BACTERIOLOGY OF CHRONIC SUPPURATIVE OTITIS MEDIA
Study of aerobic Bacteria

By
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Dedication

To my mother, father and family
with my eternal love ..
I wish to express my deep gratitude for all those who helped me throughout the course of this work.

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Finally my sincere thanks are also extended to the directors and staff working in the Khartoum Ear, Nose, and Throat (E.N.T) Teaching Hospital and the Ear, Nose, and Throat, Department of Omdurman Teaching Hospital, with special regard to Mr. Hyder Abobakr for his considerable help and encouragement.
ABSTRACT

Fifty ear swabs were collected from patients suffering from chronic suppurative otitis media (CSOM) and attended the out patients of Khartoum Ear, Nose, and Throat Teaching Hospital, and the Ear, Nose, and Throat Department of Omdurman Teaching Hospital, between December 2004 to March 2005.

All the samples were subjected to bacteriological examination to identify the causative agent of the disease. The isolated microorganisms were subjected to antimicrobial sensitivity tests using different antibiotics.

Various types of microorganisms were isolated. The common causative agents were *Pseudomonas* species 30.8%, *Staphylococcus* species 23.1%, *Proteus mirabilis* 17.4%, and *Klebsiella* species 9.7%.

From the sensitivity tests, most strains were sensitive to ciprofloxacin (94.2%), in contrast they were highly resistant to penicillin (15.4%), and ampicillin (30.8%).

Furthermore the prevalence of the disease was more common in children than its occurrence in adults.
الإعاقة

لمحة

عينة خمسية

تم دراسة هذه الفئة في "الأذن التهابي" من خلال

السوائل الوسطى والأعمر مختلفة في أشخاص

الطبية الغشاء وفاء في الأذن وفأحة

البكتريا المعتادة

الكائنات الحية المعينة كل اختبار

وتم كميات

البرغم من أن الأشكال الأخرى:

نسبة 30.8 (  ) شبيهة

الجرعة والكابوسية

الالتهابية (  ) 94.2

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ampicillin

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Chapter One

Introduction & Literature review
INTRODUCTION

The ear is one of the body organs that is usually predisposed to infection by various microorganisms including bacteria, viruses, and fungi. Bacterial infection of the middle ear (otitis media) is an inflammation of mucoperiosteal lining the middle ear cleft i.e. Eustachian tube, middle ear and mastoid antrum.

This inflammation may be classified according to its stage into acute or chronic otitis media. Chronic suppurative otitis media (CSOM) is the result of acute otitis media that fails to heal, it is usually initiated by variety of upper respiratory tract conditions leading to Eustachian tube malfunction.

Chronic otitis media is usually presented by purulent discharge through perforated eardrum. This chronically discharging ears is one of the major health problem in developing countries which may be associated with variable degree of hearing loss. Although it is not fatal and not contagious, it is a source of significant morbidity.

Full identification of the aetiological agent has received little attention despite the importance of the causative agent to make certain of drug choice.
Therefore, our study was undertaken to:

- Identify the common causative aerobic bacteria of chronic suppurative otitis media.
- Examine the sensitivity of isolated bacteria to different antibiotics.
- Screen the prevalence of CSOM among the age.
LITERATURE REVIEW

1.1 Ear Anatomy and Physiology:

1.1.1 The external ear:

The external ear consists of the auricle and the external auditory meatus.

The auricle: commonly called the ear, is formed of elastic cartilage covered with skin. This cartilage determine the external shapes of the auricle and it is projections, the free curved margin called the helix, the anthelix located parallel to it the anterior prominence, the tragus, and the antitragus situated behind it. Down word the ear terminates as the lobe which has no cartilage, this is a characteristic progressive development sing for man. In the depression on the lateral surface of the auricle (the concha auricle). Behind the tragus, is the external auditory meatus around which the reminder of the rudimentary muscles has been preserved. They are of no functional significance. Since the auricle of man is immobile, some authors consider it to be a rudimentary formation, but others disagreed with this point of view because the cartilaginous skeleton of the human ear is well defined.
The *External auditory meatus*: consist of two parts: cartilaginous and bony. The *cartilaginous auditory meatus* is a continuation of the auricular cartilage in the form of a groove open upward and to the back. Its internal end is joined by means of connective tissues with the edge of the tympanic part of the temporal bone.

The cartilaginous auditory meatus constitute two thirds of the whole external auditory meatus. The *bony auditory meatus* which constitute two thirds of the entire length of the auditory meatus opens to the exterior by means of the pours acustiens externus on the periphery of which runs a circular bony tympanic groove. The direction of whole auditory meatus is frontal in general but it does not advance in straight line, it winds in the form of letter S both horizontally and vertically. Because of the curve of the auditory meatus, the deeply situated tympanic membrane ear only be seen by pulling the auricle backward, outward and upward. The skin that covers the auricle continues into the external auditory meatus. In the cartilaginous part of the meatus the skin is very rich both in sebaceous gland and in a particular kind of glands, the ciruminous glands, which produce a yellowish secretion, cerumen (ear wax). In this part there are also a short hair growing in the skin which preventing particles from getting into the organ. In the bony part of the duct the skin thins out considerably and extends without interruption onto the
1.1.2 Middle Ear:

The ciliated pseudostratified columnar epithelium of the respiratory tract extends up the Eustachian tube as far as the anterior part of the middle ear cavity, these cells are capable of producing mucous. In addition there are goblet cells and mucous secreting glands. More posteriorly, the mucosa changes patchily into a simple cuboidal or stratified epithelium with no secretory elements. The medial aspects of the tympanic membrane and the mastoid air cells are lined by a single layer of cells ranging in shape from cuboidal to flat.

In early stages of inflammation, whatever its cause, there is vasodilatation of the submucosal tissues. Glandular secretion is stimulated with the production of a thin mucoid fluid. Some of the epithelial cells die and the bacteria that are normally in the area multiply in the denuded areas and aggravate the condition. Apolymorphnuclear reaction occurs from the neutrophils in the blood and the mucopurulent discharge results. This may remain stagnant within the middle ear and mastoid air cell system because of immobility or loss of the cilia including those of the Eustachian tube.
Most frequently, resolution will occur but the condition prolonged for some reason, such as the inability of the secretions to drain down the Eustachian tube, antibiotic treatment should be started. The number of glands and goblet cells will increase and the areas formerly covered by cuboidal or flat epithelium will change into a similar but perhaps less well differentiated pseudostratified columnar epithelium. Differentiation into squamous epithelium, most frequently non keratinized, can also occur. Granulation tissue is an end result of non resolution of an inflammatory process. Localized areas of the mucosa become hyperplastic with invasion of fibroblast, capillaries and macrophages, plasma cells and lymphocytes. Granulation tissues can be covered by all the variations in mucosal types described above but are also frequently ulcerated so that it does not have a mucosal covering (Browning, 1997).

1.1.3 Inner Ear:

The inner ear contains the organ of hearing, the cochlea, and the organ of equilibrium, the vestibular labyrinth. Both of these structures are in petrous portion of the temporal bone, not the mastoid portion. In tiny channels in the bony cochlear and vestibular labyrinth are two separate fluids: perilymph and endolymph. The endolymph, which contained in a fragile membranous tube, bathes and nourishes the end organs for hearing
and equilibrium. This tube of endolymph is surrounded by larger quantity of perilymph, which serves to cushion it. The endolymphatic fluid is contained in a continuous closed system, while the perilymphatic spaces are connected with the subarachnoid space and its cerebrospinal fluid.

The organ of corti in the cochlea serves as the end organ of hearing. From its neuroepithelium project some 24,000 hair cells. Sound waves, on entering the cochlea, mechanically bend or distort the hair cells. When this happens, sound, which has been mechanical force, is converted into an electrochemical impulse, traveling along the acoustic nerve to the temporal cortex of the brain. There it is interpreted as meaningful sound. The hair cells, the most fragile elements in the ear, are subject to injury by mechanical trauma, oxygen deprivation, and the toxic influences of drugs and infection. As long as a person has normal hair cells, he will have some hearing. The only truly deaf patient is one whose hair cells no longer function. Stated differently, we may say that a person with no ear drum and no auditory ossicles can still hear amplified sound adequately if only the inner ear and its central connections are intact. (Sanders et al, 1979).

1.2 Physiology of Hearing:

Sound is produced by vibration. The outer ear collects the sound wave vibrations from the air and directs them to the eardrum (tympanic
membrane). The ear drum vibrations are transmitted to the cochlea by the three small bones of the middle ear. The nerve impulses produced in the cochlea are carried through the auditory nerve to areas of the brain concerned with hearing and interpreting sounds.

The external auditory meatus contains many small glands that secrete wax to prevent unwanted bodies such as insects entering and damaging the middle and inner ear. (Cheesbrough, 1987).

