	31.25	3	62.5	3
	CAZ	31.25	1	62.5	1
P. aeruginosa	C	31.25	2	62.5	1
	TE	15.625	2	62.5	2
		31.25	4	62.5	4
E. coli	TE	15.625	1	62.5	2
		31.25	3	62.5	2
	Amp	15.625	1	31.25	1

Table 4.14: The MIC and MBC of the tested antibiotics against the resistant strains of the isolates from St. Luke Anglican Hospital Wusasa

Organism	Antibiotic	MIC(µg/ml)	No. of Isolates	MBC(µg/ml)	No. of isolates
S. aureus	AMP	15.625	9	31.25	9
		31.25	12	62.5	12
		62.5	1	125	1
	FOX	31.25	3	62.5	3
		62.5	2	125	2
	TE	31.25	1	62.5	1
P. aeruginosa	TE	31.25	6	62.5	6
		62.5	2	125	2
	C	15.625	1	62.5	2
		31.25	3	62.5	2
E. coli	TE	31.25	1	62.5	1
		62.5	1	125	1
	AMP	7.8125	1	31.25	1

CHAPTER FIVE

5.0 DISCUSSION

The prevalence of *Staphylococcus aureus* (82., 52.2%), *Pseudomonas aeruginosa* (47., 29.9%) and *Escherichia coli* (28., 17.8%) from the three hospital surfaces in this work could be as a result of inadequate decontamination of the surfaces in these hospitals. The prevalence of these pathogens is higher than the earlier prevalence rate of *S. aureus* (78., 30.2%), *P. aeruginosa* (31., 12.0%) and *E. coli* (30, 11.6%) as reported by Muhamad *et al*. (2013) from some hospital surfaces in Sokoto. The prevalence of the pathogens in this work is still higher than the earlier work reported by Okonko *et al*. (2009) that 20.0, 13.3 and 6.7% of *S. aureus*, *P. aeruginosa* and *E. coli* respectively were isolated from hospital environments in Abeokuta, South-western Nigeria. Aliyu *et al*. (2013) still reported lower prevalence of *S. aureus* (26.1%), *P. aeruginosa* (15.8%) and *E. coli* (12.6%). The overall prevalence of the pathogens on the surfaces in the three hospitals was 34.40%. The prevalence of *Staphylococcus aureus* and *Pseudomonas aeruginosa* occurred more on the surfaces than *Escherichia coli*. Even though there is no significant difference in the distribution of the three pathogens on surfaces in the hospitals ($P > 0.05$) at 95% confidence limit using Kruskal-Wallis test.

High prevalence of *S. aureus* on door knob or door handle, nurses table tops/staff table tops, stretcher, bedrail and cupboard, *P. aeruginosa* on toilet seat, sink, floors, operation table and stretchers and *E. coli* from toilet seats, door knobs/door handles confirmed the report of Jarvis (2007), Chikere *et al*. (2008), Wren *et al*. (2008), Dwivedi *et al*. (2009) and Ijioma *et al*. (2010) that *S. aureus*, *P. aeruginosa* and *E. coli* are the major contaminants of hospital surfaces such as room, door handles, sinks, toilet seats, floors,

operation tables, sterile packaging, mops, ward fabrics and plastics, hands of healthcare workers, keyboards and taps, stethoscopes and telephones. The high level of contamination of these pathogens could also be as a result of inadequate decontamination of the microbial load from the surfaces (Addy *et al*., 2004) even though statistically, there was no significant association between organisms isolated from the surfaces and the hospital environment that is at $P > 0.05$ Chi-Square test

This finding corroborates earlier report of Hassan *et al*. (2004) and Page *et al*. (2009) that surfaces can act as reservoirs of microbes which could in turn lead to the spread of infection upon being touched, by either healthcare workers, patients or visitors. Carvalho *et al*. (2007) reported 46.1% cases of *S. aureus* from hospital surfaces and Weber, *et al*. (2010) also in their work have reported the role play by hospital surfaces in the transmission of emerging healthcare-associated pathogens. Crowded conditions within the hospital, frequent transfer of patients from one unit to another, and concentration of patients highly susceptible to infection in one area (e.g. newborn infants, burn patients, and intensive care) all may contribute to development of nosocomial infections due to contaminated surfaces. Microbial flora may contaminate surfaces of objects, devices, and materials which subsequently contact susceptible body sites of patients (Chikere *et al*., 2008). The role of hospital environment in the distribution of nosocomial pathogen cannot be overemphasized.

The 100% prevalence of *S. aureus* from hands of the nurses from the three hospitals is higher compare to the earlier report of 42.0% of the pathogen from hand swab as reported by Boyce (2007) and 20% as reported by Ekami *et al*. (2011). High prevalence of the *S. aureus* from hand swab in this work is in agreement with earlier report of

Olalekan *et al*. (2011) that the presence of the pathogen in the hand swab might be as a result of inadequate hand hygiene and this could be one of the attributing factors of the distribution of the pathogen in the hospital environmental surfaces. A study by Ferreira *et al.* (2011) revealed that contaminated hands of healthcare workers played important role in transmission of pathogens within the hospital environment and reported that 29% of nurses working in a general hospital had *S. aureus* on their hands and 78% of those working in a hospital for dermatology patients had the organism on their hands.

This finding also confirms the reports of WHO (2006) and Hayden *et al*. (2008) that the hands of healthcare workers play an important role in the propagation of microorganism within the healthcare environment and ultimately to the patients if not properly wash and disinfected. WHO (2006) reported that despite the hand washing and disinfection, many studies continue to show poor compliance with hand hygiene in most hospitals. Therefore, hand hygiene is a constant concern and the promoting of it is necessary as part of each hospital's infection-control and safety programs.

