PREVALENCE OF AEROFLORA CONTAMINATION IN OUT-PATIENT SECTION OF COMMUNITY HEALTH CENTRES IN OGUN STATE

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CERTIFICATION

This is to certify that this research was carried out by Joshua, Opeyemi Christianah with matriculation number; 15010101006 of the department of biological sciences (Microbiology unit) in partial fulfilment of the requirement for the award of Bachelor of Science (BSc.) degree in Microbiology.

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DEDICATION

This project is dedicated to God Almighty, the Alpha and Omega and to my mother, Mrs M.O. JOSHUA.

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ABSTRACT

Microbial contamination of primary health centre is a major cause of Nosocomial infection (NI). This study assessed the level of bacterial contamination in the outpatient section of five selected Community Health Centres in Ogun State which included two private and three government owned clinics. The open plate technique was adopted for the air sampling. The Isolated bacteria were identified using their morphological characteristics and biochemical tests. Antibiotics sensitivity testing of the isolates were also done using Kirby-Bauer Disc diffusion method. There was a significant difference ($P \geq 0.05$) in the population of out-patient in the analyzed health centres during sampling which ranged from 4^{e} -32^{a}, while the environmental temperature of the sampling locations also ranged from 23^{c} – 25^{a} ^{o}C. There was a significant difference ($P \geq 0.05$) in the colony forming units (cfu/cm^3) of the sampling locations. At the end of 48 hrs of incubation for 10, 20 and 30 minutes of sampling durations, colony counts ranged from 7^{e} – 68^{a}, 14^{e} – 202^{a} and 16^{e} – 262^{a} cfu/cm^3 respectively. Moreover, from the results of morphological and biochemical tests carried out, the genera Staphylococcus, Bacillus, Pseudomonas, Klebsiella, and Citrobacter were isolated. These findings call for a thorough review of infection control measures in the primary health centre environment across the country by the authorities to forestall undesirable consequences which will help in the reduction of nosocomial infection.

Keyword: Bacteria, Antibiotics, Health Care Centre, Nosocomial Infection

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LIST OF ABBREVIATED WORDS

ABBREVIATION	MEANING
AST	Antibiotics Sensitivity Test
CNS	Central Nervous System
HAI	Hospital Acquired Infection
NA	Nutrient Agar
NI	Nosocomial Infection
SSTIs	Skin and Soft Tissue Infections
UTI	Urinary Tract Infection
WHO	World Health Organization

CHAPTER ONE

1.0 INTRODUCTION

1.1 BACKGROUND TO THE STUDY

Indoor air quality play a vital role in the level of contamination, as the airborne microbial concentration and particle mass are directly linked to human activity, the number of people and the type of cloth worn by health care personnel and patients within the clinics. The rates at which people enter and exit the clinics increase the quality of microorganisms in the clinics (Weaving *et al.,* 2008). Airborne microbial contamination can cause health problems and may also affect normal workplace activities such as clinics, pharmaceutical and cosmetics facilities and may affect the performance, morale and performance of staff (Cellini *et al.,* 2001). Bio-aerosol exposure, which contains airborne microorganisms and their by-products, can lead to respiratory disorders and other adverse health effects such as infections and toxic reactions (Gorny *et al.,* 2002). Airborne bacteria enter the atmosphere from particularly all kind of surfaces (e.g. soil, forest, water, road dust) as aerosol particles. Once in the air, the bacteria are carried upwards by air currents and can remain in the atmosphere until being removed by precipitation or direct deposition onto surfaces. (Burrows *et al.,* 2009). Air sampling is useful for monitoring airborne biological agents and can be conducted qualitatively or qualitatively (Asefa, 2009*).* There are many different methods to measure microbial air contamination and air sample can be collected in two ways: by active air samplers and passive air sampling (Settle plate). The settle plates represent an economical and simple method, are sterile, available everywhere and give results that are reproducible.

Air sampling of microorganisms is a popular method of conducting microbial examination, as it allows a direct toxicological evaluation (Velmurgan *et al.,* 2008). The number of microorganisms present in clinics will depend on the number of patient occupying the hospital, the amount of physical activity, the rate of air exchange, the ambient temperature, relative humidity, level of environmental sanitation, type of ventilation, numbers of windows available for cross ventilation amongst others. The principle of indoor air quality is very important with regards to the environments within clinics and other health care facilities; the diversity of facilities and occupants makes the complex of the hospital environment unlike that of any other commercial and industrial buildings. Different components are found in the atmosphere, which enhance or promote the survival of microorganisms in the air *(*Ekhaise*, et al.,* 2008*).* Air pollution by micro-biotic entities is a growing menace to human health throughout the world. The presence of high concentrations of airborne microorganisms within the indoor areola is of increasing concern with respect to many acute diseases, infections, and allergies, and it is an indication of degree of cleanliness of these environments.

The control of indoor air quality plays a critical part within the anticipation of contamination in clinics to secure both hospital staff and patients, who are profoundly vulnerable to the unfavorable impact of different airborne chemicals and organisms. Poor hospital indoor air quality may cause outbreak of sick clinic disorder, causing migraines, weakness, eye and skin irritations and other indications. More truly, improper control of hospital indoor air quality may cause hospital-acquired (nosocomial) diseases and word related illnesses. As stated to the study conducted by World Health organization (2005), more than 2 million premature deaths each year are ascribed to the impact of urban outdoor air contamination and indoor air contamination.

1.2 STATEMENT OF RESEARCH PROBLEMS

The problem of this study is based totally on the identity of airborne microorganism which reasons infection and infection inside the outpatient sections in various clinics, its isolation and prevention. Airborne transmission is majorly one of the routes of spreading sicknesses accountable for some of nosocomial infection (Claudette *et al.,* 2006). Nosocomial contamination is an infection acquired in a sanatorium environment, which turned into now not gift within the affected person on the time of admission (Beggs, 2003). Clinics are doubtlessly conducive for antimicrobial resistant and virulent pathogen to proliferate. Aero floral contamination in clinics can lead to health center-acquired contamination (HAI) additionally known as Nosocomial contamination, that is an infection obtained in health center, outpatient hospital or different medical placing, it's miles from time to time instead referred to as a health care-related infection. Contamination is unfolded to the susceptible affected person within the clinical setting through various approaches. Health care staffs can spread infection to one another, in addition to infected device, bed spread, or air droplets. The infection can also originate from the outside environment, some other inflamed affected person or in a few instances, the supply of the contamination cannot be decided. Although the patient can also have gotten infection from their personal pores and skin, the contamination continues to be taken into consideration, since nosocomial infection is been developed in health care center or hospitals.

1.3 JUSTIFICATION

Atmospheric contamination is one of the most drastic problems of our age. This contamination has now reached an advanced level that posse potential threat to the health and

well-being of the population. Distinct components are majorly found in the atmosphere, which enhance or promote the survival of microorganisms in the air *(*Ekhaise *et al.,* 2008).

1.4 AIM AND OBJECTIVES OF THE STUDY

The aim of the study is to isolate, characterize and identify bacteria from the out-patient section of selected Community Health Centers in Ogun State.

