MOLECULAR DETECTION OF AMINOGLYCOSIDES RESISTANCE GENES IN BACTERIA ISOLATED FROM EAR OF PATIENTS ATTENDING FEDERAL MEDICAL CENTRE KEFFI, NIGERIA.

BY

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DECLARATION

I hereby declare that this dissertation has been written by me and it is a report of my research work. It has not been presented in any previous application for a quotations are indicated and sources of information are specifically acknowledged by means of references.

Iperepolu Odunayo

Date

CERTIFICATION

This is to certify that this dissertation title; "Molecular Detection of Aminoglycosides Resistance in bacteria isolated from Ear of Patients attending Federal Medical Centre Keffi, Nigeria" meets the regulations governing the award of Master's degree of the school of postgraduate studies Nasarawa State University, Keffi and is approved for its contribution to knowledge.

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DEDICATION

This research dissertation is dedicated to the Almighty God Who gives all things, without Whom, this dissertation and my completion of a successful stay in this university would not have been possible. To God be all the glory.

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ABSTRACT

Ear infection is a common problem for both children and adults. it can be caused by viruses and fungi but the major causes of ear infection are bacterial. The aim of study was molecular detection of aminoglycosides resistance genes in bacteria isolated from ear of patients Attending Federal Medical Centre Keffi, Nigeria.Two hundred and fifteen (215) ear swab samples were collected from patients attending Federal Medical Centre Keffi. Forty eight (48) different bacteria species were isolated and identified using standard microbiological methods. The Antibiotics Resistant test for the isolates was carried out using method of Clinical and Laboratory Standards Institute. The Molecular detection of aminoglycosides of multiple antibiotic resistant isolates was carried out using agarose gel electrophoresis. The occurrence of different bacteria was 22.3%, in which *Pseudomonas* species had (7.4%). *Klebsiella* species(5.1%), *Proteus* species(3.7%), *Staphylococcus aureus* (3.2%) and *Staphylococcus* (2.7%). *Pseudomonas* species isolates were more resistant to ceftazidime (50.0%), but less resistance to chloramphenicol (37.5%), *Klebsiella* species were highly resistant to tetracycline (63.6 but less resistance to ciprofloxacin (36.3%). *Staphylococcus aureus* were more resistant to amoxicillin/clavulanic acid (71.4%), but less resistance to chloramphenicol. (28.5%). *Proteus* species were more resistant to amoxicillin/clavulanic acid (75.0%), but less resistance to ciprofloxacin (37.5%). , Streptomycin (12.5%). *Staphylococcus epidmities* were highly resistant to amoxicillin/clavulanic acid (66.6%), but less resistance to ciprofloxacin (33.3%). The most common resistant phenotype being *Pseudomonas* species with C-CIP- AMC - AMP -TE-SXT and C-CIP-CAZ- AMC -TE-SXT-S (18.7%) having occurrence of 11.5%. *Klebsiella* species C-CN-CIP-CRO-CAZ-TE- AMC-AMP-SXT (18.1%). All the MAR isolates have MAR index ≥ 0.5; the commonest with *Pseudomonas* species being 0.5 and 0.7 (25.0%); *Klebsiella* species being 0.6 (36.3%); *Staphylococcus aureus* 0.9 (42.8%); *Proteus* species 0.7 (50.0%); *Staphylococcus epidermidis* 0.8 (33.3%). Five different bacteria isolates resistant to gentamicin, three (3) were detected of having *ANT 211* resistant gene with basepair of 450bp while one (1) isolate had *aa-(c)*resistant gene with basepair of 650bp.Different bacteria species isolated from the study location were Multi-drug resistance and encode different resistant genes. The best treatment option for study is gentamicin which has the highest susceptibility rate (88.3%) for all the bacteria isolates tested.

CHAPTER ONE

INTRODUCTION

1.1 Background of the Study

Ear infection is an inflammation of the ear, and ear discharge is one of the commonest symptoms. Ear infection is a common problem for both children and adults. it can be caused by viruses and fungi but the major causes of ear infection is bacterial (Muluye *et al.*, 2013). Most infections of the ear involved the canal (otitis externa) or the middle ear cavity (otitis media) which contains the ossicles and is enclosed by bony structures and the tympanic membrane (Ryan *et al.*, 2010).

Otitis media (OM) is one of the two categories of the ear inflammation (Jido *et al.*, 2014). Otitis media is the inflammation of the mucous membrane of the middle ear cleft which includes the middle ear cavity (tympanic cavity), mastoid antrum, mastoid air cells and the Eustachian tube (Ilechukwu *et al.*, 2014), usually caused by bacteria and may be caused by fungi or viruses (Jido *et al.*, 2014). When the inflammation is associated with a discharge from the ear through a perforation in the tympanic membrane, suppurative (or discharging) otitis media occurs. It may be acute (<6 weeks) (Ilechukwu *et al.*, 2014). Otitis externa, also called swimmer's ear, which involves diffuse inflammation of the external ear canal that may extend distally to the pinna and proximally to the tympanic membrane. Otitis externa lasting three months or longer, known as chronic otitis externa, is often the result of allergies, chronic dermatologic conditions, or inadequately treated acute otitis externa. On rare occasions, the infection invades the surrounding soft tissue and bone; this is known as malignant (necrotizing) otitis externa (Schaefer and Baugh, 2012).

Otitis externa make up 5 to 20% of ear infection, most of them caused by bacteria, and from the latter 9 to 25% are caused by fungi, called fungal otitis or otomycosis (Pontes *et al.*, 2009).

Otomycosis is fungal infection of the external auditory canal and its associated complications sometimes involving the middle ear (Satish *et al.*, 2013). It results from prolonged bacterial treatment of chronic otitis media or primarily by fungal species (Abera and Kibret, 2011). The most common predisposing factors of otitis externa is swimming, especially in fresh water. Other factors include skin conditions such as eczema and seborrhea, trauma from cerumen removal, use of external devices such as hearing aids, and cerumen buildup. These factors appear to work primarily through loss of the protective cerumen barrier, disruption of the epithelium (including maceration from water retention), inoculation with bacteria, and increase in the pH of the ear canal (Schaefer and Baugh, 2012). The upper respiratory tract infections like rhinitis and nasopharyngitis usually set the stage for infection of the middle ear by allowing spread of pathogenic organisms from the nasopharynx into the middle ear via the Eustachian tube (Ilechukwu *et al.*, 2014).

Immunity play important role in ear infection, the lower immunity in children as compared to adult and the (shorter and more horizontal) Eustachian tube in children which permits easier access of microorganisms from the nasopharynx (Ogbogu *et al.*, 2013). Infection can cause irritation, pain, itching, swelling, a feeling of pressure in the ear and a purulent discharge (Kiser *et al.*, 2011). The major causes of ear infection are bacteria such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus* species, *Escherichia coli*, *Klebsiella pneumonia* and Coagulase Negative Staphylococci (Muluye *et al.*, 2013), also *Citrobacter* species cause ear infection (Ogbogu *et al.*, 2013). In otomycosis the species of Aspergillus and Candida are the most common agents of the diseases (Ho *et al.*, 2006).

About 65-330 million people suffer from ear infection worldwide and 60% of them had significant hearing impairment (Muluye *et al.*, 2013). Complication in ear infection differs and this depends on the level of severity and duration of the infection in relation to the causative

organisms (Zaria *et al.*, 2011).

Treatment include elimination of predisposing factor and the use of suitable antimicrobial agent. the antibacterial like tetracycline, penicillin, erythromycin, chloramphenicol, gentamicin, ciprofloxacin, norfloxacillin, cotrimoxazole, ceftriaxone, ampicillin and amoxicillin (Muluye, 2013), and antifungal like clotrimazole, miconazole, econazole, nystatin, tolnaftate and potassium sorbate (Khan *et al.*, 2013).

1.2 Statement of the Problem

About 65-330 million people suffer from ear infection worldwide and 60% of them had significant hearing impairment (Muluye *et al.*, 2013). This makes it difficult for people with ear infection to loss their social status, uninteractive and unproductive in the society. Ear infection differs in complication and this depends on the level of severity and duration of the infection in relation to the causative organisms (Zaria *et al.*, 2011). Inadequate provisions for molecular diagnostics outside of a selected few suitably supplied tertiary care centers, misdiagnosis of diseases and multidrug resistant (MDR) bacterial infections is likely to be a common occurrence in both urban and rural communities across Nigeria (Makanjuola *et al.*, 2020). Treatment include elimination of predisposing factor and the use of suitable antimicrobial agent.

1.3 Justification of the Study

Ear infections in children and adults are a major health problem and may be associated with hearing impairment, delayed speech and language development. Ear is one of the vital organs of the body. Over 5% of the world's population – 360 million people – has disabling hearing loss (328 million adults and 32 million children). Disabling hearing loss refers to hearing loss greater than 40 decibels (dB) in the better hearing ear in adults and a hearing loss greater than 30 dB in

the better hearing ear in children. The majority of people with disabling hearing loss live in low- and middle-income countries. Approximately one third of people over 65 years of age are affected by disabling hearing loss. The prevalence in this age group is greatest in South Asia, Asia Pacific and sub-Saharan Africa, which include Nigeria. Recently researchers have discovered that "the human middle ear is inhabited with by more diverse microbial communities than was previously thought. Alteration of the middle ear microbiome may contribute to the pathogenesis of chronic otitis media with active inflammation (Shujiro *et al.*, 2017).

The Health systems in our country are ill-equipped to contend with emerging threats of diseases and delivery of quality healthcare is still far from ideal.

1.4 Aim of the Study

The aim of this study was to detect aminoglycosides resistance genes in bacteria isolated from ear infection of patients attending Federal Medical Centre Keffi, Nasarawa State of Nigeria.

