ASSESSMENT OF STAPHYLOCOCCAL NOSOCOMIAL INFECTIONS IN NATIONAL ORTHOPAEDIC HOSPITAL, ENUGU

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PG/M.Sc./07/47127

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A PROJECT PRESENTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCES, FACULTY OF HEALTH SCIENCES AND TECHNOLOGY, COLLEGE OF MEDICINE UNIVERSITY OF NIGERIA, ENUGU CAMPUS FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE AWARD OF MASTER OF SCIENCE (M.Sc.) IN MEDICAL LABORATORY SCIENCES (PUBLIC HEALTH MICROBIOLOGY)

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DEDICATION

To Almighty God and to the memory of my late father, Nnaoma Anthony Ahanotu.

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I am indeed grateful to my supervisor Prof. N.F Onyemelukwe for her motherly and scholarly advice which made this work a success. My thanks also go to my mum Ezinne Martina Ahanotu, my brothers and sisters, my friends and my colleagues for their love and support. My special thanks go to staff and patients of National Orthopaedic Hospital, Enugu who made this dream come true by their compliance. I also wish to appreciate Dr. Oge for her assistance and encouragement during the course of this work.

May God bless and reward you all.

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ABSTRACT

In a comprehensive work to assess staphylococcal nosocomial infections in patients in National Orthopaedic Hospital, Enugu (NOHE), Nigeria and to assess the possible contribution of healthcare workers and fomites, a study was carried out between May 2013 and June 2014. This involved the analysis of a total of 578 samples, 200(35%) comprising of 100 in-patients and 100 healthcare workers) and 378 samples collected from different fomites within the theatre/ wards. Human samples (wound/nasal swabs, pus, urine and blood) and fomites(hospital equipment/ articles, floors and wall surfaces, sinks, tables, trolleys etc), 277 from wards and 89 from theatres were analyzed using standard bacteriological methods. Antibiograms were carried out on all staphylococcal isolates. Of the 100 patients studied, 85(85%) 43 males and 42 females were positive for staphyloccal nosocomial infections of which there was no statistically significant difference for sex/age ($p>0.05$). Wound infections 60 (70.4%),ranked highest, followed by urinary tract infections 20(23.5%), with bacteremia 3 (3.5%) and soft tissue infection 2(2.4%) being the least, which differences were statistically significant ($P<0.05$). Road traffic accident cases, 29 (34%) followed by flame burns cases ranked highest amongst the category of patients with staphylococcal nosocomial infections while matchet cut cases with only 1 (1.1 %) positive, was the least which differences was also stastistically significant($P< 0.05$). Of the healthcare workers, 93 ; (93%) 42(45%)males and 51(55%) females yielded a positive nasal staphylococcal carriage showjng no statistically significant differences ($P>0.05$) in the sex/age-wise comparison. Nurses were statistically significantly higher than other professional groups in staphylococcal carriage ($P<0.05$). Similarly, length of stay\ length of service were also statistically significant when the positive cases for staphylococcal infections/carriage were compared among the patients and also for healthcare workers respectively($P<0.05$). Number positive for staphylococcal nosocomial infections were also found to be statistically significant ($P<0.05$) according to total body surface area affected amongst patients with burns injury.Of the 378 fomites analyzed, 200 (53%) yielded positive *Staphylococcus aureus* isolates with floors ranking highest both for *Staphylococcus aureus 40*(11.6%) and 22(11%) methicillin resistant *Staphylococcus aureus* (MRSA).This was followed by walls 30 (8%) and bed railings19 (5%). Generally, the distribution of *Staphylococcus aureus* were not statistically significant($P >0.05$).Antibiogram results show evidence of multiple resistant *Staphylococcus aureus* and methicillin resistant *Staphyloccous aureus* in samples analysed such that 33% ,33% , and 45% *MRSA* were detected amongst patients, healthcare workers and fomites respectively. A total of 31.6% samples in the study yielded *Staphylococcus epidermidis* isolates. The need for more stringent hygienic practices and regular interventionary fumigation of NOHE and similar further studies at a higher scale in order to appreciate the full epidemiological pattern for a control regimen is hereby stressed.

CHAPTER ONE

INTRODUCTION

Staphylococcus organism belongs to the family *Staphylococeae* and genus *Staphylococcus*. So many species have envolved from this genus for example; *S.aureus, S. epidermidis* and *S. saprophyticus*. They are Gram positive cocci that are microscopically observed as organisms in pairs and irregular, grape-like clusters. In vitro, they behave as non-motile, non-sporing, catalase positive and facultative anaerobes. The organisms are resistant to temperatures as high as 50^0C, high salt concentration and to drying. These properties facilitate their survival in the environment, growth in foods and communicability (Tolan *et al*., 2008). The *Staphylococcus* specie*s* especially *S.aureus* and *S. epidermidis* are among the most important bacteria associated with nosocomial infections such as blood-stream infections, skin and soft tissue infections, endocarditis, surgical wound infections and urinary tract infections (Pitout *et al*., 2007 ; Zhanel *et al*., 2008).

Infections were regarded as nosocomial if they occurred 48 hours or longer after admission with no evidence that infection was present or incubating at the time of admission (Nejad *et al*., 2011). These include infections acquired in the hospital which may appear even after discharge. The hospital environment is a potential reservior of infectious agents as it houses both patients with diverse pathogenic microrganisms and a large number of susceptible/immunocomprised individuals (Rhomberg *et al*., 2006). In some cases, patients develop nosocomial infections due to poor hygienic conditions at a hospital or due to healthcare workers not following proper procedures (Chikere *et al*., 2008). Many of the nosocomial pathogens are bacteria which are members of the normal flora of man and are often non-invasive. These organisms become

pathogenic in immunocompromised hosts and hospitalized patients forming a pool of immunocompromised hosts for these pathogens. The nosocomial pathogens that cause nosocomial infections, e.g *Staphylococcus* species can come from either endogenous or exogenous sources. Example of endogenous source is when a patient becomes infected by his or her normal body flora following surgical manipulation, chemotherapy, and diagnostic/therapeutic procedures which in most cases suppress the natural body defense mechanism (Chikere *et al.,* 2008). Exogenous sources include those that are not part of the patient and could be animate e.g. healthcare workers, visitors and other patients while inanimate sources include the hospital environment, equipment, water etc. (Bello *et al*., 2005; Okezie and Onyemelukwe, 2007). The most important means of transmission of staphylococcal nosocomial infections is by close contact, airborne particles and via environmental routes (Cook, 1998).

National Orthopaedic Hospital Enugu (NOHE) where the study was conducted is an accident and emergency centre and also a regional burns centre for the South east, South south and parts of North central zones of Nigeria. There is high probability of staphylococcal nosocomial infections among patients in NOHE due to the fact that majority spend long time in admission e.g burns patients and patients with fracture due to road traffic accident.

Numerous epidemiological studies in the United States and Europe give evidence that *staphylococci* are now the preminent cause of infections acquired in hospitals (Muddofer and Schiffer, 2001). The organism has adapted itself to hospital environment and is established as a nosocomial pathogen. *S. aureus* prevalence in a given clinical facility can be used as a bench mark for assessing the public health status of the facility.

Nosocomial infection is a major global safety concern for both patients and healthcare professionals (Bates *et al*., 2009). These infections take a heavy toll on patients and their families by causing illness, prolonged hospital stay, potential disability, excess cost and sometimes death (Okezie and Onyemelukwe, 2007 ; Allengranzi and Pittet, 2008). The burden of nosocomial infection is already substantial in developed countries where it affects 5-15% of hospitalized patients in regular wards and as much as 50% or more patients in intensive care units (Vincent, 2003). Surveillance system exists in some developed countries and provide regular reports on national trends of endemic hospital associated infections such as National Healthcare Safety Network of the United States of America (Pittet *et al.,* 2005). In developing countries such as Nigeria, the magnitude of the problem due to nosocomial infection remains under estimated or even unknown largely because nosocomial infection diagnosis is complex and surveillance activities to guide interventions require expertise and resources (Allengranzi and Pittet, 2008). Additionally, overcrowding and understaffing in hospitals results in inadequate infection control practices and a lack of infection control policies, guidelines and trained professionals also add to the extent of the problems. In the developing countries like Nigeria, there is still a lack of knowledge of the seriousness and consequences of the risks of nosocomial infections and a lack of information that can enable control measures to be applied appropriately inspite of minimal resources (Iwuchukwu, 2005). The CDC (2011) estimates that nosocomial infections caused 49,000 deaths in the United States alone. There is no doubt that the figures will be worst for a third world country like Nigeria. Every year a great deal of financial, material and human resources are expended in an effort to treat various ailments that otherwise would have been prevented. Public health concerns focus on the safety and well being of the people. Thus, it is

expected that the hospital should not pose any risk to the patients in particular and the public in general.

Hence, this surveillance work was embarked upon to assess staphylococcal nosocomial infections in National Orthopaedic Hospital, Enugu.

1.1 **AIM AND OBJECTIVES**

1.1.1 Aim

1. To determine the prevalence of staphylococcal nosocomial infections in National Orthopaedic Hospital, Enugu

1.1.2 Objectives

1.To assess the possible contribution of fomites to staphylococcal nosocomial infections

2.To ascertain the possible contribution of healthcare workers to staphylococcal nosocomial infections in the hospital.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 The Staphylococci

Staphylococcus is a genus of bacteria containing at least 28 species that are collectively called Staphylococci (Prescot *et al*., 2004). In the Bergeyôs Manual of Systemic Bacteriology, staphylococci are placed into:-

- Kingdom *Bacteriae*
- Phylum (Division) XIII *Firmicules*
- Class I *Bacilli*
- Order I *Bacillales*
- Family VIII *Staphylococaceae*
- *Genus* *Staphylococcus*

Staphylococcus was discovered in 1880 and named in 1882 by a Scottish surgeon Alexander Ogston (1844 ï 1929). *Staphylococcus* was coined from the greek term ñstaphyle which means bunch of grapes and the latin term ñ*coccus*ò means spherical bacterium. The best known species are present in large numbers on the mucous membranes and skin of all human and other warm ï blooded animals (Prescot *et al*., 2004). According to Prescot *et al.* (2004), it is divided into two groups based on the presence or absence of the enzyme coagulase. This enzyme converts fibrinogen to fibrin, causing blood plasma to clot. The species called *Staphylococcus aureus* is coagulase positive while S*taphylococcus epidermidis* and other species are coagulase negative.

Typically, Staphylococci are opportunistic pathogens or saprophytes. They are `Gram positive cocci usually arranged in clusters like a bunch of grapes; this appearance is due to the fact that *Staphylococci* divide along two separate planes. The morphologically similar Streptococci can be differentiated from *Staphylococc*i by testing for the enzyme catalase. *Staphylococcus aureus* possess this enzyme while *Streptococc*i do not. *Staphylococci* possess both specific and type specific antigens. About 90% of *Staphylococcus aureus* isolates have protein A and this substance is capable of binding to the FC portion of immunoglobin IgG. This property helps the bacterium escape the potential lethal effect of immunoglobin action and also serves as the basis for some serological tests. (Prescot *et al*., 2004). Coagulase negative strains of Staphylococci are generally non invasive but under certain conditions may cause severe disease such as endocarditis, bacteremia and urinary tract infection. *Staphylococci* have a long association with humans and make up a major portion of skin flora. The organism generally resists host defense. Antibody may help in certain circumstances but protein A prevents opsonization and the action of complement. These organisms are ubiquitous and have virtually no possibility of elimination because they are (and have been) a significant part of human normal flora (Prescot *et al.*, 2004). *Staphylococcus epidermidis* does not synthesize the carotenoid pigment and so produces white colonies. The virulence of *Staphylococcus aureus* is significantly less than that of *Staphylococcus epidermidis*. *Staphylococcus epidermidis* produces glycocalyx and more likely to adhere to prosthetic implant materials and therefore are more likely to infect implants than strains that produce glycocalyx (Levinson, 2012).

2.2 *Staphylococcus aureus*

In the seventeen century, Anton J. Rosenbach a German surgeon isolated two strains of Staphylococci, which he named for the pigmented appearance of their colonies: *Staphylococcus aureus* from the latin word ñaurumò for gold and *Staphylococcus albus* (now called *epidermidis*) from the latin word albus for white. According to Mordic, (2008), *Staphylococcus aureus* or ñgolden staphò is the most common *Staphylococcus* species causing infection in humans including nosocomial infections. The organism is a Gram positive cocci in clusters, about one micrometer in size with the thick cell wall and thin capsule, does not form spores but it can still survive outside the body from few days to several weeks. *Staphylococcus aureus* is a facultative anaerobe i.e. it grows aerobically but can also thrive in anaerobic conditions. *Staphylococcus aureus* is non- motile, non- spore forming, catalase positive and coagulase positive. Typical colonies are yellow to golden yellow in colour, smooth, entire, slightly raised and haemolytic on 5% sheep red blood cell. However, many stains may appear dirty white and non haemolytic. *Staphylococcus aureus* produces a carotenoid pigment called staphyloxanthin which imparts a golden colour to its colonies. This pigment enhances the pathogenicity of the organism by inactivating the bactericidal effect of superoxides and other reactive oxygen species within the neutrophils (Levison, 2012). It gives a positive mannitol fermentation and deoxyribonuclease test (Turnidge *et al*., 2007). Prescot *et al* . (2004) added that it is the most pathogenic species usually identified by its ability to produce coagulase (protein that affects fibrinogen of blood ï clotting cascade). The enzyme coagulase separates the virulent pathogen *Staphylococcus aureus* from the less virulent coagulase negative *Staphylococcus* species (Turnidge *et al*., 2007). *Staphylococus aureus* can survive at temperatures as high as 50^{o}C, high salt concentration and to drying. Colonies are usually 6 to 8µm in diameter. The cell wall consists of three layers:

a. Outer polysaccaharide capsule
b. Peptidoglycan (murein) layer
c. Inner cytoplasmic membrane. Into this structure proteins and teichoic acid are embedded and protrude from the cell wall on its outer side, forming a ñfuzzy coatò. Capsule is thin and may be seen only under electronic microscope (Tolan *et al*., 2008).

2.3 Importance of *Staphylococcus* as Disease Agents

Staphylococcus aureus is one the most common human pathogens, usually opportunists and capable of causing a wide range of infection in the immunocompromised (Tolan *et al*., 2008). According to Williams (1983), staphylococcal carriage or infection occurs frequently in humans. In nosocomial transmission, there are 2 sources:(1) a person with a lesion or an asymptomatic carrier. Persons with skin lesions due to *Staphylococcus aureus* are most likely to disseminate these organisms. Direct contact is the major route of transmission, even a single boil in an occult body site (e.g. the axilla) caused by *Staphylococcus aureus* may increase the likelihood of dissemination. A close skin contact with an infected person or *Staphylococcus* carrier is needed for infection to occur (Mordic., 2008).

2.4 Carriage of *Staphylococcus aureus*

The role of the nasal carrier in the epidemiology and spread of *Staphylococcus aureus* infection is well documented. It is found that air borne transmission of antibiotic resistant *Staphylococcus aureus* from nasal carriers may result in wound infections especially in surgical and burn units. There are four prerequisites to becoming a nasal carrier of *Staphylococcus aureus*:

1. The nose has come in contact with *Staphylococcus aureus*

2. *Staphylococcus aureus* needs to adhere to certain receptors in the nasal niche
3. It needs to overcome the host defenses and
4. It should be able to propagate in the host. *Staphylococcus aureus* can survive for months on any type of surface.

The ecological niches of *Staphylococcus aureus* are the anterior nares (Williams, 1963). These studies have shown that the nares are the most consistent area from which this organism can be isolated (Williams, 1963). Sheagren (2004) added that *Staphylococcus aureus* lives as the part of the normal flora in the nasopharynx of 20 to 40% adults (carriers). When the nares are treated to eliminate nasal carriage in most cases, the organisms also disappears from other areas of the body, overtime 3 patterns of carriage can be distinguished. Hands are the main vectors for transmitting *Staphylococcus aureus* from surfaces to the nasal niche e.g. nose picking. Air borne transmission of *Staphylococcus aureus* is important for dispersal of S*taphylococcus aureus* to many different reservoirs from where via hands they reach the nose.

Approximately 20% of individuals almost always carry one type of strain and are called persistent carriers. A larger proportion of the population about 60% habour S*taphylococcus aureus* intermittently and such persons are called intermittent carriers. Few persons about 20% almost never carry S*taphylococcus aureus* and they are called non ï carriers. (Williams, 1963; Reagan *et al* .,1991) Persistent carriage seems to have a protective effect of the acquisition of other strain at least during hospitalization (Noble *et al*., 1964).

2.4.1 Prevalence of Nasal carriage of *Staphylococcus aureus*

The prevalence of *Staphylococcus aureus* nasal carriage vary according to the population studied. (Kluytmans *et al*., 1997). In a research carried out by Wenzel and Perl (1995) on the significance of nasal carriage of *Staphylococcus aureus* and incidence of post operative wound infection in Iowa, USA carrier rates of 11 to 32% were detected in the general population and a prevalence of 25% in healthcare workers. In a study done by Kluytmans *et al*,(1997) on nasal carriage of *Staphylococcus aureus* concerning epidemiology, the underlying mechanisms and associated risks in Netherlands, in the general population (13, 873), a mean carriage rate of 37% was found with healthcare workers (2, 568) being 26.6%. Also Askarian *et al.*(2009) studied the prevalence of nasal carriage of MRSA and its antibiotic susceptibility patterns in healthcare workers at Namazi hospital, Shiraz, Iran and found methicillin sensitive *Staphylococcus aureus* (MSSA) 25.7% and methicillin resistance *Staphylococcus aureus* (MRSA) 5.3%. Nur *et al.(*2009) did a study on carriage of multiresistant *Staphylococcus aureus* among health workers and 21 of 46 (46%) of the paediatric patients were carriers with most (84%) *Staphylococcus aureus* strain been multiresistant.

In the University of Nigeria Teaching Hospital Enugu,.Onyemelukwe *et al* .(1992) carried out a study on nasal carriage of *Staphylococcus aureus* in hospital staff of different categories and its antibiotics sensitivity. They observed that overall carriage was 34.42% with a significantly higher rate in females (67.53%) than in males (23.81%) with some strains showing multiple resistant to commonly used antibiotics. In a more recent work done in Nnewi South East Nigeria by Akujiobi *et al.* (2013) the overall prevalence rate of MRSA was 30% and 64% was carriage rate of *Staphylococcus aureus* among HCW. Other similar studies in Nigeria, include Abdulhadi

et al. (2008) in his study in Kano among the populace found nasal carriage more in males (61.5%) as compared to females (38.5%) . Adesida *et al.* (2007) in Lagos and in their study among healthcare workers isolated no methicillin resistant *Staphylococcus aureus* from them.

2.5 Epidemiology of Staphylococcal Nosocomial Infection.

Staphylococcus aureus as an ubiquitous organism is widely distributed in nature and carried by 20 to 40% of normal individuals in the anterior nares and skin (Sheagren, 2004). It can colonize and infect both healthy, immunologically competent people in the community and hospitalized patients with their decreased host defenses. *Staphylococcus aureus* is one of the commonest and most important gram positive hospital ï acquired organism with a high propensity to colonize abnormal skin surfaces and open wounds where it may merely reside rather than cause infection (Turnidge *et al*., 2007). Therefore treatment may not be required since recolonization can occur. It can survive on domesticated animals such as dogs, cats, and horses and can cause bumble foots in chickens. It can survive for some hours on dry environmental surfaces, but the importance of the environment in spread of *Staphylococcus aureus* is currently debated. It can host phages, such as Pantonï Valentine Leukocidin that increases itôs virulence. *Staphylococcus aureus* can infect other tissues when normal barriers have been breached, (e.g. skin or mucosal lining). This leads to furuncles and carbuncles.

In staphylococcal nosocomial infection, the patients becomes a victim of the infection either with an endogenous microbe (normal flora of the body) or through cross contamination from healthcare workers or between people through skin and hands or use of contaminated equipments such as needles, razors, towels, sport equipments. Another mode of dissemination is through environmental surfaces (e.g.) benches and mats or from an exogenous microbe. The presence of

cuts or abrasions in the skin allows the easy transmission. Conditions such as poor hygiene and sanitation and crowded living condition favour the dissemination of the infection. Also *Staphylococcus aureus* infections can be spread through contact with pus from an infected wound, skin to skin contact with an infected person by producing hyaluronidase that destroy tissues and contact with objects such as towels, sheets, lining or clothing used by an infected person. Staphylococcal nosocomial infections have been widely reported in literature. Pawun *et a*l. (2008) reported that *S. aureus* was the cause of skin infections out break in newborns in a hospital in Thailand . Also, El-Bayoumi *et al*.(2006) in a study conducted in paediatric intensive care unit of Mansoura University Childrenôs Hospital, Egypt had *S. aureus* at 12.2% and coagulase negative Staphylococci (CONS) 11.8% isolation rate. CONS and *S. aureus* were involved in bacteremia, urinary tract infection and respiratory tract infection. A number of studies in Nigeria have shown that staphylococcal nosocomial infection in post operative wounds are endemic in parts of the country. Akinjogula *et al*.(2009) reported that *S. aureus* was the leading etiologic agent of post operative wound infections in Calabar and Uyo cities of Nigeria.

The infection can also be spread through pets. An important and previously recognized means of community ï associated methicillin ï resistant *Staphylococcus aureus* colonization and transmission is during sexual contact (Heather *et al*., 2007).

2.6 Microbial Pathogenesis

A healthy human body has several defenses against infection. The skin and mucous membranes form natural barrier to infection and immune responses. (non specific and specific) are activated to resist microorganisms that are able to invade. The skin can effectively protect the body from most microorganism unless there is a physical disruption. For example some parasites can

penetrate the intact skin but bacteria and fungi cannot (Prescot, 2004). Other disrupters of the natural barrier are lesions or injury or in the healthcare setting invasive procedures and devices. In addition to breaks in the skin, other primary entry points for micro organisms are mucosal surfaces such as respiratory, gastrointestinal and genitourinary tract. (Prescot, 2004). The membranes lining these tracts comprise a major internal barrier to microorganisms due to the antimicrobial properties of their secretions.

Non pathogenic bacterial (commensal bacteria make up the normal flora and acts as protectants against invading pathogenic bacteria. Commensal bacteria are a source of infection only if they are altered by use of antibiotics e.g *Staphylococcus epidermidis* (Kolmos *et al*., 2007). Nosocomial infections are commonly caused by bacteria. It can also be caused by viruses , fungi and parasites (Johnson *et al*., 2001).