1.3 Infection:

1.3.1 Definition:

Infection is the mechanism through which microbial agents reach the potential victims and elicit pathogenic reactions (Dubos, 1958). When a microorganism or an infectious agent invades a human or animal body and disease results, whether clinical or sub clinical, the process is called infection. The microorganism is called a parasite, and the human or animal body is referred to as the host. The presence of a pathogenic microorganism in or about the body of the host does alone suffice to produce the interaction to as infection. Therefore infection is the interaction between a host and a parasite in an environment common to and effecting both. Infection whether clinically recognizable (apparent) or not recognizable (in apparent or subclinical) cannot occur without a host and a parasite and the environment (Top, 1968).
1.3.2 Effects of Infection:

The infections that matter are those causing pathological changes and disease. Many infectious agents cause little or no damage in the host. Indeed it is of some advantage to the microorganism to cause minimal host damage, when bacteria invade tissues they almost inevitably cause some damage, and this is also true for fungi, protozoa, and *Rickettsiae* (Mims, 1982).

1.3.3 Infectivity:

The infectivity of an organism is its pathogenic potential. This includes successful entry to the body, establishment in the tissues, and exertion of its pathogenic effects through invasiveness, toxigenicity or a combination of both (Ross, 1979).

1.3.4 The Infectious Process:

Infection indicates multiplication of bacteria. Prior to multiplication, bacteria must enter and establish themselves within the host. Once in the body bacteria must attach or adhere to host cells, usually epithelial cells. After the bacteria have established a primary site of infection they multiply and spread directly through tissues or via the lymphatic system to the bloodstream. This infection (bacteremia) can be transient or persistent.
Bacteremia allows bacteria to spread widely in the body and permits them to reach tissues particularly suitable for their multiplication (Brooks *et al*., 1998).

### 1.3.5 Source of Infection:

Source refer to the habitat or growth area in the human or animal. Animals are also source of infection that may transmitted to man, such infections are known as zoonosis (Ross, 1979).

Infections may be classified according to their source into:

#### 1.3.5.1 Endogenous infection:

Endogenous infections are contracted from the host himself. Many areas of the body have a normal, commensal flora characteristic of the particular area. A most important entity, this flora has many functions, including the provision of a barrier to infection in the individual. Normally the organisms that comprise the commensal flora do not cause infection in the host. There are exception to the rule. There are three common features in endogenous infections: *a/* the infections are produced some distance away from the normal habitat of the organisms; *b/* such infections are frequently a manifestation of lowered tissues resistance or tissue damage; *c/* problem of endogenous infection are generally confined to patients, in that they do not generally constitute high cross- infection risk. (Ross, 1979)
1.3.5.2 Exogenous infections:

Exogenous infections represent the greater proportion of infections and are derived from man, animals or soil, man is the most common source of exogenous infections, either when the patient suffering from clinical infection, or when the person is a carrier of infection. (Ross, 1979)

1.3.6 Transmissibility of infection:

Infection may be classified based on transmissibility into:

1.3.6.1 Localized infection:

Many infections remain localized and only become generalized: a/ if the organism become more virulent; b/ if the host resistance is decreased; or c/ if the bacteria gain access to another susceptible part of the body.

1.3.6.2 Generalized infection:

Some organism are highly invasive when they enter the body and produce generalized infection, some are acute and other are subacute. Spread can be direct by cerebrospinal fluid (CSF), by the blood, or by the lymphatic system (Ross, 1979).

1.4 Ear Infections:

The ear should considered as an integral part of the upper respiratory tract when associated with middle ear infections (otitis media), and far part of
the skin in infections of the external auditory canal (otitis externa) (Briody, 1974).

Allergies and viral infections of upper respiratory tract play a major role in predisposing patient to secondary bacterial infection of the middle ear (Garagusi, 1985).

1.4.1 Classification of ear infections:

1.4.1.1 Otitis Externa:

The lining of the external auditory canal I edematous, and superficial epithelial cells, pus, fibrins and organisms fill the lumen. Moisture, especially from swimming and warm weather, are common predisposing factors. Trauma from fingernails, bobby pins, and similar objects initiate or aggravate the disease.

Early itching or pain in the external auditory canal is followed by a purulent discharge. The patient will notice that the pain is made worse by eating or by touching the ear, as it also is by handling the pinna and the use of otoscope. The drum is usually normal, but the pre- and post- auricular glands- and some times the cervical glands- are enlarged and tender. The meatal wall I edematous, and a grayish mixture of waxy purulent material and desquamated cells is present in the canal. (Smith, 1958).
1.4.1.1 Bacteriology of Otitis Externa:

*Pseudomonas aeruginosa* is the predominant casual organism (26%), with *Staphylococcus albus* (22%), *Staphylococcus aureus* (15%), *E. coli* (8%) and *Proteus* (6%) being the next most frequent causes. (Smith, 1958).

1.4.1.1.2 Treatment of Otitis Externa:

The most important procedure is to clean the canal of wax and debris. Freedom from water is of paramount importance. Packing with sterile gauze soaked in aluminum acetate solution is beneficial in acute stages. Later an alcoholic solution of gentian violet should be used.

An acid reaction is inhibitory to most of the causative organisms, so that household vinegar dropped into the ear 6-8 times daily is a useful domestic remedy. If an antibiotic is to be used, apolymyxin ointment or a polymyxin –neomycin combination is indicated. Drops of the same type may penetrate better. (Smith, 1958).

1.4.1.2 Otitis Media:

Otitis media is an inflammatory of part or all of the *mucoperiosteal lining* of the middle- ear cleft. The middle ear cleft is the collective anatomical term for the Eustachian tube, tympanic cavity, mastoid antrum and mastoid air cells. As long as the inflammatory process is confined to the *mucoperiosteal lining* of these air spaces the disease remain uncomplicated
otitis media. If the inflammatory process affect any part of the bony walls or spreads beyond these walls into adjacent structures the disease become otitis media with a named complication. (Mawson, 1974).

1.4.1.2.1 Predisposing Factors:

Predisposing factors are broadly classified as intrinsic or extrinsic.

1.4.1.2.1.1 Intrinsic Factor:

Upper respiratory tract infections, particularly of tonsil, adenoid, maxillary and ethmoid sinus, resulting in nasopharyngeal sepsis. In children below the age of five, such attacks, which may be bacterial or viral may occur more than eight times a year, in teenagers by contrast, there are usually fewer than five pre Year. This corresponds with the higher incidence of acute suppurative otitis media in children below five year age.

Anatomic peculiarity of the Eustachian tube in infants and children. This tube is short, wide and horizontal and as low- set as that of an adult, facilitating the spread of infected materials from the nasopharynx to the middle ear.

Feeding position: Bottle or breast-feeding of The child in the recumbent position when pharyngeal reflexes are not well developed may lead to regurgitation of milk and vomits into nasopharynx, possibly reaching the middle ear via the Eustachian tube.
Lymphoid tissues: There is an abundance lymphoid tissues around the Eustachian tube opening in the nasopharynx because of physiologic hyperplasia. This may further increase in mass because of infection and edema and can produce obstruction of the Eustachian tube and hamper the draining of secretion.

Constitutional and racial factor: There is inherent tendency of certain families to develop otitis media. Person with oriented extraction an American Indians have high incidence of the disease (Chaturvedi, 1979).

1.4.1.2.1.2 Extrinsic Factors:

Seasonal variation: The incidence of otitis is greater in the cold months because of the high incidence of viral infections of the upper respiratory tract.

Environmental conditions: Poor hygiene and underwater swimming are among the environmental factors that can predispose towards development of otitis media (Chaturvedi, 1979).

1.4.1.2.2 Acute Otitis Media:

Acute otitis media often follows the upper respiratory infection such as the common cold or childhood disease such as measles and scarlet fever. It is disorder causing earache, so common during childhood. Bacterial organisms infecting the middle ear cavity almost reach it via the Eustachian
tube; infection coming from blood stream or through lymphatic channels are much less common (Sanders et al, 1979).

Edema of the Eustachian tube and mucosa of the middle ear are followed by accumulation of serous, mucoserous or purulent fluid. If untreated, the exudates may lead fibrous adhesions. (Smith, 1958).

The chief symptoms of acute otitis media is pain. If the ear drum ruptures because of the pressure of pus in the middle ear aural discharge becomes a symptom while the pain is relieved. There also is a partial loss of hearing, but because of earache, the patient usually does not complain much about his hearing. Examination of the ear drum in patient with acute otitis media shows it to red and bulging, or if the middle ear contains pus under pressure, it may look white. If it has ruptured, there will be a purulent discharge in the external auditory canal, and when this is removed by wiping or suction, a perforation is visible in the ear drum. Fever sometimes as high as 104 F. is expected. (Sanders, 1979).

1.4.1.2.2.1 Bacteriology of Acute Otitis Media:

Approximately 40% of cases of otitis media are caused by pneumococci, 25% by staphylococcus aureus, 3% by Streptococcus viridans and 16% by H. influenzae. The remaining 16% are sterile in culture. The
predominance of Gram-positive cocci or upper respiratory tract pathogens, where the infection enter via the external canal (Smith, 1958).

