ADDIS ABABA UNIVERSITY
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Ear Infections : Etiologic Agents Isolated From Patients Visiting Two Hospitals In Addis Ababa And Their Susceptibilities To Antimicrobials

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EAR INFECTIONS: ETIOLOGIC AGENTS ISOLATED FROM PATIENTS VISITING TWO HOSPITALS IN ADDIS ABABA AND THEIR SUSCEPTIBILITY TO ANTIMICROBIALS

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ABSTRACT

Information pertaining to the etiologic agents responsible for ear infection are absent in Ethiopia. Therefore, this study was initiated to identify the causative agents together with their sensitivities to antimicrobials. Microbiological samples were collected from 389 ears of 355 patients attending the E.N.T. clinics of two Hospitals (Addis Ababa). Four hundred and twenty (98.4%) bacterial strains and 7 (1.6%) fungi were isolated. Gram-negative bacteria were most frequently encountered. Of these 60.4% were members of the family Enterobacteriaceae. Of all the isolates, *Proteu spp.* (25.5%), *Pseudomonas aeruginosa* (13.1%), *Staphylococcus aureus* (12.7%), and *Klebsiella spp.* (9%) were common. All bacterial strains were tested for their susceptibility to antibiotics following a standardized method. Ampicillin, tetracyclline, penicillin, and cephalothin were the least effective antimicrobials in their respective order. *Pseudomonas aeruginosa* was the predominantly resistant organism to several antibiotics (all strains were resistant to one or more antibiotics, 93% resistant to four or more antibiotics, and 71% resistant to six or more antibiotics). Strains of *Proteus mirabilis* were resistant to tetracyclline in 97% and *Staphylococcus aureus* was resistant to penicillin G in 82%. This study has showen the polymicrobial etiology of ear infections with *P. mirabilis* being the dominating organism. Gentamycin and carbenicillin were among the effective agents agaist most of the bacterial isolates.

I. INTRODUCTION

Despite the great advances in the science of preventive medicine, infection of man with microorganisms continue to be a major medical problem. Efforts have been made to study the microbiology of different areas of the body and understand what factors predispose the colonization of specific sites of the body by microorganisms.

Infectious disease of the ear continue to be a frequent cause of morbidity inspite of advances in treatment. Otitis media is one of the most common conditions for which children receive medical attention (Stool & Bluestone, 1983). It is, next to the upper respiratory tract infection, the most common organic disease (Paradise, 1982). Most families have direct experience with this disease because it affects 85% of children at least once (Brownlee, *et al.*, 1969). It is most prevalent during the first two years of life, and maintains a high order of prevalence throughout the preschool years and even thereafter. Two-thirds of all children will experience at least one episode of otitis media by two years of age (Howie, 1975).

The basic problem underlying all forms of otitis media is the dysfunctional eustachian tube. The function of this tube is to ventilate the middle ear and to drain mucus and other debris into the nasopharynx. Tube failure can initiate middle ear inflammation, contribute to its persistence and in turn, is itself, perpetuated by the chronic otitis that results.

Appropriate choice of antimicrobial agents for treatment of otitis media is based on an understanding of the microbiology of the disease (Klein, 1981). Knowledge of which microorganisms cause the infection must be coupled with an understanding of their susceptibility patterns to the commonly used antibiotics.

Inspite of the worldwide prevalence of otitis media, there remain wide gaps in our knowledge regarding the etiology, management and prevention of the disease (Stool & Bluestone, 1983). The low socio-economic and technological development together with the geographical environment are the main factors responsible for the epidemiology and presentation of ear illnesses in the developing countries (Oburra, 1990). In Ethiopia, there

is no clearly stated prevalence of the disease since no study could be referred for this problem. This being the case, it can be stated that the prevalence is expected to be high due to several reasons. Among these reasons, socio-economic status and the level of primary health care facilities are considered as the major factors influencing the occurrence of this disease. And, both of them are low in this country.

It is difficult to get published reports on ear infections in Ethiopia except for the case reports by Firehiwot and coworkers (1990) and Lester and Juhasz (1990). There is a need to define the different etiologic agents important in ear infections along with their antibiogram status in this country. Hence, this study was initiated with the objectives to:

1. document the microbial etiologic agents responsible for ear infections;
2. determine the antibiotic susceptibility of the isolates;
3. suggest guidelines for empirical chemotherapy based on the current patterns of drug resistance obtained and;
4. give some recommendations on drug policy based on the findings of the study.

II. LITERATURE REVIEW

1. Structure and Function of the Ear

An understanding of the ear is of interest for three main reasons (Adams *et al.*, 1978) :

i. its essential functions;

ii. the variety of diseases affecting the ear; and

iii. the pleasure that hearing provides.

The ear (organ of hearing and equilibrium) is divided in to three parts, the external, middle and inner regions (Fig. 1A). The external ear includes the pinna (auricle), the external acoustic meatus (canal), and the tympanic membrane. The auricle is the most prominent portion of the external ear. It directs sound waves into the external auditory meatus. The external auditory meatus, about 2.5 cm long in the adult, passes down from the auricle to the tympanic membrane. The skin of the outer one-third of the meatus possesses numerous ceruminous or wax-secreting glands which are modified sweat glands. The wax contains lysozyme and immunoglobulins. The hairs and the cerumen help prevent small foreign objects from reaching the ear drum (Bell *et al.* 1980). It acts as a vehicle for the removal of epithelial debris and contaminants away from the tympanic membrane. The tympanic membrane (about 1 cm in diameter) separates the ear canal from the middle-ear cavity. It transmits the vibration to the ossicles of the middle ear.

The middle ear is an air-filled pocket that lies between the ear drum and the inner ear (Landau, 1980). It is connected to the nasopharynx by the eustachian tube. The tube is closed at rest but opens frequently to equalize the air pressure between the middle ear and the outside environment; that is , the eustachian tube serves as a safety valve to the middle ear. The opening and closing of the eustachian tube occurs naturally during swallowing, yawning, sneezing, and sucking. The middle ear is bridged by a chain of three tiny bones. These bones are the smallest bones in the body and consists of the hammer (malleus), anvil (incus), and stapes (stirrup). The malleus is partly incorporated into the drum membrane laterally, the stapes footplate occupies the oval window opening of the labyrinth, and the incus links the two.

Figure 1. (A) General structure of the ear and (B) inner ear

(Adopted from Landau, 1980)

Figure 1.a

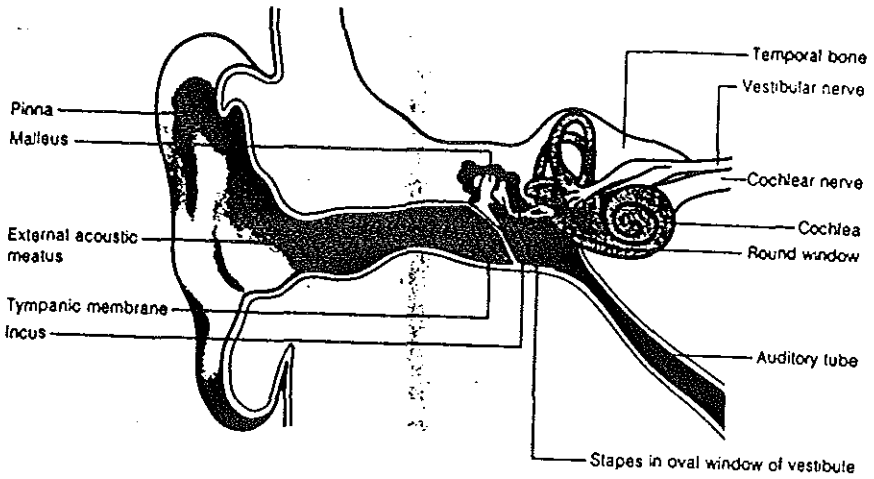
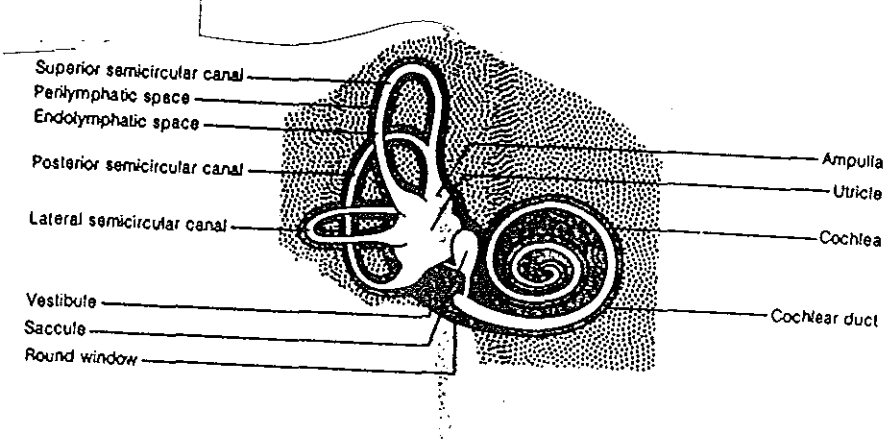


Figure 1.b



The liquid (perilymph) in the inner ear is more difficult to move than air (Horrobin, 1968). Therefore, the ossicular chain is constructed to overcome this difficulty. The vibration of the large area of the drumhead of the tympanic membrane in air is transformed to a smaller but more powerful movement of the small area of the stapes, footplate. Then the footplate transmits its motion to the perilymph of the inner ear. Hence, the pressure transmitted to the inner ear from the tympanic membrane is amplified by the movable chain of the ossicles.