The objectives of the research included:

i. Sampling of air in the out-patient sections of the selected Community Health Centre in Ogun State
ii. Isolation of bacteria from the samples using pure culture techniques
iii. Determination of the morphological and biochemical characteristics of the isolate
iv. Antibiotics sensitivity testing of the isolated bacterial species

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 DEFINITION OF NOSOCOMIAL INFECTION

The term “nosocomial” applies to any illness contracted by a patient whereas beneath therapeutic care. More absolutely, nosocomial diseases (NI) [too known as hospital associated/acquired infection (HAI)] are those infection or contamination that develop in patient during his/her remain in a hospital or other sort of clinical facilities which were not show at the time of affirmation. It may end up clinically clear either during the hospitalization or after release. Subsequently, pathogens that cause such diseases are named nosocomial pathogens. Be that as it may, an asymptomatic persistent may be considered tainted in case pathogenic microorganisms are found in a body liquid or at a body location that's ordinarily sterile, such as the cerebrospinal liquid or blood. Contaminations obtained by staff or guests to the hospital or other health care setting and neonatal disease that result from entry through the birth canal may too be considered nosocomial contaminations (Bereket *et al.,* 2012)

2.2 TYPES OF NOSOCOMIAL INFECTION

The types of nosocomial infection present in clinics are:

- Urinary Tract Infection
- Nosocomial skin and soft tissue infections
- Nosocomial Respiratory Tract Infection
- Nosocomial Central Nervous System Infection

2.2.1 URINARY TRACT INFECTION

The most common group of microbes present in urinary tract disease were *Escherichia coli, Staphylococcus aureus, Klebsiella peneumoniae, Pseudomonas aeruginosa, Citrobacter sp, Enterococci* microscopic organisms and *Proteus mirabilis*, whereas uncommon microscopic organisms causes of Urinary tract infections UTIs incorporate Ureaplasma Urealyticum and mycoplasma hominis, which are generally harmless organisms (Kastumi *et al.*, 2005). The foremost common type of UTI could be a bladder contamination which is additionally regularly called cystitis. Kidney disease is additionally a sort of UTI, known as Pyelonephritis they cause inconvenience or discomfort, the Urinary tract infection can rapidly and effortlessly be treated with a brief source of anti-microbial (Hudault *et al.*, 2001)

2.2.2 NOSOCOMIAL SKIN AND SOFT TISSUE INFECTIONS

Nosocomial infection that affect the skin and soft tissues, includes the clinical presentation of pain, warmth, cutaneous blood loss, skin sloughing, rapid evolution, edema, erythema, violaceous bullae (Steven *et al.,* 2014). Skin and soft tissue infections (SSTIs) result from invasion of the skin, and mostly occur due to trauma or surgery. SSTIs can be classified as Simple, necrotizing (Ramakrishnan *et al.*, 2015). The Risk factors of acquiring SSTIs include older age, diabetes mellitus, supprative immune-compromise, alcohol abuse and prolong hospitalization (Ki V *and* Rotsein C 2008).SSTIs is one of the most frequent infections among inpatients, with increased frequency among men (Ki V *and* Rotsein C 2008). *Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus, and Escherichia Coli* are commonly isolated from inpatient associated with SSTIs.

2.2.3 NOSOCOMIAL RESPIRATORY TRACT INFECTIONS

Nosocomial respiratory tract infections are major causes of extreme morbidity and mortality affecting five to ten of every 1,000 patients. Bacterial pneumonia accounts for 25% of all ICU infections. Ventilated Acquired pneumonia is the highest in the initial course of hospital stay. Intubation of mechanical ventilation increases the risk of nosocomial respiratory infections (American Thoracic Society 2005). The development of nosocomial respiratory tract infection is dependent on two independent pathophysiological factors: decreased immunity, and colonization of human cavities by bacteria (Blot *et al.,* 2014)

2.2.4 Nosocomial Central Nervous System Infections

Nosocomial CNS infections can be divided into surgical or device-related, and non-surgical related infections. Mycoplasma hominis is atypical pathogens that have been reported in the literature as a microorganism that causes nosocomial meningitis after surgical procedure in the brain. It is undetected by Gram Staining, and resistant to beta-lactam antibiotics (Whitson *et al.,* 2014).Other organisms observed to cause nosocomial meningitis included *Staphylococcus aureus*, coagulase negative *staphylococci, streptococcus pneumonia,* and other *streptococci* (Mace 2008). CNS infection caused by pathogens with a reduced sensitivity to drugs are a therapeutic challenge, for instance, infection caused by penicillin-resistant Pneumococci, methicillin-resistant *Staphylococci,* multi-resistant Gram-negative aerobic bacilli, or several other organisms, including *Aspergillus spp., scedosporium apiospermum, and Nocardia asteroids*, that affect primarily the central nervous system in immune comprised patients.

In addition, several antimicrobials including isoniazid, pyrazinamide, linezolid, metronidazole, fluconazole, and some fluoroquinolones, and tetracycline have demonstrated favorable CNS

penetration in adults, and macrolides and clindamycin have demonstrated poor CNS penetration in adults (Sullins *et al.,* 2013).

2.3 AGENT OF NOSCOMIAL INFECTIONS

2.3.1 Bacteria

These are the most common nosocomial pathogens. A qualification may additionally be made between It Commensalized microscopic organisms located in traditional greenery of healthy humans. These have a good sized protecting part through expecting colonization by way of pathogenic microorganisms. A few commensal microscopic organisms can also motive sickness if the herbal host is compromised. For example, cutaneous coagulase- terrible *staphylococci* purpose intravascular line disorder and intestinal *Escherichia coli* are the most frequent cause of urinary illness (Ducel, *et al.,* 2002)

Pathogenic microbes have greater outstanding harmfulness, and motive contaminations (intermittent or plague) regardless of host statue. For example: Anaerobic Gram-positive rods (e.g. *Clostridium*) cause gangrene. Gram high quality microscopic organisms: *Staphylococcus aureus* (cutaneous microbes that colonize the pores and skin and nostril of both clinic group of workers and patients) purpose a wide assortment of lung, bone, heart and blood- move contaminations and are habitually protected to anti-microbials; beta-hemolytic *streptococci* are additionally important. Gram-negative bacteria: *Enterobacteriaceae* (e.g. *Escherichia coli, Proteus, Klebsiella, Enterobacter, Serratia marcescens),* may colonize web sites when the host defenses are compromised (catheter insertion, bladder catheter, cannula insertion) and cause serious infections (surgical site, lung, bacteremia, peritoneum infection) (Ducel *et al.,* 2002).

They may also additionally be noticeably resistant. Gram terrible organisms such as *Pseudomonas spp*. are regularly isolated in water and damp areas. They may colonize the digestive tract of hospitalized patients. Selected other bacteria are a special chance in clinics. For instance, Legionella species may additionally motive pneumonia (sporadic or endemic) via inhalation of aerosols containing contaminated water (air conditioning, showers, therapeutic aerosols (Ducel, *et al*, 2002).

2.3.1.1 *Escherichia coli*

E.coli is a Gram negative, rod shaped, non-spore forming, and facultative anaerobic bacterium. The cells are about 2.0 µm lengthy and 0.25-1.0 µm in diameter and a cellphone volume of 0.6-0.7µm3 (Yu *et al.*, 2014). They are motile with peritrichous flagella and some traces are non-motile (Darnton, *et al.*, 2007). *E. coli* can survive on different types of substrates and they are fermentative underneath anaerobic conditions, this procedure produces lactate, succinate, ethanol, acetate, and Carbon dioxide (Madigan and Martinko, *et al.*, 2006). The bacterium is catalase positive, oxidase negative, Indole positive. It is a mesophile; they develop pleasant at ultimate temperature of 37 oC (Fotadar, *et al.*, 2005). They have to grow on a number defined laboratory media, they are chemo heterotrophs and they require a source of carbon and power to grow. they can develop on conventional reason media: Nutrient agar, differential medium like Cysteine-, Lactose and Electrolyte Deficient (CLED), selective and differential medium like MacConkey agar that differentiated lactose fermenter and non-lactose fermenter with the use of shade and two Eosin Methylene Blue (EMB) agar which carries dyes that are toxic to gram tremendous organism (selective towards gram high-quality organisms) and have the ability to differentiate between lactose fermenters and non-lactose fermenters. E. coli gives an inexperienced metal sheen when grown on EMB agar.