1.4.1. Specific objectives

The specific objectives of this study include to

i. Isolate and identify bacteria associated with ear infection.

ii. Determine antimicrobial susceptibility pattern of the isolates to selected antibiotics

iii. Determine the size and number of plasmids of aminoglycosides resistance isolates

1.5 Statement of Hypothesis

1.5.1 Null Hypothesis (H_0): There is no significant correlation between resistance bacteria isolated and Aminoglycosides

1.5.2 Alternate Hypothesis (H_1): There is significant correlation between resistance bacteria isolated and Aminoglycosides

CHAPTER TWO

LITREATURE REVIEW

2.1 Anatomy of the Ear

There are three parts to the ear: the external ear, the middle ear or tympanic space, and the internal ear or labyrinth (Fig 2.1). Each is prone to infection by microorganisms (Kiser *et al.*, 2011). The ear contains the receptors for two senses: hearing and equilibrium. These receptors are all found in the inner ear (Scanlon and Sanders, 2007).

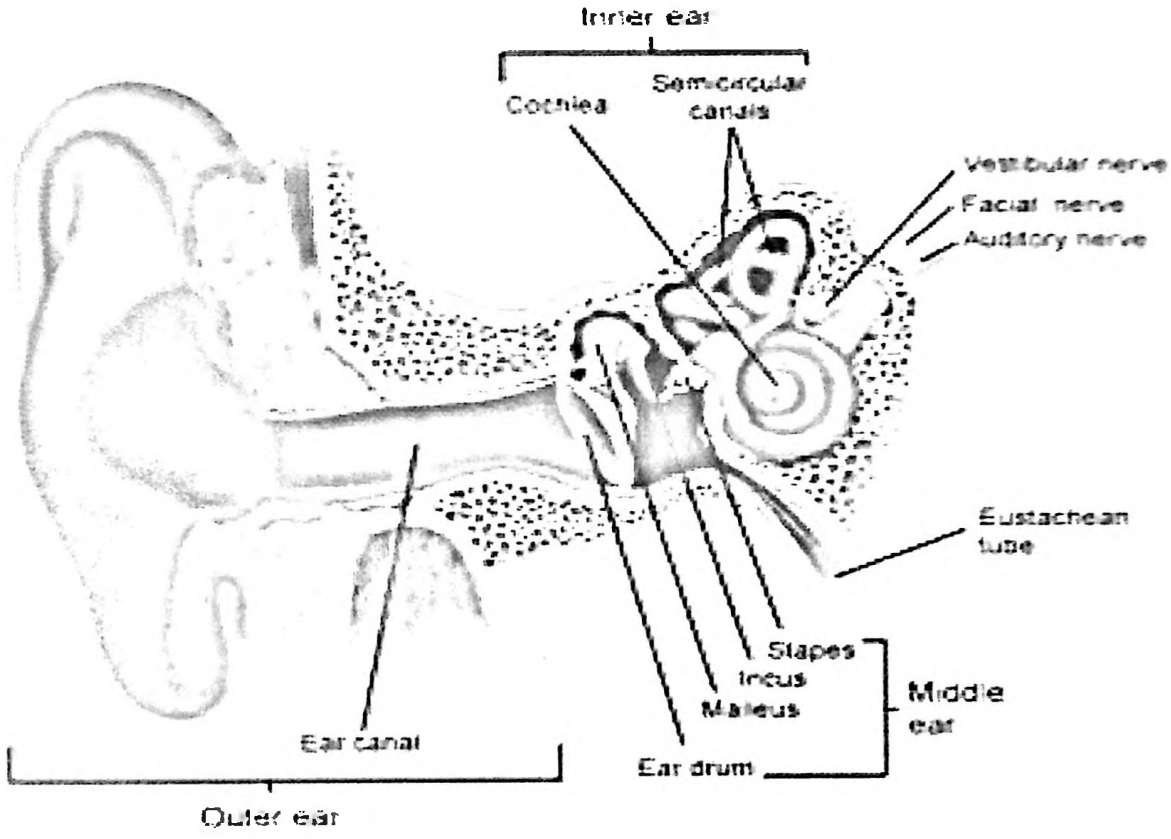


Fig (2.1): Anatomical representation of ear.

Source: Medicalhomeportal.org

2.2 Ear Infection

Ear infection is an inflammation of the ear and it is a common problem for both children and adults (Muluye *et al.*, 2013).

2.2.1 Types of Ear Infection

Otitis Media (OM)

Otitis media is the inflammation of the mucous membrane of the middle ear (Ilechukwu *et al.*, 2014). It occurs in the area between the tympanic membrane and the inner ear (Jido *et al.*, 2014), which includes the middle ear cavity (tympanic cavity), mastoid antrum, mastoid air cells and the Eustachian tube (Ilechukwu *et al.*, 2014). The infection associated with the malfunctioning of the middle ear due to pathogenic micro-organisms that are resident in the middle ear. The middle ear is usually affected as a result of colonization by pathogenic organisms (Zaria *et al.*, 2011). It is usually caused by bacteria that occurs when fluid buildup behind an eardrum. Occasionally, otitis media may be caused by fungi or viruses (Jido *et al.*, 2014). Etiologic agents for otitis media include viruses and bacteria. The notable viruses include rhinovirus, adenovirus, parainfluenza, coronavirus and respiratory syncytial virus (Ilechukwu *et al.*, 2014). While predominant bacteria that cause otitis media are *Moraxella catarrhalis, Streptococcus pneumonia* and *Hemophilus influenza*. Other pathogens responsible for Otitis Media are *Staphylococcus aureus, Escherichia coli, Klebsiella species, Pseudomonas aeruginosa* and *Proteus species*. The types of pathogens involved in otitis media have also been found to be dependent on geographical location (Okesola and Fasinam, 2012). When the inflammation is associated with a discharge from the ear through a perforation in the tympanic membrane, suppurative (or discharging) otitis media occurs (Ilechukwu *et al.*, 2014). Otitis media can be differentiated into chronic suppurative otitis media (CSOM) and acute otitis media (AOM) (Zaria *et al.*, 2011). Otitis media may be associated with infection or may be

sterile. The sterile variety is usually called Serous Otitis Media and is often attributed to allergy, but may also occur from numerous other potential sources including radiation treatment or virus (Okesola and Fasinam. 2012).

2.2.1.1 Types of Otitis Media

Acute Otitis Media (AOM)

Acute otitis media is a purulent infection of the middle ear (Nwogwugwu *et al.*, 2014). It is considered as acute infection if it continues less than 6 weeks (Ilechukwu *et al.*, 2014). Normally originates from an upper respiratory tract infection with the bacteria entering by the auditory (Eustachian) tube which is the principal portal of entry of pathogens into the ear (Okesola and Fasinam, 2012). Otitis media is one of the commonest diseases of infants and young children (Nwogwugwu *et al.*, 2014) and it is the commonest ear pathology in otorhinolaryngological practice. Otalgia followed by otorhoea are the commonest symptoms prior to presentation in the hospital. The acute form if not recognized early is commonly characterized by suppuration from the middle ear following perforation of the tympanic membrane.

b. Chronic Suppurative Otitis Media (CSOM)

Chronic suppurative otitis media is defined as a chronic inflammation of the middle ear and mastoid cavity, which presents with recurrent ear discharges through a tympanic perforation (Okesola and Fasinam, 2012), it is labeled as chronic otitis media when the symptoms are present beyond more than 6 weeks (Moorthym *et al.*, 2013). It is a massive public health problem (Singh *et al.*, 2012), and it is one of the most common diseases of all age groups, especially of childhood (Parveen and Rao, 2012). Dysfunction of the Eustachian tube and the bacterial infection are important factors in the multifactorial pathogenesis of Chronic suppurative otitis media (Lee *et al.*, 2012).

C. Otitis Media with Effusion (OME)

Otitis media with effusion is an inflammation of the middle ear in which fluid accumulates behind the eardrum, without any signs or symptoms of acute infection, and with an intact tympanic membrane. Secretory otitis media, non suppurative otitis media, serous otitis media and mucoid otitis media are synonymous with otitis media with effusion, but these terms are not as accurate (Pereira *et al.*, 2004).

The causes such as Eustachian tube dysfunction, immunodeficiency craniofacial abnormalities, infections and allergic agents are widely discussed (Tikaram *et al.*, 2012). The most frequently detected bacteria are *Streptococcus pneumoniae, Haemophilus influenzae* and *Moraxella catarrhalis* (Pereira *et al.*, 2004). The complications and sequels of otitis media externa are an important public health problem. The patients will have impaired development of speech and language, poor school performance, tympanosclerosis, retraction pockets and psychosocial problems (Tikaram *et al.*, 2012).

2.2.2. Otitis Externa (OE)

Otitis externa is a generic term for inflammation of the external auditory meatus (EAM) skin (Enoz *et al.*, 2009). Otitis externa is the most common ear infection in the clinical practice of otolaryngology and the infection of the outer ear canal with the extension to the auricle with or without involvement of the ear drum (Al Chalabi *et al.*, 2009). Usually infectious (bacterial or fungal) in etiology, the main symptoms include severe otalgia, purulent discharge and variable hypoacousia (Enoz *et al.*, 2009). Swimmers are most commonly affected with this type of infection excess moisture becomes trapped in the auditory canal and serves as the source of most infections, the external ear has several defenses to prevent infections like ear wax or cerumen is acidic preventing microorganisms from colonizing the skin, earwax is full of lipids which prevents water from reaching the skin. The dead skin cell, ear wax and debris are carried

wax is over produced or under produced infection can occur (Kiser *et al.*, 2011). *Pseudomonas aeroginosa* a water-loving organism is the most common causative agent but *Staphylococcus spp, Streptococcus spp* and fungi can cause infections (Kiser *et al.*, 2011). fungal otitis externa (Otomycosis) has typically been described as fungal infection of the external auditory canal with infrequent complications involving the middle ear. Species of *Aspergillus* and *Candida* are the most common agents of the diseases (Ho *et al.*, 2006). The other agents are *Actinomyces, Mucor, Trichophyton, Penicillium* and *Rhizopus spp*. Otomycosis found throughout the world but more common in tropical region, usually requires long-term treatment and tends to recur (Uslu *et al.*, 2005).