The inner ear is located in the temporal bone close to the middle ear (Landau, 1980). It is separated from the middle ear by the oval window. There are three areas within it, the cochlea, the vestibule (utricle and saccule) and the semicircular canals. The vestibule is an open area. The larger end of the cochlea opens on one side and the three semicircular canals on the other (Fig. 1B). The inner ear is the site of six small sensory organs that contain the hair cells (Hudspeth, 1983). The semicircular canals measure angular acceleration. The utricle and the saccule detect linear acceleration. The cochlea is the organ of hearing.

The major functions of the ear (hearing and balance) are in constant operation (Adams *et al.* 1978). During sleep the ears do not rest, but continue to function as a warning system, detecting sounds of alarm or disturbance. Besides its instinctive or protective function, the ear is integral to one of man's most highly developed functions, communication. Communication not only serves the purpose of exchanging information, but also influences our behavioral, social, and intellectual development. Hearing is also important to normal language development. If complete deafness is congenital or occurs in infancy before vocalization has reached the stage of speech, the child fails to develop the ability to speak (Bell et al. 1980).

In addition to the essential functions (protection and communication) hearing provides pleasure and a higher sense of artistic appreciation and development. These pleasing sounds include music in all its forms and the beautiful sounds of nature which can calm our anxieties or elicit our action.

The vestibular function of the ear provides a sense of orientation in space as well as a sense of motion, whether travelling along a straight or circular course.

2. Diseases of the Ear

The functions of the ear are possible when the organ is healthy and functioning properly. Good hearing requires that the entire mechanism be intact. Impairment results whenever there is a failure of any link in the auditory pathway. This impairment could be congenital or acquired. The acquired one could be from trauma, tumour, or infections. Damage to the tympanic membrane reduces its response to sound waves. The ossicles may fuse or the footplate of the stapes may become fixed at the oval window, and either will block transmission to the internal ear. These are essentially conduction problems. There is also some deterioration of reception of sound with age.

As any organ of our body, the ear is also prone to infections. When this happens, ear infections are of two clinical entities, Otitis Externa and Otitis Media. These are occasionally difficult to distinguish clinically (Mandell *et al.* 1979). It is then worthy to treat these two clinical entities separately due to their differences in anatomical predilections and the possible etiological conditions.

2.1. Otitis Externa

Otitis externa (OE) is a common skin disorder which affects the external auditory canal (Hoeprich, 1977). It could be caused by infection, other inflammatory conditions, trauma, or combination of these three (Marcy, 1985). The spectrum of infections and inflammations include both acute and chronic forms. Infection of the skin lining the external auditory canal occurs most often in hot and humid environments. Bathing in a hot climate with high humidity is a potential cause (Ware, 1969).

Healthy wax has an acid reaction with antiseptic properties, but if the meatus becomes alkaline the defense mechanism is upset. The continued removal of the lipid layer by sweat

and/or entrance of water almost certainly explains why tropical climates and bathing encourage otitis externa. The chronic form results from lack of treatment, inadequate treatment, recurrent trauma, the presence of foreign body such as hearing aid mould or draining otitis media (Adams *et al.* 1978; Yelland, 1992).

The bacteria, fungi, and viruses which give rise to otitis externa are often introduced when the skin of the external ear canal is macerated during swimming or by sweating in hot weather (Muno *et al.*, 1982). The organisms frequently isolated are *Pseudomonas aeruginosa, Staphylococcus aureus, Escherichia coli, Aspergillus species, Candida species,* herpes simplex and herpes zoster (Yelland, 1992). Bacterial infection, as a primary or secondary process, is the most frequent cause. Dibb (1991) reported a wide variety of bacteria and fungi as causative agents, the commonest being *S.aureus* (34.1%), *P. aeruginosa* (22.1%) and *S. pyogenes* (8.8%) and 9.3% of the samples contained fungi.

Malignant external otitis is a sever form of external otitis (Wilson *et al.* 1971; Zaky *et al.* 1976; Rubin *et al.* 1988; Johnson & Ramphal, 1990) occurring in elderly diabetic and immuno-compromised patients. The offending organism is usually *P. aeruginosa*, but fungi are also reported to contribute (Bickley, *et al.* 1988). If unrecognized, this can progress to serious complications including invasion of cartilage, bone, nerves, and soft tissues resulting in osteomyelitis of the skull, multiple cranial nerve paralysis, meningitis, and death. The most common complication is facial nerve palsy which is usually the first sign of neurological involvement.

2.2. Otitis Media

The middle ear is usually sterile. This remarks to consider the microflora of the nasopharynx and pharynx (Adams *et al.* 1978). The combined physiologic action of cilia and mucus-secreting enzymes and antibodies serve as a defense mechanism when these contaminants gain access in the middle ear space during the act of swallowing. Otitis media is generally caused when this physiologic mechanism is disrupted.

Otitis media is an inflammation of the middle ear, which may or may not be infectious in origin (Senturia *et al.*1980). According to this definition, it is classified in to three

categories as acute, chronic and subacute. It is arbitrarily considered the acute process to be during the initial three weeks, the chronic process after three months following onset, and the subacute phase between three weeks and three months.

2.2.1. Acute Otitis Media (AOM)

Acute otitis media is a bacterial infection of the middle ear often preceded by a viral respiratory infection (Henderson, 1982; Ruuskanen *et al.* 1989). Viruses are rarely isolated from the middle ear exudate, but the viral attack probably induces pathophysiological changes that permit the bacteria to invade the middle ear (Klein and Teel, 1976). Mycoplasma and chlamydia may possibly play a similar role like viruses. Obstruction of the eustachian tube is a basic causative factor in acute otitis media. Thus, bacterial species which do not ordinarily cause disease are able to colonize the middle ear, invade tissue, and cause infection. Results of several studies have demonstrated some correlation between nasopharyngeal flora and etiologic agents in otitis media when the tympanic membrane is intact (Howie & Ploussard, 1971; Schwartz *et al* 1979; Long *et al.*1983). It is stated that if the clinical criteria of acute otitis media are fulfilled and adequate sampling and culture techniques are used from both sites, the pathogens found in the ear exudate will be recovered from the nasopharynx in 97-98% of the cases (Howie and Ploussard, 1971). In 40-50% of the specimens more than one pathogen will be recovered from the nasopharynx and 20-30% of the exudate will be culture negative (Schwartz *et al.* 1979). Among these , 70-80% of the nasopharyngeal specimens will yield only single pathogen. This implies that a reliable bacteriological diagnosis can be obtained in 55-60% of consecutive cases of acute otitis media as their nasopharyngeal specimens yield only a single pathogen.

The etiology of AOM is well documented (Paradise, 1980; Schwartz, 1981). The causative agents in order of frequency are *Streptococcus pneumoniae* (accounting for 50-60% of the cases), *Hemophilus influenzae* (15-25% of cases), *Branhamella catarrhalis* (5-10% of cases) and group A streptococci (Coffery *et al.*, 1967; Howie *et al.*, 1970; Row, 1975; Van-Hare *et al.*,1987; McCracken, 1988; Trujllo *et al.*, 1989; Wald, 1989). Viruses, Gram-negative enteric bacilli, mycoplasma, and anaerobic bacteria are infrequent causes of acute otitis media (Klein and Teel, 1976; Klein, 1980; Osteeld and Rubin, 1980; Arola *et al.*, 1988).

2.2.2. Chronic Otitis Media.

Chronic otitis media is an indolent inflammation of the middle ear. There are two distinct forms of this disease, chronic otitis media with effusion (COME) and chronic suppurative otitis media (CSOM) (Jahn, 1991).

2.2.2.1. Chronic Otitis Media with Effusion.

Chronic otitis media with effusion is an unresolving inflammation of the middle ear with no otorrhea combined with a deficient ventilation of the middle ear (Kamme, 1985; Jahn, 1991). It is represented by a persistent hearing loss and a middle ear filled with thick mucus. In the absence of other factors, a mechanical obstruction of the tube results in negative middle ear pressure followed by the transduction of tissue fluid (serum). In many cases the effusions appear to be the result of an inflammatory response. Mediators of inflammation such as prostaglandins, kinin, histamine and protases have been demonstrated to be present in the exudate in addition to lysozyme, an antimicrobial antibodies of IgG, IgA and IgM, complement activity and immunocomplexes (Lim & Demaria, 1982; Meyerhoff & Giebink, 1982; Jahn, 1991).

The cause of this inflammation could be allergy or subacute bacterial infection (Riding *et al.*, 1978; Lim & Demaria, 1982; Bluestone, 1988). The presence of bacteria evokes an inflammatory response but does not proceed to suppuration (Brook & Finegold, 1979). Eustachian tube function is compromised in the face of the large quantity of viscid mucus, and functional obstruction may develop.