E. coli lives as an ordinary microbiota in the human gastrointestinal tract, most strains of this organism are non-pathogenic and some are pathogenic. They are released in to the surroundings through faecal depend (Russell and Jarvis, *et al.,* 2001), some strains have end up virulent due to the acquisition of virulent elements via plasmids, transposons or bacteriophages. The pathogenic strains can purpose gastrointestinal infections such as urinary tract contamination (Bruce, *et al.,* 2015), wound infection, which happens when faecal contaminates comes in contact with exterior wound.

E. coli can reason infection when they are transmitted via the faecal- oral route, and it's transferred through faecal infection of meals and water e.g. Shiga toxin producing *E. coli* (STEC) which causes foodborne diseases, it can additionally be transmitted from character to person. They act as indicator for faecal infection of meals or water.

The pathogenicity of pathogenic *E. coli* is decided by some elements such as O antigen ; which is the outer membrane of the bacterium cells which is made up of lipopolysaccharide (LPS) molecules, K antigen (the capsular polysaccharide) a thick, mucus-like layer encapsulating some pathogenic *E. coli*, H antigen; makes up the flagella, Fimbriae, Verocytotoxin etc. These elements make the pathogenic *E. coli* virulent and motive illnesses such as gastroenteritis, urinary tract infection, wound infection, neonatal meningitis, peritonitis, septicaemia (Todar, *et al.,* 2007).

Escherichia coli traces are sensitive to all antimicrobial marketers active towards the *Enterobacteriaceae* family. Due to the prevalence of R plasmids, clinic received traces may additionally be resistant to antibiotics. Therefore antimicrobial susceptibility testing must be used to guide therapy.

2.3.1.2 *Klebsiella pneumoniae*

Klebsiella pneumoniae is a gram negative, rod shaped, non-motile, and encapsulated. It is a lactose fermenting and facultative anaerobic bacterium. The size of *K. pneumonia* is measure by 2 µm by 0.5 µm because they are rod shaped. They have a thick capsule layer that encloses the bacterium. The capsule is 160 nm thick of fine fibres that extends from the outer membrane at right angles (Lawlor, *et al.,* 2005). Their optimum temperature for growth is 35^{o}C and 37^{o}C and the optimum pH is 7.2. They don't have a specified growth requirement so they can grow on a standard laboratory medium. Most strains of *K. pneumoniae* can survive when there principal source of carbon is citrate and glucose and principal source of nitrogen is ammonia. They can grow on media like MacConkey agar where they appear as a mucoid lactose fermenter (pink colour), Nutrient agar. *K. pneumoniae* are catalase positive, utilizes citrate, and urease positive.

K. pneumonia can be found anywhere, in mammals as well as in our environments (such as surface water, soil and sewage) (Brisse and Verhoef, *et al.,* 2001). They can be found as normal microbiota in the mouth, skin and intestines (Ryan and Ray, 2004*).* Some stains are pathogenic and are community-acquired or hospital acquired (nosocomial). As described by Carl Friedlander, this organism affects majorly the immunocompromised like Sick patients, alcoholics, diabetic patients etc. most of the time, the infection is hospital acquired. This organism causes diseases like pneumonia (which is in form of bronchopneumonia and bronchitis) which in turn develops into abscesses, cavity, empyema and pleural adhesions, *Klebsiella pneumonia* can also cause urinary tract infection, surgical wound site infection, diarrhoea, meningitis, respiratory tract infection, osteomyelitis, bacteraemia, and sepsis.

The mode of transmission could be from person to person contact or contact with contaminated objects or instruments. *Klebsiella pneumonia* is susceptible to few antibiotics therefore

susceptibility tests are carried out to find out the appropriate antibiotics. Antibiotics like ampicillin, Ceftazidime, cefepime, levofloxacin, norfloxacin; ciprofloaxin etc. can inhibit the growth of *Klebsiella pneumonia.*

2.3.1.3 *Staphylococcus aureus*

This is a gram positive, spherical shaped, non-motile, non-spore forming and facultative anaerobes. This bacterium is also known as *Staphylococcus Aureus.* When this organism is checked under the microscope, a grape-like cluster is seen with big, spherical, golden yellow colonies (when grown on laboratory medium containing salt). When gram stained, they are gram positive. The size of *S. aureus* ranges from 0.5-1.5µm in diameter. They reproduce asexually through binary fission (Varrone, *et al.,* 2014). The optimum growth temperature for *Staphylococcus Aureus* is 37^{o}C. They can be grown on various agars like nutrient agar, manitol salt agar (MSA). They are also haemolytic and can be grown on blood agar *(Ryan and Ray, et al., 2004).* They are catalase positive. Catalase is an enzyme produced by organisms that can grow in the presence of oxygen to neutralize toxic forms of oxygen metabolites (H_2O_2). Catalase mediates the breakdown of Hydrogen peroxide (H_2O_2) in to oxygen and water. Therefore, oxygen is given off which causes bubbles, this shows that its catalase positive. They are also coagulase positive, coagulase is an enzyme produced by *Staphylococci aureus* that converts (soluble) fibrinogen in plasma to (insoluble) fibrin.

Staphylococcus aureus can be found in the environment as well as normal microbiota on human skin nose and respiratory tract of healthy humans, but when they gain access into the blood or internal tissue they can cause skin infection such as abscesses, respiratory infection like sinusitis and food poisoning. The pathogenic strains of *S. aureus* has some factors that makes them virulent like enzymes e.g. coagulase, potent protein toxins and cell-surface proteins that binds to

antibodies and makes them inactive. This organism is mostly transmitted through direct contact with contaminated objects or person to person contact.

The resistance of *Staphylococcus aureus* to penicillin G can be predicted by the positive testing to β-lactamase; approximately 90% of *Staphylococcus aureus* produce β-lactamase. About 35% of *S. aureus* are resistant to nafcillin. Resistant strains like the Methicillin resistant *Staphylococcus aureus* can be inhibited by other antibiotics like Vancomycin, erythromycin and gentamicin. Some strains can be resistant to multiple antibiotics (Irving, *et al.*, 2005).

2.3.1.4 *Enterococcus faecalis*

Enterococcus faecalis is a gram positive, spherical shaped arranged in pairs or chains, non-motile, non-spore forming, facultative anaerobic and obligatory fermentative chemoorganotrophs. *Enterococcus faecalis* can grow in very harsh environments, even in extremely high alkaline pH and salt concentration. They can grow in temperature ranging from 10-40^{o}C but their optimum growth temperature is at 35^{o}C. They can be grown on defined media such as nutrient agar, blood agar. They are non-haemolytic.

Enterococcus faecalis are normal microbiota of the gastrointestinal tracts of healthy humans and mammals. But some strains are pathogenic can cause life threatening disease to humans, particularly in hospital environments. They can cause diseases like endocarditis, septicaemia, urinary tract infection, meningitis etc. (Hidron, *et al.*, 2008). The factors that make this organism virulent are cytolysin; a plasmid-encoded haemolysin and a plasmid-encoded adhesin called aggregation substance.

