

CHAPTER ONE

1. INTRODUCTION

1.1. Introduction

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 infection of the ear involved the external otic 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) it 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) or chronic (>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).

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).

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

1.2. Rationale

Because of distribution of ear infection in Sudan particularly in the study area and increased uncontrolled usage of antibiotics and there is no recent data that shows the magnitude of the problem. Therefore this study was conducted to determine the bacterial and fungal isolates and their drug susceptibility patterns from patients who gave ear discharge samples at Khartoum ENT Hospital and explain the increase of evidence indicating misdiagnosed cases.

1.3. Objectives

1.3.1. General objective

To determine the frequency and etiology of ear infection among patients attending Khartoum ENT hospitals, during August to October 2015.

1.3.2. Specific objectives

- i.** To isolate and identify bacterial and fungal pathogens causing ear infection.
- ii.** To study relation between ear infection and risk factors age and gender.
- iii.** To assess antimicrobial susceptibility pattern of identified pathogens.

CHAPTER TWO

2. 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 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).

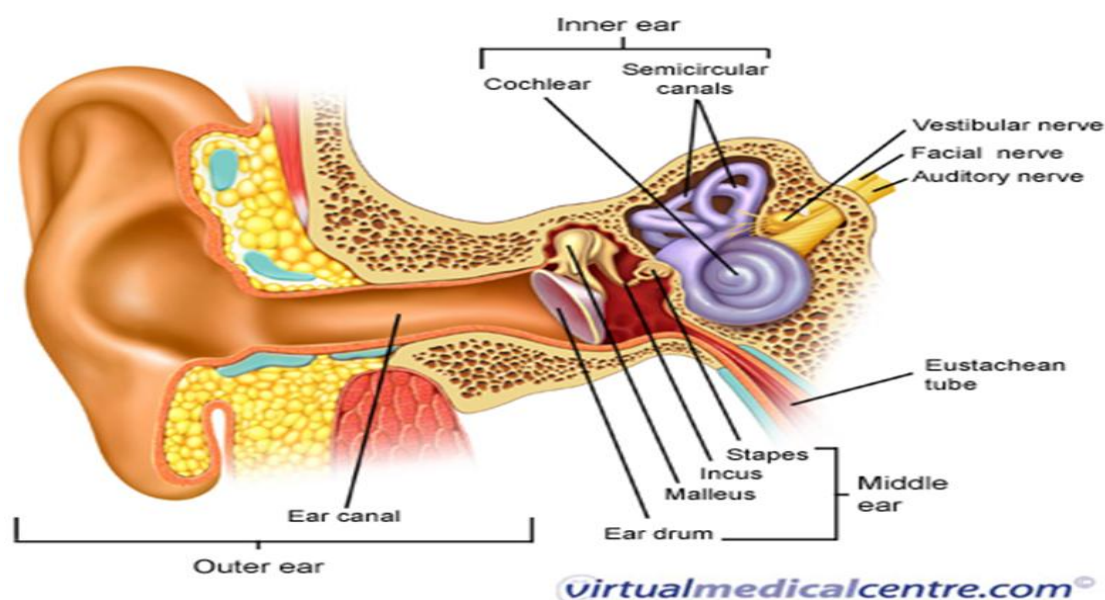


Fig (1): Anatomy of ear

2.2. Ear infection

2.2.1. Definition

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

2.2.2. Type of ear infection

2.2.2.1. 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 OM are *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella species*, *Pseudomonas aeruginosa* and *Proteus species*. The types of pathogens involved in OM have also been found to be dependent on geographical location (Okesola & 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 OM and is often attributed to

allergy, but may also occur from numerous other potential sources including radiation treatment or virus (Okesola & Fasinam, 2012).

2.2.2.1.1. Type of Otitis Media

a.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 & 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 otorrhoea 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 (Iseh and Adegbite, 2004).

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 & 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 CSOM (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 OME 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).

2.2.2.2. 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 hypoacusia (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 aeruginosa 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)

2.2.2.3. 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).

2.2.3. 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 OM 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 hypoacusia (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).

2.2.4. Pathogenesis

The pathogenesis is complex and multifactorial, resulting from a continuous interplay between host immune responses and environmental microbial load (Malmi, 2007).

The Eustachian Tube (ET) connects middle ear to the nasopharynx. The nose, nasopharynx, ET, middle ear, and mastoid gas cells compose a system of organs that play role in the development of OM. These

structures are all covered by respiratory mucosa. On the posterior wall of the nasopharynx, right above the adenoid, is the opening of the ET (Tähtinen, 2012). The Eustachian tubes (ETs), or more precisely their anatomy and function, have a crucial role in the pathogenesis of OM because microbes of the nasopharynx invade the middle ear through the ET (Malmi, 2007).

In infants, the average length of the ET is 2 cm 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 ET is about twice as long and it is angled 45° upwards (Tähtinen, 2012).

Otitis media runs through the following stages: tubal occlusion, pre-suppurative, suppurative then resolution or complication (Ilechukwu *et al.*, 2014).

2.2.5. 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 AOM 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 OM 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 OM and recurrent AOM. 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).

2.2.6. Predisposing Factors

The microbial load is influenced by such environmental factors as day-care attendance and season, whereas the host immune responses are influenced by such factors as genetic predisposition and age. Infants' and young children's immune systems are immature and their ETs are structurally and functionally different from those of adults (Malmi, 2007). Upper respiratory tract infections. 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. The presence of viral infection has been shown to increase bacterial adhesion in the nasopharyngeal tissue (Ilechukwu *et al.*, 2014).

Familial tendency include allergic rhinitis, asthma, cow's milk allergy, parental atopy, history of parental otitis media, the common postulated. Short duration of breastfeeding, bottle feeding and use of pacifiers and presence of digit sucking (Ilechukwu *et al.*, 2014).

Otitis media is a known complication of pertussis, measles, diphtheria, tuberculosis and children with congenital or acquired immunodeficiency (Ilechukwu *et al.*, 2014).

Patients with anomalies such as cleft palate and children with Down syndrome have higher incidence of Eustachian tube dysfunction and chronic otitis media with effusion (Ilechukwu *et al.*, 2014).

Several predisposing factors have been reported which include bacterial infections, use of hearing aid or a hearing prosthesis, self inflicted trauma (use of q-tips to clean the ear), swimming in contaminated pools, broad spectrum antibiotic therapy, steroids and cytostatic medication, neoplasia and immune disorder. It is seen more frequently in immunocompromised patients compared to immunocompetent persons (Khan *et al.*, 2013).

Predisposing factors such as a failure in the ear's defense mechanisms (changes in the coating epithelium, changes in pH, quantitative and qualitative changes in ear wax) (Pontes *et al.*, 2009).

Its prevalence is greatest in hot humid and dusty areas of the tropics and subtropics. Patients with diabetes, lymphoma, or AIDS and patients undergoing or receiving chemotherapy or radiation therapy are at increased risk for potential complications for otomycosis (Satish *et al.*, 2013).

The disease is predominantly unilateral with bilateral involvement more in the immunocompromised patients. *Aspergillus species* and *Candida species* are the most commonly isolated fungi among the immune-competent and immunocompromised patients respectively (Satish *et al.*, 2013).

2.2.7. Epidemiology

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 of them especially in those with poor socio-economic status. The etiologies and prevalence of ear infection is different indifferent 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 3 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 OM 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 & Fasinam, 2012).

It is estimated that 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 (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).

2.2.8. Possible Complications

Infection of otitis media accompanied by complications including brain abscess, septicaemia, 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).

Fungal infection lead to hemorrhagic granulations can cause thrombosis of adjacent blood vessels leading to avascular necrosis and perforation of tympanic membrane. Treatment of otomycosis should be vigorous to prevent complications such as hearing loss and invasive temporal bone infection (Satish *et al.*, 2013).

2.2.9. Diagnosis

The diagnosis 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 lighting is essential for sufficient visibility (Tähtinen, 2012).

Standard microbiological diagnosis methods, start by ear discharge samples were 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 bio-chemical tests done were indole, methyl red, voges – proskaver, 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).

Sabaroud Dextrose agar with antibiotics for fungal organisms fungal cultures were evaluated for isolation of any fungal growth on Sabaroud 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).

2.2.10. 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).

2.2.10.1. bacteria

2.2.10.1.1. 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 examine non motile 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 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 haemolytic. Growth may not occur on MacConkey agar. *S. saprophyticus* and *S. epidermidis* are coagulase negative (Cheesbrough, 2006).

2.2.10.1.2. 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 do this by oxidation, not by fermentation (Cheesbrough, 2006).