It is important to consider the mucus formed as potentially harmful to the middle ear. It is acidic and contains cellular debris, dead bacteria together with the inflammatory products (Jahn, 1991). If left untreated, the exudate can develop to form mucin and fibrin. As the material grows more concentrated, the middle ear space gradually collapses and becomes atlectactic.

Since several reports have described unsuccessful attempts to culture bacteria from the effusion, COME has been assumed to be sterile (Stanievich *et al.*, 1981). However, numerous studies have shown that bacteria can be found in up to 50% of the cases with COME (Liu *et al.*, 1975; Lim & Demaria, 1982; Meyerhoff & Giebink, 1982; Kamme & Nilsson, 1984). It is reported that *H. influenzae* is the most commonly isolated bacteria followed by *B. catarrhalis*, *S. pneumoniae* and group A streptococci (Giebink *et al.*, 1982; Lim & Demaria, 1982; Kamme & Nilsson, 1984; Cabenda *et al.* 1988; Diamond *et al.*, 1988).

Further evidence suggesting bacterial pathogenesis in COME is the high incidence of pathogenic flora in the nasopharynx of patients with COME. Studies have shown that *H.influenzae, S. pneumoniae, B. catarrhalis* and group A streptococci can be recovered from the nasopharynx in approximately 80% of the patients with COME (Lundgren & Rundecrantz, 1976; Kamme & Nilsson, 1984). Lundgren & Lundecrantz (1976) reported that growth of pathogenic bacteria in the nasopharynx in COME is the same as that in AOM and that it would therefore appear reasonable to assume that this bacterial situation nearly constitutes a major causal factor in the development of COME.

2.2.2.2. Chronic Suppurative Otitis Media (CSOM)

Chronic suppurative otitis media (CSOM) is a recurrent or persistent bacterial infection of the middle ear. It is characterized by chronic inflammation of the middle ear, perforated tympanic membrane and discharge (otorrhea) together with hearing loss. Two distinctly different anatomical defects in the tympanic structure predispose the patient to CSOM:

1. perforation of the tympanic membrane, which exposes the middle ear mucosa to external contamination through the external canal;
2. penetration of the tympanic and mastoid spaces by keratin; which is subject to contamination.

CSOM is most often a recurrent rather than a constant infection. Although the disease may be quiescent for long periods, it is only a matter of time before contamination occurs, and the exposed middle ear mucosa, or the accumulated cheesy debris of the cholesteatoma, becomes a culture medium for opportunistic pathogens.

Occasionally an infection may proceed to the eustachian tube from the upper respiratory tract and end up with an open perforation. This will be caused by the usual organisms such as pneumococci, streptococci, or hemophilus, encountered in acute infections. Usually the chronic draining ear is the result of an infection from the outside through the perforation (Fairbanks, 1981). Whatever bacteria are available in the outside world can become the possible pathogens. The source of infection is characteristically of faecal or water origin.

The microbiology of CSOM is usually a mixed infection with aerobic and anaerobic organisms (Jahn, 1991). *Pseudomonas aeruginosa* and *S.aureus* are the dominant aerobic organisms, although some ears may have an unexpected and unusual pathogenic flora (Hussain *et al., 1991*). The rest of the aerobes represent a mixed flora of enteric organisms and other numerous organisms including fungi (Fairbanks, 1981). The anaerobes mostly implicated in otitis media are *Bacteroides species, Fusobacterium species, Clostridium species, Propionibacterium species, Bifidobacterium species* (Fulghum *et al.*, 1977; Jokipii *et al.*, 1977).

3. Complications and Sequelae of Otitis Media

Chronic otitis media is a common otologic problem with significant complications and sequelae (Brown & Meyerhoff, 1988). A complication is defined as a second condition or disease occurring during the course of a primary disease. A sequelae is any lesion or affection following, or caused by, an attack of a disease. Otologic complications of COM include petrositis, facial paralysis, and labyrinthitis. Intracranial complications of CSOM include meningitis, and intracranial abscess. Sequelae of CSOM include osseous changes of middle ear, with associated hearing loss, cholesteatoma, and tympanoschlerosis.

Chronic suppurative otitis media may result in conductive and sensory-neural hearing loss (Paradise, 1980; Bluestone, 1981; Bluestone, *et al.*, 1983; Brown & Meyerhoff, 1988). Conductive hearing loss results from perforation of the tympanic membrane and ossicular chain fixation or discontinuity. The ossicular chain may become fixed by fibrous tissue, tympanoschelerosis, or new bone formation. Ossicular interruption occurs most commonly from necrosis of the lenticular process of the incus, or erosion of the ossicles by cholesteatoma (Meyerhoff & Giebink, 1982). Sensory-neural hearing loss may be caused

by infiltration of infectious or inflammatory agents through the semipermeable round window membrane.

Recent works are moving beyond the study of the disease per se, and beginning to describe more fully the illness experience associated with COM (Facione, 1991). The educationally handicapping nature of secondary conductive hearing loss due to COM is a good example of such kind of work. It has been suggested that language development is compromised by middle ear disease regardless of the magnitude of the hearing loss (Lewis, 1976). Even mild fluctuating hearing loss was found to interfere with a child's ability to process the acoustic signal.

Mild, intermittent hearing loss in children adversely affects articulation of consonants , while moderate and severe hearing loss adversely affects vocabulary and academic performance (Hubbard *et al.*, 1985). Various investigators have postulated that otitis media initiates a chain of events that ends in language delays and later academic underachievements, particularly in reading (Rapin, 1979; Bluestone *et al.*, 1983).

There are many ways in which otitis media can adversely affect language and intellectual development (Dibie & Berlin, 1979). Factors such as fluctuating attention and vigilance, independent of or compounded by the general malaise that goes with chronic middle ear problems, must be considered. Increased number of absences and related loss of input due to trips to the physician should also be considered as contributing variables to school difficulties.

The abilities of hearing-impaired to process, organize and store linguistic input presents the most obvious interference points for language learning (Naremore, 1979). If a child does not receive the entire auditory signal, then some information is being lost. The difficulty for hearing-impaired child with regard to environmental support is that the child may be unable to take advantage of what the environment provides. If a child's hearing loss is intermittent, as may be the case with otitis media, the connection between the language and the environmental situation to which it refers may be sufficiently inconsistent to cause the child problems with these connections. It is suggested that children who suffer from episodes of otitis media during the early years of life may be retarded

linguistically because their interaction with the environment is limited; they are not receiving consistent input and they are not consistently acting up on the environment (Holm & Kunze,1969; Menyuk, 1979).

Some studies suggest that vestibular function is affected by COM, resulting in balance disturbances. Jones *et al.* (1990) found that balance was significantly affected in 3-to 5-year-old children with otitis media. Hinjo *et al.* (1988) studied adult patients with COM and found that they inclined their heads to the affected side.

III. MATERIAL AND METHODS

1. Specimen Collection

Since our facility could not guarantee special transport and growth media for anaerobes, it was decided to restrict this study to aerobic specimens.

Samples were taken from effusions draining through a perforated tympanic membrane (chronic suppurative otitis media, CSOM). This is due to the inaccessibility of the effusion from the middle ear with intact tympanic membrane without an advanced technique, needle aspiration or tympanocenthesis. However, this is beyond the technical scope of the study. all patients who were sampled during this study were classified as having CSOM by physicians (Otorhinolaryngologists). None of the patients admitted to the study were taking antibiotics for at least 10 days befor the samples were collected.

This study took place between January, 1993 and December, 1993. Three-hundred and fifty-five patients (389 ears) with discharging ears from Ear, Nose, and Throat (E.N.T.) clinics of two hospitals, Yekatit 12 and Menelik II, were admitted to the study. Most of the patients complained for ear discharge lasting for more than 3 months. Ear swabs were collected using sterile cotton-tipped swabs by attending physicians. Samples were taken from two ears of the same patient if both ears were observed to have discharge. Samples wrere collected in duplicate. Specimens were brought to the laboratory within one hour after collection and processed in the laboratory for cultural characterization and Acid fastness. The swabs were transported in a sterile screw-cupped test tubes.

Specimens were plated on solid agar media. Blood agar (Oxoid, CM 55) was used to detect the type of haemolysis, description of colony size, colony morphology, and swarming nature. Chocolate agar (heated blood agar) was inoculated with the specimen to detect the growth of fastidious organisms. Manotol salt agar (Oxoid, CM 85) was used for the preliminary isolation of *S.aureus*. MacConkey agar (Oxoid, CM 115) was inoculated with the specimen to detect the growth of members of the family Enterobacteriaceae and non-fermentative gram-negative bacilli.

Seeded culture plates were incubated 24-48 hours at 35-37 ^{0}C before tests were discarded as negative. Specimens were also smeared on to glass slides, fixed and stained to detect acid-fastness. Stained smears were observed under the microscope to confirm for the presence of acid fast bacilli.

2. Isolation and Biochemical Identification

Colony size, shape and type of haemolysis were read from the blood agar after 24 hours of incubation of the inoculated plates at 35-37 °C. Catalase test, colony form and type of haemolysis were used for the differentiation of staphylococci from streptococci. Gram-positive cocci were identified following the methods described by Lennette *et al.* (1980).