2.2.3. Labyrinthitis

Inflammation of the inner ear labyrinth. Patients complain of dizziness with accompanying nausea and vomiting. Often the signs and symptoms appear after a recent viral upper respiratory infection such as a common cold. Viral labyrinthitis, the most common form, has a sudden onset with hearing loss and dizziness. Most cases occur in adults. Viral labyrinthitis is usually self-limiting, lasting day to week (Kiser *et al.*, 2011). Bacteria can also cause labyrinthitis but have a very low incidence. The same organisms that cause meningitis and otitis media are usually responsible. Bacterial labyrinthitis usually results in permanent hearing loss (Kiser *et al.*, 2011).

2.2.4. Clinical Features

The first symptoms of ear ache usually appear during late evening or at night, when the child is in a supine position so the pressure increases in the middle ear. The most common local symptom of otitis media is otalgia (i.e. ear ache), appearing 73% of attacks. Other local symptoms and signs include otorrhoea (i.e. discharge from the ear canal), hearing loss, vertigo,

tinnitus and in very rare cases swelling around the ear and facial paralysis. The severity of the symptoms should be also evaluated. Non severe illness is defined as mild otalgia and fever less than 39°C, and severe illness as moderate to severe otalgia and fever >39°C (Malmi, 2007). symptoms are non-specific and may include, restlessness, bouts of screaming, anorexia (Ilechukwu *et al.*, 2014), headache, vomiting diarrhea, which are also symptoms of viral upper respiratory infection (Malmi, 2007). Symptoms of otitis externa include severe otalgia, purulent discharge and variable hypoacousia (Enoz *et al.*, 2009). Infection can cause irritation, swelling and feeling of pressure in the ear (Kiser *et al.*, 2011). Otomycosis presents with nonspecific symptoms like tinnitus, hearing impairment, and sometimes discharge, and also recurrence is common (Satish *et al.*, 2013). The infection is usually unilateral and characterised by inflammation, pruritus, scaling and severe discomfort such as pain and suppuration (Khan *et al.*, 2013). Presence of greyish black coloured mass in the ear canals were the commonest signs (Uslu *et al.*, 2005). Disease other than ear infection can also cause ear pain, including cancers of any structure that shares nerves supply with the ear and shingle which can lead to herpeszoster oticus (Jido *et al.*, 2014).

2.2.5. Pathogenesis of Ear Infection

The pathogenesis is complex and multifactorial, resulting from a continuous interplay between host immune responses and environmental microbial load (Malmi, 2007). The Eustachian tube connects middle ear to the nasopharynx. The nose, nasopharynx, Eustachian tube, middle ear, and mastoid gas cells compose a system of organs that play role in the development of otitis Media. These structures are all covered by respiratory mucosa. On the posterior wall of the nasopharynx, right above the adenoid, is the opening of the Eustachian tube (Pähtinen, 2012). The Eustachian tubes, or more precisely their anatomy and function, have a crucial role in the

pathogenesis of Otitis Media because microbes of the nasopharynx invade the middle ear through the Eustachian tube (Malmi, 2007). In infants, the average length of the Eustachian tube is 2cm and the tube lies almost horizontally (Tähtinen, 2012), this allows for easier spread of infection from the nasopharynx to the middle ear (Ilechukwu *et al.*, 2014). In adults, the Eustachian tube is about twice as long and it is angled 45° upwards (Tähtinen, 2012). Otitis media runs through the following stages: tubal occlusion, presuppuration, suppuration then resolution or complication (Ilechukwu *et al.*, 2014).

2.2.6. Immunity

The immune system protects the body from foreign invaders. The immune tissue of the upper respiratory tract consists of the palatine tonsils, adenoid, lingual tonsils, and tubal tonsils. They form the Waldeyer's ring which acts as a gatekeeper, since pathogens that cause Acute Otitis Media first invade the throat or nasopharynx. The immune system also involves cells that interact with each other in order to eliminate the foreign invader (Tähtinen, 2012). The immune system may be divided into innate and adaptive immunity. The innate immunity is responsible for fast and nonspecific recognition of pathogens. The adaptive is a specific reaction to a certain antigen that results in production of antigen-specific T and B lymphocytes. The cells of the adaptive immune system remember the pathogen and create an efficient immune response in case of later re-exposition to the pathogen (Tähtinen, 2012).

The middle ear is covered by respiratory mucosa. If the microbe enters the middle ear, its immunologically active antigens induce epithelial cells, dendritic cells, and mast cells to produce a local immune response. The innate immunity is considered to be the mediator of initial host response in the middle ear. It includes barrier functions, release of antimicrobial molecules, and activity of effector cells like neutrophils, macrophages, fibroblasts, natural killer

cells, and eosinophils (Tähtinen, 2012). Toll-like receptors are situated on the surface of mucosal cells. These receptors detect specific molecules at the surface of pathogens and activate several effector molecules such as cytokines, chemokines, interferons, proteases, defensins, collectins, lysozymes, and lactoferrins. Defensins kill pathogens by forming a pore in the outer membrane. Some defensins inhibit bacterial toxins and some stimulate the inflammatory response (Tähtinen, 2012). Local immunity and B lymphocyte function have not developed fully in young children, a fact that may partly explain why children are at higher risk of Otitis Media than adult (Tähtinen, 2012). Bacteria may also prevent phagocytosis by forming a biofilm, an aggregate of bacteria in which they adhere to each other and form a thin layer on a surface. Biofilms have been found from the middle ear mucosa of children with chronic Otitis Media and recurrent Acute Otitis Media. It has been suggested that biofilms may protect the bacteria from the host's immune system and increase significantly the resistance of bacteria to antimicrobials (Tähtinen, 2012). Breast feeding is known to reduce the incidence of acute respiratory infections. It also prevents colonization with otitis pathogens through selective IgA antibody; and decreases the amount of contaminated secretions aspirated into the middle ear (Ilechukwu *et al.*, 2014).

2.2.7. Predisposing Factors of Ear Infection

Several predisposing factors have been reported to cause ear infections. The microbial load is influenced by such environmental factors as daycare attendance and season, whereas the host immune responses are influenced by such factors as genetic predisposition and age. Ear infections tend to occur more in fall and winter because upper-respiratory infections such as cold and flu are on the rise then, too. Children who are two and younger get more ear infections because of the small size and shape of their Eustachian tubes.

Other respiratory tract infections, and traumatic tympanic rupture as well as factors correlating with resource-limited living conditions such as overcrowding, poor nutrition and hygiene, and chronic infectious diseases. Otitis media is a multifactorial disease. Infectious, allergic, and environmental factors contribute to otitis media (Mittal *et al.*, 2014: Seppala, *et al*., 2016). However; normal flora of the skin such as *Pseudomonas aeruginosa, Staphylococcus aureus, Proteus mirabilis, Klebsiella pneumonia* and *Escherichia coli* that can easily enter through perforated ear have been reported as the main agents of otitis media (Abera and Kibret 2011).

i.Daycare- Children in day care tend to have more infections of any kind in general, simply because most infections and viruses are spread most easily at this age. Toddlers and infants are touching and handling everything and placing fingers in their mouths, and even with the best hand-washing techniques it is hard to control the spread of germs. Contact with multiple children and daycare providers facilitates spread bacterial and viral pathogens There are two host and environmental factors which are critical to the transmission and development of infectious diseases in day care. The first relates to age of the child and the second to the size and structure of the daycare facility. Most children with acute otitis media (70 to 90 percent) have spontaneous resolution within 7 to 14 days; therefore, antibiotics should not routinely be prescribed initially for all children (Rosenfeld *et al.*, 2003). Other aspects of age-specific personal hygiene include the potential for sharing respiratory secretions amid the many runny noses that go unchecked unless an adult hand with a disposable wipe is nearby on a minute-to-minute basis.

ii. Age- Infants and young children's immune systems are immature and their Eustachian tubes are structurally and functionally different from those of adults (Malmi, 2007).

Acute Otitis Media (AOM) usually affects children aged under 2 years, and presents with acute onset symptoms and signs of otalgia and fever, in a child that is systemically unwell. It is acute inflammation, and may be caused by bacteria or viruses.

A particular subtype of Acute Otitis Media is acute suppurative otitis media, which is characterized by the presence of pus in the middle ear. Incidence of this disease is higher in developing countries especially among low socio-economic society because of malnutrition, overcrowding, poor hygiene, inadequate health care, and recurrent upper respiratory tract infection (Kumar, 2011)

Acute otitis media is the second most common pediatric diagnosis in the emergency department following upper respiratory infections. Although otitis media can occur at any age, it is most commonly seen between the ages of 6 to 24 months (Meherali *et al.*, 2019) In contrast to Acute Otitis Media and acute mastoiditis, Otitis Media with effusion (OME) is a chronic inflammatory condition. It typically affects children between 3 and 7 years old. It is characterized by the presence of an effusion, glue-like fluid behind an intact tympanic membrane in the absence of signs and symptoms of acute inflammation (Van Zon *et al.*, 2012) for this reason the commonest reported symptom is hearing loss, which may lead to speech delay or educational problems.

The diagnosis of OME in newborns and infants is particularly difficult and inherent to the difficulty of performing the otoscopy in this age group, not only by the size of the ear canal, but also due to the patients lack of cooperation, the presence of cerumen and the difficulty in removing it (Casselbrant *et al.*, 2003)

Acute otitis media with effusion is a chronic form of otitis media in which the tympanic membrane is not perforated. Local inflammation leads to epithelial metaplasia and the collection of liquid in the cavities of the middle ear. The middle ear effusion is mucous or sero-mucous in nature but not purulent. The condition lasts for at least three months; this sets it apart from persistent effusion after acute media otitis, which disappears after two months in 90% of cases (Blanc *et al.*, 2018). Effusion in adults is treated with a greater degree of

suspicion, particularly when unilateral. Adults diagnosed with otitis media with effusion should be evaluated for additional underlying conditions and then treated accordingly.