The prevalence of *S. aureus* on nurses' table tops of the three hospitals is greatly higher that is, 83.3, 60.0 and 100% of the isolates from Gambo Sawaba hospital, Major Ibrahim B. Abdullahi memorial hospital and St Lukes' hospital respectively was higher compare with 14.3% as reported by Ekrami *et al*. (2011). The findings corroborate the report of Carvahlo *et al*. (2007) that the surfaces of hospital environments serve as a secondary reservoir for multi-resistant microorganisms, such as MRSA. This pathogen has the ability of the organisms to survive on dry surfaces.

High prevalence rate of *S. aureus* on bedrail in Gambo Sawaba general hospital and Major Ibrahim B. Abdullahi memorial hospital is in agreement with 100% prevalence on bedrail as reported by Boyce (2007). Also, 38% of *S. aureus* reported on door handle by Carvalho *et al*. (2007) is lower to the prevalence of the pathogen on door knobs/ door handles of 53.8% from Gambo Sawaba hospital and 57.1% prevalence from St Lukes' hospital but slightly higher compare to 36.7% prevalence from Major Ibrahim B. Abdullahi memorial hospital.

The prevalence of 42.2, 22.2 and 42.9% of *S. aureus* on door knobs/door handles in Gambo Sawaba hospital, Major Ibrahim B. Abdullahi memorial hospital and St. Luke's Anglican hospital respectively and the isolation of *Escherichia coli* from the door knobs/door handles confirms the early report of Nworie *et al.* (2012) and for Abuja metropolis that the contamination of door knob/door handle can be as a result of poor hand hygiene after using toilet. Bhalla *et al*. (2004) and Boyce (2007) reported that environmental contamination in healthcare settings occur when healthcare workers touch the surfaces with their hands or gloves especially after their routine patients care or when the patients come in direct contact with the surfaces.

Therefore, common surfaces within the hospital environment can become contaminated with pathogenic microbes and hands (gloved or ungloved) can become contaminated with these organisms after touching such surfaces. These surfaces of fomites and other contaminated surfaces in turn serve as vehicles for cross-infections and recontamination of washed hands (Nworie *et al*., 2012). If hand hygiene practices are suboptimal, microbial colonisation is more easily established and/or direct transmission to patients or a fomites in direct contact with the patients or fomites (Allegranzi and Pittet, 2009).

It has been reported that organisms are capable of surviving on hands of health care workers for at least several minutes following contamination (Allegranzi and Pittet, 2009). *Pseudomonas aeruginosa* was not isolated from door knobs/door handles of the three hospitals.

The 100% prevalence of *P. aeruginosa* in sinks of the three hospitals is also higher compare with a work reported by Pal *et al*. (2010) that 4.47% of the pathogen was isolated from sinks in hospital environment in Iran. The isolation of this pathogen from the sinks confirms the report of Udeze *et al*. (2012) that sinks are the most common place in hospital environment were *P. aeruginosa* are predominantly found. Also, the prevalence rate of *P. aeruginosa* on operation table of Gambo Sawaba and St Lukes' hospitals is higher compared to a work reported by Pal *et al*. (2010) that 9.6% of the pathogen was isolated from operation table in a hospital in India.

The finding also confirms the report of Silva *et al*. (2003) that hospital surfaces have been implicated in the outbreaks of *P. aeruginosa*. The higher prevalence of 100, 85.7 and 83.3% of *P. aeruginosa* from floors of Gambo Sawaba hospital, Major Ibrahim B. Abdullahi memorial hospital and St Lukes' hospital respectively is not similar to findings of Ekrami *et al*. (2011) which in their report stated that only 4.47% of this pathogen was isolated from floor in a hospital environment.

One of the most frequently used surfaces in the hospital environment that was found contaminated by *P. aeruginosa* is the toilet seat, where 44.4, 33.3 and 100% was isolated from Gambo Sawaba hospital, Major Ibrahim B. Abdullahi memorial hospital and St Lukes' hospital respectively. This corroborates the report of Sabra (2013) that *P. aeruginosa* can be isolated from toilet seats and other moist environment in hospitals and may be seeded (forming biofilm) into toilets and remain on the toilet seats for a long time even after multiple flushing and cleaning with antimicrobial agents.

Another pathogen isolated from the toilet seat in this work is *E. coli* where 55.6, 66.7 and 100% of the pathogen was isolated from Gambo Sawaba hospital, Major Ibrahim B. Abdullahi memorial hospital and St Lukes' hospital respectively. This finding corroborates the earlier report of Nworie *et al*. (2012) that *E. coli* is one of the major nosocomial isolated from toilet seats in hospital environment. The prevalence of *E.* coli O157:H7 (7.7%) from one of the toilet seats of Gambo Sawaba general hospital is below the prevalence of 13.7% as reported by Wagner *et al*. (2004). Enterohaemorrhagic strains of *Escherichia coli*, especially *E. coli* O157:H7, have been emerged as important enteric pathogens in recent years. Various serotypes have been implicated in human disease, but *E. coli* O157:H7 is the most common. The pathogen produces a toxin almost identical to that of *Shigella dysenteriae* and this is responsible for the gastroenteritis, which ranges in severity from mild to bloody diarrhoea and haemorrhagic colitis (Wagner *et al*., 2004).

High resistance of *S. aureus* to ampicillin; that is 100% resistance from Gambo Sawaba hospital, 85.7% from Major Ibrahim B. Abdullahi memorial hospital and 92.6% from St. Lukes' hospital is in agreement with 97.0% of *S. aureus* resistance to ampicillin as reported by Terry Alli *et al*. (2011) from south western Nigeria. This research confirms the earlier report of Dudhagara *et al*. (2011) that a high percentage of *S. aureus* were resistant to ampicillin and other β – lactam drugs, and is also agreement with research work carried out by Akindele *et al*. (2010) that of the 100 total number of *S. aureus* isolated from hospital environment 90% of them were resistant to ampicillin. The resistance of *S. aureus* to this antibiotic (AMP) may be as result of the ability of β-lactamase enzyme to break the β-lactam ring of the antibiotic and render it ineffective because *S. aureus* produces β-lactamase in the presence of ampicillin (Oncel *et al*., 2004). Akindele *et al*. (2010) reported in their work that β-lactamase production by staphylococci is the recognized mechanism of resistance to β-lactam antibiotics such as ampicillin and penicillin.