Acute otitis media (AOM) is one of the most frequently diagnosed infectious diseases of childhood, but becomes less common with advancing age. In children older than 6 weeks of age, it has been shown by numerous investigators that *Streptococcus pneumoniae* and *Haemophilus influenzae* are responsible for 50% of the episodes of AOM. Other important pathogens include *Moraxella catarrhalis*, *group A Streptococcus* organisms, and *Staphylococcus aureus*. About one third of the strains of *H. influenzae* and most of the *M. catarrhalis* strains have been shown to produce β-lactamase. Historically most physician believed that the incidence of *H.influenzae* in AOM decreased with advancing age leaving *S. pneumoniae* as the predominant pathogen. However, subsequent studies have shown that *H. influenzae* is as frequently grown on culture from specimens taken from older children as it is from younger children. Also *H. influenzae* is an etiologically important organism in adult sinusitis, pneumonia, and epiglottitis. Thus it is reasonable to anticipate the steadily increasing recognition of *H. influenzae* as a significant pathogen in adult otitis media. (Celin *et al*, 1991).
1.4.1.2.2 Prevention of Acute Otitis Media:

Prompt diagnosis and treatment of upper respiratory infections in young children is the best way of preventing otitis media. (Smith, 1958).

1.4.1.2.2.3 Treatment of Acute Otitis Media:

Early, adequate antibiotic therapy may bring about complete cure and recession of abnormal signs in the drum. Incision is necessary if bulging of the drum has been present for more than 24 hours. Maringotomy- has been thought necessary in 1-25% of the cases in the different series. When this is done promptly, it helps to preserve good hearing. In view of etiological bacteria initial treatment with either penicillin, erythromycin, penicillin-streptomycin or sulfonamide is indicated. (Smith, 1958).

Ear-drop to numb the eardrum or pain, relievers taken by mouth should be used if the infection is causing discomfort. A worm wash cloth on the ear can also provide comfort (MacDonald et al, 1998).

1.4.1.2.3 Chronic Suppurative Otitis Media:

Chronic suppurative otitis media (CSOM) is the result of acute otitis media that failed to heal. Of course, most cases of acute otitis media do heal so that chronic otitis media is less than acute otitis media.

In chronic otitis media there is a perforation in the eardrum through which pus run more or less continuously. This purulent discharge may
continue for months or for life, and part of the eardrum and often some of the middle ear bones are destroyed. The infectious process also extends to the air cells of the mastoid bone, since they are connected directly with the middle ear cavity. Therefore chronic otitis media and chronic mastoiditis are generally synonymous. (Sanders et al, 1979).

Following several attacks of acute otitis media, the drum ruptures, and a scanty amount of discharge is found. The latter is a thick, foul-smelling, yellowish pus in all infections except that with Pseudomonas which produce characteristic green-colored pus. Deafness of varying degree is found, and by tuning-fork test it is shown to be obstructive in type. After the canal has been cleaned, perforation of the drum is evident and granulation tissue polyps or cholesteatoma may also be seen. (Smith, 1958).

1.4.1.2.3.1 Bacteriology of Chronic Suppurative Otitis Media:

When the culture is pure the causative organism are found to be Proteus vulgaris 25%, Pseudomonas aeruginosa 20%, Staphylococcus aureus or Staphylococcus albus 12% and variety of other organisms. In case with mixed cultures the staphylococci comprise 25% of the total (Smith, 1958).

Ear swabs from 350 patients with chronic otitis media attending different orthorhinolaryngological clinics at different hospitals and health
centers in Benin City and Ekpoma in Edo state were screened for the presence of bacterial agents of chronic otitis media. Results revealed the presence of 19 different species indicating polymicrobial infections. Species isolated comprised *Staphylococcus aureus* (33.6%), *Pseudomonas aeruginosa* (19.3%), *Proteus mirabilis* (17%), *Alcaligenes faecalis* (6.2%), and *Klebsiella aerogenes* (4.3%). Others included *Escherichia coli* (3.3%), *Proteus rettgeri* (2.8%), and *Staphylococcus epidermidis* (2.2%), *Klebsiella pneumonae*, *Proteus vulgaris*, *Acinetobacter* species, *Proteus morgani*, *Haemophilus influenzae*, *Providencia* species, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Streptococcus faecalis*, non-haemolytic streptococci and Diphtheroids, each accounted for less than 2% of isolates.

The study showed that cases of chronic otitis media were highest among the age groups (0-5 years) with a prevalence rate of 50% and least among the (6-10 year) age group with a prevalence rate of 14.9% (Obi et al., 1995).

Aspiration of the exudates through the open perforation was performed in 68 children with chronic otitis media. The middle ear aspirate and swab specimens of the external auditory canal were cultured aerobically and anaerobically. Seventy-eight isolates were recovered from the middle ear, 99 from the external ear canal, and 95 were present at both sites. Aerobes only were isolated from 33 patients (48.5%), nine (13.2%) had only
anaerobes, and 26(38%) had a culture that grew both aerobes and anaerobes. There were 99 aerobic isolates. Aerobes commonly recovered were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Proteus* species, *Klebsiella pneumoniae*, and *Haemophilus influenzae*. There were 74 anaerobes isolated. Anaerobes commonly isolated were anaerobic Gram-positive cocci, *Bacteroides* species, and *clostridium* species. These findings demonstrate the polymicrobial bacteriology of chronic otitis media in children. Cultures collected from the external auditory canals prior to their sterilization can be misleading. Reliable information can be obtained from the ear exudates when collected through the open perforation in tympanic membrane (Brook, 1980).

Among other study the aerobic and anaerobic microbiology of 38 children from Surabaya- Indonesia, who suffered from chronic suppurative otitis media (CSOM) was studied using strict microbiological methodology. A total of 106 isolates (49 anaerobic and 57 aerobic) were recovered. Aerobic organism alone were isolated from 11 (29%) anaerobic bacteria only in 4 (11%) and mixed aerobic and anaerobic flora were present in 23 (60%). The predominant organisms were *peptostreptococcus* species, *Prevotella* species, *Bacteroides* species, *Staphylococcus aureus*, *pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Fusobacterium* species (Brook *et al*, 1995).
In chronic otitis media usually more than one bacterial organism is found in culture of pus. *Streptococcus*, *Staphylococcus*, *proteus*, and *Pseudomonas* organisms are most common (Sanders, 1979).

**1.4.1.2.3.2 Prevention of Chronic Otitis Media:**

Prompt treatment of acute otitis media is the best preventive. Tonsillectomy and adenoidectomy may be necessary (Smith, 1958).

**1.4.1.2.3.3 Treatment of Chronic Otitis Media:**

Antibiotic treatment systematically or locally or both is indicated only when bacteriologist testing facilities are available or I prompt response follows the intelligent choosing of agents. The ear must be kept clean and dry; all foreign bodies such as dead ossicles, granulations, polypi or cholesteatoma must be removed. Swimming not be allowed. (Smith, 1958). The treatment of acute otitis media with antibiotics is easier, quicker and more effective than the treatment of chronic otitis media. Cultures are most important in patients with chronic otitis media to make certain of drug choice (Sanders *et al*, 1979).

Although aminoglycoside eardrops, particularly gentamycin, are effective in *Pseudomonal* infections, recent reports from two retrospective studies have confirmed that ototoxicity occurs with topical gentamycin and primary affects the vestibular system. There have been a few case reports of
ototoxicity occurring in human treated with neomycin or framycetin, the other aminoglycosides in use and recent studies in animals using comparable doses to that of ear drops have confirmed this.

Ciprofloxacin and ofloxacin eardrops have several advantages over aminoglycosides (Miro, 2000).

1.5 Cultural Characteristics and Biochemical Properties of Conventional Pathogens:

1.5.1 Staphylococcus aureus:

1.5.1.1 Morphology:

Staphylococci are Gram positive cocci of uniform size, occurring characteristically in groups but also singly and in pairs. They are non-motile and non capsulated (Cheesbrough, 2000).

1.5.1.2 Culture:

Staphylococci grow well aerobically and in a carbon dioxide enriched atmosphere. Most strains also grow anaerobically, but less well. Temperature range for growth is 10-42°C, with an optimum of 35-37°C.

On blood agar and chocolate agar S. aureus produces yellow to cream or occasionally white 1-2 mm in diameter colonies after overnight incubation. Pigment is less pronounced in young colonies. Some strains are
beta hemolytic when grown aerobically. Colonies are slightly raised and easily emulsified.

On MacConkey agar (0.1-0.5 mm) colonies are produced after overnight incubation at 35-37°C. Most strains are lactose fermenting. (Cheesbrough, 2000).

1.5.1.3 Pigmentation:

Observation of the golden (orange, yellow or cream-buff) colony color is useful because it makes possible the provisional identification of *S. aureus* colonies in mixed culture also containing *albus staphylococci*. Pigmentation may be poorly developed in 24 hour on nutrient agar or blood agar and is then best seen viewing in daylight the aggregated material scraped up from several colonies on an inoculating wire. It may be enhanced by prolonged incubation at 37°C to 48 h or by the use of special pigment-enhancing media such as milk agar (Collee *et al*, 1989).

1.5.1.4 Biochemical properties:

Coagulase positive, DNase positive and catalase positive. They are able to ferment slowly many carbohydrates producing lactic acid but not gas; it attacks sugars fermentatively; indole negative, V.P positive, H₂S negative and oxidase negative. It does not ferment arabinose, cellobiose, inositol, inulin or raffinose. (Omer, 1990).
S. aureus ferment mannitol and is able to grow on agar containing 70-100g/l sodium chloride. They are novobiocin sensitive (Cheesbrough, 2000).