It is thought that between 50% and 85% of children experience at least one episode of Acute Otitis Media by 3 years of age with the peak incidence being between 6 and 15 months. Otitis Media with effusion can affect as many as 80% of children at some stage (Van Zon *et al.*, 2012) with approximately 2.2 million new cases of OME annually in the United States of America (American Academy of Family, 2004).

iii. Genetic Predisposition: It has become increasingly clear that susceptibility to infectious disease is mediated, at least in part, by a genetic predisposition of the host. Several lines of evidence indicate that there exist inherited susceptibilities to infectious disease. Before modern treatments were developed, epidemics such as the bubonic plague killed susceptible individuals and spared those more resistant. When natural resistance to certain diseases has not evolved, populations can be decimated, such as when smallpox was introduced into the New World by Spanish conquistadors (Joseph *et al.*, 2002).

Just as there is evidence that susceptibility to infectious diseases in general is genetically mediated, twin studies, epidemiologic, immunologic, and animal studies, have provided data that demonstrate there is a genetically based predisposition for the development of otitis media. Upper respiratory tract infections (URTIs) and Acute Otitis Media (AOM) are the most common reasons for doctor consultations and antibiotic use in children. The primary care incidence of URTI, including AOM, in children up to four years of age is estimated at approximately 400 per 1,000 child-years (Van Deursen *et al.*, 2012). These infections have a significant impact on the child and on family life and carry a considerable economic burden (Brouwer *et al.*, 2005).

2.2.8. Epidemiology of Ear Infection

About 65-330 million people suffer from ear infection worldwide and 60% of them had significant hearing impairment. The health-economic burden of ear infection is also severe especially in Africa and other developing nations where the disease prevalence is estimated as high as 11% (Ilechukwu *et al.*, 2014), and prevalence of chronic ear infections being up to 72 cases per 1000 inhabitants (Ahmad, 2013). Ear infection is a common problem for both children and adults but the magnitude is different in different countries, hence ear infection is a major health problem most especially in those with poor socioeconomic status. The etiologies and prevalence of ear infection is different in different geographical areas (Muluye *et al.*, 2013). By the age of 3 years, 80% of children have had at least one episode of acute otitis media, and nearly 50% have had 3 or more episodes. Incidence declines after 6 years of age (Ilechukwu *et al.*, 2014).

Otitis media is very common in childhood and is almost always accompanied by a viral upper respiratory infection (URI) with a peak incidence between 4–7 years of age. Seventy-five percent of children experience at least one episode by their third birthday. Almost half of these children will have three or more ear infections during their first 3 years. The reason for the higher frequency in these populations is the anatomic differences in skull base and Eustachian tube and biologic susceptibility. Although otitis media is primarily a disease of infants and young children, it can also affect adults. Furthermore, the incidence is higher in males than in females (Okesola and Fasinam, 2012). It is estimated that otitis externa made up 5 to 20% of ear infection, most of them caused by bacteria, and from the latter 9 to 25% are caused by fungi (Pontes *et al.*, 2009). The prevalence of otomycosis has been reported to be as low as 9% of cases of otitis externa, and as high as 30.4% in patients presenting with symptoms of otitis or inflammatory conditions of the ear. Prevalence is also influenced by the geographical area,

otomycosis is most commonly present in tropical and subtropical humid climates. Otomycosis occur more commonly in females than in males. Moreover, it usually occurs most frequently in adults, and less in children (Khan *et al.*, 2013).

2.2.9. Complications

Complications of otitis media could be life-threatening, with significant morbidity and mortality. Possible Complications Infection of otitis media may include brain abscess, septicemia, meningitis, mental retardation and facial paralysis, and it is believed to be responsible for more than two-thirds of deafness in children (Parveen and Rao, 2012). Increased antimicrobial resistant bacteria in chronic otitis media can lead to the development of complications of chronic otitis media may lead to cholesteatomas (Abera and Kibret, 2011).

2.2.10. Diagnosis

The diagnosis of ear infection is clinical and accompanied by microbiological confirmation (Satish *et al.*, 2013). Otoscopic examination is the most important method for diagnosis of ear infection, it is performed after careful removal of cerumen, should include an evaluation of tympanic membrane's position, color, translucency, light reflex, blood vessels and mobility. The speculum should be large enough for air-tight contact, and good lightning is essential for sufficient visibility (Tähtinen, 2012). Standard microbiological diagnosis methods start by ear discharge samples collected aseptically by using cotton swab techniques and transported to microbiology laboratory (Muluye, 2013). Examine the specimen microscopically but inoculate the culture media first before using the swab to make Gram smear. Inoculate the samples on MacConkey agar, Blood agar and Cooked Meat medium, add a Bacitracin disc on blood agar if Streptococci are seen in the Gram smear. Then incubate at 37°C for 24 hours. After overnight incubation examined for evidence of growth (Cheesbrough, 2006). The bacterial species isolated were identified by morphology, cultural characteristics, Gram stain and bio-chemical

reactions according to the standard techniques (Shyamala and Reddy, 2012). The Gram positive cocci were tested by Mannitol fermentation and coagulase test. Gram negative bacilli were tested for motility by hanging drop and then subjected to other biochemical and sugar fermentation test. The tests were read after incubation at 37°C at the end of 24 hours and 48 hours. The sugars were used to study the fermentation reaction with glucose, lactose, sucrose, maltose and mannitol. The biochemical tests done were indole, methyl red, Voges-Proskauer, citrate and urease. All sugar fermentation and biochemical tests were done from the subcultures made from isolated colonies picked from the primary isolation media (Shyamala and Reddy, 2012). In case of otomycosis history of ear pain and/or itching and physical findings of erythema and swelling of the external auditory meatus skin, and varied discharge (scanty white mucoid, grey, bluish-green and yellow discharges) uses as clinical diagnosis (Enoz *et al.*, 2009), and blotting paper appearance of matted mycelia can be seen on otoscopic examination while characteristic appearance of fruiting bodies or conidiophores can be seen on microscopy, but microscopic evidence can become an evidence of negativity for fungal presence and should be accompanied by culture (Satish *et al.*, 2013). Sabouraud Dextrose agar with antibiotics for fungal organisms' fungal cultures were evaluated for isolation of any fungal growth on Sabouraud Dextrose agar after incubation at 25-26°C for 2 weeks. Standard microbiological technique was used in diagnosis of isolates (Enoz *et al.*, 2009). Fungal isolates were identified on the basis of colonial morphology and lactophenol cotton blue microscopy as standard methods. The Candida species were identified using the germ-tube test and micro-morphological appearance of colonies on cornmeal agar (Ho, 2006).

2.2.11. Causative Agents

The major causes of ear infection are bacterial isolates such as *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus species*, *Klebsiella pneumonia*, *Escherichia coli* (Muluye *et al.*,

2013) and *Citrobacter species* (Ogbogu *et al.*, 2013). The bacteria cause upper respiratory tract infections like *Hemophilus influenza, Streptococcus pneumonia* and *Streptococcus pyogenes* also cause ear infection (Al-Hamadany, 2012). The *Aspergillus species, Candida species, Actinomyces spp, Trichophyton spp, Penicillium spp, Mucor spp* and *Rhizopus spp* cause Otomycosis (Uslu *et al.*, 2005).

2.2.11.1. Gram Positive Bacteria

a. *Staphylococcus aureus*

Gram positive cocci about 1µm in diameter, the cocci are mainly arranged in the grape-like clusters, but some specially when examined are non-motile and non-spore forming. Usually non capsulate, produce catalase, coagulase and DNase enzyme facultative anaerobic produce β-hemolytic colonies the organism is normal flora in skin and mucous membrane of human and animals. Staphylococcal infection depends on enterotoxin, epidermolytic toxin and toxic shock syndrome toxin (Greenwood *et al.*, 2007).

b. Coagulase Negative

Staphylococci

S. saprophyticus and *S. epidermidis* commonly found on the surface of healthy persons but can cause human infection (Greenwood *et al.*, 2007). S. *saprophyticus* and *S. epidermidis* resemble *S. aureus.* Culturally the colonies of *S. epidermidis* are white and usually non-haemolytic. The colonies of *S. saprophyticus* may be white or yellow. They are non-hemolytic. Growth may not occur on MacConkey agar. *S. saprophyticus* and *S. epidermidis* are coagulase negative (Cheesbrough, 2006).

2.2.11.2. Gram Negative Bacteria

a. *Pseudomonas aeruginosa*

P. aeruginosa is a gram negative rod, non-sporing, motile and some strains are capsulate. *P. aeruginosa* is oxidase positive and produces acid only from glucose (no gas). These features together with the typical pigments produced by most strains and the distinctive smell of cultures are usually sufficient to identify the organism. Able to Grow at 42°C. acid is formed from carbohydrate media, it does this by oxidation, not by fermentation (Cheesbrough, 2006). It causes opportunistic nosocomial infections responsible for 10% of all hospital-acquired infections. Infections caused by *P. aeruginosa* are often severe, life threatening and are difficult to treat because of limited susceptibility to antimicrobial agents and high frequency of emergence of antibiotic resistance during therapy (Jido *et al.*, 2014).

a. *Escherichia coli* Gram negative bacilli usually motile produce poly saccharide capsule aerobic and facultative anaerobe, have the ability to utilize glucose and lactose with gas and no H_2S production. Produce indole (non-oxidase, citrate and urease producer) (Greenwood *et al.*, 2007).

b. *Klebsiella pneumonae*

Gram negative coccobacilli non motile capsulated have the ability to utilize glucose and lactose with gas and no H2S production. 1-2µm long aerobe and facultative anaerobe produce citrate and urease, indole and oxidase negative (Greenwood *et al.*, 2007).

c. *Citrobacter freundii.*

Gram negative bacilli capsulated non pigmented late lactose fermenter glucose fermenter and produce H2S. Share certain somatic antigen with Salmonellae and *Escherichia coli* (Greenwood *et al.*, 2007).

d. *Proteus species*

Gram negative morphological variation non capsulate non spore forming. *Proteus vulgaris* and Proteus mirabilis strains have ability to swarm on solid media the growth spreads progressively from the edge of the colony and eventually covers the whole surface of the medium non lactose fermenter have the ability to deaminate amino acid oxidatively (Greenwood *et al.*, 2007).