The 100% susceptibility of *S. aureus* to vancomycin from the three hospitals in this finding agreed with the findings of Terry Alli *et al*. (2011) and the 100% susceptibility to linezolid is in agreement with 100% susceptibility of *S. aureus* to linezolid as reported by Kaleem *et al*. (2010) that 100% of the isolates of *S. aureus* were susceptible to linezolid and vancomycin and slightly higher than 93% susceptibility pattern as reported by Seza and Fatma (2012). The 0.0% resistance of *S. aureus* to gentamicin in this finding is not similar with report of Akindele *et al*. (2010) that 39% of this pathogen was resistant to gentamicin.

The antibacterial profile of *S. aureus* showed that 25.0, 13.0 and 18.5% of the isolates from Gambo Sawaba hospital, Major Ibrahim B. Abdullahi hospital and St. Luke's Anglican hospital respectively were resistant to cefoxitin. Resistance to cefoxitin by disc diffusion has been used for the detection of MRSA strains in routine testing because cefoxitin is a potential inducer of the system that regulates *mecA* gene (Philip and Shannon, 1993; Madhusudhan *et al*., 2011). Methicillin-resistant *Staphylococcus aureus* (MRSA) bacteria are more prevalent in the hospital environment and can be a challenge to infection control practices in most countries. Oie *et al*. (2002) and Boyce (2007) reported Methicillin-resistant *Staphylococcus aureus* (MRSA) that frequently contaminated objects included the floor, bed linens, the patient's gown, overbed tables, door knob/door handle and blood pressure cuffs.

The resistance of *Pseudomonas aeruginosa* to tetracycline from Major Ibrahim B. Abdullahi hospital (43.8%) and St. Luke's Anglican hospital (61.5%) was high. This result is not in agreement with earlier report of Pal *et al*. (2010) where they reported 36.2% resistance of *P. aeruginosa* to these antibiotics. Chloramphenicol and gentamicin were effective to *P. aeruginosa* with few strains resistant to them. *Pseudomonas aeruginosa* as reported by Harris *et al*. (2010) is resistant to most antibiotics because it is found naturally in soil; it has developed many resistances to naturally occurring antibiotics produced by bacilli, actinomycetes and moulds and their resistance to most antibiotics is attributed to efflux pumps which pump out some antibiotics before the antibiotics are able to act.

The 0.0% of the antibacterial resistance profile of *E. coli* to gentamicin, cefoxitin, ceftazidime and chloramphenicol in this finding is in agreement with research findings of Mukhtar and Saeed (2011) in Sudan, who also found that *E. coli* was 0.0% resistant to gentamicin, cefoxitin, ceftazidime and chloramphenicol. This pathogen was resistant to tetracycline (46.2%) and ampicillin (7.7%) from Gambo Sawaba hospital; from Major Ibrahim B. Abdullahi memorial hospital 71.4 and 14.3% of the isolates were respectively resistant to tetracycline and ampicillin. The resistance of *E. coli* to ampicillin could be as a result of production of β-lactamse enzyme which has the ability to deactivate the efficacy of this β-lactam drug as reported by Hassan *et al*. (2011). In this research, gentamicin, cefoxitin, ceftazidime and chloramphenicol were the most active antibiotics against *E. coli*.

The multidrug resistant *S. aureus* from Gambo Sawaba hospital (31.3%), Major Ibrahim B. Abdullahi memorial hospital (21.7%) and St. Luke's Anglican hospital (18.5%) was not higher than 87.75% multidrug resistant *S. aureus* as reported by Fagade *et al*. (2010). This finding has corroborated the report of Seza and Fatma (2012) that among the Gram-positive microorganisms, staphylococci are the most frequently resistant pathogen to antibiotics. The surfaces of the three hospital environment can serve as important secondary reservoir for multi-resistant microorganisms, such as the MRSA as reported by Carvhalo *et al*. (2007); this has to be emphasized because of the apparent ability of these pathogens to survive on dry surfaces. Therefore, the spread of multidrug resistant *S. aureus* in this research can be a great threat to everyone in the three hospital environments and the public.

The multidrug resistance of *P. aeruginosa* from Gambo Sawaba hospital (11.1%), Major Ibrahim B. Abdullahi memorial hospital (12.5%) and St. Luke's Anglican hospital (30.8%) confirms the report of Hota *et al*. (2009) that outbreaks of multidrug-resistant *P. aeruginosa* colonization or infection occurred in urology wards, a burn unit, haematology/oncology units, and adult and neonatal critical care units and that various medical devices and environmental reservoirs was implicated in the outbreak including antiseptic solutions and lotions; endoscopy equipment; ventilator apparatus, sink and hand swab. *Pseudomonas aeruginosa* has been increasingly recognized for its ability to cause significant hospital-associated outbreaks of infection, particularly since the emergence of multidrug resistant strains.

The multidrug resistant isolates of *E. coli* (7.7%) from Gambo Sawaba hospital is similar to report of Ibrahim *et al*. (2012) that 7.0% of *E. coli* isolated from hospital in Sudan were multidrug resistant. Isolates from Major Ibrahim B. Abdullahi memorial hospital had 14.3% multidrug resistant isolates and non from St. Luke Anglican hospital. The occurrence of MDR is very common and mainly in Gram negative bacteria. Multidrug resistant *E. coli* are widely distributed in hospitals and are increasingly being isolated from community. Thus, there is urgent need to find out new antimicrobial agents (Ibrahim *et al*., 2012).

The multiple antibiotic resistance (MAR) index gives an indirect suggestion of the probable source(s) of the organism. The MAR indices in this work were greater than 0.20, this indicates that the organism must have originated from an environment where antibiotics are often used as reported by Olayinka *et al*. (2004); that is MAR index gives an indirect suggestion of probable source(s) of the pathogen. Thus, the result of the

multiple antibiotic resistance indices in this work can be reported that these pathogens might have been originated from an environment where the antibiotics are used.