The growth of the fungal isolates were observed first on blood agar. The fungal colonies were subcultured to Sabouraud's Dextrose agar plates (Oxoid, CM 41). Cultural and microscopic characterstics were used to identify *Aspergillus species. Candida albicans* was identified by its characterstics microscopic appearance and germ-tube formation test.

For identification of gram negative-rods, a single colony was picked with a sterile straight wire and inoculated into tubes containing about 4 ml of tryptone broth (Oxoid, CM 87). This was incubated at 35-37 °C for 2-4 hours, or until the culture was visually turbid. The tryptone broth culture was used to inoculate (by means of a sterile Pasteur pipette) a set of biochemical tubes and the remaining portion was reserved for indole production test (Kovacs').

The tests and media employed includes: glucose, lactose and/or sucrose fermentation, H_2S production (Triple Sugar Iron Agar, Oxoid, CM 277), urea hydrolysis (Urea Agar Oxoid, CM 53), citrate utilization (Simmon's Citrate, Oxoid, CM 155), indole production (Tryptone broth, Oxoid CM 87), observation of motility (Motility test medium, Difco, B 105), fermentation in 1% inositol, sorbitol, adonitol, and rhamnose (Phenol Red broth base, Diffco, B 92), gas production from glucose (1% glucose broth with inverted Durham tube), malonate utilization (Malonate broth, Diffco, B 395), oxidation-fermentation (OF) in 1%, glucose, decarboxylation of lysine and deamination of phenylalanine (Lysine Iron

agar, Oxoid, CM 381), and oxidase reaction (Oxidase disks, BioMerieux). All biochemical tubes were incubated at 35-37 °C for 18-24 hours.

To check for the purity of broth inocula and the reliability of biochemical tests, each broth inoculum was sub-cultured on MacConkey agar (Oxoid, CM 155) ' check plate ' and incubated at 35-37 °C for 18-24 hours. For quality control of biochemical tubes, all biochemical sets were pre-incubated at 35-37 °C for 18-24 hours to ascertain sterility and quality control organisms (*Escherichia coli* and *Proteus mirabilis*) were used to check the reliability of representative tubes of each batch.

Members of the family Enterobacteriaceae were identified following the scheme of Brenner (1984), Kone *et al* (1988), and Farmer *et al.* (1991), while gram-negative non-fermenting bacilli were identified according to Lennette *et al.* (1980), Palleroni (1984) and Kone *et al.* (1988)

3. Antimicrobial Susceptibility Testing

All isolates were tested for their susceptibility to antimicrobials using the standardized agar disc diffusion technique (Bauer *et al.*, 1966). From a pure culture of each isolate, 3 to 4 colonies were randomly selected and transferred, by touching the top of each colony with the same loop, to a tube containing about 4 ml of tryptone broth. The broth was incubated at 35-37 °C until it became turbid. Using a sterile cotton swab dipped in to the broth culture, the entire surface of the Muller-Hinton agar (Oxoid, CM 337) (replaced by blood agar for streptococci) was swabbed evenly. The inoculated plate was left at room temperature for 3 to 5 minutes. Using a sterile forceps, a set of sensitivity discs (8 for gram-negative and 12 for gram-positive) were placed on the surface of each inoculated Muller-Hinton plate. These discs were pressed down gently with sterile forceps to assure even contact. Inoculated plates with sensitivity discs were then incubated at 35-37 °C for 18-24 hours.

Diameters of inhibition zones were measured by metal calibers to the nearest millimetre. The results were recorded as resistant (R), intermediate (I), and sensitive (S), according to standard interpretive charts (Buear *et al.*, 1966; Farmer *et al.*, 1991).

The sensitivity discs included (BioMerieux):

a. For gram-negative organisms:- ampicillin (10 μg), carbenicillin (100 μg), cephalothin (30 μg), chloramphenicol(30 μg), gentamycin (10 μg), kanamycin (30 μg), tetracycline (30 μg), and trimethoprim-sulphamethoxazole (Bactrim) (25 μg);

b. For gram-positive organisms:- ampicillin (10 μg), carbenicillin (100 μg), cephalothin (30 μg), chloramphenicol (30 μg), gentamycin (10 μg), kanamycin (30 μ), erythromycin (15 μg), penicillin-G (10 U/IE), methicillin (5 μg), lincomycin (2 μg), tetracyclline (30 μg), and trimethoprim-sulphamethoxazole (Bactrim) (25 μg).

For quality control purposes, *E.coli* (ATCC 25922) and *S.aureus* (ATCC 6538) [brought from the National Research Institute of Health (NRIH)] were used to detect deficiencies in media, inocula, and sensitivity discs.

IV. RESULTS

1. Patient Population.

Three-hundred fifty-five patients were included in the study. The age range was from three months to 70 years. One hundred thirty nine patients were below the age of 14 years.

Two-hundred eighty-two (79.4%) of the total patients admitted to the study were culture positive for at least one possible pathogenic organism. Out of the 355 patients, 35 (9.9%) were with bilateral tympanic membrane perforations.

2. Types and Frequencies of Microbial Isolates.

Four-hundred twenty-seven bacterial and fungal strains were isolated from 313 ears of 282 patients (Table 1). Only a single microbial species each was isolated from 169 (59.9%) patients. Two different organisms were cultured from 86 (30.5%) individuals. Three different organisms were isolated from 23 (8.2%) cultures and four organisms were found in cultures from 4 (1.4%) patients.

Out of the 35 patients with bilaterial discharge (samples from both ears), same organisms were isolated from 31 (88.6 %) patients. Different organisms from the two ears were isolated from 2 (5.7 %) patients and 2 patients were culture negative for both ears.

Of the total isolates, 420 (98.4%) were bacterial and 7 (1.6%) fungal strains. Gram-negative bacteria were most frequently encountered. Of these isolates 60.4% were members of the family Enterobacteriaceae. The rest belong to the genera of *Pseudomonas, Staphylococcus, Streptococcus, Aspergillus, and Candida. Proteus* was the predominating genera and comprised 25.5% of all the isolates. *Pseudomonas* (13.1%) and *Staphylococcus* (12.7%) species were second and third in prevalence, respectively. The bacterial species most often isolated was *P. mirabilis*. It was found from 72 (23%) ears and in 34 ears (10.9%) it was the only isolate. *Pseudomonas aeruginosa* and *S.aureus* were the species encountered next to *P. mirabilis* in frequency. They were isolated from 56 (17.9%) and 54 (17.3%) ears, respectively.

Table 1. Types and frequencies of middle ear isolates

Type of organism	Number	Percent[1]
Proteus mirabilis	72	16.9
Proteus vulgaries	37	8.7
Pseudomonas aeruginosa	56	13,1
Staphylococcus aureus	54	12.7
Klebsiella pneumoniae	20	4.7
K.oxytoca	13	3.1
K.ozoanae	3	0.7
K.rhinscheleromatis	2	0.5
Escherichia coli	27	6.3
Enterobacter cloaca	12	2.8
E.aerogens	7	1.6
E.agglomerance	4	1.0
Citrobacter diversus	16	3.8
C.freundii	6	1.4
Acinetobacter spp.	22	5.2
Providencia rettegerii	12	2.8
P.stuartii	8	1.9
Morganella morganii	11	2.6
Alcaligens spp.	10	2.4
Serratia spp.	7	1.6
Haffnia spp.	1	0.2
Streptococcus pyogens	9	2.1
S.pneumoniae	3	0.7
S.species	8	1.9
Aspergillus flavus	5	1.2
A.niger	1	0.2
Candida albicans	1	0.2
Staphylococcus epidermidis	37	8.7
No growth	35	8.1

[1]Percentage is out of the total number of strains isolated.

Fungal isolates (1.6%) were found both in pure and mixed cultures. Two isolates of *A. flavus* and one isolate of *A. niger* were encountered in pure culture. Others were found as mixed cultures either with *S. aureus* or *Streptococcus species*. None of the samples were positive for acid-fast bacilli.

3. Susceptibility to Antimicrobials

The antimicrobial susceptibilities of the bacterial isolates are shown in Table 3 and 4 for gram-negative and gram-positive organisms, respectively. gentamycin showed good *in vitro* activity (95.7% susceptibility) followed by carbenicillin and kanamycin (Table 2 and 3).

Ampicillin, tetracyclline and cephalothin were the least effective antimicrobial agents against most of the isolates (Figures 2 & 3a-h). Gentamycin and carbenicillin were the most effective agents against *P. aeruginosa*. Only 5.4% and 17.9% strains of this organism were resistant to gentamycin and carbenicillin, respectively (Figure 3b). Less than 45% of the gram-negative isolates were sensitive to ampicillin and tetracyclline and less than 60% were sensitive to cephalothin (Table 2). A 100% resistance to ampicillin was observed with *P. aeruginosa*. More than 60% of the *Pseudomonas* isolates were resistant to six antimicrobials. All isolates of *P. aeruginosa* were resistant to at least one and/or two antimicrobials (Table 4).