A microorganism requires a reservoir (a human, soil, air or water), or a host in which to live. The microorganism also needs an environment that supports itôs survival once it exits the host as well as a method of transmission. Inherent properties allow microorganisms to remain viable during transmission from a reservoir to a susceptible host. The primary routes of transmission for infections are through the air, blood (or body fluids), contact (direct or indirect) faecal oral route, food, animals or insects. Once inside a host, microorganisms thrive because of adherent properties that allow them to survive against mechanisms in the body that act to flush them out. Bacteria adhere to cell surfaces through hair ï like projections such as fimbriae or pili as well as proteins that serve as adhesins (Dantas *et al*., 2006). Receptor molecules in the body act as ligands to bind the adhesins enabling bacteria to colonize within the body. The virulence and number of the microorganism will determine whether only colonization occurs or if infection will develop. With colonization, there is no damage to local or distant tissues and no immune

reaction, with infection, bacterial toxins that break down cells and intracellular matrix are released, causing damage to local and distant tissues and prompting an immune response in the host. Bacteria continue to thrive within a host through strategies that enable them to acquire iron for nutrition and to defend against the immune response. These virulence factors enhance microrganisms potential for infection by allowing microorganisms to interrupt or avoid phagocytosis or to live inside phagocyte (Prescot *et al*.,2004)

A healthcare environment increases the risk of infection for two primary reasons. First, it is likely that normally sterile body sites will become exposed, allowing pathogens to cause infection through contact with mucous membranes, non intact skin and internal body areas (Bolyard *et al*., 1998). Secondly, the likelihood of a susceptible host is high because of the vulnerable health status of patients, especially in an era of decreased hospital stays. And increased out patients treatments. It is the most sick patient who is hospitalized, increasing the risk for infection to develop in these patients and for it to be transmitted to others. Infection is transmitted in a healthcare environment primarily through exogenous or endogenous modes. Exogenous transmission is through patient to patient or staff to patient contact. Patients who do not have infection but have bacterial colonization can act as vectors for transmission. Healthcare workers can also act as vectors because of colonization or contamination. Endogenous infection occurs within an individual patient through displacement of commensal microorganisms.

2.7 Virulence Manifestation of Staphylococcal nosocomial infection

Staphylococcus aureus causes a variety of suppurative (pus forming) infections and toxinoses in humans. In healthy individuals, *Staphylococcus* usually causes only localized skin infections which heal on their own within a few weeks. In heavily ill or immuno deficient patient it may

cause deadly sepsis (Mordic, 2008). Infections caused by *Staphylococcus aureus* ranges from minor skin disorders such as wound infections, furuncles and carbuncles and bullous impetigo through locally invasive diseases such as cellulitis, urinary tract infection, sinusitis, bacteremia, mastitis and pneumonia and deep seated infections such as endocarditis, osteomyelitis, meningitis and life threatening septicaemia (Clauditz *et al*., 2006). It is also a frequent cause of medical device related infections such as intravascular line sepsis and prosthetic joint infections although minor skin infection may resolve naturally without antibiotic intervention. Once *Staphylococcus aureus* invades deeper structures, it often spreads haematogenously to organs leading to metastic infection. Toxic shock syndrome and staphylococcal scald ï skin syndrome are due to release of super antigens to the blood stream while staphylococcal food poisoning is due to release of enterotoxin into the food (Clauditz *et al*., 2006).

Staphylococcus aureus expresses many potential virulence factors:

i. Surface proteins that promote colonization of host tissues such as laminin and fibronectin and fibrin (fibrinogen- binding protein) (clumping factor) which promotes attachment blood clots and traumatized tissue. In addition, an adhesin which promotes binding or attachment to collagen has been found in strains that cause osteomyelitis and septic arthitis (Levison., 2012).
ii. Invasins which promote bacterial spread in tissues includes leukocidin, kinases and hyaluronidase. Hyaluronidase breaks down hyaluronic acid and helps in spread *Staphylococcus aureus*. It also produces lipase which digest lipids and deoxyribonuclease (DNase) which breaks down DNA to probably provide nutrient for the bacteria. Fatty acid modifying enzyme (FAME) is also produced by some strains of *Staphylococcus aureus* which is essential in modifying antibacterial lipids and so prolong bacterial survival

(Prescot *et al*., 2004). *Staphylococcus aureus* also produces various enzymes that coat in the bacterial cell to prevent phagocytosis and immune defences of the host thereby causing localized clotting. Staphylokinase is expressed by some strains of *Staphylococcus aureus* and is thought to aid in bacterial spreading by causing fibrinolysis. Surface factors and immunological disguises that inhibit phagocytic engulfment include capsule and protein A, biochemical properties that enhance survival in phagocytes include carotenoids and catalase. *Staphylococcus aureus* produces a carotenoid pigment called staphyloxanthin which imparts a golden colour to its colonies. This pigment enhances the pathogenicity of the organism by inactivating the microbicidal effect of superoxides and other reactive oxygen species within the neutrophils (Clauditz, 2006). In mice, the pigmented strains cause lingering abscesses when inoculated into wounds whereas wounds infected with the unpigmented strains quickly heal. Drugs designed to inhibit the production of staphyloxanthin may weaken the bacterium and renew its susceptibility to antibiotics (Liu *et al*., 2008).

Depending on the strain, *Staphylococcus aureus* is capable of producing several exotoxins which are associated with specific diseases. Pyrogenic toxin superantigen have antigen activities that induce toxic shock syndrome (TSS) due to excess release of cytokines toxin TSS T ï 1 which cause TSS associated with tampon use. This is characterized by fever, erythematous rash, hypotension shock and multiple organ failure. Another form of TSS is associated with wounds from surgical procedures (Prescot *et al*., 2004). Exfoliative toxins are implicated in the disease, staphylococcal scalded skin syndrome which occurs mostly in infants and young children. It may also occur as epidemics in hospital nurseries. The protease activity of the exfoliative toxin causes the peeling of the skin observed with staphylococcal scalded skin syndrome Tolan *et al,* (2008).

Other strains of *Staphylococcus aureus* can produce an enterotoxin that is the causative agent of S*taphylococcus aureus* gastroenteritis. This is self- limiting and characterized by nausea, vomiting (projectile vomit) followed by abdominal cramps and diarrhoea which can be hemorrhagic and occurs 2 to 6 hours after ingesting the performed enterotoxin in food and recovery is within 8 to 24 hours (Prescot *et al*., 2004). The membrane damaging toxin also plays a role in pathogenesis of *Staphylococcus aureus*. Alphatoxin (alpha haemolysin) whose mode of action is likely osmoticlysis. Alpha toxin is a sphingomyelinase which damages membrane - rich in this lipid and is detected by lysis of sheep red blood cells. Delta toxin role is unknown (Tola*n et al.,*2008). Leucocidin is a protein toxin that damages membranes. The bicomponent toxin Panton Valentine Leukocidin (PVL) is associated with severe neucrotizing pneumonia in children (Tolan *et al*., 2008).

Pathogenicity of *Staphylococcus epidermidis* has been liked to resistance to antimicrobial agents, production of invasins and biofilm production (Voung *and* Otto*,* 2002). *Staphylococus epidermidis* is one of the principal skin residents present in practically all healthy and ill individuals and can be introduced into the intensive care unit by patient or medical staff and cause opportunistic infections during or after invasive procedures (Voung and Otto, 2002). Patients with lowered resistance can suffer endocarditis and septicaemia associated with catheters and prosthetic implants. Haemolytic activity has been detected in some strains.

2.8 **Virulence manifestations of Staphylococcus epidermidis**

Staphylococus epidermidis alpha haemolysin has been associated with neurotoxic activity and gamma haemolysin has been associated with a severe inflammatory response (Voung and Otto, 2002), while lipase activity has been linked with the capacity to invade dermal and epiderrmal

tissues. Proteolytic activity has been shown to play a fundamental role in tissue damage and host inflammatory response. Insertion of a prosthetic device into the human body often leads to the formation of biofilm on the surface. A characteristic of many pathogenic strains of *Staphylococcus epidermidis* is the production of slime resulting in biofilm formation. The slime is mainly a secreted teichoic acid normally found in cell wall of the Staphylococci and glycocalyx which facilitates adherence to prosthetic implant materials (Levison, 2012). Adherence factors (adhesins) also facilitate the formation of biofilms (Voung and Otto, 2002). Within the biofilm, they are protected from the bodyôs normal defense mechanism and from antibiotics since antibiotics are incapable of penetrating biofilms. Also organisms transpose resistance from one organism to another in biofilm colonies. It also provides a source of infection for other parts of the body as bacteria detach during biofilm sloughing (Prescot *et al*., 2004).

2.9 MRSA

According to Chamber, (2001), antibiotic resistance in *Staphylococcus aureus* was almost unknown when penicillin was first introduced in 1943. Indeed, the original Petri dish on which Alexander Fleming of Imperial College, London, observed the antibacterial activity of the *Penicillium* fungus was growing a culture of *Staphylococcus aureus*. By 1950, 40% of hospital *Staphylococcus aureus* isolates were penicillin resistant and by 1960, this has risen to 80%. Staphylococcal resistance to penicillin is mediated by penicillinase (a form of ß ï lactamase) production; an enzyme that breaks down the ß ï lactam ring of the penicillin molecule. Penicillinase ï resistant penicillins such as methicillin, oxacillin, dicloxacillin and flucloxacillin were able to resist degradation by staphylococcal penicillinase (Chamber, 2001; Parras *et al*., 1995). This resistance to methicillin confers on it cross resistance to other broad spectrum antibiotics including cephalosporins. The mechanism of resistance to methicillin is mediated via

the mec operon, part of staphylococcal cassette chromosome mec (SSC mec). Resistance is conferred by the mec A gene which codes for an altered penicillin ï binding protein (PBP2a or PBP2) that has a lower affinity for ß ï lactams (penicillins, cephalosporins and carbapenems). MRSA that are resistant to 3 or more non ß ï lactam antibiotics e.g gentamycin erythromycin and ciprofloxacin are regarded as multiple drug resistant MRSA while non- multi- drug resistant MRSA are resistant to less than 2 ß-lactam antibiotics. The risk factors for acquisition of MRSA include long-term use of broad spectrum antibiotics, prolonged hospital stay and previous hospitalization, crowded living conditions, patients undergoing surgery in chronic diseases, advanced cases of HIV, drug addicts etc.

Occasionally, strains of *Staphylococcus aureus* exhibits low level of methicillin resistance which may occur due to over expression of ß-lactamase, the overproduction of another low affinity PBP, PBP_4 of the genetic mutation of PBP_2 (Michelle and Gutman, 1997). These are not normally considered as MRSA and do not have spectrum of antibiotic resistance associated with true MRSA. Resistance may be heterogenous or homogenous (Tomasz *et al*., 1991). Hetergenous MRSA strains have sub populations with minimum inhibitory concentrations (MICs) ranging from intermediate to high level resistance to methicillin. It usually occurs at a low frequency but are likely to be selected for under antibiotic pressure. This heterogenous feature of MRSA has made it necessary to employ special laboratory isolation procedure to ensure that low numbers of MRSA are reliably detected and is of importance in screening and surveillance studies (Cook, 1998). The clinical significance of both the homogenous and heterogenous strains is failure of vancomycin treatment which has been well documented (Mordic, 2008). Gentamycin, Streptomycin etc were once effective against staphylococcal infections until strains evolved mechanisms to inhibit the aminoglycosides action which occurs via protonated amine and/

hydroxyl interactions with the ribosomal RNA of the bacterial 30S ribosome (Cater *et al*., 2000). There are three main mechanisms of aminoglycoside resistance mechanisms which are currently and widely accepted. They are;

a. Aminoglycoside modifying enzymes

b. Ribosomal mutation

c. Active efflux of the drug out of the bacteria. Aminoglycoside modifying enzymes are enzymes that inactivate the aminoglycoside by convalently attaching either a phosphate, nucleotide or acetyl moiety to either the amine and or alcohol functional group of the drug. This changes the charge or sterically hinders the antibiotics thereby decreasing their ribosomal binding affinity. In *Staphylococus aureus*, the best characterized aminoglycoside modifying enzyme is aminoglycoside adenyl transferase 4ô 1A (ANTC4ô) 1A (Sakon *et al*., 1993). This enzyme has been solved by X ray crystallography. This enzyme is able to attach an adenyl moiety to the 4 hydroxyl group of many aminoglycosides including gentamycin and kanamycin. Vancomycin resistant *Staphylococcus aureus* (VRSA) is a strain of *Staphylococcus aureus* that has become resistant to the glycopeptides. The first case of VRSA was reported in Japan in 2002, (*Chang et al*., 2003). Three cases of VRSA have been reported also in USA as of 2005 (Menichestti, 2005). Glycopeptide resistance is mediated by acquisition of van V gene. The gene originates from the *Enterococcus* and codes for an enzyme that produces an alternative peptidoglycan to which vancomycin will not bind.

MRSA is dangerous because of the bacterial genetic plasticity that allows it to acquire genetic material that helps them fight antibiotics i.e mec A which provides resistance against ϐ-lactams and celphalosporins. A major cause for these resistant strains development is the use of very powerful drugs for minor infections and overuse of antibiotics (Baddour *et al*., 2006). Worldwide, the prevalence of MRSA has increased. In the present decade, the rate is alarming for example in Italy (45%), United Kingdom (40%) and Greece (35%). Also MRSA related bacteremia was found to be in Ireland (41%) and Greece (44%) in 2004. MRSA is commonly transmitted in hospitals through healthcare workers (Klein *et al*., 2007).

2.10 Laboratory Diagnosis

According to Turnidge *et al*. (2007), definitive diagnosis of disease is made by isolation and identification of the species of *Staphylococcus* using biochemical or enzyme - based methods. Specimens used include skin swabs, wound swabs, pus, sputum, tracheal aspriates, urine, blood and cerebrospinal fluid. When food poisoning is suspected, faeces, vomitus and remains of food are used for analysis. Anterior nasal swabs were collected to detect *Staphylococcus aureus* carriers. A Gram stain is first performed to guide the way which should show typical Gram positive cocci in clusters.

2.10.1 Microbiological Culture:- The organism grows well on most routine bacteriological media aerobically, in enriched carbon dioxide atmosphere and less well anaerobically. Optimum temperature of growth is 37^{o}C but can grow in a range of 10 to 42^{o}C. The colonies on most media measure about 1 to 2 mm in diameter and are round, smooth, raised with entire edge. *Staphylococcus aureus* colonies are golden yellow to creamy white in colour while *Staphylococcus epidermidis* are white. Selective media used to recover *Staphylococcus aureus* from specimens include:

i. Mannitol salt agar where it appears as yellow colonies

ii. 10% Salt- cooked meat medium

iii. Lithium chloride and tellurite medium.

Staphylococcus aureus reduces potassium tellurite in the medium to produce black colonies. Other media used include deoxyribonuclease agar and phenolphthalein phosphate agar (Bascomb and Manafi. 1998;Cheesbourgh, 2004).

2.10.2 **Biochemical tests**: Furthermore, for differentiation on the species level like; catalase (positive for all species), coagulase (fibrin- clot formation), DNase (zone of clearing on DNase agar), Lipase (halo of precipitation on Tween 20 agar), haemolytic activity (halo of haemolysis on sheep blood agar), protease activity (halo of protein hydrolysis on gelatin medium). phophatase (pink colour on phenolphalein phosphate agar) are all done (Michelim *et al*., 2005). Also the capacity of the isolates to form biofilms can be evaluated by growing the organism on tryptone soy broth supplemented with 2.5 g/L glucose. It is diluted and a portion transferred to well of polystyrene tissue culture plate incubated at 37^{o}C for 24 hours. The wells are washed with buffer, air- dried and treated with alcoholic fuschin for 30 minutes to stain any adhered cells. Biofilm formation is then evaluated visually and quantified using enzyme linked immunosorbent assay (ELISA) reader (Heilmann *et al*., 1996; Michelim *et al*.,2005).

2.10.3 Bacteriophage Typing:- This is done to investigate cross infection in hospitals and staphylococcal food poisoning. Strains are generally distinguished by their patterns of susceptibility to lysis by any of the universally accepted set of 23 standard typing phage. The typing is as follows:-

a. Inoculate the strain of *Staphylococcus aureus* to be typed on nutrient agar to give a confluent growth.
b. Apply the phages drop wise at their routine test dilution (RTD) to the seeded plate
c. Incubate overnight at 30^{o}C.
d. Examine the culture for the degree of lysis by each phage. Some phages will not produce lysis. The phage type of the staphylococcal strain is reported as the combined designations of the phages which strongly lyses by 52, 52 A and 80, it is expressed as $52/52^{A}/80$. Phage typing is usually carried out by Reference Laboratory (Modric, 2008).

2.10.4 Polymerase chain reaction (PCR)

Rapid detection of *Staphylococcus aureus* is possible with tests using quantitative polymerase chain reaction (qpcr) also called real time pcr.

Principle of Pcr: In clinical samples, *Staphylococcus aureus* is present in quantities not sufficient to be exactly determined by any testing method, so bacteria have to be multiplied by culturing which usually takes 1 to 4 days.With Pcr ,a few million copies of *S. aureus* DNA can be obtained directly from clinical sample within a few hours. In this case, no culture is needed.

Procedure: A sample of the organism(staph.), DNA polymerase (an enzyme needed for DNA duplication), a sequence of nucleotides (primer) needed for the start of duplication and a large amount of free nucleotides are needed for PCR. The enzyme polymerase is obtained from a bacterium *Thermus aquatius (*Taq polymerase*)*. All the components are added into a testing tube which is placed into a thermal cycler. After some PCR cycles, agarose gel electrophoresis and

southern blotting techniques are used to determine if the obtained DNA is *Staphylococcus aureus* DNA (Mordic, 2008).

2.10.5 Commercial detection kits

Kits for detection of various species of Staphylococci or their toxins from human tissues, food, cowôs milk, water and other environmental sources are commercially available. Chromogenic media from Oxoid is available for detection of MRSA. The chromogenic plate media Chromagar *S. aureus* (CHROmagar, Paris, France) and Oxacillin resistance screening agar base (ORSAB CM 1008, Oxoid ®) are used for laboratory diagnosis. On CHROM agar ,characteristic *Staphylococcus aureus* colonies appear as pink to red colonies while on the ORSAB medium, presumptive MRSA appear as intense blue colonies. (Meruno *et al*., 2002). Other examples of kits based on agglutination for detection of MRSA in clinical samples (after culturing) are; Dry spot staphtect plus ® test, pastorex staph plus ® test, the slidex staph plus kit ® the staphaureux plus ® test and the staphylase test ®. Specificity and sensitivity of these kits were evaluated by three German Health institutions in the year 2005.

Xpert TM MRSA (SA ï Blood culture (BC) and xpert MRSA (SA ï Skin and Soft Tissue Infection (SSTI) tests are able to simultaneously detect MRSA and *Staphylococcus aureus* directly from the blood cultures and samples of infected soft tissue using PCR. Both test gives result in about 50 minutes. Food may be tested for *Staphylococcus* enterotoxin by ELISA. Presence of *Staphylococcus aureus* in the food sample may be confirmed by PCR or slidex staph kit (latex agglutination). Milk can be tested for cowôs mastitis by Bulk tank culture, California mastitis test and prostaph milk antibody test specifically for *Staphylococcus mastitis*. The multiplex real time PCR assay for simultaneous detection of *Staphylococcus uboris* takes 24

hours to be completed. Water that is to be tested for *Staphylococcus aureus* is first filtered and the filtering membrane is put on modified mannitol salt agar (Mordic, 2008).

2.11 Treatment and Management

According to (Turnidge *et al*., 2007), treatment of staphylococcal infections depends on the site and severity of infection, the antibiotic susceptibility pattern of the organism and the presence of any patient allergy or drug intolerance. Erythromycin has been used extensively for treatment of both minor and serious staphylococcal infections. It is bacterostatic against *Staphylococci* and for this reason has generally lost favour for the management of serious and life threatening infections, being largely suppressed by penicillinase resistant penicillin. Moreover, the role of erythromycin in empirical treatment is further limited because of drug resistance. Nevertheless, oral erythromycin is still suitable for minor skin infections caused by *Staphylococcus aureus* especially in penicillin allergic patients provided the strain has demonstrated susceptibility on laboratory testing.

*Staphylococ*ci colonization may be treated with mupirocin (bactroban) nasal gel and daily Hibiclen skin cleanser bath. Skin abscesses often have to be drained and deep abscesses may require surgical drainage. Artificial heart valves and vein catheters often need to be removed or replaced. Food poisoning usually heals on itôs own (Mordic, 2008). The treatment of choice for *Staphycoccus aureus* infection is penicillin in most countries however, penicillin resistance is extremely common and first line therapy is most commonly a penicillinase resistant ß ï lactam antibiotic e.g oxacillin or flucloxacillin. Combination therapy with gentamycin may be used to treat serious infections such as endocarditis but due to its risk of damage to the kidney, the use is limited (Cosgrove *et al*, 2009).

Methicillin resistant *Staphylococcus auerus* (MRSA) infections in both the hospital and community setting are commonly treated with non ï ɓ- lactam antibiotics such as clindamycin and co-trimoxazole. Resistacet to these antibiotics has also led to the use of new broad spectrum antibiotics Linezolid which have a wide variety of activity against gram positive organisms including MRSA (Betriu *et al*., 2001). It does not display cross resistance with other classes of antimicrobial agents and acts like vancomycin. First line treatment for serious invasive infections due to MRSA is currently glycopepide antibiotics vancomycin and teicoplanin). There are a number of problems with these antibiotics, mainly centered on the need for intravenous administration, toxicity and the need to monitor drug levels regularly by means of blood test. There are also concerns that glycopeptide antibiotics do not penetrate very well into infected tissues (infections of the brain, meninges and in endocarditis). Glycopeptides must not be used to treat methicillin susceptible *Staphylococcus aureus* (MSSA) infections.

Because of the high level of resistance to penicillin and potential for MRSA to develop resistance to vancomycin, the centres for disease control (CDC) and prevention have published guidelines for the appropriate use of vancomycin. In situation where the incidence of MRSA infections is known to be high, the attending physician may choose to use a glycopeptide antibiotics until the identity of the infecting organism is known. When the infection is confirmed to be due to a MSSA, then treatment can be changed to flucoxacillin or even penicillin as appropriate. A combination of quinolone such as ciprofloxacin and rifampicin can also be used in treatment of MRSA and has been shown to cure 100% of intravenous drug users with right sided *Staphylococcus aureus* endocarditis (Turnidge *et al.,* 2007).

Alternative therapies are been sought for treatment of MRSA and one area of interest is use of essential oils. MRSA is susceptible to tea tree oil (Jones *et al*.,2004) but there are concerns about itôs toxicity. Researchers from Italy have identified a bacteriophage active against *Staphylococcus aureus* including MRSA in mice and possibly in humans. Also clinical trials are on going in use of IgG antibodies to enhance opsonophagocytosis of *Staphylococcus aureus* by polymorphonuclear leucocytes (Turnidge *et al*., 2007).