1.5.2 Pseudomonas aeruginosa:

1.5.2.1 Morphology:

*Pseudomonas aeruginosa* is a Gram negative, non-sporing motile rod. Some strains are capsulated.

1.5.2.2 Culture:

*Pseudomonas aeruginosa* is an obligatory aerobe. It is usually recognized by the pigment it produces included pyocyanin a blue-green pigment, and pyoverdin (fluorescein) a yellow-green fluorescent pigment. A minority of strains are non pigment producing. Cultures have a distinctive smell due to production of 2-aminoacetophenone. *Ps. aeruginosa* grows over a wide temperature range 6-42°C with an optimum of 35-37°C.

On blood agar *Ps. aeruginosa* produces large, flat, spreading colonies which are often haemolytic and usually (90% of strains) pigment-producing. The pigment diffuse into the medium giving it a dark greenish-blue colour. Some strains produce small colonies or mucoid colonies. When culture is left at room temperature, pigment colour becomes more intense.
On MacConkey agar and CLED medium *Ps. aeruginosa* produces pale coloured colonies. Compared with blood agar, pigment production is less marked. On KIA medium a characteristic pink-red slope (often with a metallic appearance), and pink-red butt are produced. No gas is formed and no H₂S is produced (Cheesbrough, 2000).

### 1.5.2.3 Production of Pigments:

Demonstration of the presence of the blue pigment pyocyanin is absolute confirmation of a strain as *Ps. aeruginosa* and thus the major diagnostic test. Pigment enhancing media should be used. The yellow pigment fluorescein is also produced by many strains, giving the characteristic blue-green appearance of infected pus or cultures. Pyocyanin, fluorescein and the more rarely observed pyomelanin and pyorubrin are easily identified on nutrient or sensitivity test agars, particularly after prolonged incubation (Colee *et al*, 1989)

### 1.5.2.4 Biochemical properties:

*Pseudomonas aeruginosa* is oxidase positive and produces acid only from glucose. These feature together with the typical pigments produced by most strains and the distinctive smell of cultures is usually sufficient to identify the organism. Growth at 42°C differentiates *Ps. aeruginosa* from less commonly isolated *Pseudomonas, Pseudomonas putida* and *Pseudomonas*
fluorescens. When acid is formed from carbohydrate medium, *Pseudomonas*
do this by oxidation, not by fermentation (Cheesbrough, 2000).

1.5.3 *Proteus mirabilis* and *Proteus vulgaris*:

1.5.3.1 Morphology:

*Proteus mirabilis* and *Proteus vulgaris* are actively motile, non
capsulate, Gram negative pleomorphic rods. Motility is not as easily
observed at 35-37°C as at room temperature (20-28°C) (Cheesbrough, 2000).

1.5.3.2 Culture:

When cultured on blood agar aerobically, most *Proteus* cultures have
a characteristic fishy odour. On MacConkey, cystine lactose electrolyte
deficient medium (CLED) and xylose lysine deoxycolate (XLD), *Proteus*
produces individual non-lactose fermenting colonies after overnight
incubation at 35-37°C. Swarming is prevented on MacConkey agar and XLD
agar because these media contain bile salts. Swarming is inhibited on CLED
agar because it is electrolyte deficient (Cheesbrough, 2000).

When the presence of Proteus in a clinical specimen makes it difficult
to isolate in pure culture another organism such as *Streptococcus pyogenes* or
*Staphylococcus aureus* the specimen should plated on a medium that inhibits
**Dienes phenomenon:** When two identical *Proteus* cultures are inoculated at different points on the same plate of non-inhibitory medium the resulting swarms of growth coalesce without signs of demarcation. When, however, two different strains of a *Proteus* species are inoculated, the spreading films of growth fail to coalesce and remain separated by a narrow, easily visible furrow. The observation of this appearance, the Dienes phenomenon, has been used to determine the identity or non-identity of strains in epidemiological studies (Collee *et al*, 1989).

### 1.5.3.3 Biochemical properties:

Do not ferment lactose, rapidly hydrolyze urea (within 4 hours), this is an important early screening test in differentiating enteric pathogens, e.g. salmonella and *Shigellae* from *Proteus*. Phenylalanine deaminase (PDA) positive, *Beta*-galactosidase (ONPG) negative. Indole negative (*Proteus vulgaris* is indole positive (Cheesbrough, 2000). Also they are H₂S positive, methyl red positive, V.P. negative. Gelatin liquefaction positive with *Proteus vulgaris* and *Proteus mirabilis* and negative with the others (Omer, 1990).

Reaction differentiating the four species of *Proteus* are in fermenting glucose and other carbohydrates all species except *Proteus rettgeri* usually produce gas. *Proteus vulgaris* is alone in fermenting maltose, only *Proteus
mirabilis fails to form indole and only Proteus rettgeri ferments mannitol (Colee et al, 1989).

1.5.4 *Klebsiella pneumoniae*

1.5.4.1 Morphology:


1.5.4.2 Culture:

*Klebsiellae* are aerobes and facultative anaerobes. On blood agar *Klebsiellae* produce large grey-white usually mucoid colonies.

On MacCokey agar and CLED medium most *Klebsiellae* are lactose-fermenting producing mucoid pink colonies on MacConkey agar and yellow mucoid colonies on CLED medium. *Klebsiella rhinoscleromatis* is non-lactose fermenting. (Cheesbrough, 2000).

1.5.4.3 Biochemical properties

*Klebsiella pneumoniae* subspecies are indole negative, ornithindecarboxylase negative, urease positive, citrate positive and do not produce H₂S. (Cheesbrough, 2000).
MATERIALS AND METHODS

2.1 Materials:

2.1.1. Media:

For the identification of isolated pathogens, different types of media were used, including solid, semisolid and liquid media. All media were prepared according to methods described by the manufacturer such as Oxoid, Difco and etc.

2.1.1.1 Solid Media:

2.1.1.1.1 Blood agar:

Hundred ml of fresh, sterile, defibrinated blood were added aseptically to 900 ml of melted sterile nutrient agar which was cooled to 55°C, mixed and distributed into sterile Petri dishes 20 ml in each dish.

2.1.1.1.2 Nutrient Agar:

This medium was supplied by Biomark laboratories-India. It consists of yeast extract, sodium chloride, peptone and agar. It was prepared by dissolving 28 grams of powder in 1 liter of distilled water by boiling. The medium was sterilized by autoclaving (121°C for 15 minutes), cooled to 55°C and then distributed into sterile Petri dishes 20 ml in each.
2.1.1.1.3 Diagnostic Sensitivity Test Agar (D.S.T):

This medium was supplied by Oxoid- England. It consists of protease peptone, veal infusion solids, dextrose, sodium chloride, disodium phosphate, sodium acetate, adenine sulphate, guanine hydrochloride, uracil, exanthin, aneurine, and Lon agar NO-2. Forty grams of medium were dissolved completely by boiling in 1 liter of distilled water and the pH was adjusted to 7.3. It was sterilized by autoclaving (121°C for 15 minutes), cooled and distributed into sterile Petri dishes 20 ml in each dish.

2.1.1.1.4 Urea Agar:

This medium was supplied by Oxoid. It consists of peptone, dextrose, sodium chloride, disodium hydrogen phosphate, potassium hydrogen phosphate, phenol red and agar.

The medium was prepared by dissolving 2.4 grams of the powder in 95 ml distilled water by boiling. After sterilization by autoclaving at 115°C for 20 minutes the base medium was cooled to 50°C and aseptically 5 ml of sterile 40% urea solution were added. The pH was adjusted to 6.8 and distributed into screw-capped bottles 10 ml each and then was allowed to set in the slope position.
2.1.1.5 MacConkey Agar:

This medium was supplied by Hi Media laboratories- India. It consists of lactose, bile salts, peptone, sodium chloride, neutral red and agar. Fifty-two grams of the medium were dissolved in 1 liter of distilled water by boiling. The pH was adjusted to 7.4, after which the medium was sterilized by autoclaving at 121°C for 15 minutes, cooled to 55°C and distributed into sterile Petri dishes 20 ml in each.

2.1.1.6 Simmon’s Citrate Agar:

This medium composed of sodium chloride, magnesium sulphate, ammonium dihydrogen phosphate, sodium ammonium phosphate, sodium citrate, bromothymol blue and agar. Twenty-three grams of powder were dissolved in 1000 ml distilled water by boiling. The pH was adjusted to 7.0, then the medium was sterilized by autoclaving at 121°C for 15 minutes and distributed into sterile screw-capped bottles and to solidify in a slope position. The medium was supplied by Oxoid.

2.1.1.7 Cooked Meat Medium:

It consists of fat free minced meat 450 grams, distilled water 1000, and nutrient broth. The fat free minced meat was added to the distilled water and boiled or one hour. It was then filtered and pressed dry. Dried particles were added to a depth of 2-5 cm in screw-capped bottles and the nutrient
broth was added to the depth of 5cm. It was then sterilized by autoclaving at 115°C for 20 minutes.

2.1.1.1.8 **Kligler Iron Agar (KIA):**

This medium consists of peptone, Lab-Lemco powder, yeast extract, sodium chloride, lactose, glucose, ferric citrate, sodium thiosulphate, phenol red and agar. Fifty-five grams of the powder were dissolved in 1000 ml distilled water by boiling. It was cooled to 50-55°C, distributed into tubes (approx. 16-160 mm) and sterilized by autoclaving at 121°C for 15 minutes. Then it was allowed to solidify in a sloped position. The medium was supplied by Hi Media laboratories.