It is cause opportunistic nosocomial infections responsible for 10% of all hospital-acquired infections . Infections caused by *P.aeruginosa* are often severe, lifethreatening 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 H₂S production. Produce indole (non-oxidase, citrate and urease producer) (Greenwood *et al.*, 2007).

b. *Kelebsiella pneumoniae*

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 frundii*

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 capsule non spore forming. *Pr.vulgaris* and *Pr.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).

2.2.10.2. fungi

2.2.10.2.1. *Aspergilli* species

Aspergillus species are globally ubiquitous saprophytes found in a variety of ecological niches. Almost 200 species of aspergilli have been identified, less than 20 of which are known to cause human disease (Dagenais and Keller, 2009).

Aspergillosis is the most common invasive mold infection worldwide. Aspergilli are ubiquitous saprobes in nature and may be found in soil, potted plants, decaying vegetation, pepper and construction sites. *Aspergillus spp* can cause diseases in humans by airway colonization with subsequent allergic reactions, colonization of preexisting cavities (aspergilloma) or by tissue invasion. Aspergilli secrete various metabolic products, such as gliotoxins, and a variety of enzymes, including elastase, phospholipase, various proteases, and catalase, which may play a role in virulence (Murray *et al.*, 2009).

2.2.10.2.2. *Candida* species

Candida albicans is an opportunistic fungus causing various forms of candidiasis. However, under certain circumstances it is capable of becoming pathogenic (Nasution, 2013). *C. albicans*, as most pathogens, has developed an effective battery of virulence factors and specific strategies to assist the ability to colonize host tissues, cause disease, and overcome host defenses. An outstanding attribute of *C. albicans* biology is its capacity to grow in a diversity of morphological forms, ranging from unicellular budding yeast (blastospores), pseudohyphae, to true hyphae with parallel-sided walls. The yeast-hyphae transition contributes to tissue invasion and to the escape from phagocyte cells after host internalization, and is therefore considered an important virulence factor.

Additionally, several other factors have been described in association with virulence, including the production of proteins that mediate adherence, the colonization and invasion of host tissues, the maintenance of cell wall integrity, phenotypic switching, and the avoidance of the host immune response (Ferreira *et al.*, 2010).

2.2.11. Treatment

Treatment include elimination of predisposing factor and use suitable antimicrobial agents. Topical antifungals are (clotrimazole, miconazole, econazole, nystatin, tolnaftate, potassium sorbate) (Khan *et al.*, 2013), and antibacterial (systemic\topical) like tetracycline, chloramphenicol, erythromycin, ciprofloxacin, ceftriaxone, amoxicillin, norfloxacin, cotrimoxazole, gentamicin, penicilin, (Muluye, 2013), and other antibacterial like imipenem, cloxacillin, amoxicillin\ clavulanic acid, ceftazidime, and ofloxacin are also used (Ogbogu *et al.*, 2013).

a.Ciprofloxacin

Ciprofloxacin a fluoroquinolone antimicrobial agent, it is broad spectrum antibiotic 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 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. Amoxyclav (Amoxicillin\ Clavulanic acid)

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. 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. Chloramphenicol acts as an antibiotic against a wide variety of microorganisms, is also used in external treatments such as eye drops or ointment to treat bacterial conjunctivitis. Chloramphenicol stops bacterial growth by binding to the bacterial ribosome and inhibiting protein synthesis. Chloramphenicol 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).

e.Clotrimazole

Clotrimazole is the most widely used topical azole. It is available as powder, a lotion, and a solution. It is considered free of ototoxic effects. Some studies showed that clotrimazole was one of the most effective agents for management of otomycosis, with reported rate of effectiveness that varies from 90% to 100% (Khan *et al.*, 2013). Clotrimazole is a

broad-spectrum antifungal agent and effectively controls fungal isolates attributed to otomycosis (*Aspergillus* and *Candida*) (Satish *et al.*, 2013).

2.2.12. Prevention

In order to eradicate inflammation, prevent recurrence and restore middle ear structures and hearing, both medical and surgical treatment modalities are important in CSOM. 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 AOM may further decrease as the use of pneumococcal conjugate vaccine (PCV) becomes more popular. 7-valent PCV covers seven (4, 6B, 9V, 14, 18C, 19F, and 23) out of 91 *Streptococcus pneumoniae* (*S. pneumoniae*) serotypes. In randomized clinical trials, 7-valent PCV has reduced the number of AOM episodes from any cause by 6%–7% and AOM episodes caused by the serotypes present in the vaccine by 57% (Tähtinen, 2012). 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).

2.3. Background studies

Many previous studies were planned to investigate the prevalence of ear infection and etiological agents. A similar study conducted by Muluye and another co-worker in Ethiopia, 2013 the predominant isolate was proteus species (27.5%). Of individuals who had ear infection, 185 (90.7%) had single bacterial infection while 19 (9.3%) had mixed infections. Under five children were more affected by ear infection. The prevalence of ear infection was significantly high in males (63.7 vs 36.3%). Of all bacterial isolates, 192 (94.1%) had multiple antibiotic resistant pattern (Muluye *et al.*, 2013).

Another study achieved by Ogbogu and his college in Nigeria reported Gender and age did not significantly affect the prevalence of otitis media pathogens. *Pseudomonas aeruginosa* (33.33%) were the most prevalent microbial agent of otitis media followed by *Staphylococcus aureus* (23.19%) while *Citrobacter* species and *Aspergillus niger* were the least prevalent with a prevalence of 0.48% each (Ogbogu *et al.*, 2013).

Aother study achieved by Abera and Kibret in Ethiopia reported the most frequent isolates were *Proteus* spp. 223 (26.5%), *S. aureus* 203 (24.6%), *Pseudomonas* spp. 148 (18%) and *E. coli* 146 (17.7%). *Proteus* spp. were the most common isolates in children compared to adults, ciprofloxacin and gentamicin revealed high level of sensitivity Antibigrams of isolates showed that 598 (72.6%) of isolates were resistant to two and more antimicrobials (Abera and Kibret, 2011).

Other study in Nigeria, 2012 conducted by Okesola & Fasinam reported the most frequently isolated organism in acute otitis media and chronic suppurative otitis media was *Pseudomonas aeruginosa* (43.7%), followed

by *Klebsiella species* (31.0%), *Proteus species* (14.1%), *Escherichia coli* (7%), *H.influenzae* (2.8%) and *Staphylococcus aureus* (1.4%). Generally, high resistance rates were recorded against many of the antibiotics tested. However, ciprofloxacin demonstrated the highest susceptibility rates (Okesola & Fasinam, 2012).

Study achieved by Murat Enoz and others in Turkey showed that 219 cultures were positive and a total of 267 isolates were obtained. Of the isolates, 68.16% (n: 182) were aerobic or facultative bacteria, 1.12 % (3) were anaerobic bacteria, 30.71 % (82) were fungi and 17.5 % (38) were polymicrobial infections (Enoz *et al.*, 2009).

Khan and colleges in Pakistan reported that males to females ratio was 0.71:1. Patients of 15 years and above were included in the study. Adults were more affected by otomycosis than the younger age group. The efficacy of clotrimazole in treatment of otomycosis was observed in 89 (94.12%) while in 12 (5.88%) patients no efficacy was seen. Age and gender have no role in efficacy of clotrimazole in treatment of Otomycosis (Khan *et al.*, 2013).

Other partial similar study achieved by Hashim and colleges (2010) in Sudan, showed that isolates were identified as: *Aspergillus niger* (59.6%), followed by *A. flavus* (21.3%), *A. terreus* (12.8%), *Penicillium species* (2.1%), *Curvularia species* (2.1%) and *Scopulariopsis species* (2.1%). *Aspergillus species* (Hashim *et al.*, 2010).

CHAPTER THREE

3. MATERIALS AND METHODS

3.1 Study design

This was descriptive crosses sectional study.

3.2 Study type

Hospital and analytic study.

3.3 Study area

Khartoum Ear, Nose and Throat (ENT) Hospital, Khartoum, Sudan.

3.4 Study population and duration

All clinically suspected cases of ear infection (otitis media and extrnal otitis media) arrived the unit of ENT during period of the study from August- October 2015.

3.4.1 Inclusion criteria

Patients who presented with clinical diagnosis of ear infection (otitis media and extrnal otitis media).

3.4.2 Exclusion criteria

Patients with other type of ear infections other than otitis media and otitis externa and patients under antibiotic therapy were excluded.

3.5 Sampling

3.5.1 Sampling technique

Convenience non-probability sampling.