Biological control might be a new possible way to control *Staphylococus aureus* in body surfaces. Colonization of body surfaces (especially in the nose) by *Staphylococcus epidermidis* (inhibitory strain JK 16) impairs the establishment of *Staphylococcus aureus*. According to a study by (Iwase *et al*., 2010) two strains of *Staphylococcus epidermis,* that inhibits biofilm formation by *Staphylococcus aureus* were identified.

2.12 Nosocomial Infection

Nosocomial infection is defined according to the National Nosocomial Infections Surviellance (NNIS) as a localized or systematic condition that results from adverse reaction to the presence of an infectious agent(s) or its toxin(s) and that was not present or incubating at the time of admission to the hospital (Shrestha *et al.*, 2009). The common definition of nosocomial infection is an infection which occurs within 48 hours after hospitalization or 3 days from discharging or 30 days from an operation. Nosocomial infections is also called hospital acquired infection or healthcare associated infections. The word nosocomial comes from two greek words: ñNosoò which means disease and ñcomiumò which means to take care of.

Studies throughout the world document that nosocomial infections are a major cause of morbidity and mortality and health related cost (Okezie and Onyemelukwe, 2007). A high

frequency of nosocomial infections is evidence of a poor quality healthcare service delivery and leads to avoidable cost (Kelvens *et al*., 2007). Nosocomial infections affect more than 2 million patients each year or about 5 to 10% of hospitalized patients leading to approximately 90,000 deaths per year (Bearman *et al*., 2006). Many factors contribute to the frequency of nosocomial infections ie hospitalized patients are often immunocompromised, they undergo invasive examinations and treatment., patient care practices and the hospital environment may facilitate the transmission of microorganisms among patients. The selective pressure of intense antibiotics use promotes antibiotic resistance while progress in the prevention of nosocomial jnfection has been made, changes in medical practice continually present new opportunities for development of infection (Ducel and Nicolle, 2002).

Historically, hospitals have a notorious reputation for infection. The hazards of puerperal sepsis and the horror or septic infection in the prelisteran era has been well documented. Admission to hospitals in the mid 19^{th} century was associated with the fear of gangrene and death. (Green wood *et al.*, 2002). Nosocomial infections is a world wide problem and is even more alarming in the 21^{st} century. Modern medical science has managed to subdue many of the classical infections but has helped to create new ones which result from interference with normal host defence mechanisms, consequently upon medical and surgical procedures such as chemotherapy, catheterization, immunosuppression and irradiation.

Staphylococci as a Nosocomial Agent

Staphylococcus aureus is the specie with the broadest pathogenic potential among all the Staphylococci. In community acquired infection *Staphylococcus aureus* causes deep and superficial infection of the skin, pneumonia, endo carditis, septic arthritis and osteomyelitis and

can also cause toxin mediated disease such as food poisoning, toxic shock syndrome and exfoliative disease of the skin.

Hospital acquired infection due to *Staphylococcus aureus* include wound infection of surgical sites, catheter related septicemia, prosthetic valve endocarditis and septic arthritis of prosthetic joints. Nosocomial infection of both *S. aureus* and *S. epidermidis* are characterized by increasing resistance towards antibiotic which a great challenge for the management of hospital acquired infection.

Majority of infection occur in association with use of medical devices. *Staphylococcus* infect the preferentially immunocompromised patients in intensive care unit, surgical and oncological wards as well as in neonatal nurseries(Muddofer and Schiffer,2001).

Staphylococcus are regarded as pathogens associated with medical progress and these factors help in their spread:

First, the increasing number of immunocompromised patients offers the microorganism a new and very susceptible host. Second, in the course of treatment majority of these patient carry temporary or chronic medical devices which represents a suitable habitat for colonization. Finally bacteria in hospital environment are subject to a high selective pressure due to extensive use of antibiotics and disinfectants. Occupation of this ecological niche by a microorganism require special prerequisites which are obviously met by Staphylococci. These properties include special adherence mechanism, ability to gain antibiotic resistance determinants and a strong phenotypic and genotypic variability.

Adherence mechanism: Staphylococci are capable of binding to a wide range of extracellular matrix proteins, tissue components, soluble factors which coat medical devices soon after implantation. It is now known that *Staphylococcus* carry a special class of proteins on their surface that mediate adherence to collagen, fibronectin and fibrinogen and other components. Autolysin protein of *S. epidermidis* and *S. saprophyticus* play a role in attachment to polymer surfaces cell wall mediated proteins as well as surface hydrophobicity and the charge lead to an initial binding of staphylococci to smooth surfaces. In certain *S. epidermidis* and *S. saprophyticus* are strains, this primary attachment is followed by biofilm formation. In general biofilm consist bacteria which are imbedded in extracellular matrix. Biofilms protect bacteria from unfavourable environmental condition such as nutrient limitations, oxidative stress, heat and osmotic stress as well as the influence of antibiotics and are now recognized as a source of persisting and relapsing infection. It has been shown that biofilm production can be induced in response to environmental stress. (high osmolarity, detergents and alcohol and low concentration of certain antibiotics (Muddofer and Schiffer., 2001).

Antibiotics resistance: Majority of nosocomial staphylococci infections are due to multi resistance isolates whose number is on the increase. So the usual antistaphylococcal agents such as betalactams, erythromycin, clindamycin and tetracycline and aminoglycosides are not effective in these infections. Mostly, oxacillin resistance is associated with resistance to other non related antibiotic resulting in isolates which are only susceptible to glycopeptides antibiotic such as vancomycin. Oxacillin resistance has evolved by acquisition of mecA gene that encodes the additional pencillin binding protein. This protein has a low binding affinity for betalactam antibiotic and is not present in susceptible *Staphylococcus.*

Phenotypic and genetic variability: Staphylococci are known for their pronounced variability. Thus among variants of the same parent, strains-specific properties such as colony morphology, growth rate, biofilm formation and antibiotic susceptibility can differ in a wide range. Hetero genous gene expression is typically observed in clinical *S.aureus* and *S.epidemidis* and is assumed the ability is an advantage for adaptation of *Staphylococcus* to changing environmental condition. Mechanism is poorly understood but seem to include both regulatory pathways in response to environmental signals as well as genetic variation (Muddofer and Schiffer., 2001).

2.12.1 Sources of Nosocomial Infection

Generally, the sources of nosocomial infections can be categorized as being related to environmental factors (air, water, architecture), patient related factors (age, degree of illness/immune status, duration of hospitalization) and iatrogenic factors (surgery and invasive procedures). These sources have a substantial impact on the increasing incidence of nosocomial infections. World Health Organisation (WHO) notes that the rate of nosocomial infections will continue to increase as a result of these four factors viz.

i. Crowded hospital condition
ii. Increasing number of people with compromised immune systems
iii. New microorganisms
iv. Increasing bacterial resistance

2.12.1a Environmental Factors

This is not a common cause of nosocomial infections, but consideration should be given to the prevention of infection with environmental pathogens such as fungi eg. *Aspergillus* species, bacteria eg. *Legionella* species or viruses e.g *Varicell*a. In 2003, the CDC and the healthcare

infection control practices advisory committee (HICPAC) revised the guideline related to environmental factors for infection. (NNIS, 1996). The report provides clear recommendations for infections control measures according to several environment related categories including air, water and environmental services.

2.12.1b Air

Droplets of microrganisms can be transmitted in the air, causing infection either directly or indirectly (through contamination of devices or equipments). Cleaning activities such as sweeping, dry mopping, dusting or shaking linen can contribute to the transmission of airborne microorganism. Bacteria in the air primarily consist of Gram positive cocci from the skin and they can be eliminated with appropriate ventilation and circulation of air. The most common fungal spore to be transmitted through air is *Aspergillus*, which is carried through dust particles and can survive for long periods and is easily inhaled. Under normal circumstances, the level of contamination with this airborne fungal spores is not high enough to cause respiratory infection primarily pneumonia in a susceptible host. The prevalence of infection with *Aspergillus* within a healthcare setting has been strongly associated with *Asergillus* spore counts. Consequently, air conditioning systems with high efficiency particulate air (HEPA) filters are needed to minimize contamination (Auerbach, 2001). Routine inspection and cleaning of conditioning equipments is also needed to ensure proper filtration (Andreasen, *et al* .,2002).

2.12.1 C Air in the operating room

Maintaining a high quality of air in the operating rooms is an essential factor in preventing post operative wound infection. The number, movement and activity of staff within the operating room together create the primary sources of airborne bacteria. Other factors influencing airborne contamination include the type of surgery, the rate of air exchange, the initial quality of air, the

quality of staff clothing and cleaning process and the level of compliance with infection control practices. (Dulworth and Pyenson, 2004). The CDC makes several suggestions about ventilation in the operating of surgical site infection. The level 1 recommendations include:

i. Maintain positive pressure ventilation in the operating room with respect to the corridors and adjacent areas.
ii. Maintain a minimum of 15 air changes per hour of which at least 3 should be fresh air.
iii. Filter all air both recirculated and fresh through appropriate filters as recommended by the American Institute of Architects (AIA).
iv. Introduce all air at the ceiling and exhaust near the floor.
v. Do not use ultraviolet radiation in the operating room to prevent surgical site infection.
vi. Keep operating room doors closed except as needed for passage of equipments, personnel and patient (Kirkland *et al.*, 1999).

2.12.1d Air during construction

Special care must be taken to protect patients during repair or renovation of a healthcare facility as construction can facilitate the spread of airborne organisms such as *Aspergillus* species. (Mashita *et al*., 2000). It is estimated that approximately 5% of deaths attributed to nosocomial infections are related to construction and maintenance practices within the healthcare facility. Some construction issues that contribute to the spread of infection include water damaged building materials, disruption of duct work, open windows and improper setting of fans or installations of filters (Krieger *et al*., 1983). JCAHO requires an inspection process for construction on a facility which includes risk assessment and risk factors to consider include the patient population, the extent and duration of the project and the impact of the project on mechanical systems. A member of the facilities infection control team should review any

construction plan to ensure that barriers are used as appropriate and patients especially those with immunocompromised system are moved to an area away from construction (Isenberg *et al.,*1961).

2.12.1e Water

Water is a reservior for several types of microorganisms including bacteria, fungi and viruses. The quality of water within a healthcare setting must meet the standards that vary according to use. Tap water must be safe to drink and use for baths (for hygiene and therapy), according to criteria dictated by local authorities and public health standards. No bacteria, fungi, or parasites may be present in the water, the levels of disinfectants should be minimal and the turbidity should be low. The water supply to the healthcare facility can be disinfected by several including chlorination, thermal eradication, ultarviolet light and metal ionization (Wenzel, 2003). The most common pathogen identified in tap water and bath water is *Pseudomonas aeroginosa* (Stone *et al*., 2002). The effect of infection with (*Pseusomonas aeroginosa* may be mild as in folliculitis, but wound infection may be more severe. *Legionella* which causes infection of the respiratory tract is another micro organism commonly found in tap water and bath water. It colonizes hot water storage, cooling towers and condensers. Infection with *Legionella* is transmitted only through water through inhalation of contaminated water droplets from shower heads. WHO suggests that there is potential risk of nosocomial infection if tap water is used for such purposes as ice machines or devices for washing ears and eyes or for cleaning equipments. Point of care filtration may help reduce the risk of nosocomial infections related to water. Ducts, humidifers, dehumidifers and other areas of a ventilation system should be kept clean and dry as microrganisms can colonize in water that accumulates in these areas (Shitu *et al.,* 2002).

2.12.1f Architectural design

Another factor in the transmission of infection in a healthcare setting is the architectural design of the facility. The AIA and Facility Guidelines Institute (FGI) released the guidelines for design and construction of hospital and healthcare facilities in 2001 and it was updated in 2006. The latest recommendation is setting single bed private rooms as the minimum standard for new hospital construction (Mashita *et al.*, 2000). This change is based on a report of literature that showed in part, that private rooms have been associated with lower rates of nosocomial infections as well as reduction in risk factors for nosocomial infections such as prolonged hospital stay and patient transfers (Dulworth and Pyenson, 2004). The WHO guideline on infection control refers to ñarchitectural segregation according to riskò. Four areas of a healthcare facility are defined with administrative sections considered low risk, regular patient wards as moderate risk and , intensive care units, burns units or isolation units as high risk areas and operating rooms as very high risk areas (Basak *et al.,* 1992). The surfaces should be smooth, resist accumulation of dust, water resistant and easily cleaned. The use of carpet should be limited to low risk areas such as office space as bacteria can survive in carpets. Microorganisms can colonize wet acoustical tiles and so should be avoided in high risk areas. The type of sink in health care facility have been of critical concern because of the substantial role of hand washing in reducing the transmission of infection. As a result, sinks have been placed within easy access in each patientôs room. However, it is unclear that such placement promotes better hand hygiene. With the advent of alcohol based handrub, solutions as more effective hand hygiene, the placement of handrub dispensers has become more important than the placement of sinks. The CDC guideline on hand hygiene recommends placing dispenser in convenient locations such as at the entrance of each patient room or at the bed site.

2.12.1g Patient - related factors

Patient related risk factors for nosocomial infection include age, general health status of the patient and the type of procedure to be carried out. Risk can be classified as minimal, medium or high. Patients are at minimal risk if they have no significant underlying disease and have an intact immune system and will not undergo an invasive procedure. Medium risk is assigned to older patients who are susceptible to disease for a variety of reasons, including decreased immune function, co-morbid conditions and low nutritional status. Kolmos *et al.*.(2007) in a study of 185 hospitalized patients with a mean age of 82 years found the rate of nosocomial infection to be 59%. Medium risk also refers to patients who are to have non surgical invasive procedure e.g peripheral venous catheter or urinary catheter. High risk is assigned to patients who have had organ transplant, cancer or infection with human immunodeficiency virus. Also patient with multiple trauma or severe burns, undergone surgery or invasive procedure such as endotracheal intubation or insertion of a central venous catheter. Their compromised immune system puts them at a high risk for nosocomial infection.

2.12. 1h Special patient populations

The highest rates of infection are found in intensive care units, burns unit and organ transplant units. While only 15% to 20% of all hospital beds are located in intensive care units, 40 ï 60% of all life threatening nosocomial infections occur in these units. (Hassiengren *et al*., 1980). Neo natal intensive care units have been reported to have rates of nosocomial infection of 6 ï 25%, among organ transplant patients. 40% for those who received the kidney from a living related donor and 40% for those who received it a deceased donor. The etiology of the infection and types of infection vary among settings. One study found that for patients in burns units, the body surface area burned, comorbidites and use of invasive devices were significantly associated with

nosocomial infection and *S aureus* and *Pseudomonas* were the most common resistant bacteria identified. (WHO, 2002).

2.12.1i Iatrogenic factors

Three primary latrogenic factors contribute to the development of nosocomial infection; devices and equipment used in the healthcare setting, surgery and use of antibiotics. The four most common nosocomial infections ï urinary tract infection, surgical site infection, pneumonia and intravascular device ï related blood stream infection and these infections comprise approximately 80% of all nosocomial infections (CDC, 2006).

2.12.1j Devices and equipment

Nosocomial infection has been associated with several types of devices and equipment in healthcare facilities. The Spaulding classification developed in 1968 is widely used to categorize devices according to their associated risk of infection (Cruse, 1973). The system includes three categories:

i. Critical: A device that enters normally sterile tissue or the vascular system.
ii. Semi critical: A device that comes into contact with intact mucous membranes and does not ordinarily penetrate sterile tissue.
iii. Noncritical: A device that does not ordinarily touch a patient or touches only intact skin. Most nosocomial infections can be attributed to devices in the critical and semicritical categories including intravascular catheters, surgical drains, urinary catheters, and endoscopic instruments (Ducel and Nicolle, 2002). All instances of infection during endoscopy have been the result of noncompliance with established guidelines, showing

the importance of adhering to recommendations for reprocessing of endoscopy equipment. (McEwin, 2008). Noncritical devices are often overlooked by healthcare workers as vectors for infection. These devices include diagnostic equipment, sethescopes and other common place items. A systematic review of 23 studies found bacterial contamination of 87% of sampled healthcare equipments as well as diagnostic ultrasound equipment, otoscopes and auriscopes (Dantas *et al*., 2006). Most (27%) of the organism were *Staphylococcus aureus,* 15% of which were multidrug resistant.

In summary, a high rate of micro organism colonization has been found on equipment within a hospital setting but contamination is usually at low levels and risk of direct infection is low. Adequate disinfection with 70% alcohol solution can reduce the levels of contamination with one report of an 82% reduction. In general, the findings of studies have suggested the adequate cleaning of equipments can prevent as many as one third of nosocomial infection (Ikeh and Isamade,2011).

2.13 Epidemiology of nosocomial infection

As already stated, nosocomial infection is a worldwide problem. In the United States of America according to a CDC report estimates that nearly 2million patients are infected yearly and about 9,000 people died of nosocomial infections yearly. The number of infection rose from 72 to 98 per 1000 patients from 1975 through 1995 (Hanley *et al*., 1995). From 1985 to 2005, the rate of nosocomial infection had risen to 38% especially in developing countries (Ihn *et al*., 2006). As at 2003, cost of treatment of 2 million nosocomial infections in the United States exceeds 4.5 billion dollars each year (Rick, 2007). The report also indicates that one third of nosocomial infections is preventable. In France, the prevalence rate was

6.8% in 2001 and 7.5% in 2006 (Andrew,2010). The increase was due to some patients were infected twice. A study conducted in Egypt by El- Bayoumi *et al*.,(2006)put the patient infection rate at 16.9% and 78 episodes of infection per 1000 paediatric intensive care unit days. Nosocomial infections have also been reported in Nigeria (Okezie and Onyemelukwe, 2007; Oni *et al*., 2006).

The sites of nosocomial infections in order of most to least common are as follows:

- Urinary tract
- Surgical wounds
- Respiratory tracts
- Skin
- Blood
- Gastro intestinal tract
- Central nervous system

(Kelvens *et al*., 2007).

2.14 Types of nosocomial infections

2.14.1 Surgical site infection/wound infection: Surgical site infections are an important cause of increased cost, morbidity and mortality in most hospitals. They are also the second most frequent nosocomial infections (Kelvens *et al*., 2007). *Staphylococcus aureus* is a major cause of hospital acquired infection of surgical wounds. Foreign bodies such as sutures and implants (plates) are readily colonized by Staphylococci which makes infection difficult to control. *Staphylococc*i strains expresses a fibrin/fibrinogen binding protein which promotes attachment to

blood clots and traumatized tissue (Clauditz *et al*., 2006). According to CDC definition, when infection occurs within 30 days after the operative procedure and involves only skin and subcutaneous tissue, it is regarded as superficial surgical site infection and if implant is left in place and infection occurs after one year, is regard as deep incisional surgical site infection.

The signs and symptoms of wound infection include purulent discharge, pain or tenderness, redness, localized swelling etc. The presence of blood ie haematoma at the surgical site stimulates microbial proliferation by providing ferric iron with degradation of red blood cell leading to infection. The source of bacteria causing surgical site infection may be from air in the operating room or surgical equipment or breaches in the surgeonôs gloves. The rate of post operative wound infections vary from one hospital to another. Maniatis *et al*.,(1997) suggested that deep ï seated sepsis developing a few days after an operation and before the wound has been dressed reflect a theatre infection. Ward infections tend to be more superficial and frequently follow the dressing of wounds in the wards. Similarly, skin infections such as boils or abscess developing at sites other than the operation site indicate that the infection was acquired in the ward (Kirkland *et al.,* 1991). The virulence and invasive capability of the organism in the wound and immunological integrity of the host seem to be equal importance in determining whether infection occurs. Microbial densities of 10^5 or more organisms per gram of tissue indicates infection and less count reflects wound contamination (Gardner, 2010). Bacteriological studies have shown that surgical site infections is universal. In a study conducted by Joshy *et al.*(2007) in United Kingdom to identify organisms causing delayed healing and deep infection after primary total knee arthroplasty found that 36% of the infection was caused by Methicillin resistant *Staphylococcus aureus* (MRSA). Sisirak *et al*. (2010) in Bosnia also found *Staphylococcus aureus* to be the most common isolate from wound infections at 22% prevalence

rate. Elsewhere in Nigeria at University College Hospital, Ibadan, Oni *et al*.(2006) found *S. aureus* as the leading etiologic agent (29%) in surgical wound infections.

2.14.1 Urinary tract infection (UTI)

This is the most common nosocomial infection (Kleven *et al*, 2007). 80% of the infection are associated with use of an indwelling bladder catheter. Urinary tract infections are associated with less morbidity than other nosocomial infections but can occasionally lead to bactremia and death. The signs and symptoms include fever, urgency, frequency, dysuria and supra pubic tenderness. Microorganisms such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus saprophyticus*, *Escherichia coli*, *Psendomonas* species and *Klebsiella* species have been implicated in urinary tract infections (Muder *et al.,* 2006). The risk of acquiring a catherter associated urinary tract infection depends on the method and duration of catherization, the quality of catheter and host susceptibilities (Iwuchukwu., 2005). Most of the bacterial organisms responsible for urinary tract infection including *Escherichia coli* and *Kebsiella* species are part of the normal colonic flora. With alternations in the normal barriers to infections including the presence of a foreign body such as urethral catheter, these strains may colonize and infect the urinary tract. A number of potential urinary pathogens are not part of the normal colonic flora however and are acquired by patients from hospital environmental sources or other colonized patients, with the use of closed strile systems, the hands of healthcare workers have become an increasingly important source of these infections (Pittet *et al*.,1994).

2.14.2 Pneumonia

Nosocomial pneumonia occurs in several different patients groups. The most important are patients on ventilators in intensive care units where infection rate is 3% per day. Microorganism that colonize the upper air way and bronchi may cause infection within the lungs, they are often endogenous but may be exogenous often from contaminated respiratory equipments. Directly by passing primary defense barriers with endotracheal intubation predisposes the host to infection as well as use of immunosuppresive and cytotoxic drugs and radiotherapy which impair defense mechanism. The signs and symptoms of pneumonia include cough, chest pain, fever and purulent sputum *Staphylococcus aureus* still causes nosocomial pneumonia. Infection could therefore be due to patientôs own strain or those transmitted to patients by person to person contact. Patients with nosocomial pneumonia are usually the elderly and have underlying disease (Prescot, 2004).