2.1.1.1.9 **Ammonium Salt Sugars (ASS):**

This medium consists of ammonium phosphate, potassium chloride, magnesium sulphate, yeast extract, agar and bromcresol purple. It was prepared according to Barrow and Feltham (1993) by adding the solids to 1000 ml distilled water, dissolved completely by boiling and sterilized at 115°C for 20 minutes. The medium was allowed to cool to about 55°C and the appropriate sugar was added as a sterile solution to give final concentration 1%. Then the medium mixed and distributed aseptically into sterile tubes, which were inclined so that the medium sets as slope.
2.1.1.2 Semi-Solid Media:

2.1.1.2.1 Hugh and Leifson’s (O.F) Medium:

The medium was supplied by Oxoid and British Drug House (BDH)-England. It composed of peptone, potassium hydrogen phosphate, sodium chloride and agar. The medium was prepared according to Barrow and Felthman (1993) by dissolving 10.3 grams of solids in 1 liter of distilled water by heating. The pH was adjusted to 7.1, filtered bromothymol blue 0.2% aqueous solution was added and then sterilized at 115°C for 20 minutes. Sterile solution of glucose was added aseptically to give final concentration 1%, mixed and distributed aseptically into sterile tubes.

2.1.1.2.2 Motility medium:

Thirteen grams of dehydrated nutrient broth were added to 4 grams of Oxoid agar and dissolved in 1 liter of distilled water by boiling to give semi-solid agar. The pH was adjusted to 7.4, distributed in 5 ml amount in tests tubes containing Craige-tubes and sterilized by autoclaving at 121°C for 15 minutes.

2.1.1.2.3 Nutrient Gelatin:

This medium was supplied by Oxoid and BDH. It consists of Lab-Lemco, peptone and gelatin. It was prepared according to Barrow and Felthman (1993) by dissolving 128 grams of solids in distilled water, the
solids was dissolved completely by boiling, the pH was adjusted to 6.8 and distributed into screw-capped bottles. The medium was sterilized by autoclaving at 121°C for 15 minutes.

2.1.1.3 Liquid Media:

2.1.1.3.1 Nutrient Broth:

This medium consists of peptone, yeast extract and sodium chloride. It was prepared according to Barrow and Feltham (1993) by dissolving 13 g of the medium in 1 liter of distilled water. The pH was adjusted to 7.4 then the medium was distributed into screw-capped bottles 5 ml in each and sterilized at 121°C for 25 minutes, it was supplied by Biomark Laboratories.

2.1.1.3.2 Peptone Water:

The medium was prepared as described by Barrow and Feltham (1993). Fifteen grams of peptone water powder (supplied by Hi Media laboratories) were dissolved in 1 liter of distilled water. The pH was adjusted to 7.2, distributed into screw-capped bottles and sterilized by autoclaving at 121°C for 15 minutes, it was supplied by Biomark Laboratories.

2.1.1.3.3 Glucose Phosphate Broth (M.R-V.P Medium)

As described by Barrow and Feltham (1993), five grams of peptone and 5g of potassium phosphate were dissolved in 1 liter of distilled water by steaming. The pH was adjusted to 7.5 after that 5g of glucose were added and mixed
the medium was distributed into test tubes 5 ml in each and sterilized by autoclaving at 110°C for 10 minutes.

2.1.1.3.4 Peptone Water Sugars:

The medium was prepared according to Barrow and Feltham (1993). Nine hundred of peptone water was prepared and pH was adjusted to 7.1 - 7.3 before 10 ml of Andrade’s indicator was added. Ten grams of the appropriate sugar was added to a mixture of peptone plus indicator thoroughly, then distributed into tubes, 5 ml in each one. They were sterilized by autoclaving at 110°C for 10 minutes.

2.1.1.3.5 Nitrate Broth:

As described by Barrow and Feltham (1993), 1g of nitrate was dissolved in 1 liter of nutrient broth. Then it was distributed into tubes and sterilized by autoclaving at 115°C for 15 minutes.

2.1.2 Biological Materials:

2.1.2.1 Sheep blood:

Defibrinated sheep blood was used for the preparation of blood agar.

2.1.2.2 Human plasma:

This was used for the detection of coagulase.
2.1.3 Reagents:

Reagents used in this study were obtained from British Drug house Chemical (BDH), METLAB and HOPKIN & WILLIAMS Ltd - England. These were prepared according to Barrow and Feltham (1993).

2.1.3.1 Hydrogen Peroxide:

Hydrogen peroxide was prepared as 3% aqueous solution and used for catalase test.

2.1.3.2 Kovac’s Reagent:

This reagent composed of paradimethylaminobenzaldehyde, amylalcohol and concentrated hydrochloric acid. After the preparation the reagent was stored in the refrigerator at 4°C for later use.

2.1.3.3 Potassium Hydroxide:

Potassium hydroxide was prepared as 40% solution and used for Voges-Proskaur (V.P) test.

2.1.3.4 Alpha naphthol Solution:

It was prepared as 1% aqueous solution and also used for V.P test.

2.1.3.5 Oxidase Reagent:

Tetramethyl p.phenylene Diamine Dihydrochloride was prepared as 1% aqueous solution and used for oxidase test.
2.1.3.6 Methyl Red solution:

It was prepared by dissolving 0.04 g of methyl red powder in 40 ml ethanol and the volume was completed to 100 ml by distilled water. It was used for methyl red test.

2.1.3.7 Andrade’s Indicator:

This was prepared by dissolving 5 g of acid fuchsin in 1 liter of distilled water, then 150 ml of alkali solution (NaOH) was added. It was used in peptone sugars medium.

2.1.3.8 Bromothymol Blue Solution:

This was prepared as 0.2% w/v by dissolving 0.2 g of bromothymol blue powder in 100 ml distilled water. It was used for oxidation fermentation (O.F) test.

2.1.3.9 Bromcresol purple Solution:

Bromcresol purple solution was prepared as 0.9% solution, it was used in ammonium salt sugars (ASS).

2.1.3.10 Physiological Saline:

This was prepared by dissolving 8.5 g of sodium chloride in 1000 distilled water.
2.1.3.11 Nitrate Test Reagent:

This reagent composed of two types of solution:

- Solution A: Sulphanilic acid 0.33% in 5N- Acetic acid dissolved by gentle heat.
- Solution B: Dimeyhyl-α naphthylamine 0.6% in 5N- acetic acid. The complete reagent was used to detect nitrate reduction.

2.2. Methods:

2.2.1 Sterilization:

2.2.1.1 Autoclaving

Screw-capped bottles, rubber caps, media solution, normal saline etc. were sterilized in autoclave at 121°C for 15 minutes and 110°C for 10 minutes in case of sugars media.

2.2.1.2 Hot-air Oven (160°C for one hour):

Glass ware such as Petri dishes, tubes, flasks and glass rods were sterilized in hot air oven at 160°C for one hour.

2.2.1.3 Disinfection:

Solution of 70% alcohol and phenolic disinfectant were used for bench sterilization.
2.2.2 Collection of Samples:

Fifty ear swabs (pus) were collected from patients who were already diagnosed by the physician as having chronic suppurative otitis media. Such patients were the subject of the study and they were of different ages.

Purulent discharges were collected by using sterile commercial swabs and they were collected gently to avoid contamination by the normal flora. The samples were labeled and transferred immediately to the laboratory for bacteriological examination. The collection was done between December 2004 and March 2005 from Khartoum Ear, Nose and Throat (E.N.T) Teaching Hospital and the E.N.T Department of Omdurman Teaching Hospital.

2.2.3 Cultural methods:

2.2.3.1 Primary Isolation:

The collected swabs were cultured directly on blood agar and MacConkey agar. The MacConkey agar plates were incubated aerobically while the blood agar plates were incubated in carbon dioxide atmosphere (approximately 5%). All plates were incubated at 37°C for 24 hours.

2.2.3.2 Examination of Cultures:

Visual examination of all cultures on solid media was performed for detection of growth, pigmentation, colonial morphology as well as change in
the media. Plates which showed visible growth were subjected to further bacteriological tests while those did not show visible growth were incubated for further 48 hours.

2.2.3.3 Purification of isolates:

The primary isolates were subcultured on nutrient agar and blood agar (for fastidious microorganism). The subculture was repeated several times until pure colonies were obtained.

2.2.4 Identification of Isolated Pathogens:

2.2.4.1 Primary Identification:

2.2.4.1.1 Preparation of Smears:

Smears were prepared by emulsifying small inoculums of the bacterial culture in a drop of sterile normal saline and spread them on clean slide. The smears were allowed to dry and then fixed by gently flaming.

2.2.4.1.2 Gram’s Technique:

This was done as described by Barrow and Feltham (1993).

2.2.4.1.3 Microscopic Examination of Isolates:

All isolated microorganisms were subjected to microscopic examination and the shape, arrangement and Gram’s reaction were detected.
2.2.4.1.4 Catalase Test:

A drop of 3% hydrogen peroxide was placed on a clean slide, one to two colonies of tested organism was placed on the drop the solution using wooding stick. Production of air bubbles indicates positive result.