The isolates of *Staphylococcus aureus* from the three hospitals were inhibited by AMP at minimum inhibitory concentrations (MICs) from 7.8125– 62.6µg/ml, more of the isolates were inhibited at 15.625 and 31.25µg/ml by the antibiotic as reported by Dudhagara *et al*. (2011) whence they reported high resistance of the pathogen against AMP at MIC of 512-1024µg/ml. The minimum bactericidal concentrations (MBCs) of AMP were at 31.25 and 62.5µg/ml. The MICs of cefoxitin (FOX) against isolates of *S. aureus* from the three hospitals which was high at 31.25 and 62.5µg/ml shows that the pathogens were resistant to this antibiotic with MBC from 62.5µg/ml. The MIC of the antibiotic agreed with the earlier report of Salimna and Brownin (2005) which they reported the MIC of the antibiotic against *S. aureus* to be ≥20µg/ml.

The MIC of tetratcyline (TE) against *Pseudomonas aeruginosa* from the hospitals ranged from 15.625-62.5µg/ml more of the isolates were inhibited at higher concentrations of 31.35 and 62.5µg/ml with MBC more at 62.5µg/ml, therefore, the isolates were still very resistant to this antibiotic at higher concentration . The minimum concentration of chloramphenicol that inhibited *P. aeruginosa* was 15.625 and 31.25µg/ml with MBC at 62.5µg/ml. This result is slightly lower to the earlier report of Ogunleye (2012) in Ibadan that the MIC of C against *P. aeruginosa* was 32µg/ml.

Isolates of *Escherichia coli* from the hospitals were appreciably inhibited by TE at 31.25µg/ml with MBC at 62.5 and 125µg/ml. Eze *et al*. (2013) also reported MIC of TE on *E. coli* to be 32µg/ml. The MIC of ampicillin (AMP) against the isolates of *E. coli* from the hospitals was more at 15.626µg/ml with MBC at 31.25µg/ml. A higher MIC (25µg/ml) of AMP against *E. coli* has been reported earlier by Nworu and Esimone (2006). According to CLSI (2008) guideline the MIC break point of AMP against *E. coli* is recorded sensitive at ≤8µg/ml therefore, this pathogen was considered sensitive at the MIC of 7.8125µg/ml.

The widespread use of antimicrobials, especially over- or inappropriate use of antibiotics, has contributed to an increased incidence of antimicrobial-resistant organisms. Hospital-acquired infections are often caused by antimicrobial-resistant microorganisms. Resistance to antimicrobial agents is a problem in communities as well as health care facilities, but in hospitals, transmission of bacteria is amplified because of the highly susceptible population. Factors that could be associated with transmission of resistant strains of these microorganisms include poor attention to hygiene, overcrowding, lack of an effective infection control program, and shortage of trained infection control providers.

CHAPTER SIX

6.0 SUMMARY AND CONCLUSSION

The result of this study indicated that inanimate surfaces near infected patients and those frequently touched surfaces within the hospital environment are contaminated by *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* with prevalence of 33.40%. This suggests that contaminated environmental surfaces are reservoirs of these pathogens. The hands of healthcare workers can readily acquire pathogens after having contact with contaminated hospital surfaces or patients and can transfer these pathogens to other patients and inanimate surfaces; this can lower the quality of healthcare services being provided in these hospitals. Some of the isolates of *Pseudomonas aeruginosa*, *Escherichia coli* and *Staphylococcus aureus* were multidrug resistant.

However, based on this finding, it is clear that the risks of contracting a nosocomial infection in a clinical setting in developing countries is even higher than those reported in developed countries. Nosocomial infection has been a plague that torments the hospital community, prolonging the number of days patients are hospitalized and often complicates the patient's treatment. The control of microorganisms is therefore of prime importance in hospital and industrial environments.

6.1 RECOMMENDATION

In view of multiple studies indicating the environment to be an important source of bacterial transmission, more stringent routine environmental decontamination practices in healthcare facilities with regular monitoring is necessary in the MDRO containment bundle. Thorough cleaning and disinfection of the environment would remain one of the topmost effective preventive measures intended to provide reassurance that patients as well as staff are not put at unnecessary risks during their stay in the hospital setting. Cleaning remove pathogens from a surface and can be able to reduce residual organic material to a low level.

Most of these infections can be prevented with readily available, relatively inexpensive strategies by: adhering to recommended infection prevention practices, especially good hand hygiene and wearing gloves; paying attention to well-established processes for decontamination and cleaning of soiled surfaces, followed by use of disinfectants should remain the most effective means to reduce transmission of nosocomial pathogens. There is convincing evidence that improved hand hygiene can reduce infection and cross-transmission rates. Therefore, there are local as well as international guidelines for hand-hygiene practices. Healthcare workers (HCWs) should be encouraged to decontaminate (clean) their hands with an antiseptics before and after all patients' contacts.

Good health education should be organized for health workers. The hospital authorities should take the initiative by forming infection control team which continuously monitors the prevalence and incidence of such pathogens. The above measures if put in place is expected to improve the situation in the near future but for them to be effective

a proper knowledge of the prevalence/ abundance of the organisms are required such that the measures taken will be adequately structured to curb the menace of the nosocomial pathogens on surfaces.