2.2.12. Treatment of Ear Infection

Treatment include elimination of predisposing factor and use of suitable antimicrobial agents- the antibacterials like tetracycline, penicillin, erythromycin, chloramphenicol, gentamicin, ciprofloxacin, norfloxacillin, cotrimoxazole, ceftriaxone, ampicillin and amoxicillin (Muluye, 2013), and other antibacterial like imipenem, cloxcillin, amoxicillin/clavulanic acid, ceftazidime, and ofloxacin are also used (Ogbogu *et al.*, 2013). Topical antifungals are clotrimazole, miconazole, econazole, nystatin, tolnaftate and potassium sorbate (Khan *et al.*, 2013).

2.2.13. Aminoglycosides

Aminoglycosides are natural or semisynthetic antibiotics derived from actinomycetes. The vast majority of aminoglycosides are bactericidal, they have predictable pharmacokinetics, and they often act in synergy with other antibiotics, properties that make them valuable as anti-infectives. Aminoglycosides are used in the treatment of severe infections of the abdomen and urinary tract, as well as bacteremia and endocarditis. They are polycationic, they show a binding affinity for nucleic acids and possess high affinities for certain portions of RNAs, especially the prokaryotic rRNA (Lakshmi *et al.*, 2020).

Aminoglycosides inhibit protein synthesis by binding, with high affinity to the A-site on the 16S ribosomal RNA of the 30S ribosome (Kotra *et al.*, 2000). Although aminoglycoside class

members have a different specificity for different regions on the A-site. As a result of this interaction, the antibiotic promotes mistranslation by inducing codon misreading on delivery of the aminoacyl transfer RNA. This results in error prone protein synthesis, allowing for incorrect amino acids to assemble into a polypeptide that is subsequently released to cause damage to the cell membrane and elsewhere.

The most common mechanism for clinical resistance to aminoglycosides is their structural modification by specific enzymes expressed in resistant organisms. The three classes of these enzymes are: aminoglycoside phosphotransferases (APHs), aminoglycoside nucleotidyltransferases (ANTs), and aminoglycoside acetyltransferases (AACs). Within each class, there are enzymes with different regiospecificities for aminoglycoside modifications: there are four nucleotidyltransferases [ANT(6), ANT(4′), ANT(3"), and ANT(2")], seven phosphotransferases [APH(3′), APH(2"), APH(3"), APH(6), APH(9), APH(4), and APH(7")], and four acetyltransferases [AAC(2′), AAC(6′), AAC(1), and AAC(3)]. There also exists a bifunctional enzyme, AAC(6′)–APH(2"), that can acetylate and phosphorylate its substrates sequentially (Daigle *et al.*, 1999). This is a major mechanism by which clinical isolates of Gram-negative and Gram-positive bacteria cause an enzymatic modification of the amino or hydroxyl groups aminoglycosides, causing them to bind poorly to ribosomes and thus fail to trigger energy-dependent phase II, which allows bacteria to survive (Llano *et al.*, 2002). Other mechanisms include the reduction of the intracellular concentration of aminoglycosides by changes in the outer membrane permeability which is usually a non-specific resistance mechanism, inner membrane transport, active efflux or drug trapping, the alteration of the 30S ribosomal subunit target by mutation, and finally methylation of the aminoglycoside binding site (Shakil *et al.*, 2008). The efflux system has been specifically shown to be involved in aminoglycoside resistance in *Pseudomonas aeruginosa* infections in several countries (Vogne *et*

al., 2004) Even though the binding site for aminoglycosides is in rRNA, resistance to aminoglycosides is not manifested by alteration of this target in general. This is in part due to the fact that the function of the rRNA is central in the protein biosynthetic process and that this function is so well preserved across genera that it cannot be impaired by the possibility of such structural alteration. In addition, all organisms have multiple copies of the genes that encode rRNA. In order to generate an rRNA resistant to a certain antibiotic that binds to it, all these genes will have to be mutated, and the probability of the occurrence of such an event is virtually nonexistent. Moreover, it is easier for an organism either to modify the rRNA target post-translationally or to produce resistance enzymes. On the other hand, there are some reports of mutations in the ribosomal proteins that confer resistance to certain antibiotics. Aminoglycosides are potent, broad-spectrum antibiotics that act through inhibition of protein synthesis and are active against various Gram-positive and Gram-negative organisms. They are particularly potent against members of the Enterobacteriaceae family, including *Escherichia coli*, *Klebsiella pneumoniae* and *Klebsiella oxytoca*, *Enterobacter cloacae* and Enterobacter *aerogenes*, *Providencia* spp., *Proteus* spp., *Morganella* spp., and *Serratia* spp. (Aggen *et al.*, 2010; Landman *et al.*, 2010). It also has good activity against *Staphylococcus aureus*, including methicillin-resistant and vancomycin-intermediate and -resistant isolates, *P. aeruginosa* and to a lesser extent *Acinetobacter baumannii* (Aggen *et al.*, 2010). Examples of aminoglycosides includes:

a. Ciprofloxacin

Ciprofloxacin is a bacteriocidal antibiotic of fluoroquinolone drug class. It acts as interfering with microbial DNA synthesis. It is relatively non-toxic, well tolerated and has proven especially useful for oral therapy of chronic Gram-negative infections (Usman *et al.*, 2014). although ciprofloxacin is known as a safe drug, there are cases of side effects associated with

ciprofloxacin such as anaphylaxis and pulmonary edema ciprofloxacin suffers from moderate oral bioavailability as it chelates with calcium-, magnesium- and aluminium-containing salts upon concomitant administration (Abdul latip *et al.*, 2013).

b. Amoxicillin/Clavulanic acid (Amoxyclav) amoxicillin is a semi-synthetic penicillin developed from ampicillin. This compound has the same potent broad-spectrum activity (many Gram-positive and Gram-negative microorganisms) as ampicillin, but much better oral absorption. It is specifically used for otitis media, Streptococal phyaryngitis, Pneumonia, Urinary Tract Infection and animal bites. Amoxicillin is susceptible to be inactivated by bacterial enzymes (β-lactamases) and is usually combined with clavulanic acid, a β-lactam molecule produced by *Streptomyces clavuligerus*, found to be a potent inhibitor of β-lactamases but with low antibacterial activity, resulting in an enhanced antimicrobial activity against many Gram-positive aerobic and anaerobic bacteria and Gram negative aerobic bacteria (Liberato *et al.*, 2011).

C. Chloramphenicol

Chloramphenicol is an antibiotic that was derived from the bacterium *Streptomyces venezuelae*, though now is produced synthetically. It is bactericidal at clinically achievable concentration against *Streptococus pneumonia* and *Neisseria meningitides* and also bacteriostatic against gram-negative bacilli of the family Enterobacteriaceae and *Staphylococcus aureus*. Chloramphenicol is used in external treatments such as eye drops or ointment to treat bacterial conjunctivitis. It stops bacterial growth by binding to the bacterial ribosome and inhibiting protein synthesis. It loses its activity by the hydrolysis of amide group to give 2-amino-1-(4-nitrophenyl) propane-1,3-diol. This degradation product of chloramphenicol commonly occurs in pharmaceutical preparations (Sreevatsav *et al.*, 2013).

d. Gentamycin

Gentamicin an aminoglycoside antibiotic, is widely used in the treatment of suspected or proven bacterial sepsis in newborn infants. It is rapidly bactericidal. Combined with beta-lactam antibiotics, it provides synergistic activity against the most commonly encountered pathogens in the neonatal period (Chattopadhyay, 2002).

2.2.14. Prevention

Ear infection may be prevented through:

1. Keeping your ears clean by washing them and using a cotton swab carefully. Make sure you dry your ears completely after swimming or taking a shower.
2. Managing your allergies by avoiding triggers and keeping up with allergy medications.
3. Washing of hands thoroughly.
4. Avoid people who have colds or other upper respiratory problems.
5. Do not smoke, and avoid second hand smoke as much as you can.
6. Make sure your vaccines are up to date.

In order to eradicate inflammation, prevent recurrence and restore middle ear structures and hearing, both medical and surgical treatment modalities are important in chronic suppurative otitis media. It has been emphasized that selection of appropriate antibiotics should be based on the bacteriologic studies to identify the pathogens and determine sensitivities (Lee *et al.*, 2013). The incidence of acute otitis media may further decrease as the use of pneumococcal conjugate vaccine (PCV) becomes more popular.

A number of preventive measures have been recommended, including use of earplugs while swimming, use of hair dryers on the lowest settings and head tilting to remove water from the ear canal, and avoidance of self-cleaning or scratching the ear canal. Acetic acid 2% (Vosol) otic solutions are also used, either two drops twice daily or two to five drops after water

exposure. However, no randomized trials have examined the effectiveness of any of these measures (Schaefer and Baugh, 2012).

Targeting typical causal culprits of Acute Otitis Externa such as moisture and trauma, seems prudent. Some experts recommend simple techniques for keeping water out of the ears (e.g., inserting a soft, malleable plug into the auricle to block entry to the ear canal) or removing water from the ears after swimming (by positioning or shaking the head). Others advise avoiding cotton swabs because they might impact cerumen. Daily prophylaxis with alcohol or acidic drops during at-risk activities has also been suggested but not studied. Using hard earplugs should be avoided because they can cause trauma, and the use of custom ear canal molds and tight swim caps remains controversial (Hui, 2013).

CHAPTER THREE
MATERIALS AND METHODS

3.1 Study Area and Population.

The study area for this research work is Keffi. It is approximately 68km from Abuja, the Federal Capital Territory and 128km from Lafia, the Capital of Nasarawa State. Keffi is located between the latitude 8° 5¹ N of the equator and longitude 7° 8¹ E and situated on an altitude of 850m above sea level (Akwa *et al.*, 2007). The study participants are adults with ear infection accessing health care facility in Keffi who agreed to participate in the study.