2.2.4.1.5 Oxidase Test:

The tested organism was picked using sterile bent glass rod and rubbed on a filter paper, which already soaked in oxidase reagent and dried. The development of dark purple color within 10 seconds indicates positive result.

2.2.4.2 Secondary Identification:

2.2.4.2.1 Motility test:

The Craigie tube method was used to detect the motility of the organism. The growth of the microorganism outside the Craigie tube considered as motile organism.

2.2.4.2.2 Oxidation – Fermentation Test (O.F):

Tow tubes of Hugh and Leifson’s medium were inoculated with tested organism. One of them was covered with a layer of sterile paraffin. All tube were incubated at 37°C and examined daily for seven days. The fermentative organisms produced a yellow color on both tubes and the oxidative organisms produced a yellow color only on the open tube.
2.2.4.2.3 Sugar Fermentation Test:
The sugar media was inoculated with 24 hours culture of tested organism. It was incubated at 37°C and examined daily for up to seven dyes. Acid production was indicated by the development of pink color in the medium. Gas production was indicated by trapped air in the Durham’s tube.

2.2.4.2.4 Indole Test:
The tested microorganism was inoculated in peptone water and incubated for 48 hours. Two to three drops of Kovac’s reagent was added and shacked well.

Production of pink color on the upper layer of the reagent considered as indole positive.

2.2.4.2.5 Methyl Red Test (M.R):
Glucose phosphate broth was inoculated with the organism and incubated at 37°C for 48 hours. Then 3 -5 drops of methyl red solution were added and mixed gently. Red color in the medium indicated positive result.

2.2.4.2.6 Voges-Proskauer Test (V.P)
This test was carried out to detect the production of acetyl-methyl carbinol. Glucose phosphate broth was inoculated with tested organism and incubated for 48 hours. Then 0.6 ml of alpha-naphthol solution followed by 0.2 ml of 40% potassium hydroxide solution were added to 1 ml of culture,
mixed well and examine after 15 minutes and one hour. Bright red color indicated positive result.

2.2.4.2.7 Citrate Utilization Test:

Simmon’s citrate medium was inoculated with the tested organism, incubated at 37°C and examined daily for up to seven days. The development of blue color in the medium considered as positive result.

2.2.4.2.8 Urease Test:

The tested microorganisms were inoculated on a slope of urea agar medium, incubated at 37°C and examined for up to 5 days. The change of color of the medium to red or pink color indicated positive result.

2.2.4.2.9 Hydrogen Sulphide Production:

The tested organism was inoculated on a slope of Kiligler Iron Agar (KIA) by stabbing the butt and streaking the slope, incubated at 37°C and examined for blacking for up to 7 days. Blacking of the butt considered as positive reaction.

2.2.4.2.10 Gelatin hydrolysis Test (or Liquefaction):

Nutrient gelatin medium was inoculated with tested microorganism and incubated at 37°C for seven days. The culture was examined daily by incubation in the refrigerator for 30 minutes. The culture which became
liquid after the incubation in the refrigerator was considered as positive result.

2.2.4.2.11 Nitrate Reduction Test:

The tested microorganism was grown in nitrate broth and incubated at 37°C for 5 days. One ml of nitrate reagent A was added followed by 1 ml of reagent B. A deep red color produced was considered as positive reaction. To tubes that not showed red color Zinc powder was added and allowed to stand. Formation of red color indicated that nitrate was present and the tested organism did not reduce it.

2.2.4.2.12 Coagulase Test:

2.2.4.2.12.1 Slide Coagulase Test:

This test was done to detect bound coagulase. A drop of physiological saline was placed on each end of a clean slide and small amount of bacterial culture (distinct colonies) was emulsified in each of the drop to make two thick suspension. A drop of undiluted human plasma was added to one of the suspension and mixed gently. The development of Clumping within 10 second was reported as positive reaction.

2.2.4.2.12.2 Tube Coagulase Test:

This to detect free coagulase, fresh plasma was diluted 1 : 10 in physiological saline. Half ml of diluted plasma was placed in sterile test tube
and 0.5 ml of overnight growth culture of tested organism as added. The tube then was incubated at 37°C and examined after 1, 3, and 24 hours. Positive test was indicated by coagulation of the tube content.

2.2.4.2.13 Sensitivity To Novobiocin:

The disc diffusion method was used to carry out the sensitivity of tested microorganism to novobiocin. The discs was supplied by Oxoid an it was 15µg in concentration. A plate of diagnostic sensitivity test (DST) was dried for 20 minutes. Suspension of tested organism was made using sterile normal saline and compared it with the turbidity standard (Barium sulphate), when they were comparable the tested organism was cultured using sterile swab, the plate was allowed to dry and the antibiotic disc was gently applied on the plate using sterile forceps. Then the plate was incubated at 37°C for 24 hours and the zone of growth inhibition was reported as sensitive (≥ 22 mm) or (≤ 17mm).

2.2.4.2.14 Sensitivity To Bacitracin:

Bacitracin disc of 5 mg (supplied by Oxoid) was placed on blood gar inoculated by tested organism and incubated at 37°C in 5% carbon dioxide atmosphere for 24 hours. Sensitive bacteria showed inhibition zone no less than 13 mm.
2.2.4.2.15 Sensitivity To Optochin:

A disc of 5 µg of ethylhydrocuprein hydrochloride (optochin) was applied on the surface of blood agar inoculated with tested organism. The plate was incubated at 37°C for 24 hours. Sensitive organism showed zone of growth inhibition not less than 10mm.

2.2.5 Antibiotic Sensitivity Tests:

After identification of the isolated pathogens, all isolates were subjected to number of antibiotic to detect their sensitivity. The disc diffusion technique was used.

The isolate was grown on nutrient agar and incubated for 24 hours, using sterile wire loop 3-5 well isolated colonies were emulsified in 3-4 ml of sterile physiological saline, then the bacterial suspension was compared with the turbidity standard (Barium sulphate standard which equivalent to McFarland 0.5). When comparable, the tested organism was cultured on DST using sterile swab and the excess fluid was removed by pressing and rotating the swab against the side of the bottle above the level of the suspension. The swab was streaked evenly to give a confluent growth, then the plate was allowed for 5 minutes for the surface of the agar to dry. Using sterile forceps the antibiotic discs were placed and evenly distributed on the surface of the agar. Then the plate was incubated at 37°C for 24 hours. The diameter of
each zone of inhibition around each disc was measured in millimeter and result was interpreted according to the interpretative chart as sensitive or resistant.

The antibiotics used were supplied by Bioanalyse- Turkey. And they were:

- Ampicillin 10µg.
- Penicillin 10 units.
- Erythromycin 30µg.
- Gentamycin 10µg.
- Amoxicillin 20µg plus Clavulanic Acid 10µg (Amoclan).
- Ciprofloxacin 5µg.
- Cefuroxime 30µg.
RESULTS

Ear swabs from 50 patients with chronic suppurative otitis media who attended the outpatient of Khartoum Ear, Nose, and Throat (E.N.T) Teaching Hospital, and the E.N.T Department of Omdurman Teaching Hospital were collected and subjected to bacteriological examination.

Among them 42 (84%) were pure culture (unimicrobial), 5 (10%) were mixed culture (polymicrobial), and 3(6%) showed no growth. Table (1).

Thirty seven patients (74%) were suffering from recurrent chronic otitis media whilst 13(26%) were suffering from first chronic otitis media. Table (4).

Pathogenic Bacteria Isolated From Patients With Chronic Suppurative Otitis Media:

Trough aerobic and enriched carbon dioxide atmosphere cultures, different bacterial isolates were identified. The species isolated comprised *Pseudomonas* species 16 (30.8%), *Staphylococcus* species 12 (23.1%), *Proteus* species 9 (17.4), *Klebsiella* species 5(9.7%), *Streptococcus pyogenes* 2 (3.8%), *Providencia* species 2 (3.8%), *Corynebacterium* species 2 (3.8%). Others included *Escherichia coli, Enterobacter cloacae, Citrobacter freundii,* and *Hafnia alvei* each account for less than 2% . Table (2) and Figure (1).
Occurrence of Chronic Suppurative Otitis Media According To The Age:

Through the study of different group of ages the occurrence of chronic suppurative otitis media among the first group (1-15 years) was 66%, the second group (16-30 years) was 22% and the third group (31-50 years) was 12%. Table (3) and Figure (2).

Biochemical Tests of Isolated Bacteria:

The biochemical tests used to identify the different isolated Gram-positive and Gram-negative microorganism were showed in Table (6) and (7) respectively. The identification if staphylococcus species was carried out according to Elsanousi and Saeed scheme, (1995).

Antimicrobial Sensitivity Tests:

Fifty-two bacterial isolates were recovered and subjected to *invitro* antibiotics sensitivity tests. The antibiotics used were Ampicillin, Penicillin, Erythromycin, Gentamicin, Ciprofloxacin, Amoxicillin clavulanic acid (Amoclan). More than 96% of isolates were sensitive to Ciprofloxacin, and most of them were resistant to Ampicillin. Also Gram-positive organism were highly sensitive to Cefuroxime.