3.5.2 Sample size

Hundred (n=100) ear swab specimens were collected.

3.6 Study variables

Screen of ear infection (otitis media and extrnal otitis media) (dependent variable).

Etiological agents, age and sex as independent variables.

3.7 Data collection

By questionnaire contains all study variables.

3.8 Ethical Clearance

Permission of this study was obtained from the local authorities in the area of the study, the objectives of the study clearly and simply explained to all individuals participating in the study, verbal informed consent was obtained.

3.9 Experimental Work

3.9.1 Method of collection

Ear swabs were collected from patients by aid of sterile cotton tipped swabs with the help of Otologist.

3.9.2 Macroscopic examination

Color of discharge for each specimen was observed.

3.9.3 Microscopic examination

3.9.3.1 Wet preparation

The swab was rolled on clean slide and a drop of 20% potassium hydroxide was added, and covered with cover slip, then examined under microscope using X10 and X40 for head of Aspergilli, hyphae and budding yeast (Chessbrough, 2006).

3.9.3.2 Gram's stain

The swab was spread on clean slide to make smear let it to air dry, then smear was fixed by passing the slide three times over flame. The smear was covered with crystal violet stain for 1minute & washed by tap water, then covered with Lugol's iodine for 1minute & washed by tap water, then decolorized by using 70% alcohol for few seconds, Saffranin was added to the smear for 2minuts ,washed with tap water &left to dry by air. Smear was examined with oil immersion, to observed yeast and bacterial cells morphology, size, Gram positive reaction and presence of pus cells, epithelial cells (Cheesbrough, 2006).

3.9.4 Inoculation

Swab was cultured onto Blood agar ,Chocolate Blood agar ,MacConkey agar and Sabouraud Dextrose Agar (HiMedia laboratories Pvt, Ltd, India), were using sterile wire loop under aseptic conditions. Then incubated at 37°C overnight but Sabouraud Dextrose Agar (SDA) with 0.05mg/ml Chloramphenicol plate were incubated for 48-72 hours to detect *Candida species* and up to two weeks to detect of *Aspergilli species*.

3.9.5 Laboratory diagnosis of bacteria

3.9.5.1 Colonial Morphology

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

3.9.5.2 Indirect Microscopic examination

3.9.5.2.1 Gram stains

The Gram staining reaction was used to identify pathogens morphology in specimens and whether the organism was Gram positive or Gram negative morphology.

3.9.5.2.2 Wet preparation

A small drop of suspension was placed on a slide and cover with a cover glass. The preparation was examined microscopically for motile organisms, using the 10X and 40X objectives (Cheesbrough, 2006).

3.9.5.3 Biochemical tests

Biochemical activity of pure & mixed isolates was done to confirm the identification of these includes:

3.10.5.3.1 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. Then was tested for indole by adding 0.5

ml of Kovac's reagent (B.H Chemical, Japan). and shaken gently. Then was examined for a red colour in the surface layer within 10 minutes (Cheesbrough, 2006).

3.9.5.3.2 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 break down the urea (by hydrolysis) to give ammonia and carbon dioxide, with the release of ammonia, the medium becomes alkaline as shown by a change in color of the indicator to pink-red (Cheesbrough, 2006).

3.9.5.3.3 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, bright blue color in the medium was looked (Ahmed, 2008).

3.9.5.3.4 Kliglar Iron Agar (KIA)

By using of sterile sterile straight wire the KIA media (HiMedia laboratories Pvt, Ltd, India) was inoculated with organism under test. First the butt was stabbed, then the slope was streaked and the incubation was done at 37°C for overnight. A yellow butt (acid production) and red-pink slope indicates the fermentation of glucose and lactose. Blackening along the stab line or throughout the media indicates hydrogen sulphide (H₂S)(Ahmed, 2008).

3.9.5.3.5 Oxidase

The test was used to detect the ability of the organisms to produce oxidase enzyme. Oxidase discs were placed inside the Petri dish, and small inoculums were taken by using wooden stick or glass rod (not an oxidized wire loop), and then smeared on the disc by opening the the Petri dish partially. blue-purple colour , oxidase test positive (within 20

seconds), no blue- purple colour , oxidase test negative (Cheesbrough, 2006).

3.9.5.3.6 Catalase test

Two 2–3 ml of the hydrogen peroxide solution (Supplies Angles Burry, UK) was poured into a test tube. Sterile wooden stick or a glass rod was used and removed several colonies of the tested organism and immersed in the hydrogenperoxide solution. Then looked for immediate bubbling (Cheesbrough, 2006).

3.9.5.3.7 Deoxyribonuclease (DNase) test

The test organism is cultured on a medium which contains DNA (HiMedia laboratories Pvt, Ltd, India). After overnight incubation, the colonies are tested for DNA-ase production by flooding the plate with a weak hydrochloric acid solution (Shparlan, Spain). The acid precipitates unhydrolyzed DNA. DNA-ase producing colonies are therefore surrounded by clear areas due to DNA hydrolysis (Cheesbrough, 2006).

3.9.5.3.8 Manitol Salt agar

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

3.9.5.3.9 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 emulsified, then a loop ful of plasma was added to the suspensions and mixed gently. The clumping of the organism was examined within 10 seconds. Clumping within 10 seconds; positive coagulase test, no clumping or clumping in more than 10 seconds; negative coagulase test (Cheesbrough, 2006).

3.9.6 Laboratory diagnosis of *Aspergillus species*

3.9.6.1 Colonial Morphology

Growth rate, texture and color of the colony at surface and reverse were observed.

3.9.6.2 Needle Mount

By an inoculation needle small portion of the colony was removed and mounted in a drop of lactic acid or Lacto Phenol Cotton Blue (LPCB) on a clean microscope slide. The slides covered with a cover slip, squash the preparation with the butt of the inoculation needle and then the excess fluid blotted off. The mount examined using Low Power Field (LPF) and High Power Field (HPF).

3.9.7 Laboratory diagnosis of *Candida species*

3.9.7.1 Gram's stain

Gram's stain was performed for yeast suspected colonies which revealed Gram positive yeast cells.

3.9.7.2 Germ tube test (GTT)

Three drops of serum were put into small tube by using a Pasteur pipette, by sterile wire loop a colony of yeast was touched and emulsified in the serum. After incubation at 37 °C for 2-4 hours but no longer, then a drop of the serum was transferred into a slide for examination, cover slip was placed and examined microscopically using 40X objective.

Positive test: presence of short lateral filament (germ tube) for *C.albicans*, Negative test: yeast cell only for *C. non albicans* (Bhavan *et al.*, 2010).

3.9.7.3 Chrom agar pigmentation test

Chromogenic media (HiMedia laboratories Pvt , Ltd, India) was prepared according to manufacture instruction and the organism inoculated in the media, then incubated at 37 °C for 48 hours. After that the growth of *Candida spp* was observed by the change in the colour of the colonies

according to the pigment, as a result of reaction between chromogenic substrate and enzymes that secreted by different *Candida spp*, allowing organisms to be identified to the species level by their color and colony characteristics. The result was as the following: *Candida*, the product identifies *C.albicans* by growth as light to medium green colonies, *C.tropicalis* by growth as steel blue colonies accompanied by purple pigment diffusion into surrounding agar, and *C.krusei* by growth as large, fuzzy, ross-colored colonies with white edge, individual *non albicans* species was identified as : *C.glabrata* (dark pink and wet colonies), *C.krusei* (light pink and dry colonies) and *C.albicans* (green and wet colonies) (Babic and Hukic, 2010).

3.9.7.4 Zymogram (Carbohydrate fermentation test)

Fermentative yeasts recovered from clinical specimens produce carbon dioxide and alcohol. Production of gas rather than a pH shift was indicative of fermentation. Dextrose, maltose, sucrose, lactose, galactose and trehalose were used in the test.

The 5 ml of carbohydrate (pH, 7.4) containing 1 % peptone, 1 % sugar, 0.3 % beef extract and 0.5 % NaCl, 0.2 % Andrad's in distilled water medium was dispensed in sterilized Durham tube and 0.2 ml of saline suspension of the test organism was added and incubated at 37° C for 10 days (Bhavan *et al.*, 2010).

3.9.8 Anti-microbial sensitivity testing

3.9.8.1 For bacterial isolated

Anti-microbial sensitivity testing was done by using Modified Kirby-Bauer disc diffusion method.

3.9.8.1.1 Preparation of inoculums

Several colonies of similar appearance of the test organism in were emulsified a small volume of sterile peptone water. suspension was matched to the turbidity standard (Cheesbrough, 2006).