2.14.3 Bacteremia

There infections represent a small proportion of nosocomial infections (5%) (Klevens *et al*, 2007), but the case fatality rate is high. Multidrug resistant coagulase negative *Staphylococcus* (CONS) infection may occur at the skin entry site of the intravascular device or in the subcutaneous path of the catheter. Organisms colonizing the catheter within the vessel may produce bactremia without visible external infection. The resident or transit cutaneous flora is the source of infection. Purulent drainage found at the site of an intravenous catheter, inflammation of the sites and positive blood culture result is regarded as positive for bacteremia. Symptoms include fever, chills, oliguria and hypotension.

2.14.4 Gastroenteritis

Gastroenteritis of nosocomial origin occurs if vomiting or diarrhoea having its onset after admission and associated with a culture that is positive for a known pathogen. The true incidence of nosocomial gastroenteritis is unknown due to manifestations of gastro intestinal infections are nonspecific. The etiologic agents for most cases of nosocomial gastroenteritis include *Salmonella species*. *Escherichia coli* and *Staphylococcus aureus*. Staphylococcal food poisoning occurs when coagulase positive *Staphylococcus aureus* produces a heat stable enterotoxin and is allowed to proliferate to high titers in food before ingestion. The illness is an intoxication. The source is a kitchen staff employed with staphylococcal disease e.g pustule or furuncle on the hands. The incubation period is usually less than 4 hours. Symptoms include acute onset of nausea, vomiting and watery diarrhea. There is no fever. It occurs mainly in the young, elderly and those with compromised defenses (Prescot, 2004).

2.14.5 Cutaneous soft tissue infection

Many of todayôs nosocomial infection of the skin and subcutaneous tissues are caused by organisms which are usually considered non pathogenic and part of the normal body flora. Altered host responses and prolongation of life by new therapeutic regimes have been responsible for the emergence of pathogenic organisms formerly thought to be harmless. In addition, antimicrobial therapy alters the normal flora thereby enhancing the infectivity of certain organisms and allowing the overgrowth of organisms previously held in check by normal flora population. *Staphylococcus aureus* continues to be the most common single bacterial organism causing nosocomial skin infections. In hospitals, the most important route of transmission of this

organism occurs via the hand of healthcare workers, however air and fomites are less significant vectors (CDC, 2011).

2.14.6 Burns

One of the CDC definition of infection site is burns and burns infection must meet the following criteria patients must have change in burn wound appearance or character such as rapid eschar, odema at wound margin, fever or hypotension. Colonization of burn surfaces with bacteria makes sepsis an ever present threat. In addition these colonized wounds serve as reservoir for the spread of nosocomial infection to patients. A burn is a coagulative necrosis of the skin sometimes including the deeper tissues caused by dry heat, electricity, chemicals and irradiation. Burns occur most commonly in children who sustain their injuries at home Burns in adult often occur at work or as a result of road traffic accidents. Due to the fact that burned patients experience immunosuppresion due to a multitude of factors including the loss of skin as a barrier to microorganism dermal necrosis, presence of protein rich exduate, depression of neutrophilic functions depression of opsonins, complement and other defense mechanisms, prolonged hospital admission and hardship of aggressive procedures e.g blood vessel and bladder catheterization, they are susceptible to a variety of nosocomial infections (Askarian *et al*., 2009). The most frequently recovered organisms depend on patientôs normal flora, duration of hospital stay and each hospitalôs local nosocomial pathogens. Organisms involved in burns infection include *Staphylococcus aureus* and other Gram negative bacteria. (Keen *et al.,* 2010). Burns surfaces are initially sterile due to the fact that heat kills all microorganisms but within 48 hours, it is typically colonized by Gram positive skin flora such as *Staphylococcus aureus* and ß haemolytic *Streptococcus* present in the sweat gland or deep within hair follicles. After 48 hours

72 hours wounds become colonized with endogenous Gram negative bacteria from the patients respiratory and gastrointestinal tract such as *Pseudomonas* aeruginosa as well as microorganisms from the hospital environment or healthcare workers.

2.15 Prevention and control

In general, the spread of infectious disease is prevented by eliminating the conditions necessary for the microorganism to be transmitted from a reservior to a susceptible host. This can be achieved by

i. Destroying the microorganism
ii. Blocking transmission
iii. Protecting individuals from becoming vectors of transmission
iv. Decreasing the susceptibility of potential hosts.

Aseptic techniques and antibiotics will kill microorganisms, while proper hand washing will block their transmission. Gloves, gowns, caps and masks, remove the healthcare workers from the transmission cycle by protecting them from contact with microorganisms. Contact precautions and isolation techniques help patients avoid being vectors of transmission. Lastly ensuring that patients and healthcare workers are immune or vaccinated can help decrease the availability of potential host.

Nosocomial infection is a major problem for patients safety and its surveillance and prevention must be a first priority for settings and institutions committed to making health care safer.

Staphylococci infections may be prevented and controlled on many levels:

1. Presently there is no vaccine yet against Staphylococcal infections due to the possession of a microcapsule which is poorly immunogenic and has made production of effective vaccine difficult. However clinical trials are on going for use of pentastaph produced by a pharmaceutical company (Nabi). It consists of a polysaccharide conjugate vaccine and toxoid vaccine (Levinson, 2012).
2. Intra nasal mupirocin or oral antibiotic can be used to reduce persistent colonization among healthcare workers and shedders removed from high risk areas like operating theatre, nurseries, burns ward and intensive care units.
3. CDC/Health Care Infection Control Practices Advisory Committee in the United States of America recommends that either antimicrobial soap or a waterless agent e.g. alcohol-based hand washing/hand hygiene before and after each patient contact.
4. Each hospital should have a surveillance program as emphasized by the World Health Organization.
5. Training and education of staff on nosocomial infection. Healthcare waste should be properly disposed as a means to combat hospital acquired infections
6. Each healthcare facility should have antimicrobial use program and the goal is to ensure effective and economic prescription to minimize the selection of resistant organisms. This policy must be implemented through the antimicrobial use committee.
7. Each healthcare facility should have an effective infection control committee which duties are to formulate and monitor patient care policies, educate all hospital staff in the infection control programme.
8. Use of personal protective equipments, aseptic procedures and good techniques e.g. barrier nursing is known to prevent nosocomial infections

9. Isolation of patients with MRSA and terminal cleaning of their rooms is also advocated
10. Surfaces in hospitals may be cleaned with70% alcohol, quaternary ammonium or iodine. Surface sanitizing using non flammable alcohol vapour in $C0_2$ (NAV-$C0_2$) system is effective against MRSA and can be used both for routine and terminal cleaning.
11. Spread of infection (particularly staphylococcal) is most likely to occur in large opens wards. Wards should therefore be subdivided into units of 4 to 6 beds with complete separation from other areas and adequate single rooms for isolation of infected patients. Bed space should be at least 8 feet apart. Overcrowding should be avoided.
12. Lesion or wound due to Staphylococci should be carefully treated and covered with impervious dressing.
13. Sterile techniques for invasive procedures and limiting the amount of time a patient spends intubated.
14. Avoidance where possible of medical procedures that can lead to high probability of nosocomial infection.
15. Good surgical technique, reduction of traffic in the theatre, use of unidirectional laminar air flow and positive pressure ventilation systems in the operational infection.
16. Food handlers should be screened for *Staphylococcus aureus* infection
17. Only pasteurized milk should be consumed and cows checked regularly for mastitis.
18. Patients right to safe care should be introduced through health education and public health campaigns.
19. Biological control might be a new possible way to control *Staphylococcus aureus* in body surfaces. Colonization of body surfaces (especially in the nose) by *Staphylococcus epidermidis* (inhibitory strain JK 16) impairs the establishment of *Staphylococus aureus.*

A 2011 study points to this new possible way to control *Staphylococcus aureus* (Iwase *et al*., 2010). These findings open the way to a biological control therapy to help in the treatment of S*taphylococcus aureus* infections which are becoming a growing threat due to the rise of resistance to conventional antibiotic treatment (Iwase *et al*., 2010).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study Center

This epidemiological survey was carried out in National Orthopaedic Hospital, Enugu, South-east Nigeria. It is a 250-bed tertiary hospital which serves as a referral centre for the zone, south-south and parts of north-central geo political zones of Nigeria. It is also a regional burns centre established in 1972 and made up of 12 wards and 3 theatres excluding the Out patient Department and Staff Clinic.

3.1 Study Period

This study was carried out between May 2013 and June 2014.

3.2 Ethical Consideration

Ethical approval for this work was obtained from the hospitalôs ethical committee (Appendix 1). Also informed consent was obtained from the patient or their caregivers on admission as well as from the healthcare workers. The nature of the project was explained to the doctors and nurses in the wards and their co operation solicited.

3.3 Sample Size

Sample population was 100 hospitalized patients that have stayed in the hospital up to three days and above and showed no signs of infections at the point of admission. One hundred patients (comprising 50 orthopaedic and 50 burns patients) who consented to participate in the study were recruited using purposive sampling (Tables 3.1 and 3.2). Exclusion criteria for the patients

include those diagnosed of cancers, spinal cord injury, diabetes and HIV/AIDS. One hundred healthcare workers who were in frequent contact with the patients during the period of the survey which included nurses, doctors, medical laboratory scientists and health assistants were also recruited for the study(Table 3.3). For the population size, the confidence level was set at 95% and the margin of error was set at 5%. A population size of 100 was obtained.

3.4 Study Sample

Bio-data of the patients and other information related to the study were obtained using a questionnaire and folders of the patients which included sex, age, diagnosis, etc. Two hundred urine samples and wound swabs were taken, the samples were collected twice, one was taken 72 hours after admission and the other on suspicion of infection from each patient before discharge. Seven pus and twenty-two blood samples for culture were also taken from the patient upon the clinicianôs request following suspicion of sepsis. One hundred nasal swabs were taken from healthcare workers and questionnaires also filled appropriately (which included age, sex, years of service and professional group). Three hundred and seventy-eight surfaces were swabbed from twenty-two different fomites (which included oxygen machines, thermometers, floors, sinks) from seven different wards namely Trauma ward, Childrenôs ward, Burns ward, Septic ward, Arthroplasty ward, Male ward 3, Female ward I and 3 theatres- Main theatre, Burns/Emergency theatre and Septic theatre.

3.5 Sample Collection According to Cheesbrough (2004)

3.5.1 Pus Specimens

Pus specimens were collected from the patients using sterile 5 ml syringe and needle either by collecting it from the drainage tube observing aseptic precautions or from the infected site by

aspirating and transferring to a sterile universal container according to (Cheesbrough, 2004). . These were labeled and taken to the laboratory for further processing.

3.5.2 Blood Samples

Blood culture samples were taken when the patients were having fever. It was done by applying a tourniquet and locating a fixed vein by touch, then the skin around the area was sterilized by swabbing it with methylated spirit and allowed to dry. Venipuncture was performed and 10 ml of venous blood withdrawn with sterile 10 ml syringe and needle. The protective cover of the Signal Oxoid blood culture bottle containing brain heart infusion broth was removed and the needle used in collecting the blood was discarded and replaced with a new needle. The sample was dispensed into the blood culture by inserting the new needle through the rubber liner of the bottle. The top of the protective cover was cleaned with 70% alcohol and the cover replaced. The blood sample was then mixed with the blood culture broth and the container labeled with the patientôs name and date of collection. It was then incubated at 37^0C for ten days and subcultured every three days. In case of burns patients, the doctors collected the sample for blood culture by femoral tap while observing aseptic precaution.

3.5.3 Urine Samples

Mid-stream urine samples were collected from the symptomatic patients for urine culture. Male patients were advised to cleanse the area around the urethral opening with clean water and dry it. Then the first part of the urine was allowed to enter the toilet or bed pan while the middle flow was collected with a sterile universal container. It was labeled and sent to the laboratory for further processing. Female patients were also instructed to clean the area around the urethra with

clean water and dried with towel. The mid-stream urine was collected with the labia held back with the fingers and first stream of urine allowed to enter the toilet or bed pan and the middle flow to a sterile universal container. It was labeled appropriately and sent to the laboratory for further analysis. Catheter urine samples were obtained with the aid of the nurses. It was obtained by attaching a small diameter sterile needle to a 10 ml syringe and aseptically inserting it just above the location where the catheter was attached to the drainage tubing. The urine was aspirated and placed in the labeled sterile universal container and taken to the laboratory for processing without delay (Cheesbrough, 2004).

3.5.4 Wound Swab

Wound swab collection was done using Levine method (Gardner,2010)

In collecting wound swab, the hands were washed and a pair of gloves worn. Then the old dressing was removed. If the wound was fairly dry, the swab will be moistened with sterile normal saline before swabbing. The gloves were changed and then the surface of the wound was cleansed with sterile normal saline removing excessive debris and all dressing residue without unduly disturbing the wound surface. It was left for two minutes before taking the swab. The swab was taken from viable tissue where there were clinical signs of infection such as pus by rotating it over 1cm area of the wound with enough pressure to express fluid from the tissue while avoiding surrounding skin. The hands were decontaminated and swab was labeled and taken to the laboratory for processing.

3.5.5 Nasal Swabs

Nasal swabs from the healthcare workers were collected using appropriately labeled sterile swab sticks. It was taken from anterior nares of the healthcare workers, by introducing the swab 2 to

3cm in the nasal cavity and rotated 4 to 5times both clockwise and anticlockwise. It was returned to the swab container and taken to the laboratory for further processing.

3.5.6 Fomites (According to Ikeh and Isamade, 2006)

Swabs were taken from the fomites in wards and theatres by labeling the swab stick and using the cotton-tipped end to make a circular motion sweep over the surface of the fomite unti It is visibly dirty and the swab was returned to the swab container and taken to the laboratory for processing.

3.6 Microbiological Analysis

3.6.1 Microbial Isolation

All the swabs collected for bacteriological investigations were treated according to the methods of Cheesbrough, (2004).

The swabs and pus were inoculated onto well dried blood agar and mannitol salt (Oxoid) agar plates. They were then streaked to obtain discrete colonies using sterile wire loop. These were then incubated aerobically at 37^0C overnight and those without growth were left for another 24 hours. The inoculated blood culture bottles were subcultured to blood agar and mannitol agar plates as described above every three days and if there was no growth, it was further left for ten days according to the manufacturerôs instruction (Signal Oxoid system).

The appearance of the urine samples were noted. It was inoculated onto well-dried, blood agar and mannitol salt agar plates using a wire loop that can deliver 0.02 ml of urine. It was also incubated at 37^0C over night at aerobic conditions. Then the remaining samples were poured into dry labeled plastic tubes and allowed to stand for 30 minutes, the supernatant was discarded and the deposit viewed with x 10 and 40 objective and the result recorded. The presence of white

blood cells, red blood cell and yeast cell was recorded. Presence of more than 5 leucocytes per high power field indicated pyuria. For catheter specimens, colony counts of $>10^2$ cfu/ml of urine was regarded as been significant and for non-catheterized specimens, count of $>10^5$ cfu/ml of urine was regarded as significant. All suspected *Staphylococcus* isolates were sub cultured to nutrient agar slants for further characterization. Isolated colonies were identified culturally, microscopically and biochemically as described by Cheesbrough (2004).

3.7 Identification Procedure

All protocols for identification were in accordance with Cheeesbrough (2004).

1. Macroscopy:-

The primary identification of the staphylococcal species were based on colonial appearance, pigmentation, colour and odour.

2. **Gram staining**

Principle: Following staining with a triphenyl methane basic dye such as crystal violet and treatment with iodine, the dye iodine complex is easily removed from permeable cell wall of Gram negative bacteria but not from the less permeable cell wall of Gram positive bacteria.

Procedure

The dried smear on the slide was fixed by passing it through flame thrice. The smear was flooded with crystal violet stain for 30 seconds, washed off with clean water. The smear was covered with Lugolôs iodine for 30 seconds, washed off with clean water, decolourized rapidly with acetone and washed off with water. It was then counterstained with neutral red for 30

seconds and washed off with water. The back of the slide was wiped with cotton wool and placed in a draining rack for the smear to air dry . It was viewed with oil immersion objective. Gram positive bacteria appeared as dark purple cocci or rods while Gram negative bacteria appeared as pinkish red rods. *Staphylococcus* species appeared as Gram positive cocci in clusters.

3.8 Biochemical test

i. Catalase test

Principle: Catalase acts as a catalyst in the breakdown of hygrogen peroxide to oxygen and water. An organism when tested for catalase production by bringing it into contact with hydrogen perioxide. Bubbles of oxygen are released if the organism is a catalase producer.

Procedure: On a clean grease-free slide, 2 drops of 5% hydrogen peroxide were placed using a clean Pasteur pipette. A few colonies of the test organism was picked from sensitivity test agar slants using one end of a clean slide and allowed to come in contact with the reagent. Active bubbling indicated positive catalase test while absence of bubbles indicated negative test. *Staphylococcus* species were catalase positive while *Streptococcus* specie*s* were catalase negative.

ii. Coagulase test

Principle: Coagulase causes plasma to clot by converting fibrinogen to fibrin.

Procedure: A drop of distilled water was placed on each end of a grease-free slide with a clean pasteur pipette. A colony of the test organism was emulsified on each of the drops and rocked for about 10 seconds to check for autoagglutination. If absent, a loopful of pooled EDTA human Plasma was then added to the suspension and mixed gently using a sterile wire loop. Clumping

within ten seconds showed positive test while no clumping was regarded as negative test. *S. aureus* was positive while *S. epidermidis* was coagulase negative.

iii.Tube Coagulase

Procedure: Overnight broth culture of the test organism that gave negative slide coagulase test were further tested for tube coagulase. Equal volume of the broth culture of the test organism and plasma were mixed in a test tube and incubated at 37^0C. The tubes were then observed gently for clot formation every 1 hour. If negative, after 4 hours, it was left overnight and the final reading was done after 24 hours. Tubes that did not form a clot after 24 hours were regarded as negative coagulase test.

3.9 Confirmatory Cultural Characteristics

3.9.1 DNase Agar Plates

Principle:- Deoxyribonuclease hydrolyses deoxyribonucleic acid (DNA). The test organism is cultured on a medium which contains DNA. After overnight incubation the colonies are tested for DNase production by flooding the plate with a weak hydrochloric acid solution. The acid precipitates unhydrolysed DNA. DNase ï producing colonies are therefore surrounded by clear areas due to DNA hydrolysis

Using a sterile wire loop, a smear of the test organisms were made from the overnight broth culture of the *Staphylococcus* species in four sections of a freshly prepared, well dried DNase agar plates. They were incubated overnight at 37^0C. then IN HCl was used to flood the plates and the excess drained off. It was left to act for about 10 minutes after which the plates were observed for clear zones around the smears of the test organisms. The presence of clear zone indicated a positive DNase test while the absence of a clear zone indicated a negative DNase test.

S. aureus gave positive result (diameter of clear zone greater than 0.5mm) while *S. epidermidis* gave a negative result (diameter of clear zone less than 0.5mm) (Bello and Qautani, 2005).

All isolates that were Gram positive cocci in clusters or in pairs, catalase positive, coagulase positive and DNase positive were regarded as *S.aureus*.

3.10 Antibiogram

3.10.1 Disc Diffusion Technique

All the *S. aureus* isolates from patients specimens(urine, pus, blood and wound swabs) were tested for susceptibility against the following antibiotics (Oxoid single disc), - Levofloxacin 5mg, Oflaxacin 5mg, Ciprofloxacin 5mg, Pefloxacin 5mg, Erythromycin 15mg, Gentamycin 10mg, Ceftriazone 30mg, Ceftazidime 30mg and Amoxycillin/Clavunate (Augumentin) 30mg and Vancomycin 15mg. with the aid a sterile wire loop, a colony of the isolate from sub-cultured plates were streaked on the surface of freshly prepared, well-dried nutrient agar plates. The single discs were placed on the plates using sterile forceps. It was incubated overnight at 37^0C and the zone of clearing was measured using a transparent ruler (mm) and a pair of compass from the reverse side of the plate and the readings were recorded accordingly. Zones measuring less than 12 mm were regarded as resistant and those 12 mm and above as sensitive.

3.10. 2 Oxacillin screening test

Procedure:- All *S aureus* isolates were subjected to oxacillin (Iug) disc susceptibility testing using Mueller- Hinton agar supplemented with 4% sodium chloride. About 3 ï 4 colonies of each isolate from an overnight culture were picked with sterile wire loop and emulisifed in 5mls of sterile normal saline in a Bijou bottle. The emulsified suspension was compared with 0.5 mac farland standards. A sterile cotton swab was dipped into the suspension and rotated several times

and pressed firmly on the inside wall of the bottle above the fluid to remove excess inoculum from the swab. The Mueller- Hinton agar plate was inoculated by streaking the swab over the entire surface to ensure an even distribution of inoculum. Thereafter, 1µg oxacillin disc was placed onto the inoculated plate. This was incubated at 37oC for 24 hours. The zone of inhibition was read as recommended by the clinical laboratory standard institute (2012). Zones of clearing below 12 mm was regarded as resistant and 12 mm and above was regarded as sensitive.

3.10.3 Resistance to Novobiocin

Aim:- To differentiate the coagulase negative *Staphylococcus*

Procedure:- All the Staphylococcal isolates from urine culture of patients and coagulase negative isolates from fomites were tested against the drug. The freshly prepared dried sensitivity agar was streaked with the test organism and the disc placed on the inoculated plate and incubated at 37^0C overnight. A zone of inhibition of less than 12 mm indicates resistance to Novobiocin while a zone of inhibition greater than 12 mm indicates sensitivity. *S. saprophyticus* was Novobiocin resistant while *S. aureus* and *S. epidermidis* were Novobiocin sensitive.

3.11. Statistical analysis

Chi square analysis and analysis of variance (ANOVA) were used to analyse the results of the study at ($P<0.05$) level of significance.

CHAPTER FOUR

4.0 RESULTS

Among staphylococcal nosocomial infections detected by culture in NOHE as recorded in Figure1 (these figures are shown in Table 1 in the Appendix.), wound infections ranked highest with 60 (70.6%), followed by urinary tract infection (UTI) 20 (23.5%) with Bacteremia 3 (3.5%) and soft tissue infection (STI) 2 (2.4%) ranking the least. A statistically significant difference (P.<0.05) were observed when the highest 60(wound infections) were compared with 2 the least from soft tissue infection. For the wound infections, females 35 (41%) were more involved than their male counterparts with 25 (29%), UTI, males were 11 (13%) and females, 9 (11%) while for bacteremia and STI, no males were observed as only females were involved with 3 and 2 respectively. When over all sex prepondance were tested using x^2 test, no statistically significant differences were observed (P>0.05).

Overall, road traffic accident cases yielded the most of *S. aureus* nosocomial infections with 29 (34%), followed by flame burns 25 (29%), scald burns 10 (12%) with matchet cut being the least with only one isolate. A statistically significant difference (p<0.05) was observed when the number of *S. aureus* isolates from different patients categories were compared (p<0.05).