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LIST OF PLATES

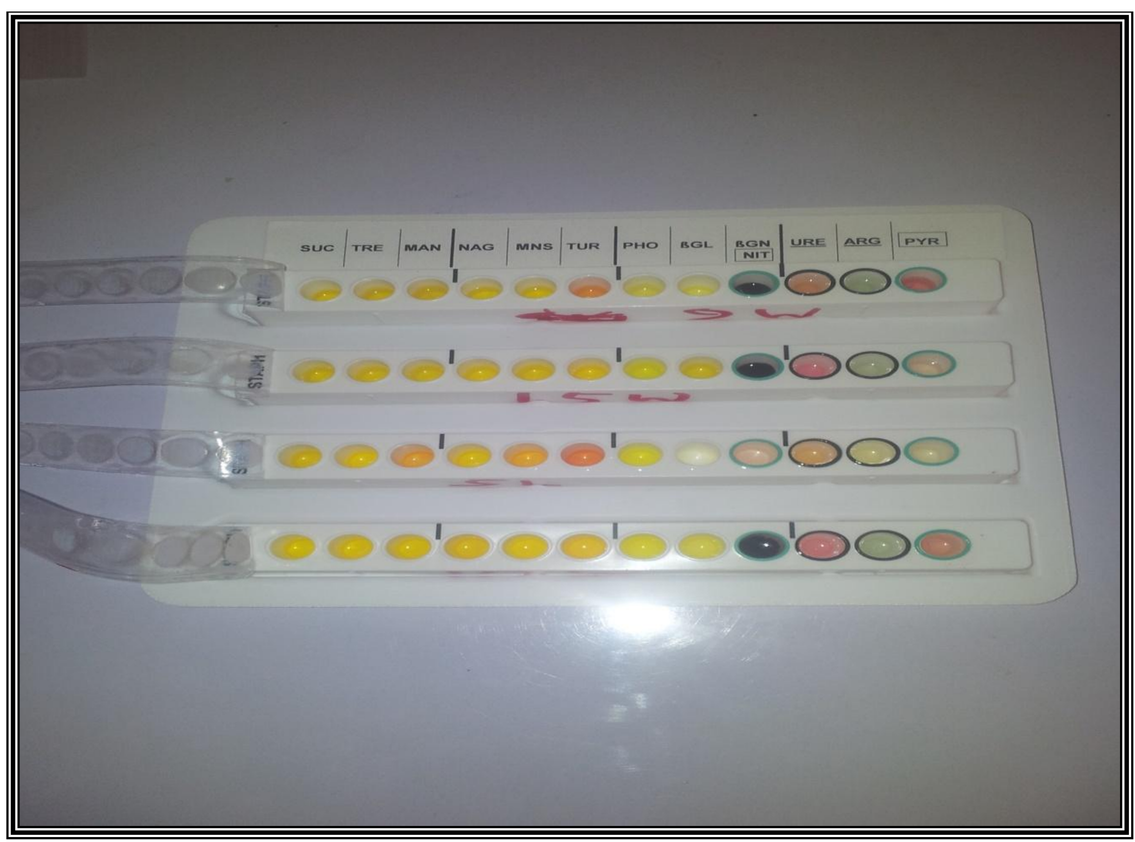

Plate 1: STAPH ID after 24 hours incubation

Plate 2: GNA-ID after 24 hours incubation

Plate 3: GNA+B- ID after 24 hours incubation

Plate 4: *Staphylococcus aureus* colonies on mannitol salt agar

Plate 5: *Pseudomonas aeruginosa* colonies on Pseudomonas CN agar

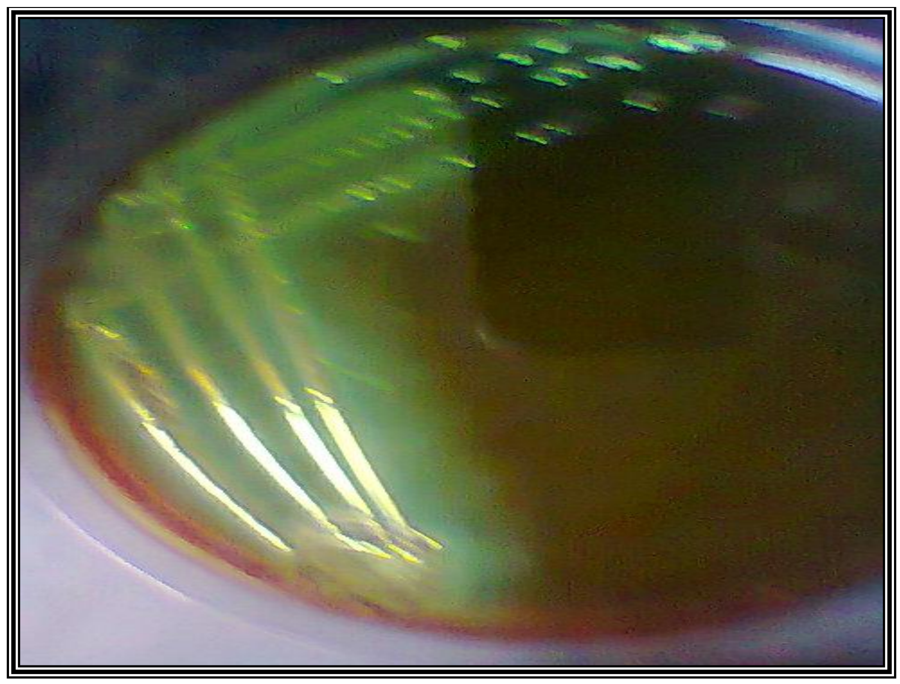

Plate 6: *Escherichia coli* colonies on Eosin Methylene Blue agar

Plate 7: The antibacterial susceptibility zonesof inhibition of *P. aeruginosa*

Plate 8: The antibacterial susceptibility zones of inhibition of *S. aureus*

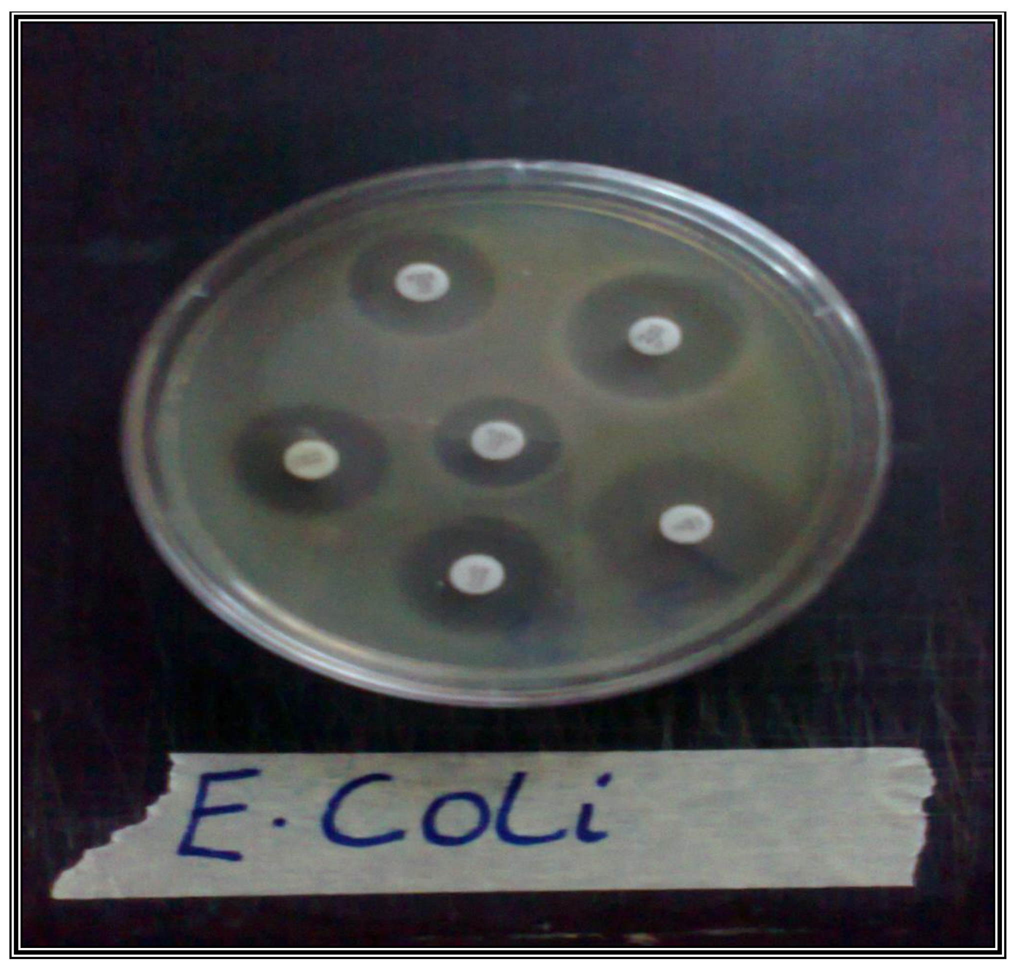

Plate 9: The antibacterial susceptibility zones of inhibition of *E. coli*

APPENDIX I: Ethical Approval

MINISTRY OF HEALTH, KADUNA STATE

All Communications to be Addressed to:
THE HON. COMMISSIONER
Quoting Reference and Date
Tel: (062) 24834
(062) 248252

Independence Way,
P.M.B. 2014,
Kaduna.
Kaduna State, Nigeria

MOH/ADM/744/VO.I/

27th March, 2012

The Medical Director,

....

....

....

ETHICAL APPROVAL

Approval has been granted Hammuel Chrinins, a post graduate Student of Department of Microbiology A.B.U. Zaria to carry out research on the topic "The prevalence and Antimicrobial susceptibility of some Nosocomial Pathogens" in some hospital environment in Zaria.

You are kindly requested to give her maximum cooperation.

A copy of the research is to be submitted to the State Ministry of Health please.

F. A. Kurah
Secretary, Ethical Committee

APPENDIX II