3.9.8.1.2 Seeding of plates

Using a sterile loop of about 4mm diameter, a loop of the test organism suspension was applied to the center of the sensitivity testing plate Mueller Hinton agar (HiMedia laboratories Pvt , Ltd, India). A sterile dry cotton wool swab was used to spread the inoculums evenly across the centre third of the plate, allowed to dry for a few minutes with the Petri dish lid in place (Cheesbrough, 2006).

3.9.8.1.3 Disc application

Using sterile forceps or a needle mounted in a holder, antimicrobial disc Ciprofloxacin, Amoxyclave, Chloramphenicol and Gentamicin, (Hi-Media laboratories Pvt, Ltd, India) were placed. The plates were inoculated aerobically at 35-37°C over night (Cheesbrough, 2006).

3.9.8.1.4 Interpretation of the result

The radius of the inhibtoition zone was measured, from the edge of the disc to the edge of the zone. The end-point of inhibition was where growth start and compared with interpretative chart. The reaction of the test organism to each antibiotic was reported as sensitive, intermediate or resistant (Cheesbrough, 2006).

3.9.8.1.5 Quality Control

Standard strains *E. coli* with ATCC NO (25922) and *S. aureus* with ATCC NO (25923) were brought from National Public Health Laboratory. Sensitivity testing were performed on Muller Hinton agar in similar way (disc diffusion method) and condition to our isolates to determine the validity of the selected antibiotics.

3.9.8.2 For fungal isolated (Disc diffusion method)

The paper disc diffusion method was used to screen the antifungal and performed by using Mueller Hinton agar (MHA). The experiment was carried out according to the National Committee for Clinical Laboratory

Standards Guidelines (NCCLS, 1999). Fungal suspension was diluted with sterile physiological solution to 10^8 cfu/ ml (turbidity = McFarland standard 0.5). One hundred micro liters of fungal suspension were swabbed uniformly on surface of MHA and the inoculum was allowed to dry for 5 minutes. Sterilized filter paper discs (6 mm in diameter) were placed on the surface of the MHA and soaked with 20 μ l of a solution of each antifungal (Clotrimazole and Naftifine hydrochloride). The inoculated plates were incubated at 37 °C for 24 -48 hours in the inverted position. The diameters (mm) of the inhibition zones were measured (NCCLS, 1999). The results were expressed in terms of the diameter of the inhibition zone: < 9 mm, inactive; 9-12 mm, partially active; 13-18 mm, active; >18 mm, very active (Alves *et al.*, 2000).

3.10 Data analysis

Data analysis will be done using Statistical Package of Social Science (SPSS) soft program version 11.5.

CHAPTER FOUR

4. RESULTS

4. Results

The results of the mycological and bacteriological studies on the 100 cases showed that microbiological culture was yielded from 96(96%) samples 80 samples (80%) had a single organism isolated from the ear discharge, while the remaining 16 (16%) had two or more organisms isolated. There were 4 samples (4%) showed no growth (Table 1).

In this study, 40% of patients were males and 60% were females (Fig 2). The mean age of the study population was 25 ± 18 years (age range, 1-75years), majority of them 37 (37%) belonged to 1 to 15 years of age group (Table 1).

Out of the total ear infection in this study 78 (78%) of patients was otitis media and 22 (22%) were otitis externa (fig 3). And most otitis externa 9 (40.9%) caused by fungi, called fungal otitis or otomycosis, 7(31.8%) were caused by bacteria and 6 (27.3%) were polymicrobial infections (Table 2). While the 64(82%) of otitis media were caused by single bacteria, 10 (13%) caused by more than one bacteria and the rest 4(5%) no organism isolated (Table 3).

The bacterial biochemical tests results are show in (Table 4,5) and the fungal examination results are show in (Table 6).

The predominant bacterial isolates *Pseudomonas aeruginosa* 35(31%), followed by *Staphylococcus aureus* 27(23.9%), *Proteus spp* 13(11.5%), *Klebsiella pneumoniae* 13(11.5%), *Staphylococcus epidermidis* 3(2.7%), *Staphylococcus saprophyticus* 2 (1.8%), *Escherichia coli* 2(1.8%) and *Citrobacter freundii* 2(1.8%) and the fungal isolates were *Aspergillus niger* 9(8%), followed by *Aspergillus flavus* 4(3.5%) and *Candida albicans* 3(2.7%) (Table 7).

45(46.4%) of bacterial isolate was resistant to Amoxayclav, followed by 17(17.5%) Gentamicin, 11(11.3%) Ciprofloxacin and 9(9.3%) Chloramphenicol, while all fungal isolates was sensitive to Clotrimazole and Naftifine hydrochloride (Table 8,9 &10).

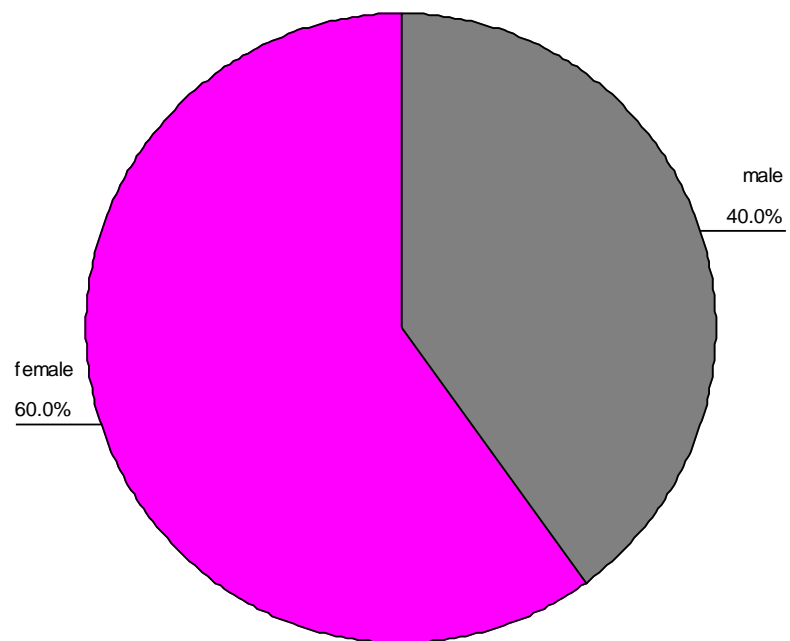


Fig (2) : Percentage of males and females among study group (n=100)

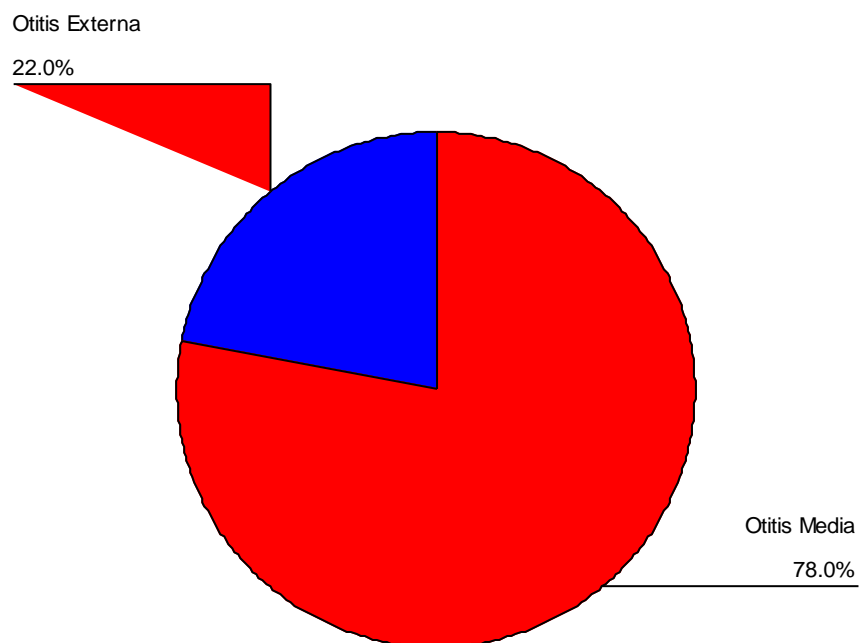


Fig (3): The type of ear infection and distribution among patient (n=100)

Table(1): Percentage of the growth among age group

Age group	Mix organisms	Single organism	No growth	Total
1 – 15	6 6%	29 29%	2 2%	37 37.0%
16 – 30	3 3%	30 30%	1 1.0%	34 34%
31 – 45	2 2%	11 11%	0 .0%	13 13%
46 – 60	4 4%	7 7%	1 1.0%	12 12%
61 – 75	1 1%	3 3%	0 .0%	4 4%
Total	16 16.0%	80 80.0%	4 4.0%	100 100%

P value= 0.633

***Sig value ≤ 0.05**

Table (2): Distribution of organism cultured among Otitis Externa

Type of isolats	Number	Percentage
Fungi	9	40.9%
Bacteria	7	31.8%
Polymicrobial	6	27.3%
Total	22	100%

Table (3): Distribution of organism cultured among Otitis media

Type of isolats	Number	Percentage
Single bacteria	64	82%
Mixed Bacteria	10	13%
No growth	4	5%
Total	78	100%

Table (4): Biochemical tests for Gram +ve cocci

Isolated organism	Catalase	MSA	Coagulase	DNase
<i>Staphylococcus aureus</i>	+ve	+ve	+ve	+ve
<i>Staphylococcus saprophyticus</i>	+ve	+ve	-ve	-ve
<i>Staphylococcus epidermidis</i>	+ve	-ve	-ve	-ve

Key: DNase: DNA hydrolysis test, MSA: Manitol Salt Ager, +ve: Positive, -ve: Negative.