The age range 16-30 years yielded the highest rate of isolates of *S. aureus* with 22 (26%) followed by 1-15, 12 (14%) are the least with 56-70 years of age with only 1 isolate. Again these differences were statistically significant (P<0.05). Length of stay showed that the highest isolates were recorded for 1-2 months 55 (65%), followed by 3-4 months with 18 (21%), 5-6 months, 8 (9%) and the least was 7-8 months with 4 (5%) which difference were statistically significant (P<0.05).

For total body surface area, Table 1 shows that the highest isolate were recorded for 16-30% with 22 (26%), followed by 1-15% with 12 (14%) while 31 to 45% and 46-55 bracketed at 2 each and 56-70% had the least with only 1 when tested. There were significant statistical differences ($P<0.05$) with ANOVA.

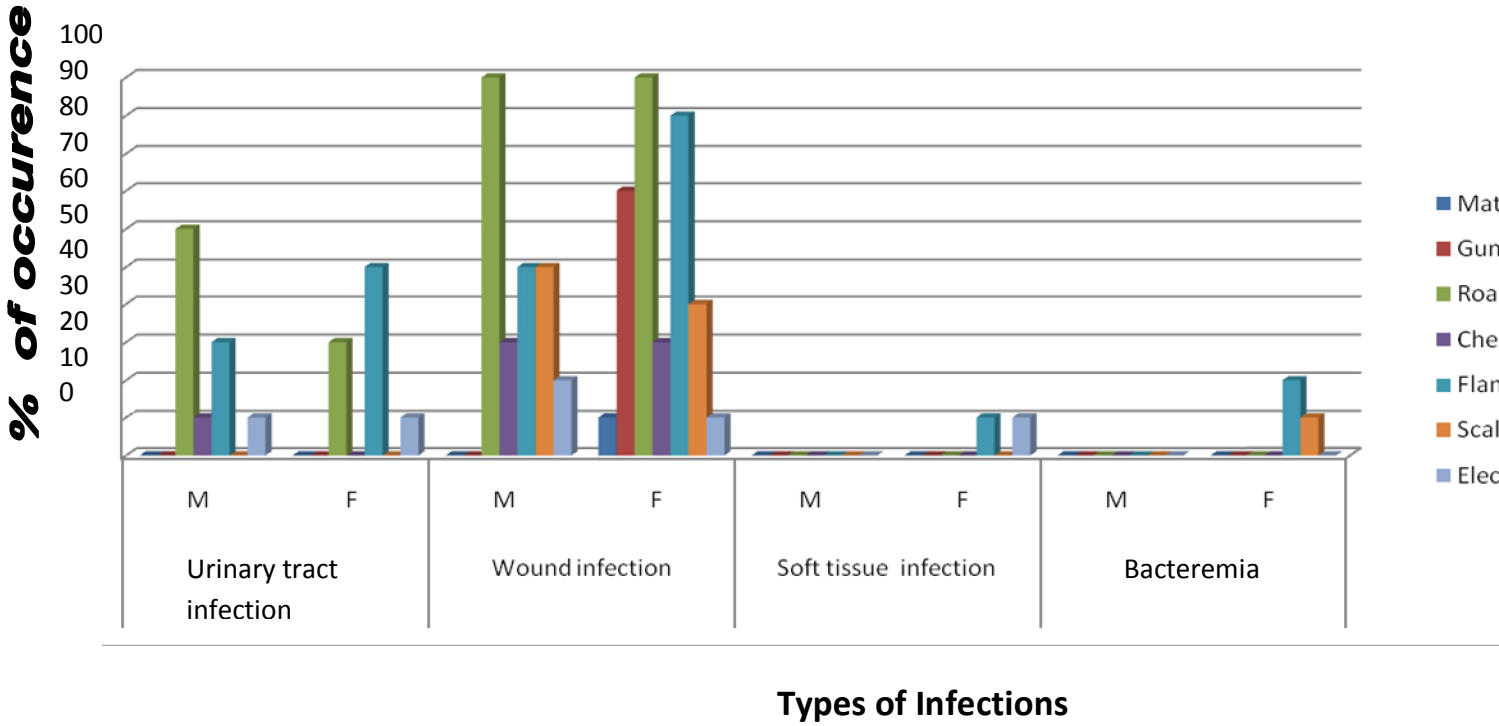


Fig. 1Distribution of staphylococcal nosocomial infections in NOHE patients

Figure 2 shows the distribution of staphylococcal nosocomial Infections according to patient category or injury. It was observed from this graph that patients that had flame burns had four types of staphylococcal nosocomial infections ; wound infection, urinary tract infection, bacteremia and soft tissue infection and ranked highest 25(29%) while patients with matchet cut had only 1 type of infection, wound infection 1(1.2%). There was significant difference between burns and matchet cut, ($P<0.05$). This was followed by patients with scald burns who recorded 3 types of infection namely wound, urinary tract infection and bacteremia. Patients with electrical burns, chemical burns, gunshot and road traffic accident had only 2 types of infections i.e wound infection and urinary tract infection, hence there was no statistical significant difference between scald burns patients and patients with electrical burns, chemical burns, gun shot and road traffic accident respectively ($P>0.05$). These figures are shown in Tables 2 and 3 in the Appendix.

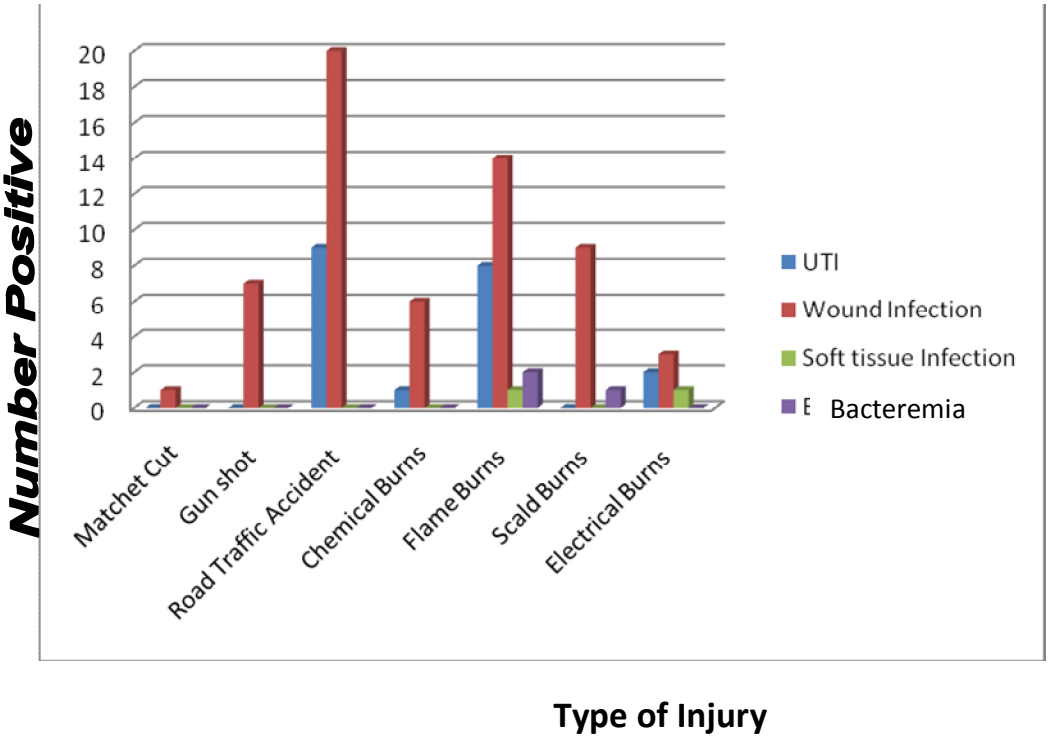


Fig. 2 : **General distribution staphylococcal nosocomial infections according to type of injury in NOHE**

Figure 3 shows the gender distribution of patients with sstaphylococcal nosocomial infections. In this graph it was observed a total of 43 males (51%) and females 42 (49%) were infected. There was no statistically significant difference between the males and females acquiring staphylococcal nosocomial infection (P> 0.05).However it was observed that more females had wound infection 35(41%) than males 25 (29%).This was found to be statistically significant (P< 0.05). 11 males and 9 females (11%) had UTI which difference was not statistically significance. No male was involved in soft tissue infection and bacteremia while only 2 female (2.3%) had soft tissue infection and 3 female (3.5%) had bacteremia. The difference was also not statistically significant (P>0.05). These figures are shown in Table1 in the Appendix.

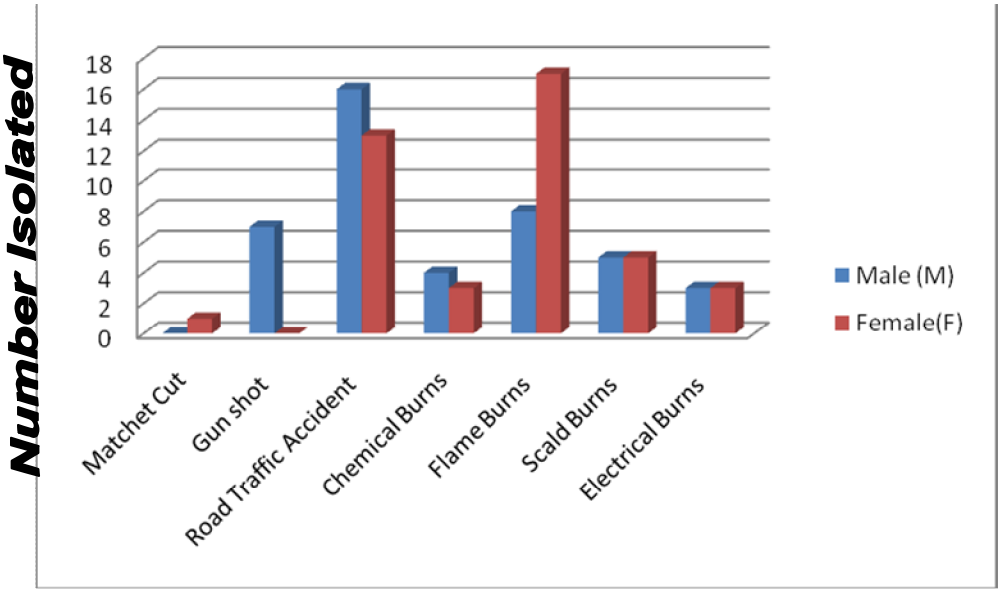


Fig. 3: Gender distribution of staphylococcal nosocomial infections according to injury sustained.

Figure 4 shows the antibiogram distribution of *Staphylococcus aureus* isolates from the patients. It was observed that Vancomycin was the most susceptible antibiotics 79 (93%) followed by Levofloxacin 73 (86%) while the most resistant antibiotics was Erythromycin 68 (80%) . Also 57(66%) of the isolates were susceptible to Oxacillin (Methicillin) while 28 (33%) were resistant (MRSA).Hence there was a statistical significant difference between Vancomycin and Oxacillin(Methicillin).($P<0.05$). These figures are shown in Table 4 in the Appendix.

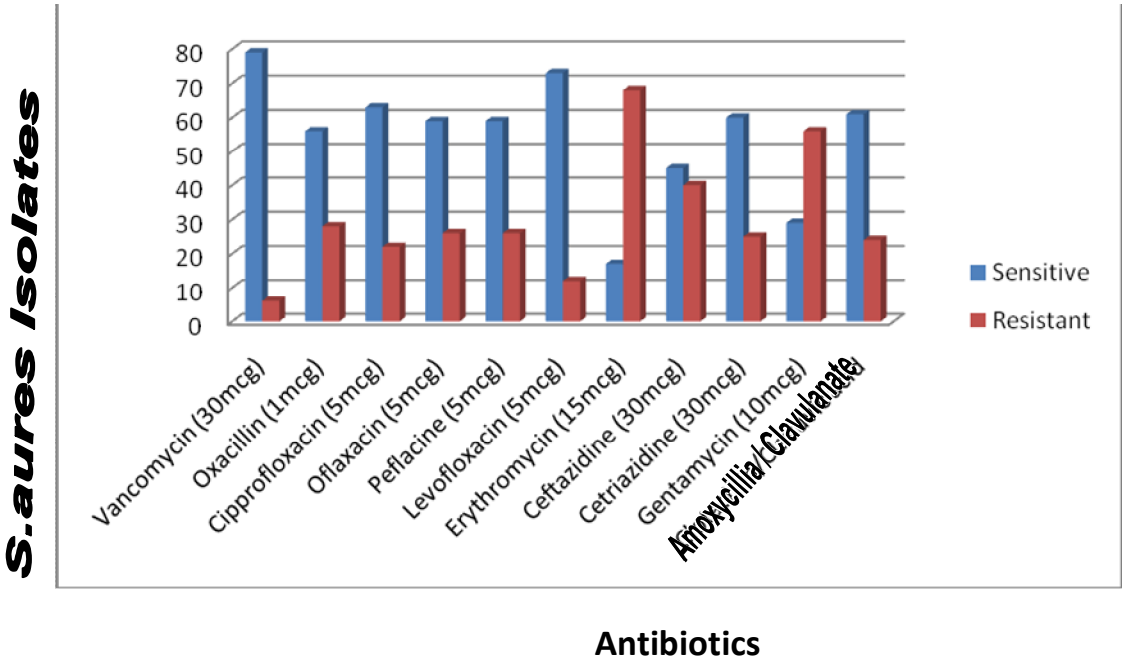
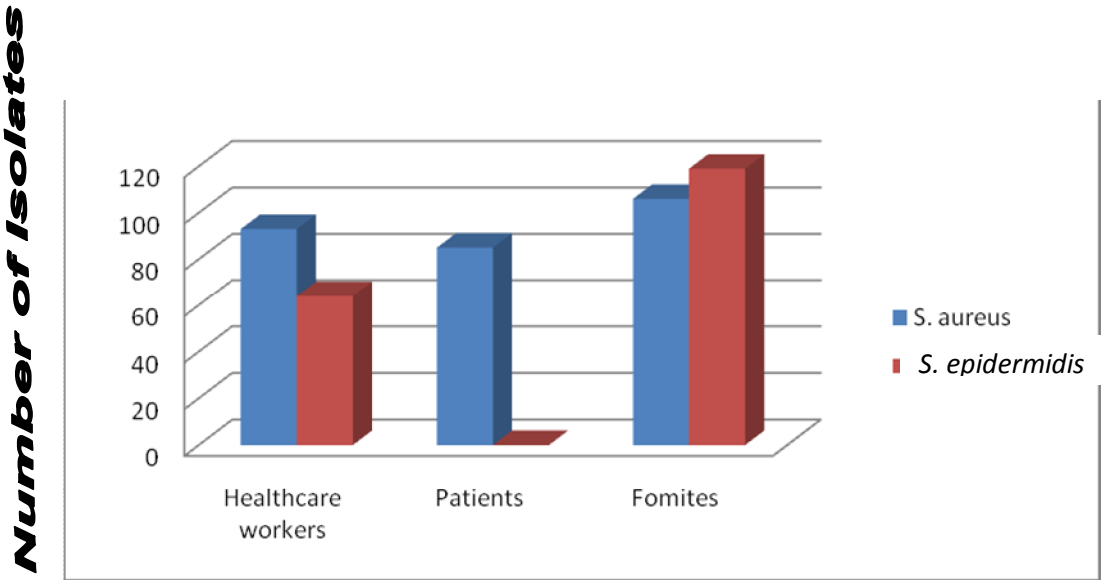


Fig. 4: **Antibiogram distribution of *Staphylococcus aureus* isolates from patients in NOHE**

Figure 5 shows the prevalence distribution of *Staphylococcus species* isolates from human and non human sources in NOHE . Both *S. aureus* and *S. epidermidis* were isolated from both fomites and healthcare workers while only *Staphylococcus aureus* was isolated from the patients. Human sources had a lower isolation rate 178(31%) while fomites (non human sources)had a higher rate of 200 (35%) which difference was not statistically significant ($P > 0.05$). These figures are shown in Table 5 in the Appendix.



Staphylococcus aureus **isolates from human non human sources**

Fig. 5: **Prevalence of *Staphylococcus aureus* from human non human sources in NOHE.**

Figure 6 shows the distribution of *Staphylococcus aureus* from fomites in NOHE (wards and theatres). The overall contribution of fomites to staphylococcal nosocomial infection was found to be 200 (53%) for *S. aureus* and 85 (42.5%) for MRSA. Floor had the highest isolation rate 40 (11%) while none was isolated from operating light handle. This is however statistically significant ($P < 0.05$). These figures are shown in Table 6 in the Appendix.

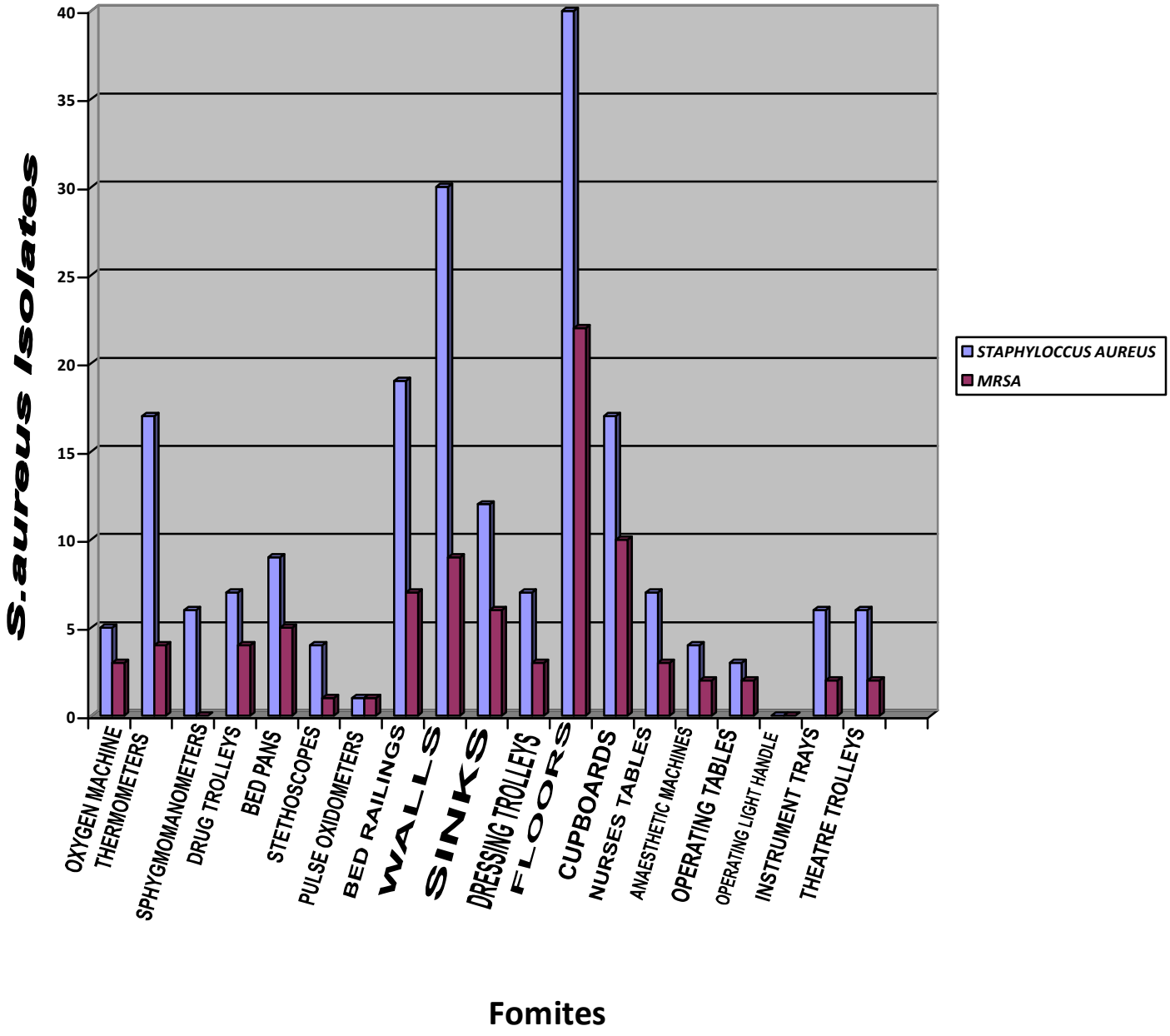


Fig.6: **Overall distribution of *Staphylococcus aureus* from fomites in NOHE**

Figure 7 shows overall distribution of *S.aureus* isolated from fomites in wards of NOHE. It was recorded from the graph that floor had the highest contamination of 20 (7.2%) while pulse oxidometer had lowest contamination 1(.36%). There was statistically significant difference between isolates from floor and that from pulse oxidometer ($P< 0.05$).

From the figure, MRSA prevalence of fomites in wards was found to be 62 (44.2%) while the overall prevalence of *S.aureus* was 140 (50.5%). This was also statistically significant ($P<0.05$) These figures are shown in Table 7 in the Appendix.