Inclusion criteria: Patients who presented with clinical diagnosis of ear infection (otitis media and external otitis media).

3.2 Sampling

The sample size was determined using the following equation and a prevalence of 25% as carried out by Fisher's Formula.

To calculate n: $n= Z^2Pq/d^2$.

Where n is the desired size when the population is greater than 10000.

230 ear swab specimens was collected out of which 215 was analysed

Z= the standard normal deviates usually set at 1.96

P= the proportion in the target population expected to have a particular characteristics.

q=1.0 - p

d=the degree of accuracy required usually set at 0.05

$$n=\frac{(1.96)^2 \times 0.25 \times 0.6}{(0.05)^2}$$

n=230.496.

n=230

Study variables-screen of ear infection (otitis media and external otitis media) (dependent variable).

3.3 Ethical Clearance

Ethical clearance for this study was obtained from the Federal Medical Center Keffi with Ref No: NHREC/21/12/2012 (see Appendix). The objectives of the study was clearly explained to all individuals participating in the study informed consent was also obtained from patients.

3.4 Sample Collection

Ear swabs were collected from patients using sterile cotton tipped swabs and labelled accordingly to avoid contamination and mix up before they are transported to the laboratory for analysis.

3.5 Isolation of the different bacteria

Swab was cultured onto enrichment media and incubated at 37°C for 18hours and later subcultured onto Blood agar, Chocolate Blood agar and MacConkey agar (HiMedia laboratories Pvt, Ltd, India), and incubated at 37°C for 18 hours.

3.5.1 Identification of the Bacteria

The identification was carried out using morphological characters, biochemical morphological characters such as Gram's stain, urease test, indole test, oxidase, citrate utilization test.

3.5.2 Colonial Morphology

Plates were examined at the end of incubation period for fermentation on MacConkey agar and hemolysis on Blood agar and the morphological characters (size, shape, color and pigment production) were recorded.

3.5.3 Gram's Staining

Gram staining of the presumptive organism was done following Cheesbrough, 2006. A smear of colonies was made on clean grease free slide and allowed to air-dry. The slide was passed three times over flame to heat fix, then poured crystal violet stain for 60 seconds and rinsed with tap water, then covered with Lugol's iodine for 60 seconds and rinsed under tap water,

then decolorized by using 70% alcohol for few seconds. Saffranin was added to the smear for 1 minute, washed with water and allow to dry by air. Smear was examined with oil immersion, to observe yeast and bacterial cells morphology, size, Gram positive reaction and presence of pus cells, epithelial cells

3.5.4 Biochemical Tests

Biochemical activity of pure and mixed isolates was done to confirm the identification organisms. These include:

3.5.4.1 Indole Test

The tested organism was inoculated in a bijou bottle containing 3 ml of sterile in peptone water (HiMedia laboratories Pvt, Ltd, India), and incubated at 37°C for 24 hours. It was tested for indole by adding 0.5ml of Kovac's reagent (B.H Chemical, Japan) and shaked gently. Then it was examined for a red colour in the surface layer within 10 minutes (Cheesbrough, 2006).

3.5.4.2 Urease Test

The test organism was cultured in urea media (BioMARK laboratories, India), then incubated at 37°C for 24 hours. When the strain is urease producing, the enzyme was breakdown the urea (by hydrolysis) to give ammonia and carbon dioxide, with the release of ammonia, the medium becomes alkaline which was shown by a change in color of the indicator to pink-red (Cheesbrough, 2006).

3. 5.4.3 Citrate Utilization Test

Organism under test was inoculated in Simmon's Citrate agar (HiMedia laboratories Pvt. Ltd. India) by sterile straight wire loop and incubate at 37°C for 24 hours, then check for bright blue color in the medium (Ahmed, 2008).

3. 5.4. 4 Oxidase

The test was used to detect the ability of the organisms to produce oxidase enzyme. Whatman paper was placed inside the Petri dish, and small inoculum was taken by using wooden stick or glass rod (not an oxidized wire loop), and smear on the disc by opening the Petri dish partially. Blue-purple colour, oxidase test positive (within 20 seconds), no blue- purple colour. oxidase test negative (Cheesbrough, 2006).

3. 5.4.5 Catalase Test

Two 2–3 ml of the hydrogen peroxide solution (Supplies Angles Burry, UK) was pour into a test tube. Wire loop was used and remove several colonies of the tested organism and immerse in the hydrogen peroxide solution. Check for immediate bubbling (Cheesbrough, 2006)

3. 5.4.6 Manitol Salt Agar

The test was done by inoculating the organism under test on MSA medium (HiMedia laboratories Pvt, Ltd, India) and then incubate the plates at 37°C for 24hrs, the change in color was observed. A yellow color colony indicates fermentation of manitol (Cheesbrough, 2006).

3. 5.4.7 Coagulase Test

On clean slide a drop of normal saline (Industry LCD, Saudi Arabia) was placed and emulsified a colony of the tested organism was emulsifying, then a loopful of plasma was added to the suspensions and mix gently. The clumping of the organism was examined within 10 seconds. Clumping within10 seconds; positive coagulase test, no clumping or clumping in more than 10 seconds; negative coagulase (Cheesbrough, 2006).

3.6 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility of the isolates was determined using the disk diffusion method on Mueller–Hinton agar plates. The standard procedure of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2015) was strictly followed throughout the testing procedure.

Briefly, a sterile swab stick was soaked in standardized bacteria suspension and streaked on Mueller-Hinton agar plates and the antibiotic disc was aseptically placed at the center of the plates and allow to stand for 1 hour for pre-diffusion time. The plates were incubated at 37°C for 24 hours. The diameter zone of inhibition in millimeter was measured and the result of the susceptibility was interpreted in accordance with the susceptibility break point earlier described by Clinical and Laboratory Standards Institute (CLSI, 2015).

Preparation of agar plates: The medium (Muller Hinton Agar) was prepared according to the manufacturer's instructions and was cooled and poured into Petri dishes of approximate 4 mm depth. The plates were used within a week after the preparation.

Inoculation of test plates: A well-isolated colony was inoculated into Mueller-Hinton broth (MHB) and incubated overnight at 37°C. The density of the culture was adjusted to a turbidity equivalent to that of a BaSO4 turbidity standard (0.5 McFarland standard) by adding sterile MHB. A sterile swab was then immersed in the suspension, pressed on the inside wall of the tube to remove excess inoculums from the swab and streaked over the entire surface of Muller Hinton Agar plates, rotating the plate approximately 60 degrees after each application to ensure an even distribution of the inoculum swabbed all around the edge of the agar surface.

Dispensing of discs: The working antimicrobial disks were stored in the refrigerator (4°C) in a container with desiccant. The container containing antimicrobial disks was left unopened at room temperature for approximately 1 hour before using the disks to allow the temperature to equilibrate. After inoculation (no longer than 15 min), the antimicrobial disks were applied to the plates with sterile forceps and then gently pressed down onto the agar. To prevent overlapping of the zones of inhibition and possible error in measurement, disks were distributed to be apart from so that no closer than 20 mm from center to center of the disks and from the edge of the plate. The plates were then incubated at 37°C for 16-18 hour.

Measurement of zones: Inhibition zones were measured with a ruler and recorded to the nearest millimeter and isolates were classified as susceptible, intermediate, or resistant according to the zone diameter interpretative standards recommendations from the Clinical and Laboratory Standards Institute (CLSI, 2015) and recorded as susceptible, intermediate, or resistant to each antibiotic tested.

3.7 Molecular Detection of Aminoglycoside Genes

3.7.1 DNA Extraction

Following purification on MacConkey agar, Zymoresearch, 2018 manufacturer's instruction bacterial was isolated from a 24hours culture bacterial cells were suspended in 200µl of sterile water in ZR BashingBead™ Lysis Tube. containing BashingBead™ Buffer to the tube. Secured in a bead beater fitted with a 2ml tube holder assembly and vortexed at maximum speed for ≥ 5 minutes. Then centrifuged the ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm) in a microcentrifuge at 10,000rpm for 1 minute then transferred up to 400µl supernatant to a Zymo-Spin™ III-F Filter in a Collection Tube and centrifuged again at 8,000 rpm for 1 minute. 1,200µl of Genomic Lysis Buffer was added to the filtrate in the Collection Tube from previous step. 800 µl of the mixture from the collection tube was harvested into a Zymo-Spin ™ IIC Column and centrifuged at 10,000 rpm for 1 minute. The flow through from the Collection Tube was discarded and repeated again. 200 µl DNA Pre-Wash Buffer was added to the filtrate in Zymo-Spin™ IIC Column in a new Collection Tube and centrifuged at 10,000 rpm for 1 minute after which 500 µl g-DNA Wash Buffer was added to the Zymo-Spin™ IIC and centrifuged at 10,000rpm for another 1minute. Transferred the Zymo-Spin™ IIC Column to a clean 1.5 ml microcentrifuge tube and add 100 µl (35 µl minimum) DNA Elution Buffer directly to the column matrix centrifuged at 10,000 rpm for 30 seconds to the elute the DNA. The pure DNA was ready for use and later stored in the freezer.

Estimation of concentration, purity and yield of DNA sample was carried out using absorbance method with the spectrophotometer (Nanodrop 1000). For the concentration of DNA, absorbance readings were performed at 260nm (A_{260}) A and the readings were observed to be within the linear range (0.1-1.0). DNA purity was estimated by calculating the A_{260}/A_{280} ratio and this was done with spectrophotometer's computer software (where A_{260}/A_{280} ratio ranges from 1.7-1.9)

3.7.2 DNA Amplification

The presence of genes (aa-*C*, *ANT*, *APH*) were tested using primer sets and conditions were listed in Table 3:1. PCR cocktail of 25µl was prepared (4µl of DNA, 2µl of forward primer, 2µl of reverse primer, 12.5µl of Master mix, 4.5µl of Nuclease free sterile H_2O)in tubes then placed in a thermocycler (Thermofisher Scientific, Finland) Initial denaturation temperature 95°C for 5min, 35 cycles of amplification took place at second denaturation at 94°C for 45min, annealed at 60°C for 40sec, extended at 35°C 1min, final extension at 72°C for 5min and hold 4°C ∞

3.7.3 Agarose Gel Electrophoresis

10µl of the PCR products and 10µl DNA ladder (Purple 100 bp DNA ladder, New England BioLabs) was analyzed by electrophoresis on 1% TAE agarose gel containing 0.5µl of ethidium bromide pipetted into well created with comb. Electrophoresis was run at 100 volts for 1 hour, after which DNA amplicons were then viewed on a UV trans-illuminator.