Table (5): Biochemical tests of Gram –ve rods

Isolate	Motility	Oxidase	Indole	Urease	Citrate	KIA			
						Slope	butt	H ₂ S	Gas
<i>P.aeruginosa</i>	+	+ve	-ve	D	+ve	R	R	-	-
<i>Proteus vulgaris</i>	+	-ve	+ve	+ve	D	R	Y	++	D
<i>Proteus mirabilis</i>	+	-ve	-ve	+ve	+ve	R	Y	++	+
<i>Klebsiella pneumonia</i>	–	-ve	-ve	+ve	+ve	Y	Y	-	+
<i>Escherichia coli</i>	+	-ve	+ve	-ve	-ve	Y	Y	-	+
<i>Citrobacter freundii</i>	+	-ve	-ve	D	+ve	Y/R	Y	D	+

Key: Y: yellow, R: Red, D: Different strains give different results, +ve: Positive, -ve: Negative

Table (6): Results of *Aspergillus species* and *Candida albicans*

Isolated organism	Direct microscopy by KOH	Direct Gram's stain	SDA	Chrome agar	Needle mount	GTT	Zymogram				
							Glucose	Lactose	Sucrose	Maltose	Galactose
<i>C.albicans</i>	Budding yeast	G+ve yeast	White & pasty	Green & wet	—	+ve	+ve	-ve	-ve	+ve	+ve
<i>A.flavus</i>	Hypahae	—	Greenish yellow	—	Septated hyphae, Rough conidiophore, globose vesicle, biseriated & with radial conidia	—	—	—	—	—	—
<i>A.niger</i>	Hypahae	—	Black	—	Septated hyphae, globose vesicle, biseriated & with radial conidia	—	—	—	—	—	—

Key: +ve: Positive, -ve: Negative

Table (7) : Distribution of organism cultured from ear discharge

Isolates	mix organisms	single organism	Total
<i>S.aureus</i>	7 6.2%	20 17.7%	27 23.9%
Coagulase negative <i>Staphylococci</i>	0 .0%	5 4.5%	5 4.5%
<i>Ps.aeruginosa</i>	10 8.8%	25 22.1%	35 31.0%
<i>E.coli</i>	1 .9%	1 .9%	2 1.8%
<i>K.pneumonia</i>	5 4.4%	8 7.1%	13 11.5%
<i>P.vulgaris</i>	0 .0%	1 .9%	1 .9%
<i>P.mirabilis</i>	3 2.7%	9 8.0%	12 10.6%
<i>C.freundii</i>	0 .0%	2 1.8%	2 1.8%
<i>C.albicans</i>	2 1.8%	1 .9%	3 2.7%
<i>A.niger</i>	2 1.8%	7 6.2%	9 8.0%
<i>A.flavus</i>	3 2.7%	1 .9%	4 3.5%
Total	33 29.2%	80 70.8%	113 100%

P value= 0.459

***Sig value ≤ 0.05**

Table (8): Antibiotic susceptibility pattern of bacterial pathogens of ear infection

Isolates	Total	Ciprofloxacin		Amoxyclav		Gentamicin		Chloramphenicol	
		R	S	R	S	R	S	R	S
<i>S.aureus</i>	27 27.8%	5 18.5%	22 81.5%	6 22.2%	21 77.8%	0 .0%	27 100%	3 11.1%	24 88.9%
Coagulase negative staphylococci	5 5.2%	1 20%	4 80%	0 .0%	5 100%	0 .0%	5 100%	0 .0%	5 100%
<i>Ps.aeruginosa</i>	35 36.1%	5 14.3%	30 85.7%	28 80%	7 20%	13 37%	22 63%	3 8.6%	32 91.4%
<i>E.coli</i>	2 2.1%	0 .0%	2 100%	2 100%	0 .0%	1 50%	1 50%	0 .0%	2 100%
<i>K.pneumonia</i>	13 13.4%	0 .0%	13 100%	5 38.5%	8 61.5%	0 .0%	13 100%	2 15.4%	11 84.6%
<i>Proteus spp</i>	13 12.4%	0 .0%	13 100%	3 23.1%	10 76.9%	3 23.1%	10 76.9%	1 7.7%	12 92.3%
<i>C.freundii</i>	2 2.1	0 .0%	2 100%	1 50%	1 50%	0 .0%	2 100%	0 .0%	2 100%
Total	97 100%	11 11.3%	86 88.7%	45 46.4%	52 53.6%	17 17.5%	80 82.5%	9 9.3%	88 90.7%

Table(8): Antifungal susceptibility pattern of fungal isolated

Isolates	Total	Clotrimazole		Naftifine hydrochloride	
		R	S	R	S
<i>C.albicans</i>	3 18.75%	0 .0%	3 100%	0 .0%	3 100%
<i>A.niger</i>	9 56.25%	0 .0%	9 100%	0 .0%	9 100%
<i>A.flavus</i>	4 25%	0 .0%	4 100%	0 .0%	4 100%
Total	16 100%	0 .0%	16 100%	0 .0%	16 100%



Fig (5): Antifungal susceptibility pattern

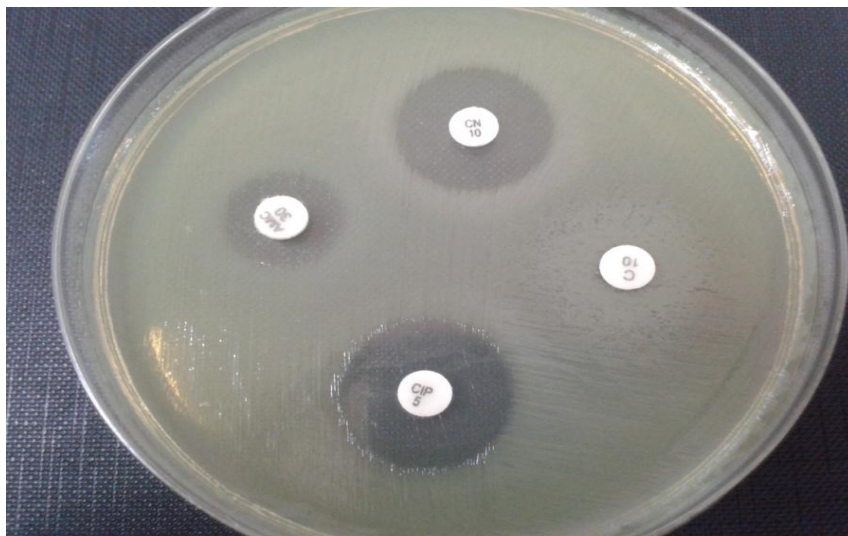


Fig (4) : Antibiotic susceptibility pattern of bacteria

CHAPTER FOUR

5. DISCUSSION

5.1 Discussion

Ear swab is one of the most frequently ordered samples for microbiological analysis in the study area. This indicates that ear infection is a common problem in the given area.

An analysis of the age groups revealed that ear infection can affect any age from one year through 75 years.

The present study results revealed that infection was high in the age-group 1-15 years when compared with similar study in Ethiopia showed that the 1- 18 years age group had the highest prevalence of ear infection (Abera and Kibret, 2011). However, 40% of patients were males and 60% were female, while in Ethiopia the study carried out by Muluye *et al.*, 2013, 66.2% were males and 33.8% were females.