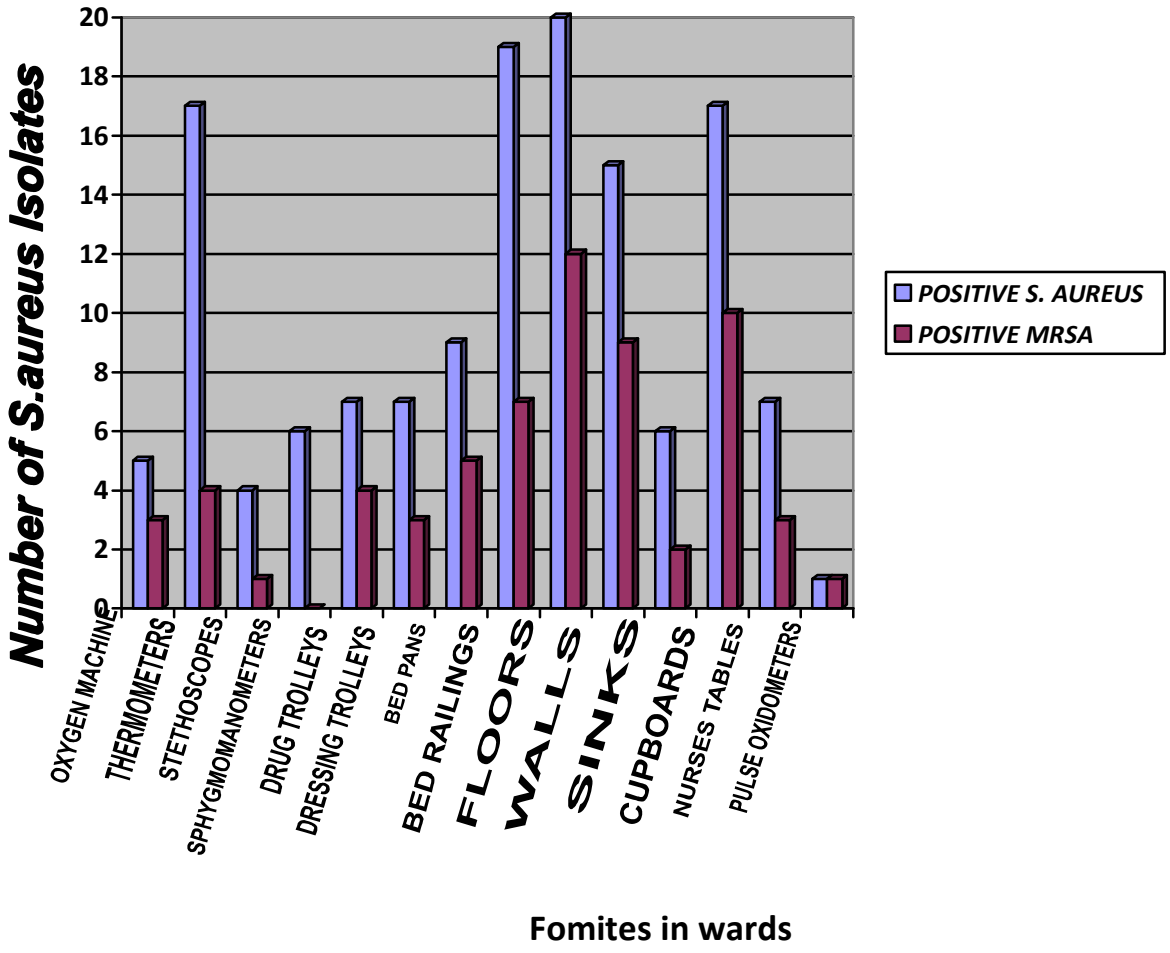


Fig.7:Overall distribution of *Staphylococcus aureus* and MRSA in wards in NOHE

Figure 8 showed the contamination of fomites in the theatres of the hospital. It was also observed that floor had the highest contamination 20 (22.4%) while non was recovered from operating light handle. The overall prevalence of *Staphylococcus aureus* in the theatre was recorded to be 60 (67.4%) while the prevalence of MRSA was 23 (38,3%),This was however statistically significant ($P< 0.05$). These figures are shown in Table 8 in the Appendix.

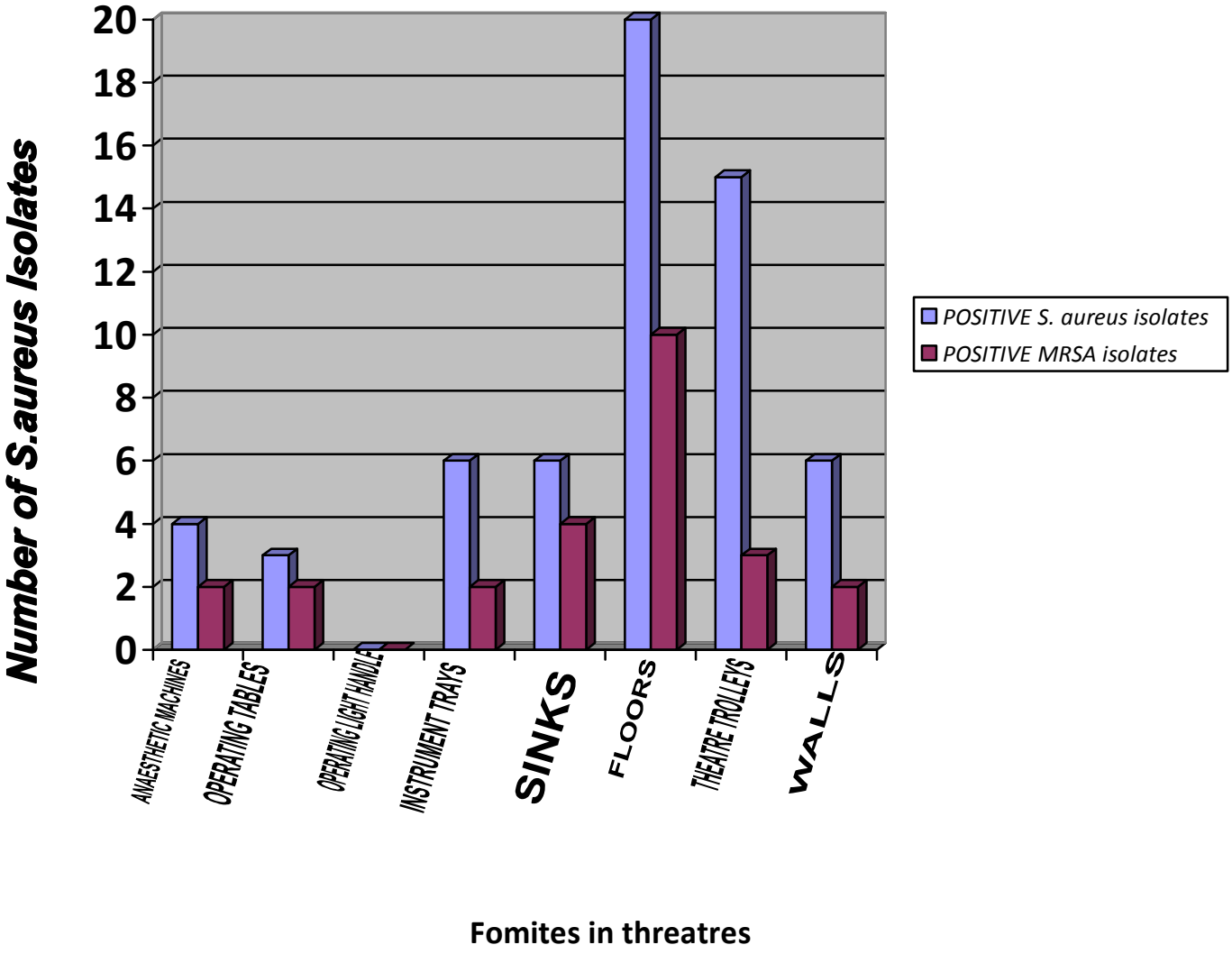


Fig. 8: Distribution of Staphylococcus aureus and MRSA isolated from fomites in threatres of NOHE

Figure 9 showed the distribution of nasal carriage of *S.aureus* and MRSA among the healthcare workers.

Out of the 100 healthcare workers, 93 (93%) had their nasal cavity colonized by *Staphylococcus aureus*. Among the 93 nasal carriers of *S aureus*, 42 (45%) were males while females recorded a higher carriage rate of 51 (53%) which difference was not statically significant (P> 0.05). it was also observed that nurses as a professional group ranked highest in nasal carriage of *S. aureus* 46 (49.5%) while health assistants had the lowest rate 10 (10.8%). Using ANOVA, this was found to be statistically significant (P< 0.05).

The overall prevalence of methicillin ï resistant *Staphylococcus aureus* (MRSA) among the healthcare workers was found to be 31 (33%). Nurses also had the highest MRSA carriage of 17 (18.2%) and medical laboratory scientist 2 (2.1%). This was also not statistically significance (P>0.05). These figures are shown in Tables 9 and 10 in the Appendix.

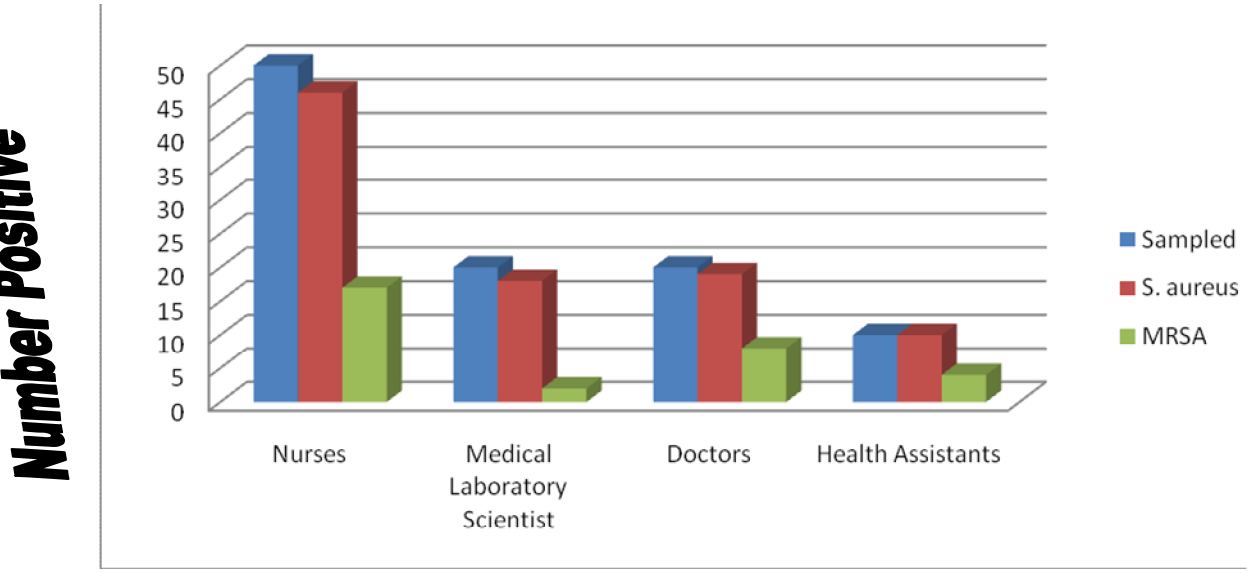


Fig. 9: **Distribution of nasal carriage of S. aureus and MRSA among Healthcare Workers in NOHE**

CHAPTER FIVE

5.0 DISCUSSION,CONCLUSION AND RECOMMENATIONS

5.1Discussion

The overall prevalence of staphylococcal nosocomial infections among in- patients of National Orthopaedic Hospital, Enugu was recorded as 85 (85%). Sixty (70.5%) had wound infections, 20 (24%) had urinary tract infection, 3 (4%) bacteremia and 2 (3%) had soft tissue infection (Figure 1 and Table 1) . The patient infection rate obtained in this study (85%) does not agree with the work done by El Bayoumi *et al*. (2006) in Egypt in their clinical and microbiological study of nosocomial infections in paediatric intensive care unit of University of Mansoura Paediatric Hospital, rated respiratory tract infection 30.9%, urinary tract infection 16.7% and bacteremia 27.3%. The results obtained in this study however agree with that of Oladeinde *et al*. (2013) in Okada near Benin (70.1%). Nworie *et al*.(2013) in Abakaliki studied clinical specimen and found that prevalence rate of *Staphylococcus* infection was 60.4%. The disparity in the infection rate could be attributed to differences in geographical location and possible differences in hygienic practices. Hospitals in developing countries especially Nigeria have rudimentary and highly compromised infection control programme due to lack of awareness of the problem, lack of personnel, poor water supply, instability of electricity, poor laboratory back- up, funding and professional rivalry. (Samuel *et al*, 2010). Another reason for high infection rate may be due to the population sampled ie burns patients and orthopaedic patients and it is known that *Staphylococcus aureus* is a major organism causing nosocomial infection among burns patients (Jones *et al*. 2004).

Also we observed that in National Orthopaedic Hospital, Enugu, patients on admission stay long in overcrowded wards and therefore are exposed to cross infection.

Figure 1 shows distribution of staphylococcal nosocomial infections in National Orthopaedic Hospital Enugu patients. In this figure, it was observed that wound infection had the highest number than the soft tissue infection. The reason may be due to the fact that wounds are exposed to aerosols both indoors and outdoors, unlike the soft tissue infections that are always localised. There was a statistically significant difference between wound infection and soft tissue infection ($P<0.05$). This then agrees with the work of Zerovs *et al*. (2012) in Europe who found that nosocomial infections in wounds results from aerosols. Also this agrees with the work of Mohammed *et al*. (2013) in Kano, Nigeria who also found out that nosocomial infections are higher for wound infection. This however was not in agreement with the findings of Sisirak *et al*. (2010) in Bosnia who studied MRSA as a cause of wound infections and had (16%) infection rate and Hamid *et al*. (2011) in Saudia Arabia who reported a lower rate of 38.5% in their study of where they investigated prevalence of bacteria causing nosocomial infection. Another reason may be that aspesis may often be overlooked in the rush of crisis care which is a common occurrence in my facility due to the nature of services it renders ï emergencies from burn cases and road traffic accident victims. Also, nosocomial infection of the urinary tract 20 (24%) was less than that found in wound infection 60(70%). The difference was also statistically significant ($P<0.05$). The reason may still be due to the fact that wounds are more exposed to aerosols than the urinary tract. Urinary tract infection ranked second in the study 20(24%). This agrees with the work of Nworie *et al*. (2013) in Abakaliki south east Nigeria where they obtained (21%) infection rate. However, this result disagrees with that of Olowe *et al*.(2004) in Osogbo, south west Nigeria in their study on antimicrobial susceptibility of *Staphylococcus aureus* isolates from

human clinical specimen including urine where the rate was 1.5%. The reason for the higher rate obtained in the study may be due to the fact that the sampled population as already stated stay long in the hospital and as such are exposed to colonization and subsequent infection. Also the use of urinary catheter for majority of the patients involved in fracture predisposes them to urinary tract infection (Iwuchukwu, 2005).

Low rate of bacteremia 3 (2.4%) and soft tissue infection 2 (2,4%) occurred in the study (Figure1). This agrees with the work of Ige *et al*. (2011) in University College Hospital Ibadan, south west Nigeria. In their audit surveillance of hospital acquired infectious in University College Hospital Ibadan between 2005 and 2009, where they also recorded low bacteremia and soft tissue infection rate. It was also observed that both bacteremia and soft tissue infection occurred only among burns patients and females only. The reason could be the skin barrier has been destroyed due to burns allowing bacterial colonization and infection. The overall distribution of staphylococcal nosocomial infections among the various patients categories was shown in Figure 2. This showed that among the patients category, burns patients 48 (56.5%) had four types of staphylococcal nosocomial infections (UTI, wound, bacteremia and soft tissue infection) while orthopaedic patients had only wound infection and UTI 37 (43.5%). This is however not statistically significant (P>0.05). The reason for this observation may be due to the fact that burns patients experience immunosuppresion due to a multitude of factors including the loss of skin as a barrier to micro organisms, dermal necrosis, presence of a protein ï rich exudate, depression of neutrophilic functions, depression of opsonins, complement and other defense mechanisms. Also prolonged hospital admission and catherization coupled with overcrowding makes them susceptible to a variety of nosocomial infection (Askarian *et al* .,2009).

In Figure 3 which shows the gender distribution of staphylococcal nosocomial infection among the patients, it was observed that more males 43 (50.6%) were infected than females 42 (49.6%). However, this is not statistically significant (P>0.05). This goes to show that gender does not predispose to nosocomial infection. Other workers in Nigeria made similar observations,e.g.Mohammed *et al*.(2013) in Kano in their study of wound infection and Akinjounla *et al*.(2009) in Calabar south south Nigeria also in their study of wound infections due to automobile accident. However more females had wound infection 35 (41%) than males 25 (29%).This could be due to more females using skin lightening cosmetics which contains hydroquione, mecury and corticosteriod for long duration, on large body surface area and under hot humid condition so percutaneous absorption is bound to occur and this could result in impaired wound healing, wound dehiscence and predisposition to infection (Olumide *et al.,* 2008).

Figure 4 shows a graph of sensitivity profile of isolated *S aureus* from the patients. It was observed that vancomycin was the most susceptible antibiotic tested 79(93%) while erythromycin was the most resistant 68 (80%). However, some researchers did not isolate any vancomycin resistant strain of *S aureus* from their study. Askarian *et al*. (2009) in Iran in their study of prevalence of MRSA among healthcare workers observed no resistance to vancomycin and Alghaithy *et al* .(2004) in Saudia Arabia in a study among healthcare workers and Nyasulu *et al* .(2012) in South Africa in a study of antimicrobial resistance pattern among nosocomial pathogens including *Staphylococcus aureus* recorded no resistance to vancomycin. However Ibe *et al*. (2013) in Abia State in his study recorded a high sensitivity pattern in vancomycin (65%) and Olowe *et al*.(2004) also had 93% susceptibility to vancomycin. The rate of MRSA was also found to be 28 (33%) among the patients. From the foregoing, multiple resistance was observed

in the study. This could be due to exposure to new antibiotic as noted by Hanley *et a*l. (1982) who opined that exposure of the organism to new antibiotics often results in further selection of homologous resistant strains. In our environment, drugs are purchased directly off the counter without serious regulation and prescription. Therefore, misuse and abuse is bound to occur commonly and leads to development of bacterial resistance over time. (Omoregie *et al*., 2010).

The results of this study showed that both *Staphylococus aureus* and *Staphylococcus epidermidis* (Figure 5) were isolated from both human and non ï human sources in National Orthopaedic Hospital, Enugu, 378(65%) for *S. aureus* and 183(32%) for *S. epidermidis* respectively. This agrees with the work of Chikere *et al*.(2008) in Port Harcourt,South south, Nigeria where they isolated both *Staphylococcus* species from both animate and inanimate sources in a hospital environment. Aminu *et al*. (2014) in his study of antibiotic pattern fomites had *Staphylococcus aureus* 31% and *Staphylococcus epidemidis* 8.7%. The reason for this may be because *Staphylococcus* are members of the body flora of both asymptomatic and sick persons. These organisms can be spread by hands, expelled from the respiratory tract and transmitted by animate or inanimate objects. The characteristic features that allow the persistence of *Staphylococus* in hospital environment include itôs ability to acquire resistance to a variety of antibiotics, withstand harsh physical environment, e.g high temperature and high salt concentration (Prescot, 2004).

Figure 6 shows the general distribution of *Staphylococcus aureus* and MRSA contamination from fomites in National Orthopedic Hospital, Enugu. From the figure, overall contribution of fomites to staphylococcal nosocomial infection in NOHE was put at 200 (53%) and for MRSA 85 (42.5%). It was also observed that floor 40 (11%) had the highest contamination while none

was isolated from operating light handle. This is however statistically significant. ($P<0.05$). This is in agreement with the work of Ikeh and Isamade (2011) in Jos University Teaching Hospital where they obtained 55.3% rate for S *aureus* and 22.6% for MRSA in their study where assessed the bacterial flora of fomites and they did not recover any organism from fluorescent light. Also, Uneke *et al*. (2009) also suggested that stethoscopes have the potential to transmit hospital acquired infection. It also agrees with this work where stethoscopes are also contaminated. The isolation of *S. aureus* from almost all the fomites show their ubiquitous nature and it can survive in dry environment surfaces between 10 to 98 days in fabrics found in hospitals and can be sources of infection to patients (Chako *et al*.,2003 Ikeh and Isamade 2011). Also MRSA strains have been shown to be transmitted from fomites to skin (Desai *et al.,* 2011). The main source of colonization of fomites might likely be nasal carriage by healthcare workers (Graham *et al*. 2006) and this is likely facilitated by hand to mouth or hand to nose contact while using these fomites and poor hand washing habits (ASM, 2005). It was observed from this result that the efficacy of disinfection and sterilization in the hospital is not very effective. Also modern sanitizing methods such as Non ï flammable vapour of alcohol in carbon dioxide (NAV ï CO2) from our observation is not used in the hospital which has been clinically proven to effectively reduce infection rate and risk of acquisition of MRSA (Otter and French, 2009). Also there are no policies specifying the frequency of cleaning and cleaning agent (disinfectant) used for non - critical items and environmental sources in NOHE.

The high rate of MRSA isolation from both the wards and theatres may be due to use of disinfectant at sub minimal concentration and this can induce resistance. Also disinfectants not discarded as at when due also contribute to emergency of multidrug resistant organisms. An

organism exposed to such agents over a long period of time develops resistance as they mutate (Opaluwa 2008).

Also, water shortage in our environment also contributed to poor sanitation and hygiene in the hospital. It was also observed that from Figure 7 showing distribution of *S. aureus* and MRSA from wards, the prevalence of *S. aureus* was found to be 140 (50.5%) and MRSA 62 (44.4%) and that of theatres as shown in Figure 8, *S. aureus* ï 60 (67.4%) and MRSA 23 (38.3%). There was no statistically significant difference between the two locations. Also in both, floor still had the highest frequency of contamination. In my facility, the cleaners employed by contractors and have no knowledge of their job and sometimes they even lack materials such as disinfectants to do the work. They are also overworked and so do not perform optimally.

In the work of Njoku-Obi and Ojiegbe (1993), they isolated no pathogen from the theatre sampled which disagrees with that found in National Orthopaedic Hospital, Enugu. State of the theatres and wards in National Orthopaedic Hospital, Enugu is very unsatisfactory as have been reported by other workers (Atata *et al*., 2006s). Also this poor level of hygiene and unrestricted movement of people in and out of these important hospital environments may contribute to the presence of pathogenic bacteria in the surgical theatre and poses a health risk because of their potential to cause nosocomial infection in patients undergoing surgery (Oni *et al*., 2006).

The prevalence of nasal carriage among the healthcare workers was shown on Figure 9. Out of the 100 screened, 93 (93%) were nasal carriers. This agrees with the work of Akujobi *et al.(* 2013) in Nnewi, South east Nigeria where they got 64% among healthcare workers and Iyeri *et al*. (2014) in Jeddah where the rate was 76% among healthcare workers. However, lower rates were obtained in the work of Onyemelukwe *et al*. (1992) in Enugu Nigeria, 34.3% among

healthcare workers, Nsofor *et al*. (2015) in Owerri obtained 47.4% among healthcare workers. The variation may be due to population studied as noted by Kluytmans *et al*. (1977) in America who reported that prevalence varies according to population studied. The high carriage rate among the healthcare workers in National Orthopaedic Hospital, Enugu may be due to poor hygiene and non compliance to universal basic precautions.

Also in Figure 9, it was observed that amongst the HCW, nurses were more prone to transmitting nosocomial staphylococal infections. The reason may be due to the fact that nurses have more contact with patients than any other HCW, hence there was a statistically significant difference between nurses 46 (49%) and health assistants 10(10%) ($P<0.05$). This agrees with the work of Onyemelukwe *et al*. 1992) in Enugu who reported that the carriage rate of *S. aureus* was highest in nurses. This also agrees with the work of Zer *et al*.(2012) in Turkey who also recorded highest frequency of nasal carriage of *Staphylococcus aureus* among the nurses.