Penicillin was less effective against *S. aureus*. Thirteen (24.1%) of the isolates were sensitive to penicillin, 51.8% sensitive to tetracyclline and 98.1% sensitive to methecillin (Table 3). *Streptococcus* spp. were sensitive to most of the antimicrobials tested. Seventy five per cent of the isolates were sensitive to tetracyclline, 95% sensitive to bactrim and erythromycin. All streptococci isolates were sensitive to the rest of antimicrobials tested.

Table 2. Antimicrobial Susceptibility of Gram-negative Middle ear isolates.

Type of bacteria	No. tested	Percentage sensitive to[1]							
		Amp[2]	Car	Cep	Chl	Gen	Kan	Tet	Sxt
Proteus	109	59	96	67	83	99	92	20	90
Pseudomonas	56	0	82	14	11	95	25	21	8.9
Klebsiella	38	32	55	74	90	92	92	76	90
Escherichia	27	56	59	82	74	100	89	68	82
Enterobacter	23	31	83	35	87	100	96	83	91
Citrobacter	22	36	59	64	95	100	86	77	100
Acinetobacter	22	55	77	59	64	73	77	45	82
Providencia	20	60	90	50	65	90	85	35	80
Others[3]	29	48	100	52	79	100	76	45	93
Total	346	42	82	52	70	96	88	43	76

[1]Percentage is out of number of strains tested.

[2]Abbreviations: Amp., ampicillin; Car, carbenicillin; Cep.,cephalothin; Chl., chloramphenicol; Gen., gentamycin; Kan., kanamycin; Tet., tetracyclline; Sxt., trimethoprim-sulphamethoxazol.

[3]Includes: Morganella, Alcaligens, Serratia, and Haffnia

Table 3. Antimicrobial Susceptibility of Gram-positive Middle-ear isolates

Type of bacteria		*S.aureus*	*Streptococcus spp.*	Total
No. tested		54	20	74
Percentage sensitive to[1]	Amp[2]	_[3]	100	100
	Car	100	100	100
	Cep	100	100	100
	Chl	88.9	100	91.1
	Gen	100	100	100
	Kan	96.3	100	97.3
	Tet	51.8	75	58.1
	Sxt	90.7	95	91.9
	Ery2	96.3	95	95.9
	Lin	87	-	90.5
	Met	98	100	98.6
	Pen	23.1	100	54.6

[1]Percentage is out of the strains tested

[2]Abbreviations: Ery, erythromycin; Lin, lincomycin; Met, methicillin; Pen, penicillin; see table 3, for other abbreviations.

-[3]: not tested.

Fig.2

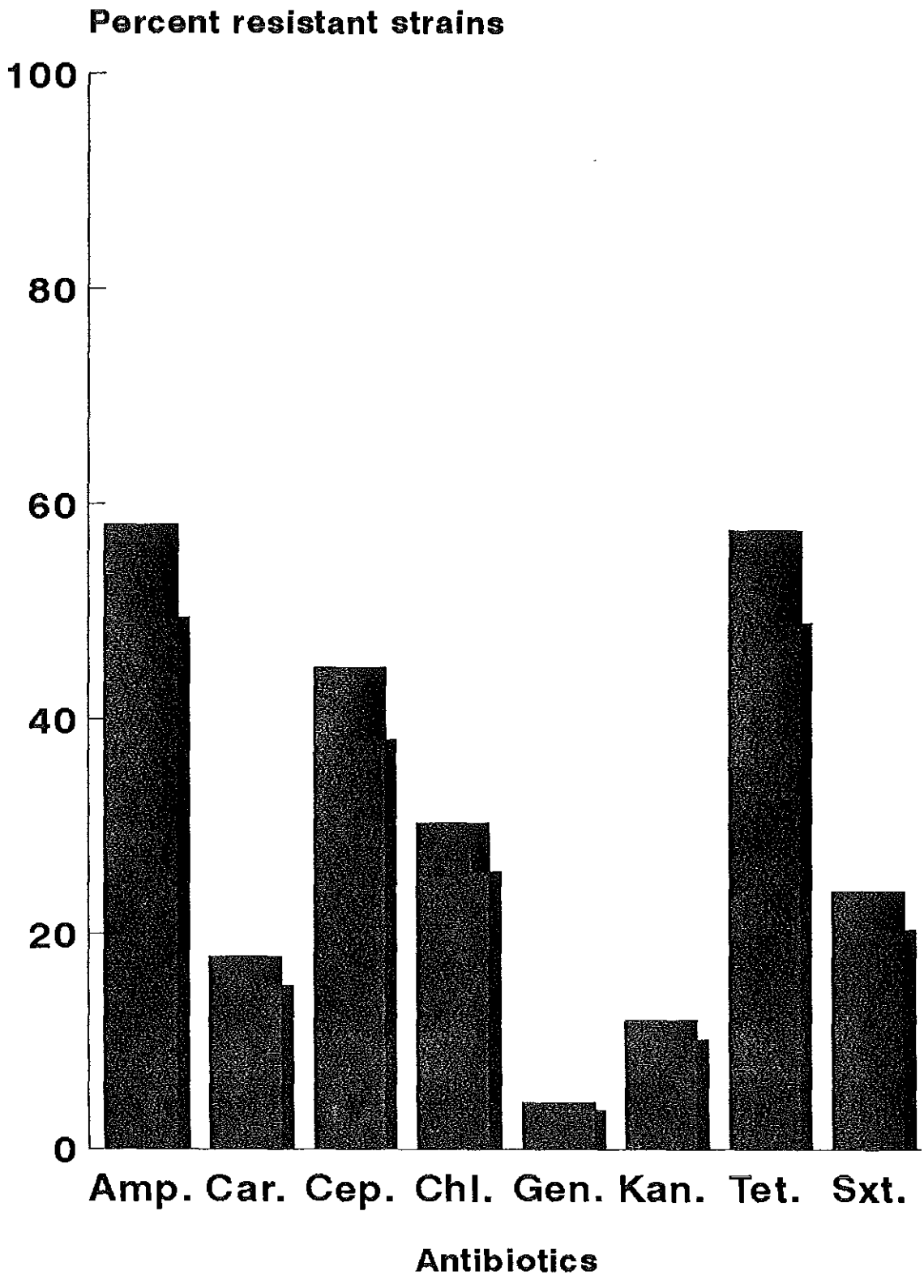

Percent resistant strains
100
80
60
40
20
0
Amp.
Car.
Cep.
Chl.
Gen.
Kan.
Tet.
Sxt.
Antibiotics

Fig.3A. Proteus spp.

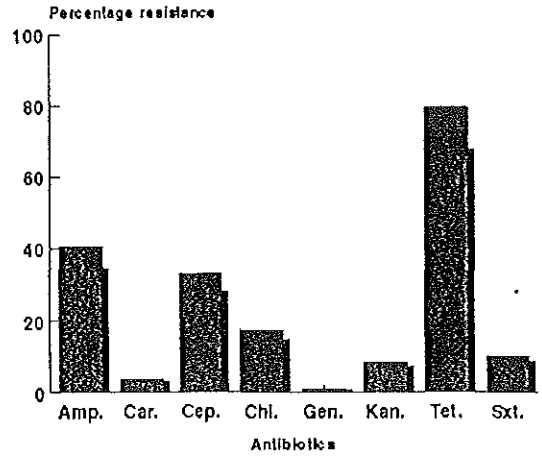


Fig.3B P. aeruginosa

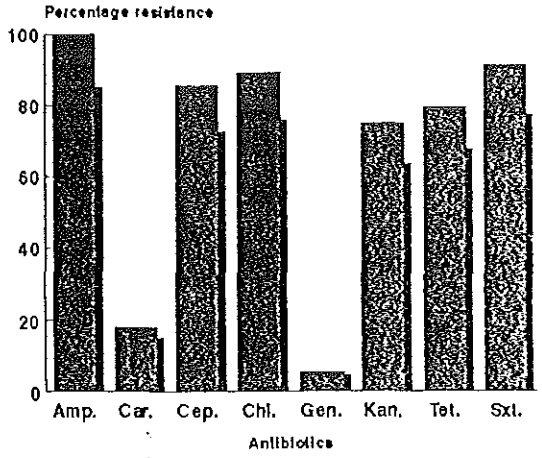


Fig.3C Klebsiella spp.

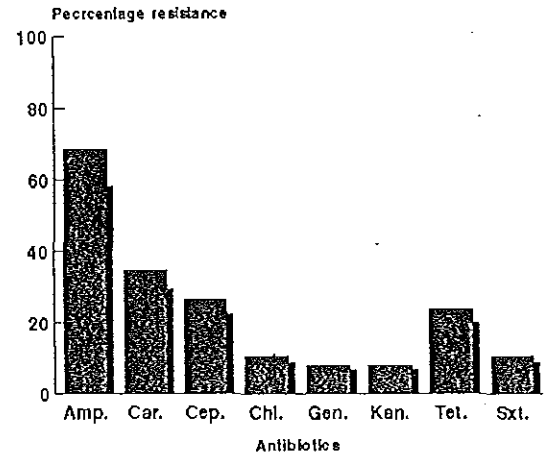


Fig.3D E. coli

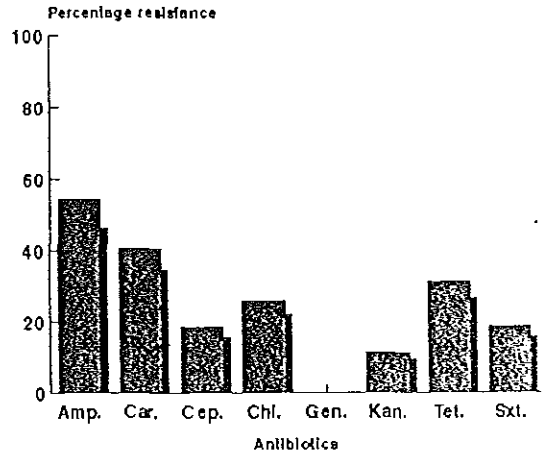


Fig.3E Enterobacter spp.