Identification and isolation of the pathogens using conventional biochemical method.

Colonial morphology	Gram reaction	Catalase	Citrate	Methyl Red (MR)	Coagulase	Indole	Voges-Proskauer (VP)	Oxidase	Probable isolate
Yellow, creamy colonies, with yellow zones on MSA, ferment manitol	Gram positive, pairs, clusters, cocci in shape	+	NT	NT	+	NT	NT	NT	*S. aureus*
Green metallic shine colonies on EMB agar and ferment lactose on MacConkey agar	Gram negative, short rods shape	NT	+	+	NT	+	-	NT	*E. coli*
Colonies produced green pigment on Pseudomonas CN agar	Gram negative rods in shape	NT	+	NT	NT	NT	NT	+	*P. aeruginosa*

KEY: NT = Not tested, + = Positive, - Negative

APPENDIX III

Identification profile of *S. aureus* using Microgen STAPH-ID kit

Substrate	LAT	CPG	Nitrate	Sucrose	Trehalose	Mannitol	N-acetyl Glucoseamine	Manose	Turanose	Alkaline Phosphatase	Glucosidase	Glucuronidase	Urease	Argenine	PYR
Results	+	+	+	+	+	+	+	+	+	+	+	+	+	+	-
Reaction Index	4	2	1	4	2	1	4	2	1	4	2	1	4	2	0
Sum of positive reaction		7			7			7			7			6	
Profile No.								77776							

PYR = L-Pyrrolidonyl-α-naphthylamide CPG = Colony Pigmentation, LAT = Latest Agglutination Test(Coagulase Test)

APPENDIX IV

Identification profile of *E. coli* using Microgen GNA kit

Substrate	Lysine	Ornithine	H_2S	Glucose	Mannitol	Xylose	ONPG	Indole	Urease	VP	Citrate	TDA
Results	+	+	-	+	+	+	+	+	-	-	-	-
Reaction Index	4	2	1	4	2	1	4	2	1	4	2	1
Sum of positive reaction		6			7			6			0	
Profile number						6760						

KEY: VP = Voges-Proskauer, ONPG = Ortho-Nitrophenol-β-galactoside, TDA = Tryptophan deaminase, H_2S = Hydrogen sulphide