Enterococcus faecalis is resistant to many antimicrobial agents such as aminoglycosides, aztreonam, cephalosporin, clindamycin, Oxacillin etc. *Enterococcus faecalis* is becoming more

resistant to Vancomycin (Amyes, *et al.,* 2007*;* Courvalin, *et al.,* 2006). Other antibiotics like nitrofurantoin, linezolid and daptomycin can be used against Vancomycin resistant *Enterococcus faecalis.*

2.4 Reservoirs and transmission

Microscopic organisms that cause nosocomial contaminations can be obtained in a few ways:

1. The lasting or transitory vegetation of the quiet (Endogenous contamination) Microbes show within the ordinary vegetation cause disease since of transmission to locales exterior the common living space, harm to tissue or in suitable anti-microbial treatment that permits over development (C. difficile, yeast spp).For example, Gram-negative microscopic organisms within the stomach related tract regularly cause surgical location contaminations after stomach surgery or urinary tract contamination in catheterized patients (Ducel, *et al.,* 2002).

2. Greenery from another quiet or part of staff (Exogenous cross-infection) Microbes are transmitted between patients through: coordinate contact between patients (hands, spit beads or other body Liquids), within the discus (beads or tidy sullied by a patient's microscopic organisms), by means of staff sullied through persistent care (hands, dress, nose and throat) who gotten to be transitory or changeless carriers, hence transmitting microscopic organisms

2.5 Factors influencing the development of nosocomial infections:

2.5.1 The microbial agent:

The patient is exposed to a variety of microorganisms during hospitalization. Contact between the patient and a microorganism does not by itself necessarily result in the development of

clinical disease other factors influence the nature and frequency of nosocomial infections. Many different bacteria, viruses, fungi and parasites may cause nosocomial infections. Infections may be caused by a microorganism acquired from another person in the hospital (cross-infection) or may be caused by the patient's own flora (endogenous infection). Some organisms may be acquired from an inanimate object or substances recently contaminated from another human source. Before the introduction of basic hygienic practices and antibiotics into medical practice, most hospital infections were due to pathogens of external origin (food borne and airborne diseases, gas gangrene, tetanus, etc.) or were caused by microorganisms not present in the normal flora of the patients (e.g. diphtheria, tuberculosis) (Ducel, *et al.,* 2002).

Progress in the antibiotic treatment of bacterial infections has considerably reduced mortality from many infectious diseases. Most infections acquired in hospital today are caused by microorganisms which are common in the general population, in whom they cause no or milder disease than among hospital patients such as *Staphylococcus aureus*, coagulase negative *Staphylococci, enterococci* and *Enterobacteriaceae* (Ducel, *et al.,* 2002).

2.5.2 Patient susceptibility

Important patient factors influencing acquisition of infection include age, immune status, underlying disease, and diagnostic and therapeutic interventions (Ducel, *et al.,* 2002). The extremes of life infancy and old age are associated with a decreased resistance to infection. Patients with chronic disease such as malignant tumors, leukemia, diabetes mellitus, renal failure, or the acquired immunodeficiency syndrome (AIDS) have an increased susceptibility to infections with opportunistic pathogens (Ducel, *et al.,* 2002).

The latter are infections with organisms that are normally innocuous, e.g. part of the normal bacterial flora in the human, but may become pathogenic when the body's immunological defenses are compromised. Immunosuppressive drugs or irradiation may lower resistance to infection. Injuries to skin or mucous membranes bypass natural defense mechanisms. Malnutrition is also a risk (Ducel, *et al.*, 2002).

Many modern diagnostic and therapeutic procedures, such as biopsies, endoscopic examinations, catheterization, ventilation and suction and surgical procedures increase the risk of infection. Contaminated objects or substances maybe introduced directly into tissues or normally sterile sites such as the urinary tract and the lower respiratory tract (Ducel, *et al*., 2002).

2.5.3 Environmental factors

Health care settings are an environment where both infected persons and Persons at increased risk of infection congregate. Patients with infections or carriers of pathogenic microorganisms admitted to hospital are potential sources of infection for patients and staff. Patients who become infected in the clinics are a further source of infection (Ducel, *et al.*, 2002).

Crowded conditions within the hospital, frequent transfers of patients from one unit to another and concentration of patients highly susceptible to infection in one area (e.g. newborn infants, burn patients, and intensive care) all contribute to the development of nosocomial infections. Microbial flora may contaminate objects, devices, and materials which subsequently contact susceptible body sites of patients. In addition, new infections associated with bacteria such as water borne bacteria (atypical mycobacteria) and/or viruses and parasites continue to be identified (Ducel, *et al.*, 2002).

2.5.4 Bacterial resistance

Many patients receive antimicrobial drugs. Through selection and exchange of genetic resistance elements, antibiotics promote the emergence of multidrug resistant strains of bacteria; microorganisms in the normal human flora sensitive to the given drug are suppressed, while resistant strains persist and may become endemic in the hospital. The wide spread use of antimicrobials for therapy or prophylaxis is the major determinant of resistance (Ducel, *et al.,* 2002). Antimicrobial agents are, in some cases, becoming less effective because of resistance. As an antimicrobial agent becomes widely used, a bacterium resistant to this drug eventually emerges and may spread in the health care setting. Many strains of *pneumococci, staphylococci, enterococci,* and tuberculosis are currently resistant to most or all antimicrobials which were once effective. (Ducel, *et al.,* 2002)

Multi resistant *Klebsiella* and *Pseudomonas aeruginosa* are prevalent in many clinics. This problem is particularly critical in developing countries where more expensive second-line antibiotics may not be available or affordable.

Nosocomial infections are wide spread. They are important contributors to morbidity and mortality. They will become even more important as a public health problem with increasing economic and human impact because of: Increasing numbers and crowding of people, more frequent impaired immunity (age, illness, and treatments), new microorganisms, increasing bacterial resistance to antibiotics (Ducel, *et al.,* 2002).

2.6 THE PREVENTION OF NOSOCOMIAL INFECTION

Nosocomial infection can be prevented using the following methods:

2.6.1 CLEANING

One of the most basic measures for the maintenance of hygiene, and one that is particularly important in the hospital environment, is cleaning. The principal aim of cleaning is to remove visible dirt. It is essentially a mechanical process: the dirt is dissolved by water, diluted until it is no longer visible, and rinsed off. Soaps and detergents act as solubility promoting agents. The microbiological effect of cleaning is also essentially mechanical: bacteria and other microorganisms are suspended in the cleaning fluid and removed from the surface. The efficacy of the cleaning process depends completely on this mechanical action, since neither soap nor detergents possess any antimicrobial activity. Thorough cleaning will remove more than 90% of microorganisms. However, careless and superficial cleaning is much less effective; it is even possible that it has a negative effect, by dispersing the microorganisms over a greater surface and increasing the chance that they may contaminate other objects. Cleaning has therefore to be carried out in a standardized manner or, better, by automated means that will guarantee an adequate level of cleanliness.

Diluting and removing the dirt also removes the breeding-ground or culture medium for bacteria and fungi. Most non-sporulating bacteria and viruses survive only when they are protected by dirt or a film of organic matter; otherwise they dry out and die. Non-sporulating bacteria are unlikely to survive on clean surfaces.

The effectiveness of disinfection and sterilization is increased by prior or simultaneous cleaning.