The antibiotics sensitivity patterns were showed in Table (8). Table (4) showed the effectiveness of antibiotics in isolated bacteria in percentage.
Table (1): Number of microorganisms isolated from samples

<table>
<thead>
<tr>
<th>Cause Of Infection</th>
<th>No. of Sample</th>
<th>Percentage %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unimicrobial</td>
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</tr>
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Table (2): The number and percentage of isolated bacteria from CSOM

<table>
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<tr>
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</tr>
<tr>
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<td>17.4</td>
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<tr>
<td><em>Klebsiella pneumoniae sub.sp. aerogenes</em></td>
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<tr>
<td><em>Streptococcus pyogenes</em></td>
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</tr>
<tr>
<td><em>Pseudomonas stutzeri</em></td>
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</tr>
<tr>
<td><em>Pseudomonas fluorescens</em></td>
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<td><em>Klebsiella oxytoca</em></td>
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<tr>
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</tr>
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</tr>
<tr>
<td><em>Providencia stuartii</em></td>
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<td>1.9</td>
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<td><em>Corynebacterium striatum</em></td>
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Table (3): Occurrence of CSOM in different ages

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<th>Number of patients</th>
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<td>1-15 years</td>
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Table (4): The number and percentage of CSOM according to the type of infection

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<th>Number</th>
<th>Percentage %</th>
<th>Children</th>
<th>Adult</th>
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<tr>
<td>First CSOM</td>
<td>37</td>
<td>74</td>
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<td>Recurrent CSOM</td>
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<td><strong>Total</strong></td>
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<td><strong>100</strong></td>
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Table (5): Frequency of infection according to the side of the ear

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<td>Left</td>
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<td>Bilateral</td>
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Table (7): Biochemical reaction of Gram-negative isolated pathogens

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<th><em>P. putida</em></th>
<th><em>K. aerogenes</em></th>
<th><em>K. oxytoca</em></th>
</tr>
</thead>
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<td>+</td>
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</tr>
<tr>
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<td>O</td>
<td>F</td>
<td>F</td>
</tr>
<tr>
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<td>+</td>
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Table (7): - continued

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<th>Hafnia alvei</th>
<th>Cit. freundii</th>
<th>E. coli</th>
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<td>Amc</td>
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Amp: Ampicillin.
Pen: Penicillin.
Amc: Amoxicillin clavulanic acid.
Ery: Erythromycin.
Cip: Ciprofloxacin.
Gent: Gentamicin.
Cxm: Cefuroxime.
Fig. 1: Percentage of pathogenic bacteria isolated from CSOM

<table>
<thead>
<tr>
<th>Type of microorganism</th>
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<tbody>
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<td>Pseudomonas sp.</td>
<td>30.80%</td>
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<tr>
<td>Staphylococcus sp.</td>
<td>23.10%</td>
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<tr>
<td>Proteus mirabilis</td>
<td>17.40%</td>
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<tr>
<td>Klebsiella species</td>
<td>9.70%</td>
</tr>
<tr>
<td>Streptococcus pyogenes</td>
<td>3.80%</td>
</tr>
<tr>
<td>Providencia sp.</td>
<td>3.80%</td>
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<tr>
<td>Corynebacterium sp.</td>
<td>1.90%</td>
</tr>
</tbody>
</table>
Fig. 2: Occurrence of CSOM in different age groups

- 1–15: 66%
- 16–30: 22%
- 31–50: 12%

Age (in years)
Fig. 3: Percentage of infection according to the side of the ear

- Right: 36%
- Left: 46%
- Bilateral: 18%
Fig. 4: Effectiveness of antibiotics in isolated bacteria in percentage
Figure (5) Staphylococcus aureus In Nutrient Agar

Figure (6) Proteus mirabilis In Nutrient Agar
Figure (7) Staphylococcus epidermidis In Blood Agar

Figure (8) Staphylococcus intermedius In Blood Agar
Figure (9) antibiotic sensitivity test for *Pseudomonas aeruginosa*

Figure (10) antibiotic sensitivity test for *K. pneumoniae subsp. aerogenes*
Figure (11) antibiotic sensitivity test for staphylococcus aureus

Figure (12) antibiotic sensitivity test for proteus mirabilis
Chapter Four
DISCUSSION

Chronic suppurative otitis media (CSOM) is a long standing purulent infection of middle ear cleft presented by ear discharge which may be associated with variable degree of hearing loss. Aslam et al, (2004). It is a real problem in babies and young children. Chronic ear infection may be more dangerous than an acute infection because its effects are prolonged or repeated and it may cause permanent damage to the ear. Repeated infection occurs after short period of antibiotic treatment, and the organisms isolated showed resistant to most common used antibiotics. Paul, (2001). Complication of otitis media include meningitis, nasopharyngitis, mastoiditis, and sinus thrombosis. Coffey, (1967).

In this study we tried to evaluate and detect the causative aerobic bacteria and its sensitivity to different antibiotics among the patients suffering from chronic suppurative otitis media. The studied people included were from different ages and sex.

The study was done during winter (2004-2005) and all patients complaining from the symptoms of chronic otitis media. Sanders et al, (1979) said that it’s a common disease in the winter while otitis externa is common in the summer. In our results and from 50 samples which were subjected to bacteriological examinations a percentage of 84% were pure
culture (Unimicrobial), 10% were mixed culture (polymicrobial), and 6% showed no growth.

Aslam et al, (2004) carried out similar study in Pakistan. Their study showed that 76% of their cultures were pure, and 23.9% were found to be mixed culture. Also identical results were obtained by Campos et al, (1995) who studied the etiology and therapy of chronic suppurative otitis media in Spain. Their study revealed that 86% of cultures were monomicrobial whilst 14% were polymicrobial. With regard to the occurrence of CSOM in different ages we found that the incidence of infection was 66% in children, and 34% in adults. When we consider the type of infection we found that 74% were suffering from first CSOM and 26% were suffering from recurrent CSOM. These findings are in agreement with Obi et al, (1995). Their results showed that 64.6% were children whilst 35.4 were adults. Also Moshi et al, (2000) found that most patients (82.7%) who suffering from CSOM were under 19 years old. In addition Coker et al, (1982) and Birell, (1976) found that the age group (0 -5 years) recorded the highest percentage of occurrence (49%).

Among the bacterial pathogens isolated in this study, our findings showed that Pseudomnas aeruginosa was the commonest causative agent (21.3%)and all pseudomonas species showed rate of 30.8%. The next common microorganism was staphylococcus aureus (17.4%) and all Staphylococcus species showed rate of 23.1%. These results are
comparable and in accordance with Brook et al, (1979) who recovered *Ps. aeruginosa* from 36 out of 50 patients (72%), also Aslam et al, (2004) reported that *Ps. aeruginosa* was the most common isolate (50.5%) followed by *S. aureus* (23.6%). Jang et al (2004) who carried out similar study in 88 patients in South Korea, recovered *Pseudomonas aeurginosia* from all patients (100%). In addition to that and according to Kenna et al, (1986) it was found that the two microorganism accounted for 37% and 11% respectively.

On the other hand our findings disagree with Obi et al, (1995) who found that *S. aureus* was the commonest isolated bacteria (32%), and the second common one was *Ps. aeruginosa*. Also it is not in agreement with Ahmad (2003) who reported that 37.8% of isolates were *S. aureus* and 28.9% were *Ps. aeruginosa*. Our results were comparable with Smith, (1958) who reported in his manual that *Ps. aeruginosa* account for 20% whilst *S. aureus* account for 12%, but in case with mixed culture the *staphylococci* comprise 25% of the total.

The high ratio of these organisms may be related to the nature of specimen, which were pus samples, as it is known that *staphylococci* are pyogenic organisms. Although other organisms are not pyogenic they were isolated repeatedly in pure and mixed culture, these organisms were *Proteus mirabilis, Klebsiella pneumoniae, Streptococcus pyogenes, Providencia* species, *Corynebacterium* species, *Escherichia coli,*
Enterobacter cloacae, Citrobacter freundii, other pseudomonas species, and other staphylococcus species.

The third rate of isolation in this study was recorded for Proteus mirabilis, which accounted for 17.4%. This result is similar to that obtained by Ahmad (2003) who studied the CSOM in Gombe, Nigeria. Further more Moshi et al (2000) found that the Proteus mirabilis was the third isolate in a ratio of 13.2%.

The fourth microorganism isolated from CSOM was Klebsiella pneumoniae sub species aerogenes and Klebsiella oxytoca. These organisms are not common like those types mentioned above. Their ratio was 9.7%. However this result correlates well with Brook et al, (1995), Aslam et al, (2004) and other studies.

In our study Streptococcus pyogenes were isolated in a ratio of 3.8%, although its always recovered from acute otitis media beside Streptococcus pneumoniae such organism was also isolated from chronic infection as the acute infection can become chronic, and as a complication of tonsillitis. Therefore it was found that 2.2% of isolates were Streptococcus pyogenes Aslam et al, (2004), and less than 2% was reported by Obi et al, (1995).

In our study Providencia species including Providencia rettgeri, and Providencia. Stuartii were isolated in the same ratio 3.8%. Ochei & Kolhatkar, (2000), and Brooks et al, (1998) consider them as secondary
invader like *Proteus* species. However this result is in agreement with Obi *et al.*, (1995).