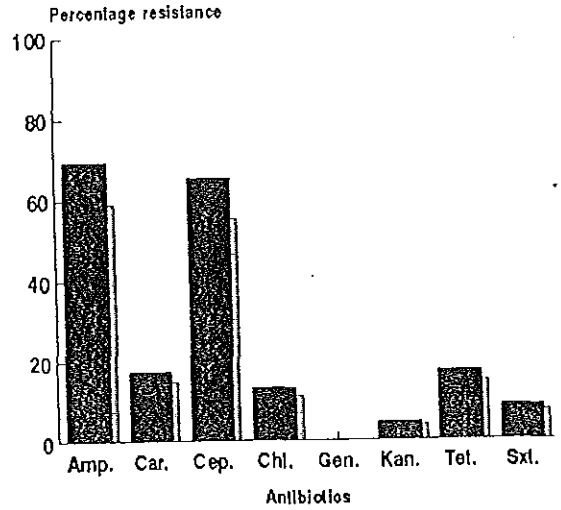


Fig.3F Citrobacter spp.

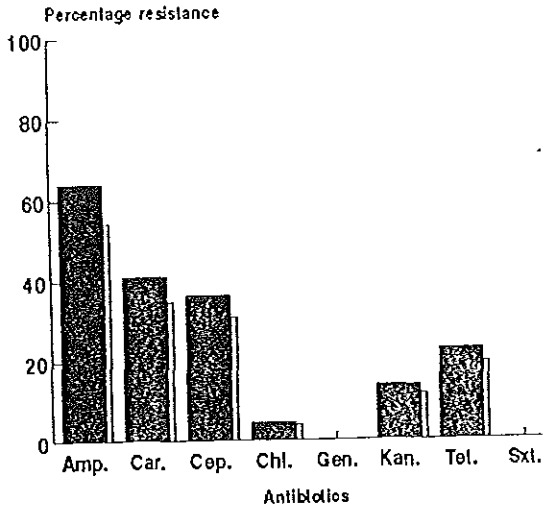


Fig.3G. Acinetobacter spp.

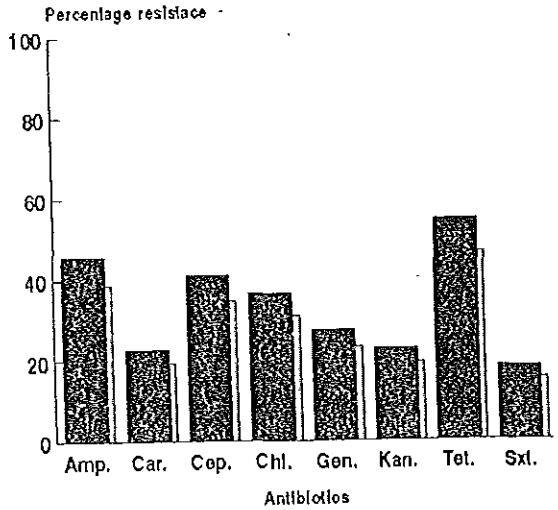


Fig.3H. Providenoia spp.

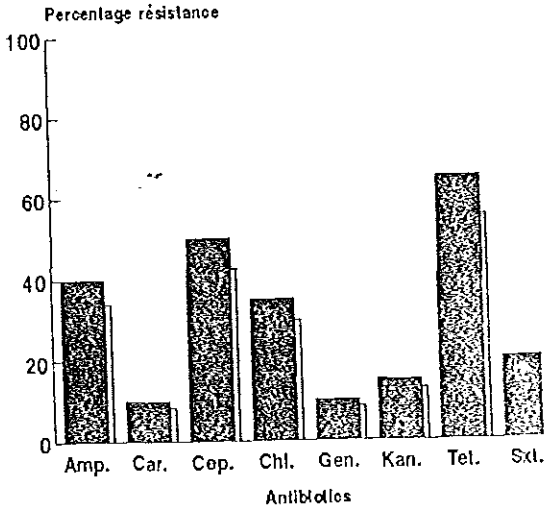


TABLE 4. Single and Multiple Antibiotic Resistance of Bacterial Isolates from Patients with Chronic Suppurative Otitis Media

Type of Bacteria	No. of Resistance, with Percentage In Bold.									
	1	2	3	4	5	6	7	≥ 1	≥ 2	≥ 3
Proteus spp. (109)[1]	56 **51.4**	20 **19.6**	16 **15.7**	6 **5.9**	5 **4.6**	3 **2.9**	0	106 **97.3**	50 **45.9**	30 **27.5**
P. aeruginosa (56)	0	1 **1.8**	3 **5.4**	9 **16.1**	4 **7.1**	36 **64.3**	3 **5.4**	56 **100**	56 **100**	55 **98.2**
S. aureus (54)	22 **40.7**	14 **25.9**	8 **14.8**	1 **1.9**	0	2 **3.7**	0	47 **87.0**	25 **46.1**	11 **20.4**
Klebsiella spp. (38)	8 **21.1**	10 **26.3**	5 **13.2**	4 **10.5**	1 **2.6**	1 **2.6**	1 **2.6**	30 **78.9**	22 **57.9**	12 **31.6**
E. coli (27)	3 **11.1**	1 **3.7**	4 **14.4**	2 **7.4**	3 **11.1**	1 **3.7**	1 **3.7**	15 **55.6**	12 **44.4**	11 **40.7**
Enterobacter spp. (23)	5 **21.7**	10 **43.5**	4 **17.4**	2 **8.7**	0	0	0	21 **91.3**	16 **69.6**	6 **26.1**
Citrobacter spp. (22)	4 **18.2**	6 **27.3**	5 **22.7**	0	2 **9.1**	0	0	17 **77.2**	13 **59.1**	7 **31.8**

Table 4 (Continued)

Acinetobacter spp. (22)	3 **13.6**	5 **22.7**	1 **4.6**	2 **9.1**	2 **9.1**	3 **13.6**	1 **4.6**	17 **77.2**	14 **63.6**	9 **36.4**
Providentia spp. (20)	4 **20**	3 **15**	4 **20**	1 **5**	2 **10**	1 **5**	1 **5**	16 **80**	12 **60**	9 **45.5**
Streptococcus spp. (20)	7 **35**	0	0	0	0	0	0	7 **35**	0	0
Others[2] (29)	6 **20.6**	2 **6.9**	11 **37.9**	3 **10.4**	0	1 **3.5**	0	23 **79.3**	17 **58.6**	15 **51.7**
Total (420)	118 **28.1**	72 **17.1**	61 **14.5**	30 **7.1**	19 **4.5**	48 **11.4**	7 **1.7**	355 **84.5**	237 **56.4**	165 **39.3**

[1]Number of strains tested.

[2]*Morganella, Alcaligens, Serratia, and Haffnia.*

Only 14.3% of the strains tested were sensitive to all antimicrobials and 6.9% were with intermediate sensitivities (Table 5). The sensitive strains included : 13 (65%) strains of *Streptococcus* spp., 12 (44.4%) isolates of *E.coli*, 5 (22.2%) strains of *Acinetobacter* species. All these covered 50% of all the sensitive strains to all antimicrobials tested. No *P. aeruginosa* was sensitive to all or resistant to only one antibiotics.

Table 5. Sensitivities of Isolates of Intermediate and Sensitive to All Antimicrobials Tested.

Type of bacteria	No. tested	No., with percentage[1] in parentheses	
		Intermediate	Sensitive to all antimicrobials
Proteus	109	5 (4.6)	3 (2.9)
Pseudomonas	56	4 (7.1)	0
Staphylococcus	54	3 (5.6)	6 (11.1)
Klebsiella	38	9 (23.7)	6 (15.8)
Escherichia	27	0	12 (44.4)
Enterobacter	23	2 (8.7)	2 (8.7)
Citrobacter	22	3 (13.6)	3 (13.6)
Acinetobacter	22	2 (9.6)	5 (22.7)
Providentia	20	0	4 (20)
Streptococcus	20	0	13 (65)
Others[2]	29	1 (3.5)	6 (20.6)
Total	420	29 (6.9)	60 (14.3)

[1]Percentage is out of the tested strains.

[2]*Morganella, Alcaligens, Serratia, Haffnia.*

A wide variety of resistance patterns (Antibiograms) were observed among the different isolates. The resistance antibiograms are shown in Table 6. Seventy seven different resistance antibiograms were observed among 400 isolates without considering streptococci and fungi. Out of these, 17 different combinations were observed in the triple resistance antibiogram. And 15 and 14 different combinations were seen in four and double resistance antibiograms, respectively.