APENDIX V

Identification profile of *Pseudomonas aeruginosa* using Microgen GNA +B kit

Substrate	Oxidase	Motility	Nitrate	Lysine	Ornithine	H_2S	Glucose	Mannitol	Xylose	ONPG	Indole	Urease	VP	Citrate	TDA	Gelatine	Malonate	Inositol	Sorbitol	Rhamnose	Sucrose	Lactose	Arabinose	Adonitol	Raffinose	Salicin	Arginine
Result	+	+	+	+	-	-	+	+	+	-	-	+	-	+	-	+	+	-	-	-	-	-	-	-	-	-	+
Reaction index	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1	4	2	1
Sum of positive reaction		7			4			7			1			2			6			0			0			1	
Profile number														747126001													

KEY: VP = Voges- Proskauer, ONPG = Ortho-Nitrophenol-β-galactoside, TDA = Tryptophan deaminase, H_2S = Hydrogen sulphide

APPENDIX VI

The antibacterial susceptibility profile of standard organisms

Organism	VA	AMP	TE	IPM	C	FOX	CAZ	CN	LZD
S. aureus(ATCC25923)	18(S)	29(S)	19(S)	NT	NT	23(S)	20(S)	25(S)	26(S)
E. coli(ATCC 25922)	NT	17(S)	25(S)	NT	24(S)	25(S)	26(S)	28(S)	NT
P. aeruginosa(ATCC9027)	NT	NT	20(S)	28(S)	24(S)	NT	31(S)	26(S)	NT

KEY: NT = Not tested, ATCC = American Type Culture Collection.

APPENDIX VII

The statistical analyses

Crosstabs
Case processing summary

	Case					
	Valid		Missing		Total	
	N	Percent	N	Percent	N	Percent
Hospital *HS	9	100.0%	0	0%	9	100.0%
Hospital *DH(DK)	9	100.0%	0	0%	9	100.0%
Hospital * NTT	9	100.0%	0	0%	9	100.0%
Hospital *OT	9	100.0%	0	0%	9	100.0%
Hospital * Sink	9	100.0%	0	0%	9	100.0%
Hospital *Stretcher	9	100.0%	0	0%	9	100.0%
Hospital *TS	9	100.0%	0	0%	9	100.0%
Hospital *Floor	9	100.0%	0	0%	9	100.0%
Hospital *BR	9	100.0%	0	0%	9	100.0%
Hospital * CB	9	100.0%	0	0%	9	100.0%

Hospital* HS

Crosstab

		HS		
		Negative	*S. aureus*	Total
Hospital A	count % within hospital	2 66.7%	1 33.3%	3 100.0%
B	count % within hospital	2 66.7%	1 33.3%	3 100.0%
C	count % within hospital	1 33.3%	1 33.3%	3 100.0%
Total	count % within hospital	5 55.6%	3 33.3%	9 100.0%

Chi- Square tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.400[a]	2	.663
Likelihood ratio	2.634	2	.621
N of valid cases	9		

a. 9 cells (100%) have expected count less than 5
The minimum expected count is .67.

Hospital* DH (DK)

Crosstab

	DH(DK)			
	Negative	*S. aureus*	*E. coli*	Total
Hospital A count % within hospital	1 33.3%	1 33.3%	1 33.3%	3 100.0%
B count % within hospital	1 33.3%	1 33.3%	0 0%	3 100%
C count % within hospital	0 0%	1 33.3%	1 33.3%	3 100.0%
Total count % within hospital	2 22.2%	3 33.3%	2 22.2%	9 100.0%

Chi- Square tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.000[a]	4	.809
Likelihood ratio	4.866	4	.561
N of valid cases	9		

a. 12 cells (100%) have expected count less than 5.
The minimum expected count is .67.

Hospital* NTT

Crosstab

		NTT			
		Negative	*S. aureus*	*E. coli*	Total
Hospital A	count % within hospital	0 0%	1 33.3%	1 33.3%	3 100.0%
B	count % within hospital	0 0%	1 33.3%	1 33.3%	3 100.0%
C	count % within hospital	2 66.7%	1 33.3%	0 0%	3 100.0%
Total	count % within hospital	2 22.2%	3 33.3%	2 22.2%	9 100.0%

Chi- Square tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.000[a]	4	.423
Likelihood ratio	7.638	4	.266
N of valid cases	9		

a. 12 cells (100%) have expected count less than 5.
The minimum expected count is .67.

Hospital * OT

Crosstab

		OT				
		Negative	*S. aureus*	*P. aerugionsa*	*E. coli*	Total
Hospital A	count % within hospital	1 33.3%	1 33.3%	0 0%	1 33.3%	3 100.0%
B	count % within hospital	1 33.3%	0 0%	1 33.3%	1 33.3%	3 100.0%
C	count % within hospital	2 66.7%	0 0%	1 33.3%	0 0%	3 100.0%
Total	count % within hospital	4 44.4%	1 11.1%	2 22.2%	2 22.2%	9 100.0%

Chi- Square tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.500[a]	6	.409
Likelihood ratio	5.912	6	.433
N of valid cases	9		

a. 6cells (100%) have expected count less than 5.
The minimum expected count is .67.

Hospital * Sink

Crosstab

		Sink		
		Negative	*Pseudomonas aeruginosa*	Total
Hospital A	count % within hospital	2 66.7%	1 33.3%	3 100.0%
B	count % within hospital	2 66.7%	1 33.3%	3 100.0%
C	count % within hospital	3 100.0%	0 0%	3 100.0%
Total	count % within hospital	7 77.8%	2 22.2%	9 100.0%

Chi- Square tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	1.286[a]	2	.526
Likelihood ratio	1.897	2	.387
N of valid cases	9		

a. 12 cells (100%) have expected count less than 5.
The minimum expected count is .67.