2.6.2 STERILIZATION

Sterilization can be achieved by both physical and chemical means. Physical methods are based on the action of heat (autoclaving, dry thermal or wet thermal sterilization), on irradiation (g-

irradiation), or on mechanical separation by filtration. Chemical means include gas sterilization with ethylene oxide or other gases, and immersion in a disinfectant solution with sterilizing properties (e.g. glutaraldehyde). Sterilization results in damage of all forms of microbial life, whereas disinfection results in destruction of specific pathogenic microorganisms. In medical practice, an object should be sterilized depending on its uses. Thing that come in direct contact with mucous membranes, such as endoscope; require a high level of disinfectant (Mohammad and Ali, 2009)

2.6.3 DISINFECTION

Disinfection is been accomplished by the use of liquid chemicals or wet pasteurization in healthcare settings, The efficacy of disinfection is affected by a number of factors, each of which may nullify or limit the efficacy of the process (Rutala and Weber, 2004).

- High-level disinfection: can be expected to destroy all microorganisms, with the exception of large numbers of bacterial spores.
- Intermediate disinfection: inactive ates Mycobacterium tuberculosis, vegetative bacteria, most viruses, and most fungi; does not necessarily kill bacterial spores.
- Low-level disinfection: can kill most bacteria, some viruses, and some fungi; cannot be relied on to kill resistant microorganisms such as tubercle bacilli or bacterial spores.

There is no ideal disinfectant and the best compromise should be chosen according to the situation. A disinfectant solution is considered appropriate when the compromise between the antimicrobial activity and the toxicity of the product is satisfactory for the given application. Another consideration may well be the cost. The more active disinfectants are automatically the more toxic ones; potentially toxic products can be applied to inanimate objects or surfaces,

whereas for disinfection of human tissues only the less toxic disinfectants can be considered. For antisepsis, different disinfectants are used for application to the intact skin (e.g. alcoholic solutions) and to mucous membranes or wounds (only aqueous solutions of non-toxic substances). Cost is a less important consideration for an antiseptic than for a disinfectant.

The principal requirements for a good antiseptic are absence of toxicity and rapid and adequate activity on both the natural flora and, especially, pathogenic bacteria and other microorganisms after a very short exposure time. Essential requirements for a disinfectant are somewhat different: there must be adequate activity against bacteria, fungi, and viruses that may be present in large numbers and protected by dirt or organic matter. In addition, since disinfectants are applied in large quantities, they should be of low ecotoxicity.

In general, use of the chosen disinfectant, at the appropriate concentration and for the appropriate time, should kill pathogenic microorganisms, rendering an object safe for use in a patient, or human tissue free of pathogens to exclude cross-contamination.

2.7 Importance of the standard precautions to be used in the care of all patients

A. Hand washing

- Wash hands after touching blood, secretions, excretions and contaminated items, whether or not gloves are worn.
- Wash hands immediately after gloves are removed, between patient contacts.
- Use a plain soap for routine hand washing.
- Use an antimicrobial agent for specific circumstances.

B. Gloves

- Wear gloves when touching blood, body fluids, secretions, excretions, and contaminated items.
- Put on clean gloves just before touching mucous membranes and non-intact skin.
- Disposable gloves should not be reused.
- Hands must be washed when gloves are been removed or changed.

C. **Mask, eye protection, face shield**

- Wear a mask and eye protection or a face shield during procedures and patient care activities that are likely to generate splashes or sprays of blood, body fluids, secretions, and excretions.

D. **Gown**

- Wear a gown during procedures and patient-care activities that are likely to generate splashes or sprays of blood, body fluids, secretions, or excretions.

E. **Patient-care equipment**

- Ensure that reusable equipment is not used for the care of another patient until it has been cleaned and reprocessed appropriately.

F. **Environmental control**

- Ensure that the hospital has adequate procedures for the routine care, cleaning, and disinfection of environmental surfaces.

G. **Linen**

- Handle used linen, soiled with blood, body fluids, secretions, and excretions in a manner that prevents skin and mucous membrane exposures, and that avoids transfer of microorganisms to other patients and environments.

H. **Occupational health and blood borne pathogens**

- Take care to prevent injuries when using needles, scalpels, and other sharp instruments or devices
- Use ventilation devices as an alternative to mouth-to-mouth resuscitation methods.

Surveillance of Nosocomial infections is important to reduce health care stay, cost, and quality of life. In addition, carrying out control measures to reduce morbidity and mortality in the clinics is mandatory

CHAPTER THREE

3.0 MATERIALS AND METHODOLOGY

3.1 Materials

The materials used includes; Sterile Petridishes, Distilled water, Cotton wool, Alcohol (70% ethanol), Test tube, Cornical flask, Inoculating loop, slide, Durham tubes, Beaker, Aluminum Foil, Dropper, Measuring Cylinder.

3.2 Culture media

Nutrient Agar (NA) for the isolation of bacteria, Sensitivity Agar, Peptone Broth, Simmon Citrate Agar, Methyl red Vogues Proskauer Agar.

3.3 Equipment and Reagent

The equipment used include; Oven, Laminar flow, Incubator, Weighing balance, bursen burner, Autoclave, Water bath, Magnetic Sterile, Thermometer, Colony Counter, Microscope while the reagents used include: Gram iodine, Safranine, Kovacs Reagent, Crystal Violet, Methyl Red

3.4 Study Area

The work was carried out in five selected Primary Health Centers. At different hospital located within community of Ogun state. The study sites were the outpatient section of clinics.

3.5 Study duration

Air Samples were collected twice a week for one (1) month in the wet season (June, 2019) using the settle plate method.

3.6 Sample Size

Three (n=3) air samples were collected randomly from 5 different clinics.

3.7 Air Sampling Techniques

Air sample was carried out using settle plate's methods. Petri dishes containing Nutrient agar were transported to clinics in sealed plastic bags. The plates were labelled with sample number, time and date of collection. The plates were placed at one chosen place in the indoor of the hospital. All the plate was exposed for 30minutes. After the exposure, the plate were covered with the lids and taken to laboratory in sealed plastic bags and the bacteria were incubated at 37^0c for 24hours (Napoli *et al.*, 2012).

3.8 Pure Culture Technique

Different isolates from primary plates were aseptically sub-cultured by streaking onto prepared nutrient agar plates. Plates were incubated at 37 for 24hours. These gave pure culture of isolated organism. The pure culture of the isolates were streaked on prepared sterile set agar slant in MacConkey bottles and kept in the refrigerator at 4 to 6 for further test and identification.

3.9 Preparation of the Nutrients Agar Slants

The Himedia nutrient agar medium was prepared according to the manufacturers instruction (28g of nutrient agar was measured on a weighing balance into a sterile conical flask; 1000ml of distilled water was dispensed into the conical flask). The solution in the conical flask was swirled, to dissolve the medium. After mixing properly, the solution was boiled on the hot plate to ensure homogenization. After boiling, the medium was dispensed into sterile bijou bottle; the caps were placed loosely on the bottles and autoclaved for 15ins at 121 ^oC. Immediately after autoclaving, the bottle was tilted on a rack so that the medium in the bottle will be slanted. The medium was allowed to solidify in this position. When the medium was cool, the caps were tightened. The bottles were stored in the refrigerator.

3.10 Morphological Characteristics of the Isolates

Morphological characterizations were done using colonial, cellular and pigment appearances on culture plates

3.10.1 Biochemical Characterization of the Isolates

The test carried out in identifying the isolates are: Gram Staining, Catalase test, Oxidase test, Methyl red/ Voges Proskauer test, Coagulase test, Starch hydrolysis test, Sugar Fermentation Test, Simmon citrate test, Indole test, and Antibiotics Sensitivity test.