Also Figure 9 shows the distribution of MRSA among the HCW in National Orthopaedic Hospital, Enugu where 31 (33%) is the overall prevalence. This agrees with other workers who reported MRSA among healthcare workers ï Zer *et al*.(2012) in Turkey, Akou-Koffi *et al*.(2004) in Cote Dô Ivoire, Olowe. *et al* .(2004) in Ilorin and Akujobi *et al*.(2013) in Nnewi. This however disagrees with the works of Hassan *et al*.(2008) in their study of nasal carriage of *Staphylococcus aureus* among surgical personnel in Sudan isolated no MRSA, Dimitrov *et al*. (2007) in their study of nasal carriage of *Staphylococcus aureus* among medical students in an infectious disease hospital isolated no MRSA in Kuwait and Adesida *et al*.(2007) in Lagos who did not isolate MRSA from healthcare workers.

The 33% rate observed here may be due to inappropriate usage or abuse of antibiotics in the population studied. Also, it indicates poor hand washing habits and these personnel may constitute an infection risk for hospitalized patients who are now exposed to staphylococcal nosocomial infections.

5.2 Conclusion

- Four types of staphylococcal nosocomial infections were found among the patients sampled.
- There was also an increased rate of wound infection among the patients.
- Vancomycin followed by Levofloxacin were the most susceptible antibiotics in the study.
- Fomites in the hospital were contaminated especially, the floors.
- The rate of nasal carriage of *S. aureus* among the healthcare workers was also high.

5.3 Recommendations

With the fact that there was drastic increase in rate of staphylococcal nosocomial infections among burns patients, stringent care measures should be taken by the healthcare workers like:

- Reducing the population of patients relatives on visit ,
- Improvement of personal hygiene and behavior.This could be achieved through regular water supply,training and retraining of staff and initial job orientation on infection prevention and control by infection control unit.
- Also the hospitals infection control committee should be more active and strictly enforce compliance of healthcare workers to infection control measures such as hand washing.
- Establishment of an effective antibiotics policy for the hospital to oversee drug prescription and slow down the development of resistant strains. Vancomycin and Levofloxacin should be included in the hospital drug list.
- Both healthcare workers and patients should be screened to establish their carrier status and carriers should be treated. Patients with nosocomial infections should be separated from other patients to avoid cross ï infection.
- More wards should be constructed to avoid overcrowding
- Upgrading of the theatres to meet the required standards.
- Use of more effective disinfectants e.g Lysol, 10% hypochlorite or concentrated hydrogen peroxide in the cleaning of the hospital wards and surfaces.
- Non-critical equipments should be thoroughly cleaned and disinfected after use.
- To minimize wound infections, adherence to strict surgical procedure and proper management of wounds is required.

- Wounds should not be exposed for prolonged period unduly during the course of dressing.
- Surveillance system should be initiated and maintained.

Establishment of patient safety initiative in the hospital as recommended by W H O(2006).

- Regular intrerventionary fumigation of NOHE is needed using non-flammble vapour of alcohol in carbon dioxide.
- Further studies at a higher scale in order to appreciate the full epidemiological pattern for control regimen is stressed.

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Table 3.1: Distribution of orthopaedic patients sampled

Number sampled				**Age range (years)**							**Length of stay(Months)**			
Type of injury	Sex			1-10	11 - 20	21-30	31-40	41-50	51-60	61-70	1-2	3-4	5-6	7-8
	Male	Female	Total											
Road Traffic Accident	19	16	35	1	6	9	7	8	3	1	30	1	3	1
Machet cut	2	1	3	0	0	1	1	1	0	0	2	1	0	0
Gun shot	11	0	11	0	0	6	0	2	3	0	7	4	0	0
Fall	1	0	1	0	0	1	0	0	0	0	1	0	0	0
Total	33	17	50	1	6	17	8	11	6	1	40	6	3	1

Table 3.2: Distribution of burns patients sampled

Number sampled				**Age range(years)**							**Length of stay (months)**				**Total bo**
Types of burns	Sex			1-10	11-20	21-30	31-40	41-50	51-60	61-70	1-2	3-4	5-6	7-8	1-15
	Male	Female	Total												
Chemical	4	3	7	0	0	5	0	1	0	1	3	3	1	0	5
Flame	10	20	30	5	9	7	2	2	1	4	19	9	0	2	8
Scald	6	4	10	8	1	1	0	0	0	0	10	0	0	0	2
Electrical	2	1	3	0	0	1	2	0	0	0	1	0	1	1	0
Total	22	28	50	13	10	14	4	3	1	5	33	12	2	3	15

Table 3.3: Distribution of healthcare workers sampled

Number sampled				**Age range (years)**							**Length of service (years)**				
Professional groups	Sex														
	Male	Female	Total	20 - 25	26 -30	31-35	36-40	41-45	46-50	>50	1-5	6-10	11-15	16-20	21-25
Nurses	11	39	50	10	19	8	6	0	4	3	31	13	1	1	2
Medical Laboratory Scientist	9	11	20	2	11	6	1	0	0	0	16	4	0	0	0
Doctors	18	2	20	0	1	2	10	5	0	2	9	7	2	2	0
Health Assistant	7	3	10	0	5	3	0	2	0	0	7	0	2	1	0
Total	45	55	100	12	36	19	17	7	4	5	63	24	5	4	2

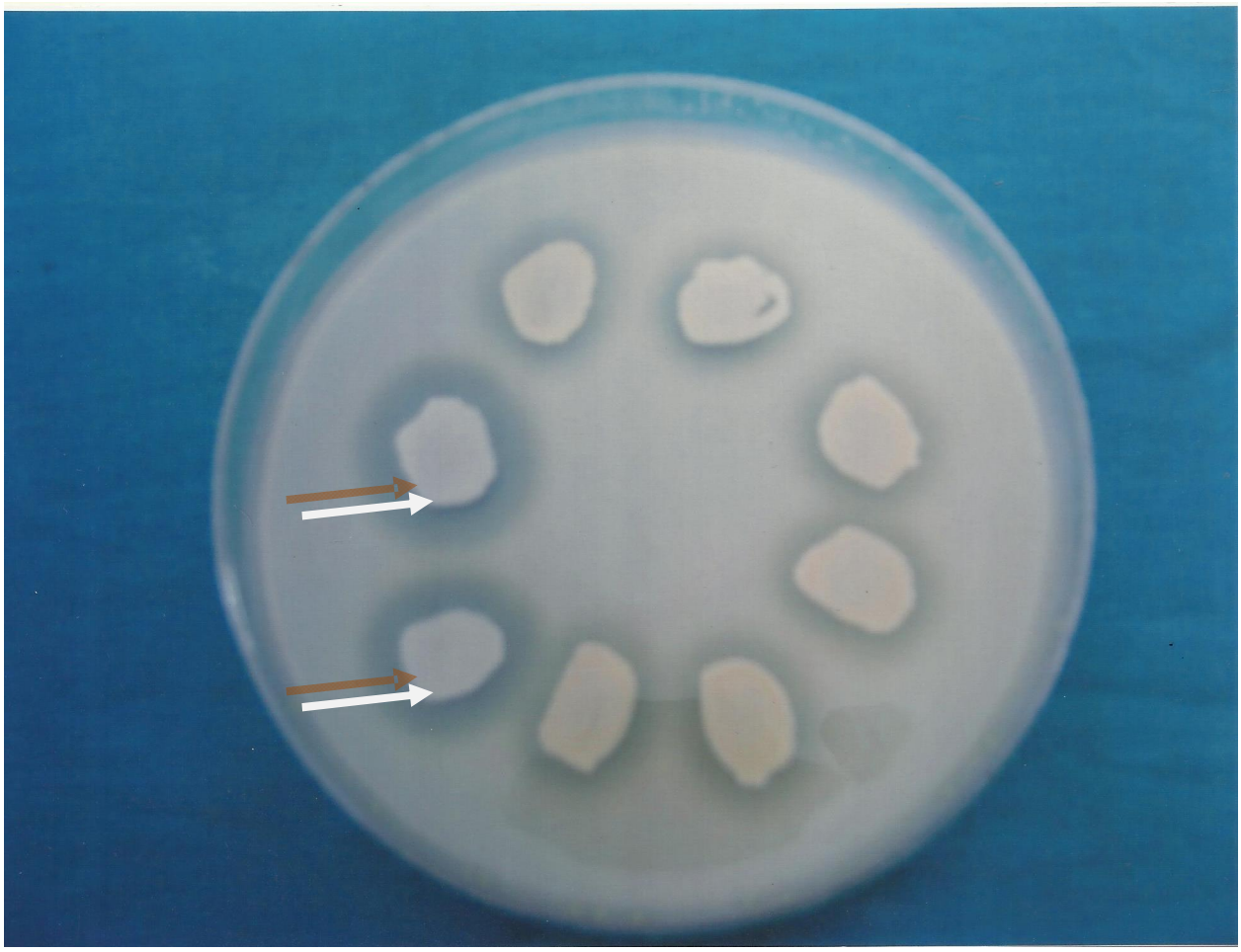

PLATE1: Showing DNAse isolate

Note the zone of clearing

Table 1: General distribution of staphylococcal nosocomial infections among patients in NOHE

Patients category	Sex			Types of staphylococcal nosocomial infections												Age rang			
	Male (M)	Female (F)	Total	UTI			Wound infection			Soft tissue infection			Bacteremia						
				M	F	Total	M	F	Total	M	F	Total	M	F	Total	1-15	16-30	31-45	46
Matchet Cut	0	1	1	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0
Gun shot	7	0	7	0	0	0	0	7	7	0	0	0	0	0	0	0	0	0	0
Road Traffic Accident	16	13	29	6	3	9	10	10	20	0	0	0	0	0	0	0	0	0	0
Chemical Burns	4	3	7	1	0	1	3	3	6	0	0	0	0	0	0	4	1	0	2
Flame Burns	8	17	25	3	5	8	5	9	14	0	1	1	0	2	2	5	10	1	0
Scald Burns	5	5	10	0	0	0	5	4	9	0	0	0	0	1	1	3	6	1	0
Electrical Burns	3	3	6	1	1	2	2	1	3	0	1	1	0	0	0	0	5	0	0
Total	43	42	85	11	9	20	25	35	60	0	2	2	0	3	3	12	22	2	2

Table 2: General distribution of staphylococcal nosocomial infections among orthopaedic patients in NOHE

Types of injuries	Sex			Types of infections				Age range (years)							Length of stay (months)			
	Male	Female	Total	Urinary Tract Infection		Wound Infection		1-10	11-20	21-30	31-40	41-50	51-60	61-70	1-2	3-4	5-6	7-8
				Male	Female	Male	Female											
Matchet cut	0	1	1	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0
Gun shot	7	0	7	0	0	0	7	0	0	3	0	2	2	0	3	4	0	0
Road Traffic Accident	16	13	29	6	3	10	10	1	2	10	6	5	3	2	21	3	4	1
Total	23	14	37	6	3	10	18	1	2	13	7	7	5		24	8	4	1

Table 3: General distribution of nosocomial infections among burns patients in NOHE

Types of burns	Sex			Types of infections								Age range(years)			
	Male	Female	Total	UTI		Wound Infection		Bacteremia		Soft Tissue Infection		1-10	11-20	21-30	31
				Male	female	Male	Female	Male	Female	Male	Female				
Chemicals	4	3	7	1	0	3	3	0	0	0	0	0	0	5	1
Scald	5	5	10	0	0	5	4	0	1	0	0	7	1	2	0
Electrical	3	3	6	1	1	2	1	0	0	0	1	0	0	1	5
Flame	8	17	25	3	5	5	9	0	2	0	1	5	16	5	2
Total	20	28	48	5	6	15	17	0	3	0	2	12	17	13	8

Table 4: Prevalence of *Staphylococcus* species isolated from human and non human sources in NOHE

Sources	No tested	Types of isolates and prevalence			
		***S. aureus* (No +ve)**	**Prevalence (%)**	***S. epidermidis* (No +ve)**	**Prevalence**
Healthcare workers	100	93	16%	64	11%
Patients	100	85	14.7%	-	-
Fomites	378	200	34.6%	119	20.5%
Total	578	378	65.3%	183	31.6%

Table 5: Antibiogram of *S. aureus* isolates from patients in NOHE

Antibiotics used	**No tested**	**No (%) susceptible**	**No (%) resistant**
Vancomycin (30mcg)	85	79 (93%)	6 (7%)
Oxacillin (1mcg)	85	57 (67%)	28 (33%)
Ciprofloxacin (5mcg)	85	63 (74%)	22 (26%)
Oflaxacin (5mcg)	85	59 (69%)	26 (31%)
Peflacine (5mcg)	85	59 (69%)	26 (31%)
Levofloxacin (5mcg)	85	73 (86%)	12 (14%)
Erythromycin (15mcg)	85	17 (20%)	68 (80%)
Ceftazidine (30mcg)	85	45 (53%)	40 (47%)
Ceftriazidine (30mcg)	85	60 (71%)	25 (29%)
Gentamycin (10mcg)	85	29 (34%)	56 (66%)
Amoxycillin/Clavulanate (Augumentin) (30mcg)	85	61 (72%)	24 (28%)

Table 6: Overall distribution of *S.aureus* isolates from fomites in wards and theatres of NOHE

Fomites	**No.sampled**	**No. of S. aureus isolated**	**No. isola**
Oxygen machines	7	5	
Thermometers	40	17	
Sphygmomanometer	7	6	
Drug trolleys	10	7	
Bed pans	34	9	
Sethescopes	7	4	
Pulse oxidometers	1	1	
Bed railings	42	19	
Walls	44	30	
Sinks	16	12	
Dressing trolleys	10	7	
Floors	54	40	
Cupboards	55	17	
Nurses Tables	7	7	
Anasthestic Machines	7	4	
Operating Tables	7	3	
Operating Light Handles	6	-	
Instruments Trays	6	6	
Theatre Trolleys	6	6	
TOTAL	**378**	**200**	

TABLE 7:- DISTRIBUTION OF *STAPHYLOCOCCUS AUREUS* AND MEITHICILLIN RESISTANT STAPH. AUREUS ISOLATED FROM FOMITES IN WARDS OF NATIONAL ORTHOPAEDIC HOSPITAL, ENUGU.

FOMITES	NO SAMPLED	NO OF POSITIVE S. AUREUS	NO OF POSITIVE MRSA
Oxygen machines	7	5	3
Thermometers	40	17	4
Stethoscopes	7	4	1
Sphygmomanometers	7	6	0
Drug Trolleys	10	7	4
Dressing Trolleys	10	7	3
Bed Pans	34	9	5
Bed Railings	42	19	7
Floor	27	20	12
Walls	22	15	9
Sink	8	6	2
Cupboard	55	17	10
Nurse's Table	7	7	3
Pulse Oxidometer	1	1	1
Total	277	140	62

Table 8: Distribution of *S. aureus* and MRSA isolated from fomites in threatres of NOHE

Fomites	**No Tested**	**No of positive *S. aureus* isolates**	**No of positive *MRSA* isolates**
Anaesthetic machine	7	4	2
Operating table	7	3	2
Operating light handle	6	0	0
Instrument tray	6	6	2
Sink	8	6	4
Floor	27	20	10
Theatre trolley	22	15	3
Wall	6	6	2
Total	89	18	23

Table 9: Distribution of nasal carriage of *S.aureus* among Healthcare Workers in NOHE

Healthcare workers category	No +ve Sex			Age range (Years)			
	Male	Female	Total	20-25	26-30	31-35	36-
Nurses	9	37	46	9	20	5	5
Medical laboratory scientists	9	9	18	2	9	6	1
Doctors	17	2	19	-	1	2	10
Health assistants	7	3	10	-	5	3	-
Total	42	51	93	11	35	16	16

Table 10: Distribution of MRSA among Healthcare Workers in NOHE

No sampled				Age range (years)				
Healthcare Workers category	No positive for MRSA Sex			20 - 25	26 -30	31-35	36-40	41-4
	Male	Female	Total					
Nurses	5	12	17	1	9	2	2	-
Medical Laboratory Scientist	1	1	2	1	1	-	-	-
Doctors	7	1	8	-	1	1	3	1
Health Assistants	2	2	4	-	1	2	-	1
Total	15	16	31	2	12	5	5	2

MATERIALS AND EQUIPMENTS

The following materials and equipments were used for the collection and processing of samples:

1. EQUIPMENTS

   Incubator

   Autoclave

   Refrigerator

   Hot air oven

   Timer

   Wireloops

   Forceps

   Sterile swab sticks/gloves

   Universal containers

   Petri dishes

   Transparent ruler (Millimeter)

   10ml syringe, 5ml syringe and needle

   GLASSWARES

   Slides

   Test tubes

   Bijou bottles

   Measuring cyclinder

Beakers

Conical flask

Screw cap containers

REAGENTS/MEDIA

Mannitol salt Agar (Oxoid)

Nutrient Agar(Lab M)

Blood Agar (Lab M)

Nutrient Broth(Lab M)

Muller Hinton Agar(Oxoid)

DNAase Agar(Oxoid)

Barium chloride salt

1% Concentrated sulphuric acid

Oxoid single antibiotics disc

Oxoid signal blood culture system.

IN HCL

Hydrogen perioxide

Pooled human EDTA plasma and normal saline

Gram staining reagents

Lugolôs iodine solution

Acetone

Crystal violet

Neutral red

Lugol's iodine solution

Formular

Potassium iodide 20g

Iodine 10g

Distilled water 1000mls

NB:- A commercially prepared one was used

Neutral red 1g/L (0.1% w/v)

Formular

Neutral red 1g

Distilled water 1000mls

NB:- A commercially prepared one was used.

Acetone

Formular

Acetone 500mls

Distilled water 10mls

NB: A commercially prepared one was used.

IN HCL

Formular

Concentrated Hydrochloric acid 86mls

Distilled water 1000mls

Preparation

The flask was filled up to the half mark with distilled water and 85mls of the concentrated HCL was added to it and mixed well and then the volume was made up to 1000mls. It was transferred to screw-cap bottles and labeled appropriately.

Preparation of media

Nutrient broth (LAB m)

Formular

Peptone	10g
Sodium chloride	5g
Meat extract	10g
Distilled water	1000mls

25g of the media was weighted and dissolved in one litre of distilled water. It was then distributed to bijou bottles and sterilized by autoclaving at 121^0C for 15minutes. It was allowed to cool and stored in the refrigerator.

(II). Mannitol salt agar (LAB M).

Formular

Beef extract	1.0g
Balanced peptone	10g
Sodium chloride	75g

D-mannitol	10g
Agar No 2	12.0g
Phenol red	0.025g
Distilled water	1000mls

Preparation

108g of the media was weighted and dissolved in 1000mls of distilled water and sterilized by autoclaving at 121^0c for 15 minutes. It was allowed to cool to about 50^0C, mixed well and poured aseptically into sterile Petri dishes.

(III) **Nutrient agar**

Formula:

Peptone	5g/L
Meat extract	1g/L
Yeast extract	2g/L
Sodium chloride	5g/L
Agar	15g/L
PH	7.4

Preparation

28g of the medium was dissolved in one litre of distilled water and sterilized by autoclaving at 121^oc for 15 minutes. The medium was allowed to cool, mixed well and dispensed aseptically into sterile Petri dish. This was allowed cool and stored in the refrigerator. Also about 4mls of

the media was poured into sterile bijou bottles and allowed to solidify in a slanting position to make agar slants.

Blood agar

10% of sterile defribinated blood was added to a known volume of the nutrient agar after cooling to 50^0C and poured into sterile Petri dishes.

Muller Hinton Agar

Formular(g\l)

Beef dehydrated infusion from 300.0g

Casein hydrolyate 17.5g

Strach 1.5g

Agar 17,0g

Preparation: 38g of agar powder was aseptically weighed and suspended in 1 of distilled water in a conical flask. It was sterilized by autoclaved at 121^oc for 15 min, allowed to cool to about 50^0C and poured aseptically to sterile Petri dish about 4mm. Plates were flamed to remove air bubbles and stored in the fridge until is ready for use.

Preparation of turbidity standard equivalent to Mcfarland 0.5

Formula

1% concentrated sulphuric acid

Dihydrate barium chloride 0.5g

Preparation

1. Add 1m1 of concentrated sulphuric acid to 99m/s of water and mix well.
2. Then weigh 0.5g of Dihydrate barium (1% barium chloride and dissolve in 50 mls of distilled water. (0.5g Bacl2 in 50mls of H_20).
3. 0.6 ml of the barium chloride solution was added to 99.4 m/s of H_2S0_4 and mixed.
4. It was then stored in screw ï capped container in a dark cupboard at room temperature.

DNAse Agar (Oxoid)

Formula(g/L)

Tryptose 20.0g

Sodium chloride 5.0g

Agar 12.0g

Deoxyribonucleic acid 2.0g

Preparation

39g of the medium was dissolved in 1 litre of distilled water and sterilized by autoclaving at 121oC for 15 minutes.It was allowed to cool, mixed well and dispensed aspectically into sterile Petri dish.The surface of the media was flamed to remove air bubbles.It was allowed to solidify and stored in the refrigerator until ready for use.