In most of the frequent resistance antibiograms, there was also resistance to ampicillin, cephalothin, and /or tetracycline. Single resistance antibiogram was observed in 111 (28.8%) strains. The most frequent of this type of resistance was the one associated with tetracyclline. This was demonstrated by 51 out of 56 (91.1%) strains of *Proteus species*.

Double resistance antibiograms were detected in 72 (18%) strains of the total isolates (excluding the streptococcal and fungal isolates). The commonest of this type was ampicillin-cephalothin combination. This comprises 30.6% of the double resistance antibiogram. This type of antibiogram was showed by 38.1% strains of *Enterobacter* followed by 9.4% strains of *Proteus*.

Triple resistance antibiogram was found in 61 (15.3%) of the 400 isolates. Ampicillin-cephalothin-tetracyclline was the most frequent combination. This comprised 27 (44.3%) of the triple resistance antibiograms and was demonstrated by 10.4% of the *Proteus* strains.

Resistance antibiograms to four were found in 7.5%, to 5 in 4.8%, and to 6 in 12% of the strains. The resistance antibiograms to 6 was mainly by *P.aeruginosa*. Thirty-six (64.2%) of the isolates of this organism were resistant to six antimicrobials. The commonest combination of this type of resistance involved ampicillin-cephalothin-chloramphenicol-kanamycin-tetracyclline-bactrim. This combination comprised 88.9% of the resistance antibiograms to six antiinicrobials. Resistance antibiograms to 7 were observed in 1.8% of the strains while no resistance was observed to combinations of 8 or more antibiotics.

Table 6. Resistance Antibiograms of the Resistance Middle Ear Isolates.

Type of bacteria	Antibiogram[1]		Isolates	
			No.	%[2]
Proteus spp. (106)[3]	Single	Amp	1	0.9
		Cep	3	2.8
		Chl	1	0.9
		Tet	51	48.1
	Two	AmpCep	10	9.4
		AmpTet	5	4.7
		AmpSxt	1	0.9
		CepTet	1	0.9
		ChlTet	3	2.8
	Three	AmpCarCep	1	0.9
		AmpCarSxt	1	0.9
		AmpCepTet	11	10.4
		CepChlTet	1	0.9
		ChlTetSxt	1	0.9
		GenKanTet	1	0.9
	Four	AmpCepChlKan	1	0.9
		AmpCepChlTet	3	2.8
		AmpChlTetSxt	1	0.9
		AmpKanTetSxt	1	0.9
	Five	AmpCepChlKanTet	2	1.9
		AmpCarChlTetSxt	1	0.9
		AmpChlKanSxt	2	1.9
	Six	AmpCarCepChlTetSxt	1	0.9
		AmpCepChlKanTetSxt	2	1.9

Table 6. (continued)

Type of bacteria	Antibiogram		Isolates	
			No.	%
P.aeruginosa (56)	Single	-	0	0
	Two	AmpCep	1	1.8
	Three	AmpCarSxt	1	1.8
		AmpCepTet	1	1.8
		AmpCepSxy	1	1.8
	Four	AmpCarCepChl	2	3.6
		AmpCarChlTet	1	1.8
		AmpCepChlSxt	5	8.9
		AmpCepKANSxt	1	1.8
	Five	AmpCepChlKanSxt	1	1.8
		AmpCepChlTetSxt	2	3.6
		AmpChlKanTetSxt	1	1.8
	Six	AmpCarChlKanTetSxt	3	5.4
		AmpCepChlKanTetSxt	32	57.1
		AmpCepGenKanTetSxt	1	1.8
	Seven	AmpCarCepChlKanTetSxt	1	1.8
		AmpCarChlGenKanTetSxt	2	3.6
E.coli (15)	Single	Apm	1	6.7
		Cep	1	6.7
		Tet	1	6.7
	Two	CepTet	1	6.7
	Three	AmpCarCep	2	13.3
		AmpCarTet	2	13.3
	Four	AmpCarChlTet	1	6.7
		AmpCarChlSxt	1	6.7
	Five	AmpCarChlKanSxt	1	6.7
		AmpCarChlTetSxt	2	13.3
	Six	AmpCarChlKanTetSxt	1	6.7
	Seven	AmpCarCepChlKanTetSxt	1	6.7

Table 6. (continued)

Type of bacteria	Antibiogram		Isolates	
			No.	%
Klebsiella spp. (30)	Single	Amp	6	20.0
		Cep	1	3.3
		Tet	1	3.3
	Two	AmpCar	8	26.7
		AmpCep	1	3.3
		CepTet	1	3.3
	Three	AmpCarCep	1	3.3
		AmpCarKan	2	6.7
		AmpCepTet	2	6.7
	Four	AmpCarCepGen	1	3.3
		AmpCarChlTet	1	3.3
		AmpCarKanTet	1	3.3
		AmpCepTetSxt	1	3.3
	Five	AmpCarChlTetSxt	1	3.3
	Six	AmpCarCepChlGenSxt	1	3.3
	Seven	AmpCarCepChlGenTetSxt	1	3.3
Enterobacter spp. (21)	Single	Amp	2	9.5
		Car	1	4.8
		Cep	2	9.5
	Two	AmpCar	1	4.8
		AmpCep	8	38.1
		CepTet	1	4.8
	Three	AmpCepChl	1	4.8
		AmpCepTet	1	4.8
		AmpCepSxt	1	4.8
		ChlTetSxt	1	4.8
	Four	AmpCarCepKan	1	4.8
		AmpCarChlTet	1	4.8

Table 6. (continued)

Type of bacteria	Antibiogram		Isolates	
			No	%
Citrobacter spp. (17)	Single	Amp	3	17.7
		Cep	1	5.9
	Two	AmpCar	3	17.7
		AmpCep	1	5.9
		CarCep	1	5.9
		CepKan	1	5.9
	Three	AmpCarCep	2	11.8
		AmpCarTet	1	5.9
		AmpCepTet	2	11.8
	Five	AmpCarCepKanTet	1	5.9
		AmpCarChlKanTet	1	5.9
Acinetobacter spp. (17)	Single	Cep	1	5.9
		Chl	1	5.9
		Sxt	1	5.9
	Two	AmpChl	1	5.9
		CarCep	1	5.9
		CepTet	1	5.9
		ChlTet	1	5.9
		GenTet	1	5.9
	Three	AmpCepTet	1	5.9
	Four	AmpCarChlTet	2	11.8
	Five	AmpCepKanGenTet	2	11.8
	Six	AmpCarCepChlKanTet	1	5.9
		AmpCepChlGenTetSxt	1	5.9
		AmpCepGenKanTetSxt	1	5.9
	Seven	AmpCarChlGenKanTetSxt	1	5.9

Table 6. (Continued)

Type of bacteria	Antibiogram		Isolates	
			No.	%
Providentia spp. (16)	Single	Amp	1	6.3
		Tet	3	18.8
	Two	CepTet	1	6.3
		CepSxt	1	6.3
		TetSxt	1	6.3
	Three	AmpCepTet	2	12.5
		CepChlTet	1	6.3
		ChlGenTet	1	6.3
	Four	AmpCarCepChl	1	6.3
	Five	AmpCepChlKanTet	1	6.3
		AmpCepChlTetSxt	1	6.3
	Six	AmpCepChlGenKanTet	1	6.3
	Seven	AmpCarCepChlKanTetSxt	1	6.3
Others[4] (23)	Single	Kan	4	17.4
		Tet	2	8.7
	Two	AmpCep	1	4.4
		CepTet	1	4.4
	Three	AmpCepKan	1	4.4
		AmpCepTet	7	30.4
		AmpCepChl	1	4.4
		AmpChlTet	1	4.4
		ChlKanTet	1	4.4
	Four	AmpCepChlTet	2	8.7
		AmpChlTetSxt	1	4.4
	Six	AmpCepChlKanTetSxt	1	4.4

Table 6. (continued)

Type of bacteria	Antibiogram		Isolates	
			No.	%
S.aureus (47)	Single	Lin	1	2.1
		Pen	18	38.3
		Tet	3	6.4
	Two	LinPen	2	4.3
		PenTet	11	23.4
		TetSxt	1	2.1
	Three	ChlPemTet	2	4.3
		ChlTetSxt	1	2.1
		LinPenTet	3	6.4
		PenTetSxt	2	4.4
	Four	ChlLinPenTet	1	2.1
	Six	ChlEryKanPenTetSxt	1	2.1
		ChlEryKanMetPenTet	1	2.1

[1]See Tables 2 & 3, for abbreviations.

[2]Percentage is out of the total resistant strains of the genera.

[3]Numbers in parentheses indicate the total number of resistant strains of that genera.

[4]Includes: *Morganella*, *Alcaligens*, *Serratia*, and *Haffnia*.

V. DISCUSSION.

The rate of ear infection (79.4%) in this study may be an underestimation of the magnitude of the problem. This is because anaerobic infections of the middle ear were not included in the study. Anaerobes are, however, reported to cause ear infections (Brook, 1985).