From the 100 ear swabs collected 96 developed culture growth, majority of the patients 80 (80%) had a single organism isolated, while the remaining 16 (16%) had two or more organisms isolated. There were 4 (4%) samples who had a sterile culture with no organism isolated, this was similar to the result reported in Ethiopia by Abera and Kibret (2011), which showed (88%) had a single organism isolated, while the remaining (12%) had two or more organisms isolated and also similar to the result reported in Ethiopia by Muluye and his colleges which showed (90.7%) had a single organism isolated from the ear discharge, while the remaining (9.3%) had two or more organisms isolated (Muluye *et al.*, 2013).

In the present study the the predominant bacterial isolates were *Pseudomonas* 35(31%), followed by *Staphylococcus aureus* 27(23.9%), *Proteus spp* 13(11.5%), *Klebsiella pneumoniae* 13(11.5%), Coagulase Negative *Staphylococci* 5 (4.5%), *Escherichia coli* 2(1.8%) and *Citrobacter frundii* 2(1.8%), and the frequency of fungal isolates was

Aspergillus niger 9(8%), followed by *Aspergillus flavus* 4(3.5%), *Candida albicans* 3(2.7%), compared with similar study conducted in Nigeria the predominant bacterial isolates were *Pseudomonas aeruginosa* (33.3%), followed by *Staphylococcus aureus* (23.2%), *Proteus spp* (15%), *Klebsiella spp*(7.3%), *Escherichia coli* (2.4%) and *Citrobacter spp* (0.5%) while *C. albicans* (6.3%) showed high frequency among other fungi isolated followed by *Aspergillus niger* (0.5%), (Ogbogu *et al.*, 2013)

Also the present study partially similar to other study achieved by Muluye and his colleges in Ethiopia showed *Proteus spp* (27.5%), followed by, *Staphylococcus aureus* (26.5%), *Pseudomonas aeruginosa* (8.8%), *Escherichia coli* (6.9%), *Klebsiella spp* (5.9%) (Muluye *et al.*, 2013),

According to our study 78 (78%) of the patient had Otitis media infection, while 22 (22%) had otitis externa, and the 64(82%) of otitis media were due to single bacteria, 10 (13%) was due to mixed bacteria and the rest 4(5%) no organism isolated, while the most otitis externa 9 (40.9%) due to fungi, called fungal otitis or otomycosis, 7(31.8%) were due to by bacteria and 6 (27.3%) were polymicrobial infections, this result was different from the result reported by Pontes and his colleges in Brazil showed in otitis externa most of them were caused by bacteria (80.6%) while 19.4% caused by fungi (Pontes *et al.*, 2009). and also different from other study in Turkey Enoz *et al.*, 2009 showed, (68.16%) were aerobic or facultative bacteria, (30.71 %) were fungi and (17.5 %) were polymicrobial infections (Enoz *et al.*, 2009).

These difference may be attributed to differences in culture, socioeconomic standard of possibly the environment and hygiene.

The sensitivity test carried out using modified Kirby-Bauer disc diffusion method and the result revealed that 45(46.4%) of bacterial isolate was resistant to Amoxayclav, followed by 17(17.5%) Gentamicin, 11(11.3%)

Ciprofloxacin and 9(9.3%) Chloramphenicol, this result was different from the result reported by Abera and Kibret in Ethiopia which showed (47.1%) of bacterial isolate was resistant to chloramphenicol has highest resistance rate followed by gentamicin (10.4%) and ciprofloxacin (6.4 %) (Abera and Kibret, 2011).

On the other hand *P.aeruginosa*, was highly resistant to amoxyclav (80%), followed by gentamycin (37%), ciprofloxacin (14.3%), and chloramphenicol (8.6%), while *S. aureus* was highly resistant to amoxyclav (22.2%), followed by ciprofloxacin (18.5%) and chloramphenicol (11.1%) but was show the most effective with high sensitivities (100%) against *S. aureus*, this result partially agree with result of another study in Nigeria by Okesola and Fasina (2012) showed *P.aeruginosa*, was highly resistant to amoxyclav (100%), followed by gentamycin (35.5%) and ciprofloxacin (22.6%), but gentamycin, ciprofloxacin & amoxyclav were show the most effective with high sensitivities (100%) against *S. aureus* .Whereas disagree with another study conducted in Ethiopia by Kibret and Abera (2011) showed *P.aeruginosa* was highly resistant to chloramphenicol (69.7%) followed by ciprofloxacin (55%) and gentamycin (7.7%) and *S. aureus* was highly resistant to chloramphenicol (32%) followed by gentamycin (10%) and ciprofloxacin (4.6%).

This variation may be due to abuse of these antibiotic in our country.

For the antibiotics commonly available locally as topical ear drops chloramphenicol , (90.7%) was shown to be the most effective with high sensitivities for the most commonly isolated organisms followed by ciprofloxacin (88.7%), gentamicin (82.5%), compared with other study conducted in India the ciprofloxacin (89%) was shown to be the most effective with high sensitivities for the most commonly isolated

organisms followed by gentamicin (76.6%) and chloramphenicol (59.4%) (Singh *et al* 2012).

In the present study clotrimazole and naftifine hydrochloride showed good activity against all fungal isolates, this result agree with other study conducted in Pakistan by Khan and his colleges which showed the clotrimazole is effective in the treatment of otomycosis (Khan *et al.*, 2013).

5.2 Conclusion

the overall prevalence of bacterial isolates was higher than fungi and the predominant isolates were *Pseudomonas aeruginosa* and *S.aureus*. The isolates was highly resistance rate to Amoxayclav antibiotic, followed by gentamicin, ciprofloxacin and chloramphenicol. clotrimazole and naftifine hydrochloride have a good activity against all fungal isolates.

5.3 Recommendations

1. Diagnosis of infection should not be made on clinical diagnosis only, it should be confirmed by laboratory diagnosis.
2. Specimens must be collected by experienced health workers by aiding of otoscope.
3. Large sample size should needs.
4. Retraction of antibiotic perceptions by only physician.

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بسم الله الرحمن الرحيم

Appendix (1)

Questionnaire

Sudan University of Science and Technology

College of Graduate Studies

**Etiology of ear infection among Sudanese Patients attending
Khartoum ENT hospitals**

(A) General information:

1. Code-number
2. Patient's name
3. Sex
4. Age
5. Type of ear infection.....

(B) Laboratory investigation:

1. Direct microscopy
2. Culture
3. Colonial morphology
4. Gram's stain
5. Needle mount
6. Biochemical tests.....
7. Isolated organism.....

(C) Antimicrobial evaluation:

1. Sensitive.....
2. Resistance.....
3. Intermediate

Appendix (2)

2.Media:

To prepare the following culture media do to the following:

- 1- Dissolve the ingredient in the appropriate amount to distilled water.
- 2- Adjust the pH to 7.2
- 3- Sterilize by an autoclave 121 for 15 minutes.

2.1. Blood Agar:

Nutrient agar	500ml
Sterile defibrinated blood	25g

2.2. Chocolate Blood Agar

Nutrient agar	500ml
Sterile defibrinated blood	25g

2.3. MacConKey Agar:

Peptone	3g
Lactose	10g
Bile salt	1.5g
Sodium chloride	5g
Neutral red	0.3g
Agar	15ml
Distilled water	1000ml

2.4. Sabouraud dextrose agar:

Mycological peptone	10g
Dextrose	40g
Agar No.1	15g
Distilled water	1 liter

2.5. Nutrient Agar:

Lab-lemco	5g
Yeast extracts	10g
Pepton	25g
Sodium chloride	25g
Distilled water	500ml

2.6.Muller Hinton:

Beef infusion	300g
Casein hydrolysate	1.5g
Starch	105g
Agar	10g
Distilled water	100ml

2.7.Manitol Salt Agar:

Lab-lemco powder	1g
Peptone	5g

Manitol	10g
Sodium chloride	75g
Phenol red	0.025g
Agar	15g
Distilled water	1000ml

2.8.DNase:

Tryptose	20g
DNA	2g
Sodium chloride	5g
Agar	12g
Distilled water	100ml

2.9.Kligler Iron Agar:

Lab-lemco powder	3.0 g
Yeast extract	3.0g
Peptic	15.0g
Sodium chloride	5.0g
Lactose	10.0g
Dextrose (glucose)	1.0g
Ferric sulphate	0.2g
Phenol red	0.024g

Sodium thiosulphate	0.3g
Agar	15.0g
Distilled water	1000ml

PH within the range 2.7-7.6 at room temperature

2.10. Peptone water:

Peptone	2g
Sodium chloride	1g
Distilled water	200ml

2.11. Simmon s citrate:

Sodium ammonium phosphate	1.5g
Potassium dihydrogen phosphate	1.0g
Magnesium sulphates	0.2g
Sodium citrate	2.5g
Bromothymol blue	0.08g
Agar	15g
Distilled water	1000ml

2.12. Urea medium

Peptone	1.0
Glucose	1.0
Sodium	5.0

Disodium sulphates	1.0
Potassium hydrogen phosphate	0.8
Phenol red	0.012
Agar	15.0
Distilled water	1000ml

2.12.Chromogenic agar medium

For differentiation between *Candida spp.* This medium is based on sabouraud dextrose agar (Oxoid CM41) and contain (per liter) 40.0 g of glucose, 10.0g of mycological peptone, and 15.0g of agar along with a novel chromogenic glucosaminidase substrate, ammonium 4-{2-[4-(2-acetamido-2-deoxy- β -o-glucopyranosyloxy)-3-methoxyphenyl]-vinyl}-1-(propan-3-yl-oate)-quinolium bromide (0.32g/liter).