3.8 Data Analysis

Data analysis was done using Statistical Package of Social Science (SPSS) software (version 11.5). A p-value of <0.05 was considered statistically significant.

CHAPTER FOUR

DATA PRESENTATION AND ANALYSIS

4.1 Data Presentation

4.1.1 Isolation and Identification of Bacteria from Ear

The cultural, morphological and biochemical characteristics of different bacteria isolated from ear of the patients is as shown in Table 4.1. From the total bacterial isolates (48), 2(56.4%) were gram positive bacteria (*Staphylococcus aureus* and *Staphylococcus epidermidis*) while the remaining bacteria were gram negative rods and all the isolates were citrate positive (*Pseudomonas* species, *Klebsiella* species and *Proteus* species).

4.1.2 Occurrence of Different Bacteria

Out of 215 ear swab samples, the occurrence of bacteria isolates was 48 (22.3%). The occurrences of different bacteria were *Pseudomonas* species 16(7.4%), *Klebsiella* species 11(5.1%), *Proteus* species 8(3.7%), *Staphylococcus aureus* 7(3.2%), *Staphylococcus epidermidis* 6(2.7%) are as shown respectively in Table 4.2. The occurrences of bacteria isolates from patients ear were statistically insignificant (P>0.05).

4.1.3 Antibiotic Resistance

The antibiotic resistance of bacteria isolated from ear is as shown in Table 4.3. The *Pseudomonas* species isolates were more resistant to Ceftazidime (50.0%), but less resistance to chloramphenicol, amoxicillin/clavulanic acid and ampicillin (37.5%), cefotaxime and streptomycin (31.2%), gentamicin and sulphamethoxazole/trimethoprim (25.0%) as shown in Table 4.3. *Klebsiella* species isolates were highly resistant to Tetracycline (63.6%), cefotaxime and amoxicillin/clavulanic acid (54.5%), ceftazidime(45.4%) but less resistance to ciprofloxacin, streptomycin and ampicillin (36.3%). *Staphylococcus aureus* isolates were more resistant to amoxicillin/clavulanic acid (71.4%), ceftazidime (57.1%), ciprofloxacin and

ampicillin (36.3%) but less resistance to chloramphenicol, cefotaxime, tetracycline and streptomycin (28.5%), gentamicin and sulphamethoxazole/trimethoprim (14.2 %). *Proteus* species isolates were more resistant to amoxicillin/clavulanic acid (75.0), chloramphenicol (50.0%) but less resistance to ciprofloxacin and ceftazidime (37.5%), cefotaxime (25.0%), streptomycin (12.5%). *Staphylococcus epidermidis* isolates were highly resistant to amoxicillin/clavulanic acid (66.6%), cefotaxime (50.0%), but less resistance to ciprofloxacin, tetracycline and ampicillin (33.3%), ceftazidime and streptomycin (16.6 %) as shown in Table 4.3.

4.1.4 Antibiotic Resistance Phenotypes

The antibiotic resistance phenotypes of the different bacteria isolates are as shown in Table 4.5. The *Pseudomonas* species isolates were distributed into different antibiotic resistant phenotypes and the most common phenotypes were C-CIP- AMC - AMP -TE-SXT and C-CIP-CAZ- AMC -TE-SXT-S (18.7%). *Klebsiella* species isolates were distributed into different antibiotic resistant phenotypes and the most common phenotypes were C-CIP-CRO- AMC -TE-SXT-S and C-CN-CIP-CRO-CAZ-TE- AMC-AMP-SXT (18.1%). *Staphylococcus aureus* isolates were distributed into different antibiotic resistant phenotypes and the most common phenotypes was C- AMC - AMP -CAZ-TE (28.5%). *Proteus* species isolates were distributed into different antibiotic resistant phenotypes and the most common phenotypes were C-CN-CRO-CAZ-TE- AMC -S- AMP and CN-CIP-CRO-CAZ-TE- AMC -SXT-S (25.0%) as shown in Table 4.6.

4.1.5 Multiple Antibiotic Resistance (MAR) Index

Multiple antibiotic resistances, defined here as resistance by the isolates to more than two antibiotics tested, were observed in all (100%) the isolates. The MAR indices of the isolates are as shown in Table 4.6 were > 0.2 with the commonest among *Pseudomonas* species being 0.5 and 0.7 (25.0%); *Klebsiella* species being 0.6 (36.3%); *Staphylococcus aureus* 0.9 (42.8%);

Proteus species 07 (50.0%); *Staphylococcus epidermidis* 0.8 and 0.9 (33.3%).

4.1.6 Molecular Detection of Aminoglycosides Resistance

The genotypic detection of aminoglycoside resistance to gentamicin resistant bacteria isolates are as shown in Table 4.4, of all the 5 resistant gentamicin resistant bacteria isolates 3 were *AAT* positive and 1 was aa-(c) positive.

Table 4.1: Cultural, Morphological, Biochemical characteristics of bacteria isolated from ear of patients

Cultural characteristics	Morphological characteristics	Biochemical Characteristics							
	Gram reaction	Morphology	IND	MR	VP	CT	UR	TDA	NT	Inference
Golden yellow colonies on NA	+	Cocci in cluster	+	+	-	+	-	+	+	*Staphylococcus aureus*
Milkfish colonies on NA	+	Cocci in cluster	+	-	-	+	-	-	-	*Staphylococcus epidermidis*
Brown mucoid colonies on NA	-	Rod	-	+	+	+	+	+	-	*Proteus* species
Pinkish colonies on MCA	-	Rod	+	+	-	+	-	-	+	*Klebsiella* species
Greenish on NA	-	Rod	-	-	+	+	-	-	+	*Pseudomonas* species

+ = Positive, - = negative, IND = Indole; MR = Methyl red; VP= Voges-Proskauer. CT = Citrate, UR = Urease, NT = Nitrate, TDA = Phenylalanine deaminase

Table 4.2: Bacteria isolated from ear of patients attending ENT clinic in FMC

Bacteria isolated	No samples	No isolated	Percentage (%)
Pseudomonas species	215	16	7.4
Klebsiella species	215	11	5.1
Staphylococcus aureus	215	7	3.2
Proteus species	215	8	3.7
Staphylococcus epidermidis	215	6	2.7
Total	215	48	22.3

X^2: 6.4718
P- value: 0.1699

Table 4.3: Antibiotics resistance bacteria isolates

Antibiotics	Conc. (µg)	No Resistance				
		Pseudomonas species n=16	*Klebsiella* species n=11	*Staphylococcus aureus* n=7	*Proteus* species n=8	*Staphylococcus epidermidis* n=6
Chloramphenicol	30	6(37.5)	3(27.2)	2(28.5)	4(50.0)	6(00)
Gentamicin	15	4(25.0)	0(00)	1(14.2)	0(00)	0(00)
Ciprofloxacin	30	2(12.5)	4(36.3)	3(42.8)	3(37.5)	2(33.3)
Cefotaxime	30	5(31.2)	6(54.5)	2(28.5)	2(25.0)	3(50.0)
Ceftazidime	30	8(50.0)	5(45.4)	4(57.1)	3(37.5)	1(16.6)
Tetracycline	15	3(18.7)	7(63.6)	2(28.5)	0(00)	2(33.3)
Amoxicillin/clavulanic acid	10	6(37.5)	6(54.5)	5(71.4)	6(75.0)	4(66.6)
Streptomycin	30	5(31.2)	4(36.6)	2(28.5)	1(12.5)	1(16.6)
Sulphamethoxazole/trimethoprim	30	4(25.0)	3(27.2)	1(14.2)	0(00)	0(00)
Ampicillin	30	6(37.5)	4(36.3)	3(42.8)	0(00)	2(33.3)

Table 4.4: Antibiotics resistant pattern profiling against bacteria isolates

Antibiotics Resistance phenotype	P n=16	K n=11	S n=7	Pr n=8	SE n=6
CIP-CRO-AMP-TE-SXT	2(12.5)	1(9.0)	0(00)	0(00)	0(00)
C- AMC - AMP -CAZ-TE	0(00)	0(00)	2(28.5)	0(00)	0(00)
CIP-AMC- AMP -CRO-TE-S	1(6.2)	0(00)	0(00)	0(00)	1(16.6)
C-CIP- AMC - AMP -TE-SXT	3(18.7)	0(00)	0(00)	0(00)	0(00)
C-CIP-CRO- AMC -TE-SXT-S	0(00)	2(18.1)	0(00)	1(12.5)	0(00)
C-CIP-CAZ- AMC -TE-SXT-S	3(18.7)	0(00)	0(00)	0(00)	0(00)
C-CIP-CAZ- AMC -TE- AMP –SXT	0(00)	0(00)	1(14.2)	0(0)	0(00)
C-CN-CRO-CAZ-TE- AMC -S- AMP	0(00)	3(27.2)	0(00)	2(25.0)	0(00)
C-CIP-CRO-CAZ- AMC - AMP -SXT-S	1(6.2)	0(00)	0(00)	0(00)	1(16.6)
C-CIP-CRO-CAZ- AMC - AMP -SXT-S	0(00)	1(6.2)	0(00)	1(12.5)	0(00)
C-CIP-CRO-TE- AMC-AMP -SXT-S	2(12.5)	0(00)	1(14.2)	0(00)	0(00)
CIP-CRO-CAZ-TE- AMC -AMP -SXT-S	0(00)	1(6.2)	0(00)	0(00)	1(16.6)
C-CAZ-CN-CRO-TE- AMC - AMP-SXT	2(12.5)	0(00)	1(14.2)	1(12.5)	0(00)
C-CIP-CAZ-CRO- AMC - AMP-SXT-S	1(6.2)	0(00)	0(00)	0(00)	1(16.6)
CN-CIP-CRO-CAZ-TE- AMC -SXT-S	0(00)	1(6.2)	1(14.2)	2(25.0)	0(00)
C-CN-CIP-CRO-CAZ-TE- AMC -SXT-S	0(00)	0(00)	0(00)	0(00)	1(16.6)
C-CIP-CRO-CAZ-TE- AMC - AMP -SXT-S	0(00)	0(00)	0(00)	0(00)	1(16.6)
C-CN-CIP-CRO-CAZ-TE- AMC-AMP-SXT	1(6.2)	2(18.1)	0(00)	1(12.5)	0(00)
C-CN-CIP-CRO-CAZ-TE-AMP-AMC-S-SXT	0(00)	0(00)	1(14.2)	0(00)	0(00)