3.10.1.1 Gram staining

Gram staining was done to find reactions of the bacterial isolates to Gram reagents. A smear was prepared and heat fixed. The crystal violet (primary stain) stain was flooded over the fixed culture for 60 seconds; the stain was washed with water. The iodine solutions were added onto the smear for 60 seconds, pour off and rinsed with water. A few drops of decolourizer (ethyl alcohol) was added and washed with water immediately after 5 seconds and finally safaranin (secondary stain) was added for 60 seconds and washed, the smear was allowed to air dry. After drying the slide was mounted under the microscope and observed. The stain differentiates bacterial species into two groups; Gram positive bacteria, which take up crystal violet dye (primary stain) and are stained violet and Gram negative, which pick up safaranin (secondary stain) are thus stained red after decolourization with alcohol.

3.10.1.2 Catalase test

Catalase test is used to differentiate between *Staphylococci* (which produce catalase enzyme) from *streptococci* (which cannot produce catalase enzyme). One ml of hydrogen peroxide Solution was placed in a test tube, and small amount of bacteria growth was added by wood stick. The formation of air bubbles indicated positive result (Cheesbrough, 2000).

3.10.1.3 Coagulase test

This test was carried out for all Gram positive cocci only. Coagulase test is a test used to differentiate between *Staphylococci aureus* (coagulase positive) and other *Staphylococci specie* (coagulase negative).This enzyme can clot the plasma by converting fibrinogen to fibrin. The test was done by placing drop of plasma on slide and then the organism under test was added and mixed gently. Positive was detected by clumping of bacterial cells within 10 seconds (Cheesbrough, 2000). For the Gram negative Bacteria. Some biochemical tests are also carried out to classify them.

3.10.1.4 Oxidase test

This test is carried out with the use of an oxidase strip that has already been soaked in few drops of oxidase reagent. A sterile wire loop is used to pick up colonies from the 24 hours old pure culture, and a smear is made on the oxidase strip each. Appearance of a purple colour within 10 seconds indicates a positive result and no color change indicates a negative result. The results were recorded. On a strip of 2-3 drops of oxidase reagent were placed. With the use of a glass spreader a moderate amount of the organism was taken and streaked on the moist surface of the paper. The presence of Pseudomonas was confirmed by the appearance of intense purple colouration (Hossain *et al.,* 2006)

3.10.1.5 Indole Test

By using of sterile wire loop the organism under test was inoculated in peptone water. The tubes were incubated over night at 37˚C .A drop of kovacs reagent was added after incubation period (Cheesbrough, 2006)

3.10.1.6 Simmons citrate test

12.14gm of Simmons citrate agar was dissolved in 500ml of distilled water gently homogenized using magnetic stirrer while gently swirling to dissolve the medium completely. Afterwards, the

medium was sterilized by autoclaving at 121^0C for 15 minutes and allowed to cool to 50^0C and poured in sterile test tubes. The tubes were then stabbed with a loopful of each isolates into each test tube and then transferred to the incubator and incubated at 37^0C for 24hours. After 24 hours, the tubes were observed. (Olutiola *et al.*, 2000)

3.10.1.7 Methyl red / Voges Proskauer (mrvp) test

8.5gm of the MRVP broth was dissolved in 500ml distilled water, gently homogenized to dissolve the medium completely. 10ml of the broth was distributed into each test tube, covered with corks and the sterilized by autoclaving for 15 minutes afterwards was allowed to cool to room temperature. Each isolate was inoculated into each test tube while labeling them accordingly. The tubes were incubated at 37^0C and observed after 24 hours. Afterwards, 5 drops of methyl red solution was added to each solution. Appearance of red colour indicated positive reaction while appearance of yellow colour indicated negative reaction. (Olutiola *et al.*, 2000)

3.10.1.8 Urease

Urea is a diamide of carbonic acid. It is hydrolyzed with the release of ammonia and carbon dioxide. The aim of the test is to identify organisms that have a urease enzyme which is able to split urea in the presence of water to release ammonia and carbon dioxide. The urea broth medium will be inoculated with a loopful of a pure culture of the test organism; the surface of the agar slant will be streaked with the test organism. The cap will be on loosely and incubated in the test tube at 35 °C in ambient air for 18 to 24 hours; unless specified for longer incubation. If organism produces urease enzyme, the color of the slant changes from light orange to magenta. If organism do not produce urease the agar slant and butt remain light orange (medium retains original color).

3.10.1.9 Sugar fermentation:

5g of peptone, 0.5g of NaCl, 5g of the fermentable sugar (Glucose, Sucrose, Galactose, Maltose and Lactose) and a pint of bromocresol purple was measured into a conical flask and 500ml of distilled water was added, homogenized, dispensed to 19 test tubes. Inverted Durham tubes were placed in each tube, covered with corks and sterilized for 15 minutes. Afterwards, each isolates were inoculated into each test tube respectively and incubated at 370C. After 24 hours, the results were observed. (Olutiola *et al.,* 2000)

3.10.1.10 Starch hydrolysis test

20ml of molten starch agar was aseptically poured into each sterile petridishes, allowed to set and was inverted in an incubator at 37^0C. The organism was streaked across the surface of the plate and incubated at 37^0C for 24-48 hours. Afterwards, the plates were flooded with some quantity of Gram's Iodine. (Olutiola *et al.,* 2000)

3.11 Antibiotics Sensitivity Disc

Sensitivity agar was prepared and poured in petridishes aseptically and allowed to solidify. Afterwards an isolate was streaked all over the surface of agar and the antibiotics disc was placed on the surface using sterile forceps. The Plates were then incubated for 24hours.The presence of a zone of inhibition around the discs showed that the organism is resistant to the antibiotics. (Olutiola *et al.,* 2000)

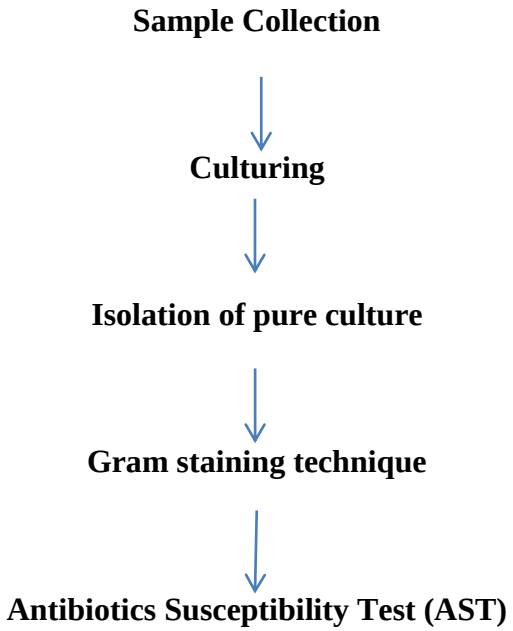

Figure 3.1: Identification of isolated bacteria flow chart

CHAPTER FOUR

4.0 RESULT AND DISCUSSION

4.1 RESULT

Air sampling were carried out from the out-patient section of five (5) selected Primary Health Care Centers (two Private and three Government owned Clinics) in Ogun State, which included Mountain Top University Clinic, Prayer City Clinic, Ibafo Health Center, Ofada Health Center, Wawa Health Center. The open plate method was adopted for the research. Three sterilized nutrient agar plates were exposed at each location for duration of 10 – 30 minutes respectively.

Table 4.1 summarizes the physical conditions of sampling locations such as population of out-patient and environmental temperature during sampling. There was a significant difference in the population of out-patient in the analyzed health centres during sampling which ranged from 4^{e} - 32^{a}, where Ofada had the highest population while Wawa health centre had the lowest. Moreover, environmental temperature of the sampling locations also ranged from $23^{c} – 25^{a}$ ^{o}C.