Appendix (3)

3.Stains:

3.1.Crystal violet:

Crystal violet	20g
Ammonium oxalate	9.0g
Ethanol (absolute)	95g
Distilled water	1 liter

3.2.Lugol's Iodine:

Iodine	10.0g
Potassium iodide	20.0g
Distilled water	1 liter

3.3.Safranin:

Safranin	10.0g
Distilled water	1 liter

Appendix (4)

4.Reagents:

4.1.Alcohol 70%:

Alcohol absolute	70ml
Distilled	30ml

4.2.Hydrogen Peroxide 3%:

Hydrogen peroxide	3ml
Distilled water	100ml

4.3.Normal Saline:

Sodium chloride	8.5g
Distilled water	1000ml

4.4.Hydrochloric acid:

Hydrochloric acid	8.3ml
Distilled water	100ml

4.5.Kovac's Reagent:

4(p)dimethyl amino benzaldehyde	2g
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4.6.Mac Farland standard:

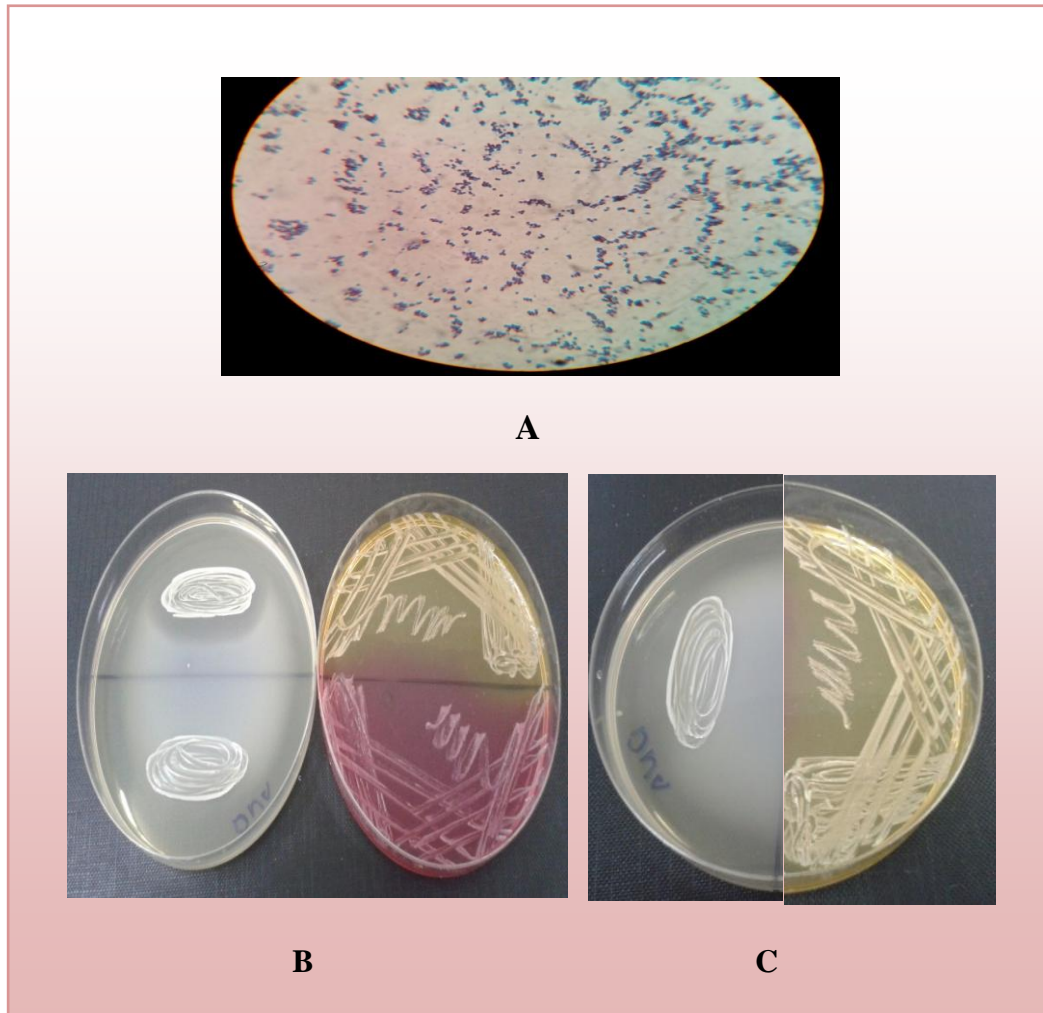
Sulphuric acid solution	99.5ml
Barium chloride solution	0.5ml

4.7.Potassium hydroxide, 200 g/l (20% w/v)

Potassium hydroxide (KOH). 10 g

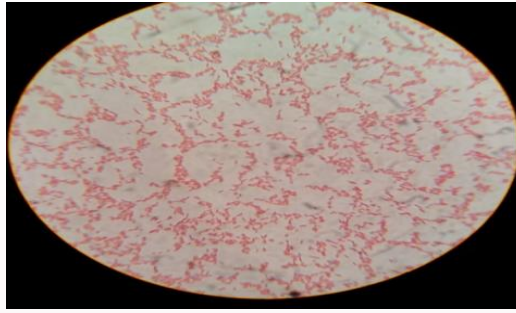
Distilled water 50 ml

Appendix (5)



Fgi (6): Show biochemical tests of *Staphylococcus*

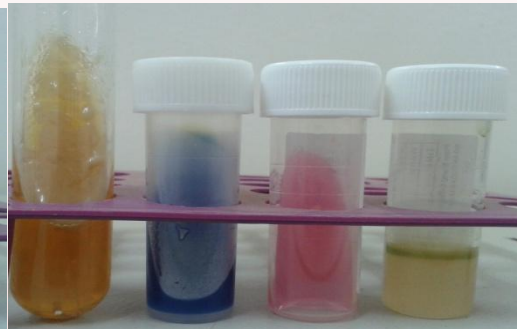
(A) Gram +ve cocci. (B) In upper part *Staphylococcus aureus* in DNase: clear zone, in MSA: ferment Manitol and give golden yellow color, and in lower part *Staphylococcus epidermidis* in DNase: no clear zone, in MSA: no Manitol ferment. (C) *Staphylococcus saprophyticus* in DNase: no clear zone and in MSA: ferment Manitol and yellow color



A



B



C



D



E



F



G

Fig (7): Show biochemical tests of gram –ve rods

(A)Gram –ve rods. (B) *Escherichia coli* from left to right **KIA**: yellow slop, yellow butt with gas, without H₂S, **Urease**:-ve, **Citrate**:-ve, **Indole**:+ve .