Hospital* Stretcher

Crosstab

		Stretcher			
	Negative	*S. aureus*	*P. aerugionsa*	*E. coli*	Total
Hospital A count %	1	1	1	0	3
within hospital	33.3%	33.3%	33.3%	0%	100.0%
B count %	1	1	1	1	3
within hospital	33.3%	33.3%	33.3%	33.3%	100.0%
C count %	2	1	1	0	3
within hospital	66.7%	33.3%	33.3%	0%	100.0%
Total count %	4	3	3	1	9
within hospital	44.4%	33.3%	33.3%	11.1%	100.0%

Chi- Square tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	4.500[a]	6	.409
Likelihood ratio	5.912	6	.433
N of valid cases	9		

a. 12 cells (100%) have expected count less than 5.
The minimum expected count is .67

Hospital TS

Crosstab

		TS			
		Negative	*E. coli*	*P. aeruginosa*	Total
Hospital A	count % within hospital	1 33.3%	1 33.3%	1 33.3%	3 100.0%
B	count % within hospital	1 33.3%	1 33.3%	1 33.3%	3 100%
C	count % within hospital	3 100.0%	0 0%	0 0%	3 100.0%
Total	count % within hospital	5 55.6%	2 22.2%	2 22.2%	9 100.0%

Chi- Square tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	3.600[a]	4	.463
Likelihood ratio	4.727	4	.316
N of valid cases	9		

a. 9 cells (100%) have expected count less than 5.
The minimum expected count is .67

Hospital* Floor

Crosstab

		Floor			
		Negative	*P. aeruginosa*	*S. aureus*	Total
Hospital A	count % within hospital	2 66.7%	1 33.3%	0 0%	3 100.0%
B	count % within hospital	1 33.3%	1 33.3%	1 33.3%	3 100.0%
C	count % within hospital	2 66.7%	1 33.3%	1 33.3%	3 100.0%
Total	count % within hospital	5 55.6%	3 33.3%	2 22.2%	9 100.0%

Chi- Square tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.400[a]	4	.663
Likelihood ratio	2.634	4	.621
N of valid cases	9		

a. 9 cells (100%) have expected count less than 5.
The minimum expected count is .33

Hospital * BR

Crosstab

		BR				
		Negative	*S. aureus*	*P. aerugionsa*	*E. coli*	Total
Hospital A	count	2	1	0	0	3
	% within hospital	66.7%	33.3%	0%	0%	100.0%
B	count	2	1	0	0	3
	% within hospital	66.7%	33.3%	0%	0%	100.0%
C	count	0	1	1	1	3
	% within hospital	0%	33.3%	33.3%	33.3%	100.0%
Total	count	4	3	1	1	9
	% within hospital	44.4%	33.3%	11.1%	11.1%	100.0%

Chi- Square tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	6.000[a]	6	.423
Likelihood ratio	7.638	6	.266
N of valid cases	9		

a. 12 cells (100%) have expected count less than 5.
The minimum expected count is .33

Hospital * CB

Crosstab

	CB			
	Negative	*S. aureus*	*P. aerugionsa*	Total
Hospital A count %	3	1	0	3
within hospital	100.0%	33.3%	0%	100.0%
B count %	3	1	1	3
within hospital	100.0%	33.3%	33.3%	100.0%
C count %	2	1	0	3
within hospital	66.7%	33.3%	0%	100.0%
Total count %	8	1	1	9
within hospital	88.9%	11.1%	11.1%	100.0%

Chi- Square tests

	Value	df	Asymp. Sig. (2-sided)
Pearson Chi-Square	2.250[a]	4	.325
Likelihood ratio	2.460	4	.292
N of valid cases	9		

a. 12 cells (100%) have expected count less than 5.
The minimum expected count is .67.

KEY

Hospital A = Gambo Sawaba general hospital

Hospital B = Major Ibrahim B. Abdullahi Memorial Hospital

Hospital C = St Luke’s Anglican hospital.

Descriptive statistics using Kruskal-Wallis test

	N	Mean	Std. Deviation	Minimum	Maximum
Orgtest **A**	30	2.1333	2.40306	.00	7.00
Orgfactor **A**	30	18.5000	2.92138	14.00	23.00

Ranks

Or...		N	Mean Rank
Orgtest **A**	14	3	18.67
	15	3	21.83
	16	3	17.83
	17	3	13.50
	18	3	13.00
	19	3	14.00
	20	3	13.00
	21	3	13.00
	22	3	14.17
	23	3	16.00
	Total	30	

Kruskal-Wallis Test

	Orgtest
Chi-Square	3.508
Df	9
Assmp. Sig.	.941

Descriptive statistics using Kruskal-Wallis test

	N	Mean	Std. Deviation	Minimum	Maximum
Orgtest **B**	30	1.6000	1.86806	.00	6.00
Orgfactor **B**	30	5.5000	2.92138	1.00	10.00

Ranks

Or...		N	Mean Rank
Orgtes **B**	1	3	16.33
	2	3	21.00
	3	3	12.00
	4	3	9.67
	5	3	12.0
	6	3	14.33
	7	3	14.17
	8	3	19.50
	9	3	22.83
	10	3	13.17
	Total	30	

Kruskal-Wallis Test

	Orgtest
Chi-Square	7.059
Df	9
Assmp. Sig.	.631

Descriptive statistics using Kruskal-Wallis test

	N	Mean	Std. Deviation	Minimum	Maximum
Orgtest **C**	30	1.5667	1.81342	.00	6.00
Orgfactor **C**	30	31.5000	2.92138	17.00	36.00

Ranks

Or...	N	Mean Rank
Orgtes **C** 27	3	18.33
28	3	22.00
29	3	14.17
30	3	11.17
31	3	15.83
32	3	16.33
33	3	12.83
34	3	16.83
35	3	13.33
36	3	14.17
Total	30	

Kruskal-Wallis Test

	Orgtest
Chi-Square	3.620
Df	9
Assmp. Sig.	.935

KEY

A = Gambo Sawaba general hospital

B = Major Ibrahim B. Abdullahi Memorial Hospital

C = St Luke's Anglican hospital.