2way ANOVA of Gender Variation of Wound Nosocomial Staph:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D
Table Analyzed	Gender Variation of Wound Nosocomial Staph			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Gender	5.47	0.3235		
Injury type	88.05	0.0686		
Source of Variation	P value summary	Significant?		
Gender	ns	No		
Injury type	ns	No		
Source of Variation	Df	Sum-of-squares	Mean	F
Gender	1	13.50	square	1.688
Injury type	2	217.3	13.50	13.58
Residual	2	16.00	108.7	
			8.000	
Number of missing values	0			
Bonferroni posttests				
MALE vs FEMALE				95% CI of
Injury type	MALE	FEMALE	Difference	diff.
Matchet cut	0.0	1.000	1.000	-29.60 to 31.60
Gun shot	7.000	0.0	-7.000	-37.60 to 23.60
Road Traffic Accident	16.00	13.00	-3.000	-33.60 to 27.60
Injury type	Difference	t	P value	Summary
Matchet cut	1.000	0.2500	P > 0.05	ns
Gun shot	-7.000	1.750	P > 0.05	ns
Road Traffic Accident	-3.000	0.7500	P > 0.05	ns

2way ANOVA of Variation in Nosocomial Staph of Infection type in diff Conditions:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D
Table Analyzed	Data 2			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Interaction	7.00	0.3424		
Nosocomial infection	16.63	0.0482		
Injury type	60.06	0.0097		
Source of Variation	P value summary	Significant?		
Interaction	ns	No		
Nosocomial infection	*	Yes		
Injury type	**	Yes		
Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	2	12.67	6.333	1.288
Nosocomial infection	1	30.08	30.08	6.119
Injury type	2	108.7	54.33	11.05
Residual	6	29.50	4.917	
Number of missing values	0			
Bonferroni posttests				
UTI vs Wound Infection				
Injury type	UTI	Wound Infection	Difference	95% CI of diff.
Matchet cut	0.0	0.5000	0.5000	-6.789 to 7.789
Gun shot	0.0	3.500	3.500	-3.789 to 10.79
Road Traffic Accident	4.500	10.00	5.500	-1.789 to 12.79
Injury type	Difference	t	P value	Summary
Matchet cut	0.5000	0.2255	P > 0.05	ns
Gun shot	3.500	1.578	P > 0.05	ns
Road Traffic Accident	5.500	2.480	P > 0.05	ns

2way ANOVA of Variation in Nosocomial Staph Infections in Burn Patients:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D
Table Analyzed	Data 5			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Interaction	0.18	0.9929		
Type of Nosocomial Infection	51.23	0.0004		
Gender	1.41	0.4057		
Source of Variation	P value summary	Significant?		
Interaction	ns	No		
Type of Nosocomial Infection	***	Yes		
Gender	ns	No		
Source of Variation	Df	Sum-of-squares	Mean square	F
Interaction	3	0.2500	0.08333	0.02985
Type of Nosocomial Infection	3	72.75	24.25	8.687
Gender	1	2.000	2.000	0.7164
Residual	24	67.00	2.792	
Number of missing values	0			
Bonferroni posttests				
UTI vs Wound Infection				95% CI of diff.
Gender	UTI	Wound Infection	Difference	-1.241 to 6.241
Male	1.250	3.750	2.500	-0.9908 to 6.491
Female	1.500	4.250	2.750	Summary
				ns
Gender	Difference	t	P value	ns
Male	2.500	2.116	P > 0.05	
Female	2.750	2.328	P > 0.05	
				95% CI of diff.
UTI vs Bactremia				-4.991 to 2.491
Gender	UTI	Bactremia	Difference	-4.491 to 2.991
Male	1.250	0.0	-1.250	
Female	1.500	0.7500	-0.7500	Summary
				ns
Gender	Difference	t	P value	ns
Male	-1.250	1.058	P > 0.05	
Female	-0.7500	0.6348	P > 0.05	

UTI vs Soft Tissue Infection				95% CI of diff.
				-4.991 to 2.491
Gender	UTI	Soft Tissue	Difference	-4.741 to 2.741
Male	1.250	Infection	-1.250	
Female	1.500	0.0	-1.000	Summary
		0.5000		ns
Gender	Difference		P value	ns
Male	-1.250	t	P > 0.05	
Female	-1.000	1.058	P > 0.05	
		0.8464		
Wound Infection vs Bactremia				
	Wound Infection		Difference	95% CI of diff.
Gender	3.750	Bactremia	-3.750	-7.491 to -0.009174
Male	4.250	0.0	-3.500	-7.241 to 0.2408
Female		0.7500		
	Difference		P value	
Gender	-3.750	t	P<0.01	Summary
Male	-3.500	3.174	P < 0.05	**
Female		2.962		*
Wound Infection vs Soft Tissue Infection	Wound Infection		Difference	
		Soft Tissue Infection		95% CI of diff.
Gender				-7.491 to -0.009174
Male	3.750	0.0	-3.750	-7.491 to -0.009174
Female	4.250	0.5000	-3.750	
Gender	Difference	t	P value	Summary
Male	-3.750	3.174	P<0.01	**
Female	-3.750	3.174	P<0.01	**
Bactremia vs Soft Tissue Infection	Bactremia	Soft Tissue	Difference	
Gender	0.0	Infection	0.0	95% CI of diff.
Male	0.7500	0.0	-0.2500	-3.741 to 3.741
Female		0.5000		-3.991 to 3.491
	Difference		P value	
Gender	0.0	t	P > 0.05	Summary
Male	-0.2500	0.0	P > 0.05	ns
Female		0.2116		ns

2way ANOVA of Variation in Nosocomial Staph Infections amongst different Burn-ty:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D
Table Analyzed	Data 6			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Interaction	17.61	0.0280		
Type of Nosocomial Infection	51.23	< 0.0001		
Burn types	20.60	0.0005		
Source of Variation	P value summary	Significant?		
Interaction	*	Yes		
Type of Nosocomial Infection	***	Yes		
Burn types	***	Yes		
Source of Variation	Df	Sum-of-squares	Mean	F
Interaction	9	25.00	square	2.963
Type of Nosocomial Infection	3	72.75	2.778	25.87
Burn types	3	29.25	24.25	10.40
Residual	16	15.00	9.750	
			0.9375	
Number of missing values	0			
Bonferroni posttests				
UTI vs Wound Infection				95% CI of
Burn types	UTI	Wound	Difference	diff.
Chemicals	0.5000	Infection	2.500	-1.050 to 6.050
Scald	0.0	3.000	4.500	0.9496 to 8.050
Electrical	1.000	4.500	0.5000	-3.050 to 4.050
Flame	4.000	1.500	3.000	-0.5504 to 6.550
		7.000		
Burn types	Difference		P value	Summary
Chemicals	2.500	t	P > 0.05	ns
Scald	4.500	2.582	P<0.01	**
Electrical	0.5000	4.648	P > 0.05	ns
Flame	3.000	0.5164	P < 0.05	*
		3.098		
UTI vs Bactremia				
Burn types	UTI		Difference	95% CI of
Chemicals	0.5000	Bactremia	-0.5000	diff.
Scald	0.0	0.0	0.5000	-4.050 to 3.050
				-3.050 to 4.050

Electrical	1.000	0.5000	-1.000	-4.550 to 2.550
Flame	4.000	0.0	-3.000	-6.550 to 0.5504
		1.000		
Burn types	Difference		P value	Summary
Chemicals	-0.5000	t	P > 0.05	ns
Scald	0.5000	0.5164	P > 0.05	ns
Electrical	-1.000	0.5164	P > 0.05	ns
Flame	-3.000	1.033	P < 0.05	*
		3.098		
UTI vs Soft Tissue Infection				
Burn types	UTI		Difference	95% CI of diff.
Chemicals	0.5000	Soft Tissue	-0.5000	-4.050 to 3.050
Scald	0.0	Infection	0.0	-3.550 to 3.550
Electrical	1.000	0.0	-0.5000	-4.050 to 3.050
Flame	4.000	0.0	-3.500	-7.050 to 0.05040
		0.5000		
Burn types	Difference	0.5000	P value	
Chemicals	-0.5000		P > 0.05	Summary
Scald	0.0	t	P > 0.05	ns
Electrical	-0.5000	0.5164	P > 0.05	ns
Flame	-3.500	0.0	P<0.01	ns
		0.5164		**
Wound Infection vs Bactremia		3.615		
	Wound Infection		Difference	95% CI of diff.
Burn types	3.000		-3.000	-6.550 to 0.5504
Chemicals	4.500	Bactremia	-4.000	-7.550 to -0.4496
Scald	1.500	0.0	-1.500	-5.050 to 2.050
Electrical	7.000	0.5000	-6.000	-9.550 to -2.450
Flame		0.0		
	Difference	1.000	P value	Summary
Burn types	-3.000		P < 0.05	*
Chemicals	-4.000	t	P<0.01	**
Scald	-1.500	3.098	P > 0.05	ns
Electrical	-6.000	4.131	P<0.001	***
Flame		1.549		
		6.197		
Wound Infection vs Soft Tissue Infection	Wound Infection		Difference	95% CI of diff.
	3.000		-3.000	-6.550 to 0.5504
Burn types	4.500	Soft Tissue	-4.500	-8.050 to -0.9496
Chemicals	1.500	Infection	-1.000	-4.550 to 2.550
Scald	7.000	0.0	-6.500	-10.05 to -2.950
Electrical		0.0		
Flame	Difference	0.5000	P value	
	-3.000	0.5000	P < 0.05	Summary
Burn types	-4.500		P<0.01	*

Chemicals	-1.000	t	P > 0.05	**
Scald	-6.500	3.098	P<0.001	ns
Electrical		4.648		***
Flame		1.033		
	Bactremia	6.713	Difference	
Bactremia vs Soft Tissue	0.0		0.0	95% CI of
Infection	0.5000		-0.5000	diff.
Burn types	0.0	Soft Tissue	0.5000	-3.550 to 3.550
Chemicals	1.000	Infection	-0.5000	-4.050 to 3.050
Scald		0.0		-3.050 to 4.050
Electrical	Difference	0.0	P value	-4.050 to 3.050
Flame	0.0	0.5000	P > 0.05	Summary
	-0.5000	0.5000	P > 0.05	ns
Burn types	0.5000		P > 0.05	ns
Chemicals	-0.5000	t	P > 0.05	ns
Scald		0.0		ns
Electrical		0.5164		
Flame		0.5164		
		0.5164		

1way ANOVA of Variation in Nosocomial Staph Infections amongst different Burn-ty:Ta

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Data 7				
One-way analysis of variance					
P value	0.0850				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	3				
F	3.283				
R square	0.4218				
	SS	df	MS		
ANOVA Table	100.5	2	50.25		
Treatment (between columns)	137.8	9	15.31		
Residual (within columns)	238.3	11			
Total					
					95% CI of diff
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	-2.475 to 12.97
1-2mths vs 3-4mths	5.250	2.684	No	ns	-0.9747 to 14.47
1-2mths vs 5-6mths	6.750	3.451	No	ns	-6.225 to 9.225
3-4mths vs 5-6mths	1.500	0.7668	No	ns	

1way ANOVA of Variation according to Burn degree:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Data 8				
One-way analysis of variance					
P value	0.0077				
P value summary	**				
Are means signif. different? (P < 0.05)	Yes				
Number of groups	5				
F	5.222				
R square	0.5820				
	SS	df	MS		
ANOVA Table	83.20	4	20.80		
Treatment (between columns)	59.75	15	3.983		
Residual (within columns)	143.0	19			
Total					
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
1-15% vs 16-30%	-2.500	2.505	No	ns	-6.858 to 1.858
1-15% vs 31-45%	2.500	2.505	No	ns	-1.858 to 6.858
1-15% vs 46-55%	2.500	2.505	No	ns	-1.858 to 6.858
1-15% vs 56-70%	2.750	2.756	No	ns	-1.608 to 7.108
16-30% vs 31-45%	5.000	5.010	Yes	*	0.6421 to 9.358
16-30% vs 46-55%	5.000	5.010	Yes	*	0.6421 to 9.358
16-30% vs 56-70%	5.250	5.261	Yes	*	0.8921 to 9.608
31-45% vs 46-55%	0.0	0.0	No	ns	-4.358 to 4.358
31-45% vs 56-70%	0.2500	0.2505	No	ns	-4.108 to 4.608
46-55% vs 56-70%	0.2500	0.2505	No	ns	-4.108 to 4.608

2way ANOVA of Variation in Staph spp Contamination of Sources in NOHE:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D
Table Analyzed	Data 9			
Two-way ANOVA				
Source of Variation	% of total variation	P value		
Sources in NOHE	54.35	0.3303		
Staph species	18.85	0.3574		
Source of Variation	P value summary	Significant?		
Sources in NOHE	ns	No		
Staph species	ns	No		
Source of Variation	Df	Sum-of-squares	Mean square	F
Sources in NOHE	2	4901	2451	2.028
Staph species	1	1700	1700	1.407
Residual	2	2417	1209	
Number of missing values	0			
Bonferroni posttests				
Healthcare workers vs Patients				
Staph species	Healthcare workers	Patients	Difference	95% CI of diff.
S. aureus	93.00	85.00	-8.000	-543.2 to 527.2
S. epidermiidis	64.00	0.0	-64.00	-599.2 to 471.2
Staph species	Difference	t	P value	Summary
S. aureus	-8.000	0.1627	P > 0.05	ns
S. epidermiidis	-64.00	1.302	P > 0.05	ns
Healthcare workers vs Fomites				
Staph species	Healthcare workers	Fomites	Difference	95% CI of diff.
S. aureus	93.00	106.0	13.00	-522.2 to 548.2
S. epidermiidis	64.00	119.0	55.00	-480.2 to 590.2
Staph species	Difference	t	P value	Summary
S. aureus	13.00	0.2644	P > 0.05	ns
S. epidermiidis	55.00	1.119	P > 0.05	ns
Patients vs Fomites				
Staph species	Patients	Fomites	Difference	95% CI of diff.
S. aureus	85.00	106.0	21.00	-514.2 to 556.2
S. epidermiidis	0.0	119.0	119.0	-416.2 to 654.2
Staph species	Difference	t	P value	Summary
S. aureus	21.00	0.4271	P > 0.05	ns
S. epidermiidis	119.0	2.420	P > 0.05	ns

t test of Gender Variation in Nasal S. aureus Healthcare Professional carriers

	Data Set-A
Table Analyzed	Variation in Nasal S. aureus Healthcare Professional carriers
Column A	Male
vs	vs
Column B	Female
Unpaired t test	
P value	
P value summary	0.8006
Are means signif. different? (P < 0.05)	ns
One- or two-tailed P value?	No
t, df	Two-tailed
	t=0.2640 df=6
How big is the difference?	
Mean SEM of column A	10.50 2.217 N=4
Mean SEM of column B	12.75 8.230 N=4
Difference between means	-2.250 8.523
95% confidence interval	-23.11 to 18.61
R square	0.01148
F test to compare variances	
F,DFn, Dfd	13.78, 3, 3
P value	0.0586
P value summary	ns
Are variances significantly different?	No

t test of Variation of Isolates from formites in the Theatre

	Data Set-A
Table Analyzed	Variation of Isolates from formites in the Theatre
Column A	No of S aureus isolates
vs	vs
Column B	No of MRSA isolates
Unpaired t test	
P value	0.0165
P value summary	*
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2.722 df=14
How big is the difference?	
Mean SEM of column A	2.250 0.4532 N=8
Mean SEM of column B	0.7500 0.3134 N=8
Difference between means	1.500 0.5510
95% confidence interval	0.3182 to 2.682
R square	0.3462
F test to compare variances	
F,DFn, Dfd	2.091, 7, 7
P value	0.3515
P value summary	ns
Are variances significantly different?	No

1way ANOVA of Variation in Nasal S. aureus Healthcare Professional carriers:Tabular

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Data 12				
One-way analysis of variance					
P value	0.0675				
P value summary	ns				
Are means signif. different? (P < 0.05)	No				
Number of groups	4				
F	3.173				
R square	0.4639				
ANOVA Table	SS	df	MS		
Treatment (between columns)	157.0	3	52.33		
Residual (within columns)	181.4	11	16.49		
Total	338.4	14			
Tukey's Multiple Comparison Test	Mean Diff.	q	Significant? P < 0.05?	Summary	95% CI of diff
Nurses vs Medical Laboratory Scientists	7.000	3.447	No	ns	-1.642 to 15.64
Nurses vs Doctors	6.750	3.324	No	ns	-1.892 to 15.39
Nurses vs Health Assistants	8.167	3.724	No	ns	-1.168 to 17.50
Medical Laboratory Scientists vs Doctors	-0.2500	0.1231	No	ns	-8.892 to 8.392
Medical Laboratory Scientists vs Health Assistants	1.167	0.5319	No	ns	-8.168 to 10.50
Doctors vs Health Assistants	1.417	0.6459	No	ns	-7.918 to 10.75

1way ANOVA of Variation of MRSA in Healthcare Professionals:Tabular results

	Data Set-A	Data Set-B	Data Set-C	Data Set-D	Data Set-E
Table Analyzed	Data 14				
One-way analysis of variance					
P value					
P value summary	0.0837				
Are means signif. different? (P < 0.05)	ns				
Number of groups	No				
F	4				
R square	2.825				
	0.4139				
			MS		
ANOVA Table	SS	df	10.06		
Treatment (between columns)	30.19	3	3.563		
Residual (within columns)	42.75	12			
Total	72.94	15			
			Significant? P <	Summary	95% CI of
Bonferroni's Multiple Comparison	Mean	t	0.05?	ns	diff
Test	Diff.	2.810	No	ns	-0.4577 to
Nurses vs Medical Laboratory Scientist	3.750	1.686	No	ns	7.958
Nurses vs Doctors	2.250	2.060	No	ns	-1.958 to
Nurses vs Health Assistants	2.750	1.124	No	ns	6.458
Medical Laboratory Scientist vs Doctors	-1.500	0.7493	No	ns	-1.458 to
Medical Laboratory Scientist vs Health Assistants	-1.000	0.3746	No		6.958
Doctors vs Health Assistants	0.5000				-5.708 to 2.708
					-5.208 to 3.208
					-3.708 to 4.708

t test of Gender Variation of MRSA in Healthcare Professionals

	Data Set-A
Table Analyzed	Data 15
Column A	MALE
vs	vs
Column B	FEMALE
Unpaired t test	
P value	0.9365
P value summary	ns
Are means signif. different? (P < 0.05)	No
One- or two-tailed P value?	Two-tailed
t, df	t=0.08305 df=6
How big is the difference?	
Mean SEM of column A	3.750 1.377 N=4
Mean SEM of column B	4.000 2.677 N=4
Difference between means	-0.2500 3.010
95% confidence interval	-7.616 to 7.116
R square	0.001148
F test to compare variances	
F,DFn, Dfd	3.780, 3, 3
P value	0.3036
P value summary	ns
Are variances significantly different?	No

t test of Variation of Staph contamination between Theatre&Wards formites

	Data Set-A
Table Analyzed	Data 17
Column A	S.aureus Theatre
vs	vs
Column B	S. aureus Wards
Unpaired t test with Welch's correction	
P value	0.0045
P value summary	**
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
Welch-corrected t, df	t=3.914 df=8
How big is the difference?	
Mean SEM of column A	2.250 0.4532 N=8
Mean SEM of column B	9.778 1.869 N=9
Difference between means	-7.528 1.923
95% confidence interval	-11.96 to -3.093
R square	0.6569
F test to compare variances	
F,DFn, Dfd	19.14, 8, 7
P value	0.0008
P value summary	***
Are variances significantly different?	Yes

t test of Variation of Isolates from formites in Hospital Wards

	Data Set-A
Table Analyzed	Variation of Isolates from formites in Hospital Wards
Column A	S. aureus isolates
vs	vs
Column B	MRSA isolates
Unpaired t test	
P value	0.0065
P value summary	**
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=2.957 df=26
How big is the difference?	
Mean SEM of column A	6.286 1.324 N=14
Mean SEM of column B	2.071 0.5290 N=14
Difference between means	4.214 1.425
95% confidence interval	1.284 to 7.145
R square	0.2516
F test to compare variances	
F,DFn, Dfd	6.261, 13, 13
P value	0.0022
P value summary	**
Are variances significantly different?	Yes

t test of Variation of MRSA contamination between Theatre&Wards formites

	Data Set-A
Table Analyzed	Data 18
Column A	Theatre MRSA
vs	vs
Column B	Ward MRSA
Unpaired t test	
P value	0.0055
P value summary	**
Are means signif. different? (P < 0.05)	Yes
One- or two-tailed P value?	Two-tailed
t, df	t=3.237 df=15
How big is the difference?	
Mean SEM of column A	0.7500 0.3134 N=8
Mean SEM of column B	3.222 0.6620 N=9
Difference between means	-2.472 0.7637
95% confidence interval	-4.100 to -0.8447
R square	0.4113
F test to compare variances	
F,DFn, Dfd	5.020, 8, 7
P value	0.0469
P value summary	*
Are variances significantly different?	Yes

Contingency of Nurses vs MLS

	Data Set-A	Data Set-B	Data Set-C
Table Analyzed	Data 10		
Fisher's exact test			
P value	1.0000		
P value summary	ns		
One- or two-sided	Two-sided		
Statistically significant? (alpha<0.05)	No		
Strength of association			
Relative Risk	1.022		
95% confidence interval	0.8646 to 1.209		
Odds ratio	1.278		
95% confidence interval	0.2148 to 7.601		
Difference between proportions			
Fraction of top, bottom row in left column	See Overview		
Difference between fractions			
95% confidence interval of difference			
Data analyzed	Carrier	Non-Carrier	Total
Nurses	46	4	50
Medical laboratory scientists	18	2	20
Total	64	6	70

Contingency of Drs vs MLS

	Data Set-A	Data Set-B	Data Set-C
Table Analyzed	Data 19		
Fisher's exact test			
P value	1.0000		
P value summary	ns		
One- or two-sided	Two-sided		
Statistically significant? (alpha<0.05)	No		
Strength of association			
Relative Risk	1.056		
95% confidence interval	0.8840 to 1.260		
Odds ratio	2.111		
95% confidence interval	0.1757 to 25.36		
Difference between proportions			
Fraction of top, bottom row in left column	See Overview		
Difference between fractions			
95% confidence interval of difference			
Data analyzed	Carrier	Non-carrier	Total
Doctors	19	1	20
Medical laboratory scientists	18	2	20
Total	37	3	40

Contingency of Nurses vs Drs

	Data Set-A	Data Set-B	Data Set-C
Table Analyzed	Data 20		
Fisher's exact test			
P value	1.0000		
P value summary	ns		
One- or two-sided	Two-sided		
Statistically significant? (alpha<0.05)	No		
Strength of association			
Relative Risk	0.9825		
95% confidence interval	0.8551 to 1.129		
Odds ratio	0.7368		
95% confidence interval	0.06228 to 8.718		
Difference between proportions			
Fraction of top, bottom row in left column	See Overview		
Difference between fractions			
95% confidence interval of difference			
Data analyzed	Carrier	Non-carrier	Total
Nurses	28	2	30
Doctors	19	1	20
Total	47	3	50

Contingency of Data 21

	Data Set-A	Data Set-B	Data Set-C
Table Analyzed	Data 21		
Fisher's exact test			
P value	1.0000		
P value summary	ns		
One- or two-sided	Two-sided		
Statistically significant? (alpha<0.05)	No		
Strength of association			
Relative Risk	1.087		
95% confidence interval	1.002 to 1.180		
Odds ratio	2.032		
95% confidence interval	0.1014 to 40.75		
Difference between proportions			
Fraction of top, bottom row in left column	See Overview		
Difference between fractions			
95% confidence interval of difference			
Data analyzed	Carrier	Non-Carrier	Total
Health Assistants	10	0	10
Nurses	46	4	50
Total	56	4	60

Contingency of Data 22

	Data Set-A	Data Set-B	Data Set-C
Table Analyzed	Data 22		
Fisher's exact test			
P value	0.5402		
P value summary	ns		
One- or two-sided	Two-sided		
Statistically significant? (alpha<0.05)	No		
Strength of association			
Relative Risk	1.111		
95% confidence interval	0.9601 to 1.286		
Difference between proportions			
Fraction of top, bottom row in left column	See Overview		
Difference between fractions			
95% confidence interval of difference			
Data analyzed	Carrier	Non-Carrier	Total
Health Assistants	10	0	10
Medical laboratory scientists	18	2	20
Total	28	2	30