(C) *Klebsiella pneumonia*, **KIA**: yellow slop, yellow butt with gas, without H₂S, **Citrate**:+ve, **Urease**:+ve , **Indole**:-ve

(D) *Proteus mirabilis*, **KIA**: red slop, yellow butt with gas & H₂S, **Citrate**:+ve, **Urease**:+ve, **Indole**:-ve

(E) *Proteus vulgaris*, **KIA**: red slop, yellow butt with gas & H₂S, **Citrate**:+ve, **Urease**:+ve, **Indole**:+ve

(F) *P.aeruginosa*, **KIA**: red slop, ,red butt without H₂S & gas, **Citrate** +ve, **Urease**: weak +ve, **Indole** +ve

(G) *Citrobacter freundii*, **KIA**: yellow slop, yellow butt with H₂S & gas, **Citrate**+ve, **Urease**+ve , **Indole** -ve

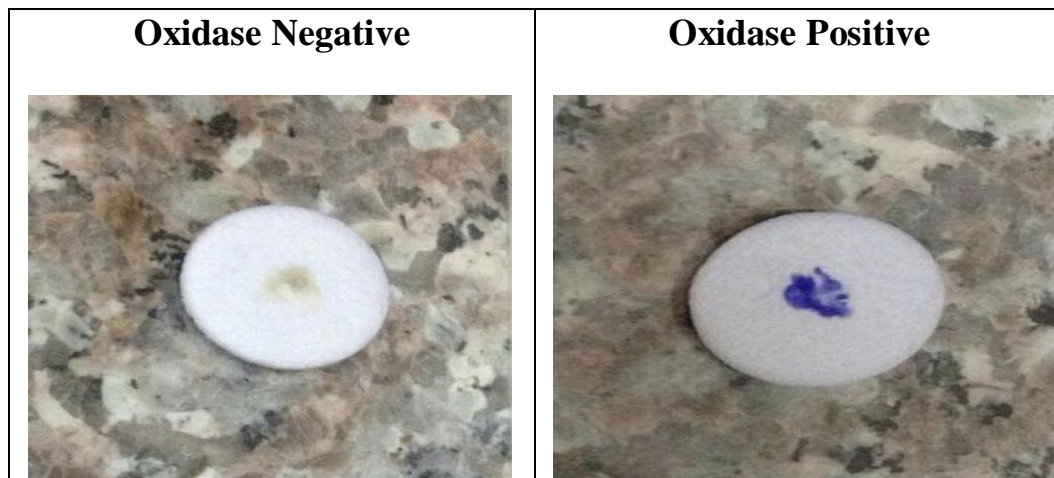
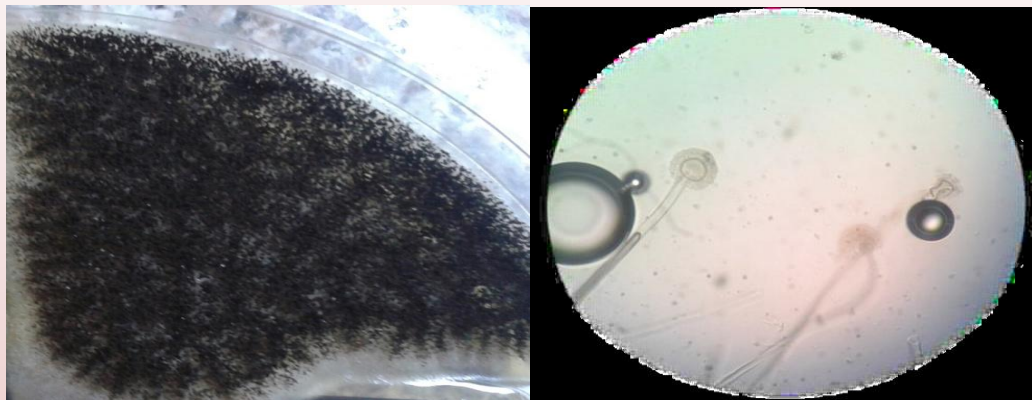


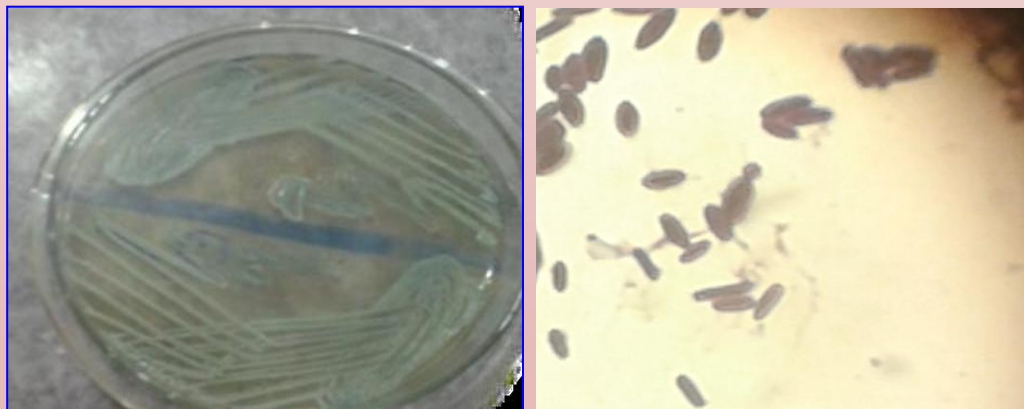
Fig (8): Show Oxidase test



A



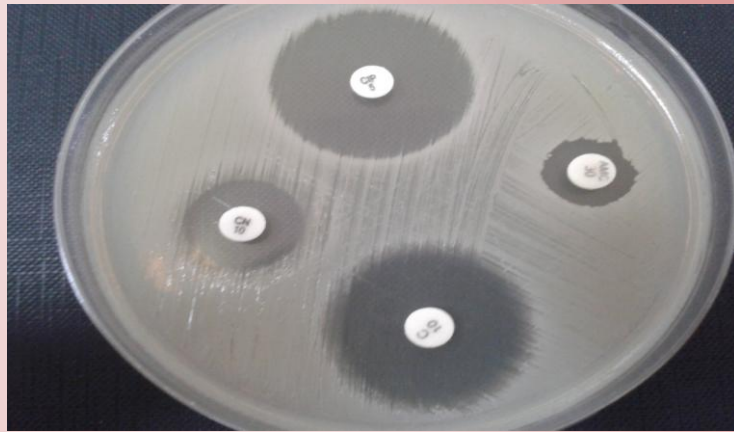
B



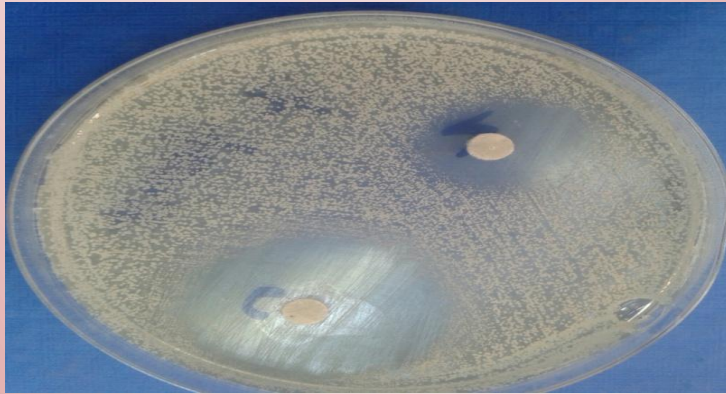
C

Fig (9) : Show different technique for fungal isolates

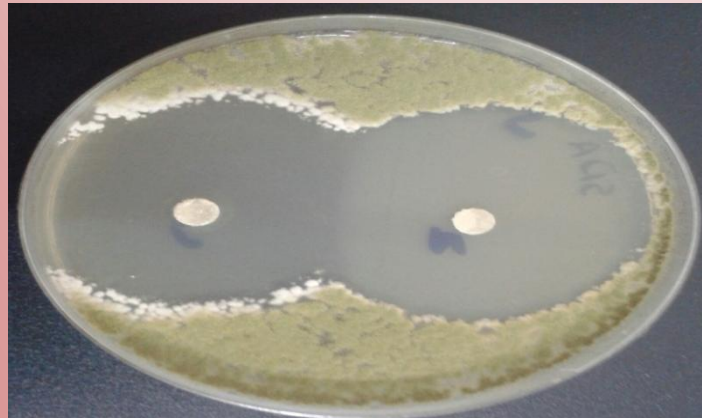
- (A) *A.flavus* in **SDA**: Greenish yellow color, in **Needle mount**: head and hyphae
 (B) *A.niger* in **SDA**: black color, in **Needle mount**: head and hyphae
 (C) *C.albicans* in **Chrome agar**: Green & wet, in **Gram's stain**: G+ve yeast,



A



B



C

Fig (10): Show antimicrobial susceptibility pattern

(A): antimicrobial susceptibility pattern for Bacteria

(B): antimicrobial susceptibility pattern for *C.albicans*

(C): antimicrobial susceptibility pattern for *A.flavus*

Table (10): Quality control of antibiotics

Standard Strain	Antibiotics	NCCL Sensitivity Result	Standard Strain Sensitivity Result
<i>E. coli</i> (ATCC 25922)	Gentamicin	19-26mm	20mm
	Ciprofloxacin	30-40 mm	32mm
	Amoxyclav	18-24mm	22mm
	Chloramphenicol	21-27mm	25mm
<i>S. aureus</i> (ATCC 25923)	Gentamicin	19-27mm	23mm
	Ciprofloxacin	22-20 mm	28mm
	Amoxyclav	28-36 mm	30mm
	Chloramphenicol	19-26mm	21mm