Key: AMP = Ampicillin; AMC = Amoxicillin/Clavulanic acid; S = Streptomycin; CN = Gentamicin, SXT = Cotrimoxazole; CAZ = Ceftazidime; CTX = Cefotaxime; CIP = Ciprofloxacin

Key: P= *Pseudomonas* species, K= *Klebsiella* species, S= *Staphylococcus aureus*, Pr= *Proteus* species, SE= *Staphylococcus epidermidis*

Table 4.5: Multiple Antibiotic Resistance Index (MARI) of bacteria isolates

No. of Antibiotic Resistance to (a)	No. of Antibiotic tested (b)	MAR Index (a/b)	Frequency (%) MAR Isolates				
			P n=16	K n=11	S n=7	Pr n=8	SE n=6
10	10	1.0	0(00)	0(00)	1(14.2)	0(00)	0(00)
9	10	0.9	1(6.2)	2(18.1)	3(42.8)	2(12.5)	2(33.3)
8	10	0.8	1(6.2)	1(9.0)	2(28.5)	2(37.5)	2(33.3)
7	10	0.7	4(25.0)	0(00)	2(28.5)	4(50.0)	0(00)
6	10	0.6	3(18.7)	4(36.3)	0(00)	0(00)	1(16.6)
5	10	0.5	4(25.0)	3(27.2)	1(14.2)	0(00)	1(16.6)

Key: P= *Pseudomonas* species, K= *Klebsiella* species, S= *Staphylococcus aureus,* Pr= *Proteus* species, SE= *Staphylococcus epidermidis*

Table 4.6: Molecular detection of aminoglycoside resistant bacteria from ear of patients

Isolates	No. (%) Aminoglycosides genes			
	ANT 211	*ANT 3*	*aa-C*	*APH*
Bacteria (n=5)	3 (60.0)	0(0.0)	1(20.0)	0(0.0)

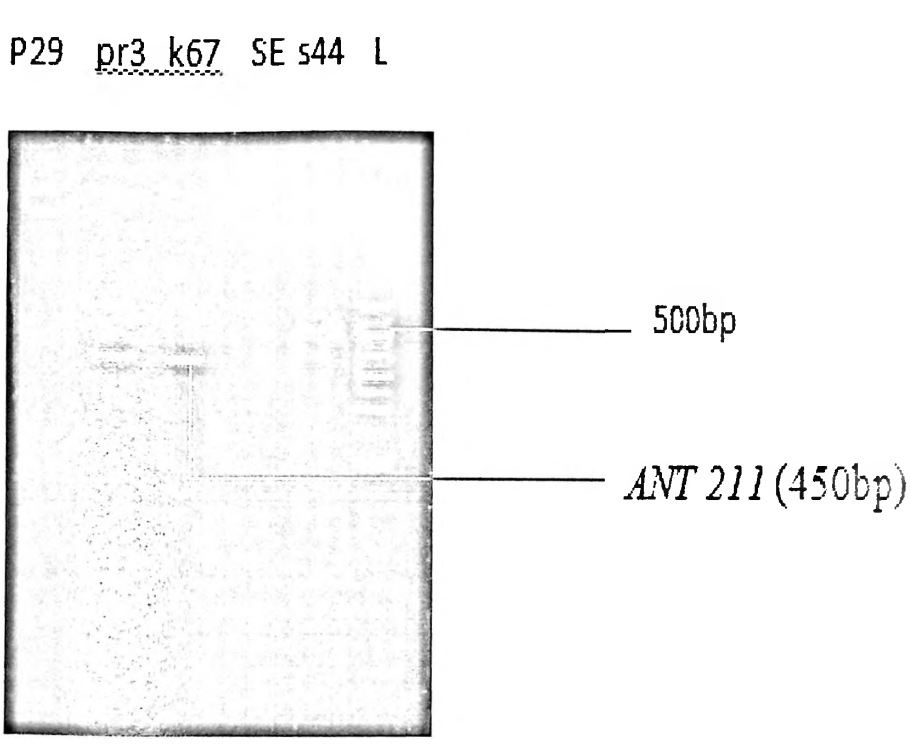


Plate 1: Agarose gel electrophoresis of the amplified *ANT 211* genes from the bacteria isolates from Ear. Lanes 1 = p29- *Pseudomonas* species, 2=pr3- *Proteus* sp, 3=k67- *Klebsiella* species,4= SE, *Staphylococcus epidermidis* and Lane 5= s44 - *Staphylococcus aureus*. Lane 2.3 and 5 represent the *ANT 211* bands, Lane L represents the 500bp molecular ladder, while Lane 1 and 3 showed no band

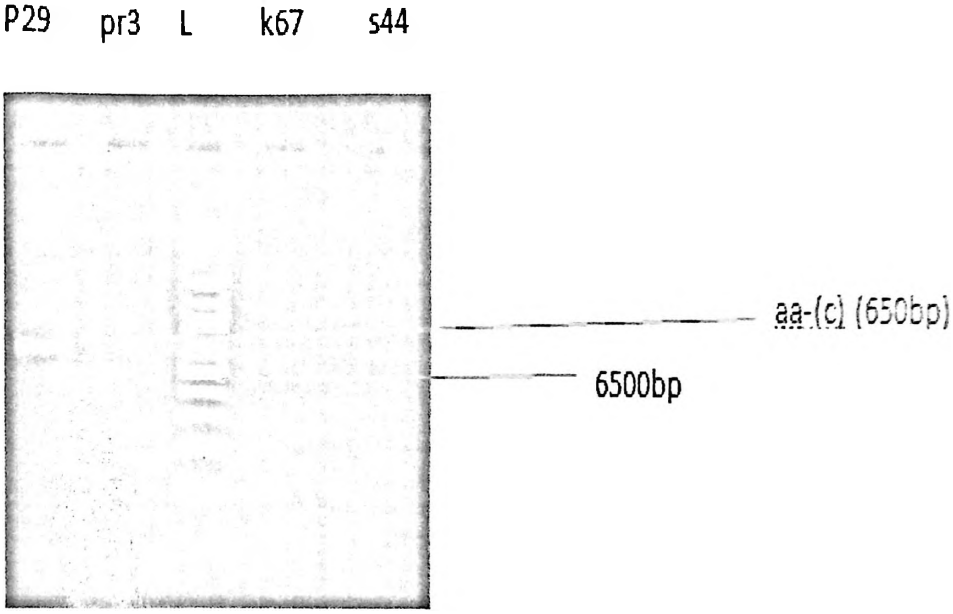


Plate 2: Agarose gel electrophoresis of the amplified *ANT 211* genes from the bacteria isolates *from Ear. Lanes 1 = p29- *Pseudomonas* species, 2= pr3- *Proteus* sp, 3= k67- *Klebsiella* species,4= SE, *Staphylococcus epidermidis* and Lane 5= s44 - *Staphylococcus aureus*. Lane 2. represent the *aa-(c)* bands, Lane L represents the 6500bp molecular ladder, while Lane 2,3 and 4 showed no band

4.2 Discussion of Findings

Aminoglycoside resistance in bacteria has been reported to emerge in developing and developed countries (Zinnat *et al.*, 2011). Studies on molecular detection of aminoglycoside resistance gene in different bacteria isolated in the study location were carried out. The occurrence of *Pseudomonas* species, *Klebsiella* species, *Staphylococcus aureus*, *Proteus* species, *Staphylococcus epidermidies* from ear of patients with suspected ear infection in the study was not new as this finding is similar to that research earlier reported by (Alhashash *et al.*, 2013). The percentage of occurrence of the bacteria isolates observed in this study was 22.3% less than 98.2%, 97.6% and 87% as earlier reported by (Wasihun and Zemene, 2015; Hailu *et al.*, 2016; Tesfa *et al* 2020). This may be due to the difference in climate and geographical variations in different countries and the number of sample tested. The occurrence of these bacteria such as *Pseudomonas* species, *Klebsiella* species, *Staphylococcus aureus, Proteus* species. *Staphylococcus epidermidis* in ear of suspected ear infection was an indication that such organism may be responsible for ear infection and this finding is in agreement with the study earlier reported by (Zinnat *et al.*, 2011) that bacteria is the most common etiological agent of ear infections. In this study *Pseudomonas* species is the most frequently isolated gram negative organism as earlier reported by (Mohammed *et al.*, 2016). This trend conforms to the findings of Tesfa *et al*, 2020 that bacterial pathogens like *Pseudomonas aeruginosa* and *Staphlococcus aureus* are majorly responsible for otitis media in sub-Saharan Africa and these isolates are highly resistant to commonly used drugs for the management of otitis media.

The high resistance of bacteria isolates to ceftazidime, tetracycline, cefotaxime and amoxicillin/clavulanic acid, ceftazidime, ciprofloxacin observed in this study was not new. This may be due to indiscriminate use of these antibiotics in the study location. The percentage resistance of the isolates to ceftazidime, tetracycline, ciprofloxacin observed in this

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