*Corynebacterium* species including *Corynebacterium. amycolatum* and *Corynebacterium striatum* were isolated in pure cultures from ear discharge. Information from literature review such as Ochei *et al.*, (2000) mentioned that *corynebacterium* species comprise one of the normal flora of the ear, however our findings are supported by results from previous similar studies such as Campos *et al.*, (1995), Obi *et al.*, (1995). They reported it generally as diphtheroid, but we carried out full identification to the species level. According to Brooks *et al.*, (1998) it was found that the two mentioned species were found to be of medical importance.

Other microorganisms which were isolated in very low ratio were *Escherichia coli, Citrobacter freundii, Enterobacter cloacae, and Hafnia alvei*, each account for 1.9%. These findings are comparable with Aslam *et al.*, (2004) who recovered *Escherichia coli* and *Cirobacter freundii* from CSOM in a ratio of 5.3% and 1.1% respectively. Also Moshi *et al.*, (2000) reported similar results in Tanzania.

In addition *Enterobacter Cloacae* was reported by Richard *et al.*, (1981) as causative agent of acute otitis media, however this means that it can be isolated also from CSOM.

With regard to *Hafnia alvei (Enterobacter alvei)* the isolation of such organism does not represent big argument as many previous studies
reported some of their findings as unidentified coliforms for example Moshi et al, (2000).

Other *Staphylococcus* species isolated in very low ratio were *Staphylococcus epidermidis*, *Staphylococcus intermedius*, and *Staphylococcus auricularis*, each account for 1.9%.

Brooks et al, (1998) mentioned that *S. epidermidis* was a ubiquitous commensal in human that normally inhabit the respiratory tract. Therefore such organism considered as opportunistic pathogen. *S. intermedius* which a coagulase positive was also isolated from CSOM, and these results are in accordance with Ibrahim, (2002), who isolated the same species from chronic otitis media.

One of the interesting observations in our study is the isolation of *S. auricularis* from chronic otitis media in pure culture as a single causative agent. This organism was reported by Ibrahim, (2002) as one of the ear normal flora.

Another interesting observation is the isolation of *Pseudomonas fluorescens*, *Pseudomonas stutzeri* and *Pseudomonas putida*. The first one is very similar to *Ps. aeruginosa* in its biochemical reactions Brooks et al, (1998) considered them as microorganism of medical importance, therefore, they were isolated in our study as causative agent of CSOM.

The pathogenic bacteria isolated in this study were subjected to invitro antimicrobial sensitivity tests using disc diffusion method. The
standards of zone of inhibition recommended by the National Committee for Clinical Laboratory Standards (NCCLS) were used to classify the isolates as sensitive or resistant.

*Pseudomonas aeruginosa* strains were found to be highly resistant to the most antibiotics used except for ciprofloxacin 90.9% and gentamicin 81.8%.

*Pseudomonas fluorescens* and *Pseudomonas putida* were like *Pseudomonas aeruginosa* but they were more susceptible to ciprofloxacin (100%) and gentamicin (100%).

In addition to amoxicillin clavulanic acid (55.6%) *S. aureus* were highly sensitive to ciprofloxacin (100%), Gentamicin (100%), erythromycin and Cefuroxime (88.9%), while fully resistant to Ampicillin (100%) and penicillin (77.8%).

Among the *Proteus mirabilis* isolates they showed sensitivity to ciprofloxacin, Cefuroxime (100%), and Ampicillin, amoxicillin clavulanate (88.9%) and moderate sensitivity to Gentamicin (55.6%), while 100% resistant to penicillin and erythromycin.

*Streptococcus pyogenes* showed marked sensitivity to all antibiotics used (100%) except for penicillin (50%) and ultimately resistant to ampicillin.

*Klebsiellae* species were found to be resistant to ampicillin (100%), penicillin (100%), erythromycin (100%), and 100% sensitive to
ciprofloxacin, Gentamicin, and Cefuroxime. With exception to amoxicillin clavulanate which showed moderate activity against *K. aerogenes* (66.7%), *K. oxytoca* was complete resistant to it.

Unexpected results were recorded for *Pseudomonas stutzeri*, which was 100% sensitive to ampicillin, ciprofloxacin, Gentamicin, and Cefuroxime. While moderately sensitive to amoxicillin clavulanate (50%) and 100% resistant to penicillin and erythromycin.

Other microorganisms isolated in very low ration (1.9%) including *Staphylococcus epidermidis* and *Staphylococcus intermedius* which were highly sensitive (100%) to all antibiotics used except for ampicillin. *S. epidermidis* alone was 100% resistant to penicillin. *S. auricularis* was the most susceptible (100%) to all antibiotics used, perhaps its not usually isolated as a conventional pathogen. *Providencia rettgeri* was resistant (100%) to all antibiotics used except for ciprofloxacin, gentamicin, and cefuroxime, which showed high (100%) activity against it. In addition *Providencia stuartii* was sensitive to ampicillin, amoxicillin clavulanate, ciprofloxacin, and Cefuroxime. On the other hand it was 100% resistant to penicillin, erythromycin, and Gentamicin. Brooks *et al*, (1998) reported that *Providencia* species are often resistant to antimicrobial therapy.

*Enterobacter cloacae* was 100% sensitive to ampicillin, amoxicillin, ciprofloxacin, Gentamicin, and Cefuroxime. In contrast it was resistant to penicillin, and erythromycin (100%).
*Citrobacter freundii* was found to be 100% resistant to ampicillin, penicillin, amoxicillin clavulanate, and 100% sensitive to erythromycin, ciprofloxacin, Gentamicin, and Cefuroxime. With regard to *Hafnia alvei* it was 100% sensitive to all antibiotics used, also perhaps it is not usually isolated as conventional pathogen.

*Corynebacterium amycolatum* and *Corynebacterium striatum* were found to be 100% susceptible to ampicillin, penicillin, amoxicillin clavulanate, and cefuroxime. On the other hand the two isolates were resistant to Gentamicin. With concern to ciprofloxacin and erythromycin *Corynebacterium amycolatum* was 100% resistant, in contrast *Corynebacterium striatum* was 100% sensitive. *Escherichia coli* which was single isolate was fond to be resistant to all antibiotics used, however, Obi *et al*, reported that *Escherichia coli* was resistant to 61.5% of antibiotics used in his study. Collectively the effectiveness of antibacterial agents against the isolated bacteria were found to be ampicillin 30.8%, penicillin 15.4%, amoxicillin clavulanic acid 50%, erythromycin 30.8%, ciprofloxacin 94.2%, Gentamicin 80.8%, and Cefuroxime 65.4%. Vide figure (4). These findings are agree with Oguntibeju, (2003) in that the Gentamicin showed marked sensitivity against the isolates, but disagree with him in that in our result *Klebsiellae* species were 100% resistant to ampicillin while he reported that they were 50% sensitive to it.
Our results are also in accordance with Ahmad (2003) who reported that Gentamicin (88.5%), and Cefuroxime (63.7%) were active against the majority of isolates.

Here also ciprofloxacin was the most effective antibacterial gent, this was previously reported by Ghosh et al, (2000) and Obi et al, (1995) who reported that over 90% of isolates were sensitive to ciprofloxacin.

In our results *S. aureus* were moderate sensitive to amoxicillin clavulanate (55.6%) this finding is not in accordance with Campos et al, (1995) who reported that *S. aureus* strains were highly sensitive to it. However this means that there is development of resistance to such antibiotic.

The antibiotics with less activity were penicillin (15.4%) and ampicillin (30.8%). This result is supported by many other studies such as Aslam et al, (2004) & Brook, (1985).

On the other hand Erythromycin was found to be effective against *S. aureus* (88.9%), this is in agreement with Oguntibeju, (2003) who mentioned that 90% of *S. aureus* were sensitive to erythromycin. Also in our findings Cefuroxime was 100% active against Gram-positive bacteria while 52.8% of Gram-negative were susceptible to it.
CONCLUSION AND RECOMMENDATIONS

• In conclusion, the present results in this study have demonstrated that the bacteriology of chronic suppurative otitis media in Khartoum state is very similar to that observed elsewhere.

• *Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Proteus mirabilis* were the most common microorganisms isolated from chronic suppurative otitis media. In addition it showed a high prevalence of the disease among the children under 15 years old, also in children untreated upper respiratory tract infection lead to otitis media.

• With concern to antimicrobial sensitivity pattern, most isolated strains were resistant to commonly used antibiotics such as penicillin, ampicillin and erythromycin, whereas ciprofloxacin was active against the majority of isolates.

• Furthermore the study revealed that there is a great development of resistance against the antibiotic amoxicillin clavulanic acid which is a modified and improved amoxicillin.

  Based on these finding we came out with the following recommendations:

• Patients presenting with chronic suppurative otitis media should have their ear swab for culturing and antimicrobial sensitivity to make proper selection of treatment.
• Full identification of causative agent is recommended as unusual microorganisms were isolated as causing of chronic suppurative otitis media.

• Further studies particularly that based on molecular biology are needed to through a light of aerobic and anaerobic organisms causing chronic otitis media.
REFERENCES