Table 4.2 summarizes the colony counts in each of the sampling locations for the duration of 10 - 30 minutes. There was a significant difference in the colony forming units (cfu/cm^3) of the sampling locations. At the end of 48 hrs of incubation for 10, 20 and 30 minutes of sampling, colonies ranged from the $7^{e} – 68^{a}$, $14^{e} – 202^{a}$ and $16^{e} – 262^{a}$ cfu/cm^3respectively where the highest was recorded in Prayer city clinic while the lowest was recorded in Wawa Health centre.

Table 4.3 shows the morphological characteristics of each of the isolates. The colour, shape, edge and elevation of each of the isolates were recorded. At the end of incubation periods, the observed colour included yellow, pink, brown and cream; the shape included circular,

filamentous and rhizoid; edge included flat, entire, lobate and rhizoid; the elevation included flat, raised, convex and umbonate; while the surface appearances included smooth, dull and rough.

Table 4.4 shows the biochemical test carried out on the bacterial isolates and their reaction to the test whether positive or negative. The biochemical tests carried out were Catalase test, Coagulase test, Indole test, Citrate test, Mrvp test, Starch hydrolysis and Sugar fermentation.

Table 4.5 shows the antibiotics sensitivity test Antibiotics (Amoxicillin, Augmentin, Erythromycin, chloramphenicol and Pefloxacin) were found to be more resistance to *staphylococcus aureus* whereas antibiotics (Cotrimazole, Gentamicin, ciprofloxacin) were found more sensitive to *Citrobacter spp, Klebsiella, Enterococci.*

Table 4.1: shows the population and physical condition of the out-patient sections of the sampling locations during sampling

Sampling Locations	Population of Out-Patient during sampling	Environmental Temperature of Sampling location (^{O}C)
Ibafo Health Center	18^{b}	23^{c}
Mtu Clinic	5^{d}	23^{c}
Ofada Health Center	32^{a}	24^{b}
Prayer City Clinic	10^{c}	25^{a}
Wawa Health Center	4^{e}	23^{c}

Mean followed by different superscript within a column are significantly different (P ≥0.05)

Table 4.2: shows the variations in colony counts per exposure time on the nutrient agar plate for each of the sampling locations

Sampling Location	Plate exposure time (Minutes)					
	10 min		20 mins		30 mins	
	24 hrs incubation (cfu/cm^3)	48 hrs incubation (cfu/cm^3)	24 hrs incubation (cfu/cm^3)	48 hrs incubation (cfu/cm^3)	24 hrs incubation (cfu/cm^3)	48 hrs incubation (cfu/cm^3)
Wawa health centre	1^{e}	7^{e}	3^{e}	14^{e}	6^{e}	16^{e}
Prayer city clinic	22^{b}	97^{a}	46^{c}	202^{a}	69^{c}	262^{a}
Ofada Health Center	6^{d}	80^{b}	10^{d}	104^{b}	12^{d}	132^{d}
Mountain Top University Clinic	16^{c}	52^{d}	54^{b}	66^{d}	132^{b}	142^{c}
Ibafo Health Center	40^{a}	68^{c}	63^{a}	82^{c}	152^{a}	170^{b}

Mean followed by different superscript within a column are significantly different (P ≥0.05)

Table 4.3: shows the morphological characterizations of the isolates

Sampling Locations	Isolates	Colour on Nutrient Agar	Shape	Edge	Elevation	Surface
Wawa health center	OWN10	cream	circular	rhizoid	Flat	smooth
	OWN20	yellow	circular	entire	Raised	smooth
	OWN30	cream	circular	entire	Raised	dull
Mountain top university clinic	OMN10	brown	circular	rhizoid	Umbonate	rough
	OMN20	yellow	circular	entire	Flat	smooth
	OMN30	cream	rhizoid	lobate	Flat	rough
Ofada Health Center	OFN10	yellow	circular	rhizoid	Raised	rough
	OFN20	pink	filamentous	lobate	Raised	dull
	OFN30	yellow	circular	lobate	Raised	smooth
Prayer city clinic	OPN10	pink	filamentous	entire	Convex	smooth
	OPN20	yellow	circular	lobate	Convex	dull
	OPN30	cream	rhizoid	rhizoid	Flat	rough
Ibafo health Center	OBN10	yellow	circular	entire	Convex	Dull
	OBN20	cream	circular	entire	Flat	dull
	OBN30	cream	circular	flat	Flat	smooth

Table 4.4: shows the biochemical characteristics of the isolated bacteria

Location	Isolation	Gram Staning	Shape	Catalase	Oxidase	Methyl Red	VP Test	Urease	Indole	Citrate	Coagulase	Starch Hydrolysis	Glucose	Galactose	Bacterial Isolates
IBAFO HEALTH CENTER	OBN101	+	Cocci	+	-	-	+	-	-	-	-	+	AG	AG	*Staphylococcus*
	OBN102	+	Cocci	+	-	-	+	+	-	-	-	-	AG	-	*Staphylococcus*
	OBN103	+	Rod	+	-	-	+	-	-	+	-	+	-	AG	*Bacillus*
	OBN201	+	Rod	+	-	-	+	-	-	+	-	+	-	AG	*Bacillus*
	OBN202	-	Clustered short rod	+	-	-	+	+	-	+	+	-	AG	AG	*Klebsiella*
	OBN203	+	Cocci	+	-	-	+	-	-	-	-	+	AG	AG	*Staphylococcus*
	OBN301	+	Cocci	-	-	+	+	-	-	-	+	-	AG	-	*Enterococci*
	OBN302	+	Cocci	-	-	+	+	-	-	-	+	-	AG	-	*Enterococci*
	OBN303	+	Rod	+	-	-	+	-	-	+	-	+	-	-	*Bacillus*
MOUNTAIN TOP UNIVERSITY	OMN101	+	Cocci	+	-	-	+	-	-	+	-	+	-	-	*Bacillus*
	OMN102	-	Rod	+	-	-	+	-	-	-	+	-	AG	A	*Citrobacter*
	OMN103	-	Rod	+	-	-	+	+	-	+	+	-	AG	AG	*Klebsiella*
	OMN201	+	Cocci	+	+	-	+	+	-	-	-	-	AG	-	*Staphylococcus*
	OMN202	+	Cocci	+	+	-	+	+	-	-	-	-	AG	-	*Staphylococcus*
	OMN203	+	Cocci	+	+	-	+	+	-	-	-	-	AG	-	*Staphylococcus*
	OMN301	-	Rod	+	-	+	+	-	-	+	-	+	AG	-	*Klebsiella*
	OMN302	-	Rod	+	-	+	+	-	-	+	-	+	AG	-	*Klebsiella*
	OMN303	+	Cocci	+	+	-	+	+	-	+	-	-	AG	AG	*Staphylococcus*
OFADA HEALTH CENTER	OFN101	+	Short rod	+	+	+	-	-	-	+	-	+	AG	-	*Bacillus*
	OFN102	+	Short rod	+	+	+	-	-	-	+	-	+	AG	-	*Bacillus*
	OFN103	-	Rod	+	-	-	+	-	-	-	+	-	AG	A	*Citrobacter*
	OFN201	+	Cocci	-	-	+	+	-	-	-	+	-	AG	-	*Enterococci*

	OFN202	-	Rod	+	-	-	+	-	-	-	+	-	AG	A	*Citrobacter*
	OFN203	+	Rod	-	+	+	-	+	-	+	-	+	AG	+	*Bacillus*
	OFN301	+	Rod	-	+	+	-	+	-	-	-	-	AG	-	*Bacillus*
	OFN302	-	Rod	+	-	+	-	-	-	-	+	+	AG	-	*Klebsiella*
	OFN3O3	-	Cocci	-	-	+	+	-	-	-	+	-	AG	-	*Enterococci*
PRAYERCITY CLINIC	OPN101	+	Short rod	+	+	-	+	-	-	+	-	-	AG	-	*Bacillus*
	OPN102	+	Short rod	+	+	-	+	-	-	+	-	-	AG	-	*Bacillus*
	OPN103	+	Cocci	+	-	-	+	-	-	+	-	-	-	AG	*Staphylococcus*
	OPN201	+	Cocci	+	-	-	+	-	-	+	-	-	-	AG	*Staphylococcus*
	OPN202	+	Cocci	+	-	-	+	-	-	+	-	-	-	AG	*Staphylococcus*
	OPN203	+	Cocci	+	-	-	+	-	-	+	-	-	-	AG	*Staphylococcus*
	OPN301	+	Cocci	-	-	-	+	-	-	-	+	-	AG	AG	*Enterococci*
	OPN302	-	Rod	+	+	+	-	-	-	+	-	+	-	-	*Pseudomonas*
	OPN303	+	Cocci	+	+	-	+	+	-	-	-	-	AG	-	*Staphylococcus*
WAWA HEALTH CENTER	OWN101	+	Cocci	+	+	-	+	-	-	+	-	+	AG	-	*Staphylococcus*
	OWN102	+	Cocci	+	+	-	+	+	-	-	-	-	AG	-	*Staphylococcus*
	OWN103	+	Cocci	+	+	-	+	+	-	-	-	-	AG	-	*Staphylococcus*
	OWN201	-	Rod	+	-	+	-	+	+	-	+	+	AG	AG	*Klebsiella*
	OWN202	+	Cocci	+	+	-	+	+	-	-	-	-	AG	-	*Staphylococcus*
	OWN203	+	Cocci	+	+	-	+	+	-	-	-	-	AG	-	*Staphylococcus*
	OWN301	-	Rod	+	-	-	+	-	-	-	+	-	AG	A	*Citrobacter*
	OWN302	-	Rod	+	+	+	-	-	-	-	+	+	-	-	*Pseudomonas*
	OWN303	-	Rod	+	+	+	-	-	-	+	-	+	-	-	*Pseudomonas*

(+)= Positive, (-)= Negative; A= Gas only; AG = Acid and Gas Production (NO) = No Gas Production

Table 4.5 shows the antibiotics sensitivities of the bacterial isolates

BACTERIA ISOLATES	ANTIBIOTICS									
	APX	AU	AM	CPX	SXT	S	E	PEF	CN	CH
STAPHYLOCOCCUS	R	R	R	S	R	R	R	R	R	R
BACILLUS	R	I	R	R	S	S	S	R	S	S
ENTEROCOCCI	S	R	S	S	R	R	R	S	S	R
KLEBSIELLA	R	R	R	S	I	S	I	R	S	R
PSEUDOMONAS	R	R	S	S	S	R	R	S	I	R
CITROBACTER	R	R	S	R	R	S	R	S	R	R

Codes: (R)- Resistance, (S)-Sensitive, (I)-Intermediate, (AM)- Ampicillin, (APX)-Amoxicillin, (Cpx)- Ciprofloxacin,(E)- Erythromycin, (AU)-Augmentin, (SXT)-Cotrimoxazole, (PEF)-Pefloxacin, (S)-Septrin, (CH)-Chloramphenicol, (CN)-Gentamicin

4.2 DISCUSSION

Recently there have been increased in the reported cases of nosocomial infections and a lot of factors have been found to contribute to this upsurge. One of them is the increase rate of out-patients visiting the hospitals compared to the available facilities and man-power (Ekhaise *et al* 2008). In this study, the colony counts per each sampling locations was found to correspond to the population of the outpatients during sampling.

Various morphological appearances of the isolates were observed such as colour, elevations, edges, surface appearance. This observation has been previously reported by (Olutiola *et al* 2000). Moreover, the populations of gram positive bacteria were found to out-weigh that of gram negative. This observation has also been reported by (Varrone *et al.*, 2014). Furthermore, the genera Staphylococci and Bacillus were found to be more significant in all the sampling locations compared to other genera such as *Pseudomonas spp, Klebsiella spp, Citrobacter spp*. This finding was also supported by Shiaka *et al.* (2011) that Staphylococcus appeared to be a normal floral of air especially where the air is highly contaminated. *Staphylococcus aureus* belong to normal flora of the human skin; it is likely that these microorganisms may be originated from the skin flora of the lab workers, guard, patients. The high concentration of *Staphylococcus aureus* in the hospital environment should not be seen as a blessing but as a pathogen as they can cause disease through invasion and toxin production such as abscess, pneumonia, diarrhoea and the most feared toxic shock syndrome.

Moreover, the presence of Bacillus species in the sampling location was not surprising as it can survive in the air for longer period of time since it produces spores which are resistant against severe environmental conditions. However, Bornehag *et al.* (2004) was of the opinion that since

air does not contain any nutrients and has low moisture content it is not conducive to the growth and survival of microorganisms but it can act as important medium for carrying and spreading of biological agents. Additionally, in non-industrial indoor environments, the most important source of airborne bacteria is the presence of human (Stetzenbach, 2007). Specific activities like talking, sneezing, coughing, walking, washing and toilet flushing can generate airborne biological particulate matter.

CHAPTER FIVE

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 CONCLUSION

The result of microbial assessment of outdoor air of the five selected Primary Health Care (MTU Clinic, Prayer City Clinic, Ibafo Health Center, Ofada Health Center, Wawa Health Center) revealed that outdoor air of the hospital environments were not hygienic when compared to the W.H.O standard values where the maximum limit for air borne bacterial count is placed at 100 cfu/m^3. As such, the outdoor air may pose health risk to patients, healthcare workers and visitors who may spend long period of time in the hospital.

The genera Staphylococcus, Bacillus, Pseudomonas, Klebsiella, and Citrobacter were isolated. Staphylococcus and Bacillus implicated in food poisoning were the predominant outdoor air contaminants. The unavoidable contact with these microbes in the air by patients directly or indirectly could cause nosocomial infection leading to their increase stay in the hospital or undesirable health effects. It may also pose serious threat to the health of health care workers and visitors to the hospital wards/units. These findings call for a review of infection control measures in the hospital environment and necessary steps should be taken by the authorities to forestall undesirable consequences.

5.2 RECOMMENDATION

The results of this study can be further worked on to isolate and determine other air borne microbes in the outstation and other areas of the clinic or hospital to improve the environmental condition of the clinics and create a safer environment for the worker and the patient. Moreover, as observed in this study, Staphylococcus aureus isolates and Bacillus spp were found to be the predominant isolates from Primary Health Center premises. As such, environmental sanitation

should be carried out on a regular basis so as to maintain clean Primary Health Care Center environment.

More concentration should be given on maintenance of air conditioners because they can transmit biological agents such as allergen, mycotoxin, volatile organic compounds etc., into the air which can cause health problems. In order to develop the quality of outdoor air in the clinic or hospitals first overcrowding has to be avoided and a good ventilation system has to be design. Implementation of comprehensive infection control programs and surveillance of infection, in hospitals by infection control committee. Health Talk program to Primary Health Center staff, so as to protect themselves and the patients from the contaminating bacteria, as well as from spreading pathogenic bacteria to others.

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