There has not been any study regarding the microbial etiology of chronic suppurative otitis media in Ethiopia. Hence, comparison of our results with previous studies was not possible.

Pseudomonas aeruginosa is the most common aerobic organism isolated from CSOM in many studies (Brook, 1980; Brook & Finegold, 1979; Kenna & Bluestone, 1986; Kenna,1988; Nelsson, 1988). Diphtheroides, *S. aureus* and *S. epidermidis* were also reported to be very common, too (Karma *et al.* 1978). Friedmann (1954) put *S. aureus* higher on the list.

In the present study *Proteus* spp. was predominant. It comprised 25.5% of all isolates; *P. mirabilis* comprised 16.9%. This was followed by *P. aeruginosa* (13.1%), *S. aureus* (12.7%), and *Klebsiella* spp. (9%). These results reflected the poly-microbial etiology of CSOM as described in many studies (Coker *et al.* 1983; Ibekwe & Okafor, 1983; Kenna & Bluestone, 1986; Amadasun, 1991). The frequency of the isolates, especially for *Proteus* spp. and *P. aeruginosa*, did not correspond with findings of other workers (Brook & Finegold, 1979; Mohoney, 1980; Coker *et al.*, 1983; Ibekewe & Okafor, 1983; Kenna & Bluestone, 1986; Stolp & Swart, 1989; Amadasun, 1991). In all these reports *P. aeruginosa* was the dominant isolate. However, reports from the Sudan (Yagi, 1990; Hussain *et al.* 1991) were consistent with the present findings. *Proteus* spp. was the dominant isolate in both cases.

The difference in prevalence of different organisms in different studies from the same site of infection could be due to the difference in the source of infection. That is to say, the natural habitat of the infecting organism from which the individual acquires the infection may determine the prevalence of the pathogen. Despite the difference in the awareness of the factors that predispose to infection of the middle ear among communities, mere

geographic factors do not seem to affect the prevalence rate of an organism (Mohoney, 1980).

The predominance of *Proteus* spp. could be related to the late presentation of patients after the onset of middle ear drainage. The relation between the type of organism isolated and duration of discharge in patients suffering from CSOM has been described by Mohoney (1980). He showed that *Proteus spp*. was the most common pathogen in patients visiting hospitals after two months or more after the onset of ear drainage. From one week to two months *P. aeruginosa* was prevalent. *S. aureus* was common during the first seven days.

Our results were in aggrement with Mohoney's (1980) report. The majority of our study patients from which our samples were collected reported to the hospitals three or more months after the onset of middle ear discharge. This delay could be due to long distances to the hospitals or due to other social and/or economic factors.

The prevalence of enteric bacilli, gram-positive cocci, and non-fermenters other than *Pseudomonas* in this study were in line with other reports (Kenna, 1988; Brook & Yocum,1989).

Otomycosis (*ie.*; fungal infection of the ear) is generally considered to be infrequent. It may result from topical application of corticosteroids, antimicrobials or manipulation of the ear canal with contaminated devices (Thank *et al.*, 1980). The fungal isolates (1.64%) in this study were also reported from patients with CSOM in previous studies (Young, *et al.*, 1970; Talwar *et al.*, 1988; Amadasun, 1991). Thank *et al.* (1980) reported *A. niger, A. flavus, A. fumigatus*, and *C. albicans* comprising more than 50% of all isolates. Although *Mucor* spp. and *C. parapsilosis* were also reported (in addition to the above fungal strains) by other researchers (Ibekwe & Okafor, 1983; Talwar *et al.*, 1988), both species were not isolated in this study.

Tuberculous otitis media, although a rare entity, is reported to affect the middle ear (Stone, 1967; Mumtaz *et al.*, 1983, Giebink, 1989; Firehiwot *et al.*, 1990). Although we did not get a positive result in this study, it is important to consider its diagnosis. This is

because of the great damage it could cause to the middle ear and surrounding structures if left untreated.

By comparing our results with other studies of the nasophryngeal carrier rates (Howie & Plousard, 1971; Long *et al.*, 1983; Tewodrose & Gedebou, 1983; 1984; Mengistu & Gedebou; 1986), it is possible to say that the source of infection of CSOM could be both from the environment and from the nasopharynx since our isolates from the middle ear discharge have been also isolated from the nasophryngeal cultures by these workers.

The extent of antimicrobial susceptibility of bacterial strains depends on the therapeutic practice in the particular region and on the period of the study (Bulger *et al.*, 1970; Finland, 1972). Inspite of the expected regional variations in the rate of susceptibilities of bacterial strains, it is important to compare the findings of this study with reports from other countries and with reports from Ethiopia which are non-ear drainage isolates.

Gentamycin, carbenicillin, and kanamycin were the most effective antimicrobial agents against most of the isolates in this study in their respective order. However, 75% of the *P. aeruginosa* strains were resistant to kanamycin. Carbenicillin was also not active against *Klebsiella* spp. (44.7%), *Citrobacter* spp. (40.9%), and *E. coli* (40.7%).

The *in vitro* effectiveness of gentamycin in our study was in line with other reports (Coker et al., 1983; Moellering, 1983; Mengistu & Gedebou, 1986; Amadasun, 1991; Hussain et al., 1991). This is supported by the fact that the emergence of resistance to gentamycin is slow and gentamycin resistant organisms are mostly limited to the hospital environment (Block, 1978; Moellering, 1983).

A high percentage of our isolates were resistant to ampicillin, tetracylline and penicillin. This agrees with reports from elsewhere (Coker *et al.*, 1983; Mengistu & Gedebou, 1986; Hussain *et al.*, 1991). The high rate of resistance of *P.aeruginosa* to ampicillin, trimethoprim-sulphamethoxazole, chloramphenicol, and tetracylline in this study was strongly in agreement with the findings of Neu (1981). He reported a 100% resistance to ampicillin, trimethoprim-sulphamethoxazole,and tetracylline. He also observed a 100% resistance to tetracylline by *P. mirabilis* which is more or less similar to our results

(97.2% resistance). The increased degree of resistance of *P. mirabilis* to tetracyclline is supported by the fact that most strains of this species are reported to express inducible high level tetracyclline resistance from a chromosomal locus (Foster, 1983). Ampicillin was least effective against *Pseudomonas, Klebsiella, Escherichia, Enterobacter* and *Citrobacter*. Tetracyclline was ineffective against *Proteus, Pseudomonas, Providentia*, and *Acinetobacter* species. It was observed to have an intermediate effectivity against the enteric bacilli (*Klebsiella, Escherichia, Enterobacter*, and *Citrobacter*).The observed *in vitro* ineffectiveness of ampecillin and cephalothin against the gram-negative isolates were in line with reports from the pharyngeal isolates (Mengistu & Gedebou, 1986)

The *in vitro* effectiveness of trimethoprim-sulphamethxazole and kanamycin were more or less similar. Both were not effective against *P. aeruginosa* (79% & 91% resistance, respectively). A 100% and 86% of the *Citrobacter* spp. were sensitive to trimethoprim-sulphamethoxazole and kanamyccin, respectively.

The susceptibility of all *Streptococcus* spp. (20 strains) to penicillin was consistent with the known efficacy of this antimicrobial against all group A and most other beta-haemolytic streptococci. Twenty-five per cent of the streptococci were resistant to tetracyclline. The susceptibilities of this organism to penicillin and tetracyclline are similar to what has been reported by Gedebou (1983).

Penicillin and tetracyclline were not effective against *S. aureus* in this study. This finding is, therefore, similar to other reports by Neu (1981), Coker (1983) and Gedebou (1983). In line with reports of none-ear discharge isolates from this country (Tewodrose & Gedebou, 1983; 1984), the staphylococcal isolates in this study were resistant to penicillin and tetracyclline. However, the level of resistance of this organism to tetracyclline in this study (48.2%) was lower than the report by these workers. This could be due to the selection pressure of antibiotics in the hospital environment favouring the growth of resistant organisms.

The frequency of single and multiple antimicrobial resistance of the middle ear pathogens, especially for *P.aeruginosa*, is alarming. Sixty four per cent of the isolate were resistant to six antimicrobials. This is larger than the 43% reported from urinary tract infections of

hospital patients (Gedebou, 1983). Ampicillin-cephalothin-kanamycin-tetracycline-trimethoprim-sulphamethoxazole were the frequent combination in both studies. Regardless of the drawbacks of comparing susceptibility results obtained from hospital and community acquired infections, the rate of multiple drug resistance by *P. aeruginosa* has increased from 43% to 64% in 11 years duration. This may reflect the indiscriminate therapeutic practice in this country.

There seems to be a strong tendency to misuse antibiotics for therapy in this country, mainly because of inadequate bacteriological facilities and lack of published information on the types and antibiotic susceptibility profiles of the bacteria commonly isolated (Gedebou, 1983; Gedebou *et al.*, 1983). The choice of antibiotics has to depend, among other factors, on a knowledge of the types of likely pathogens in the site of infection and their current antibiotic sensitivities. Hence, this study will provide part of such information for chronic suppurative otitis media.

In this study CSOM has a poly-microbial etiology with *Proteus* spp. being the most common. Gentamycin and kanamycin were among the most effective antimicrobials against most of the isolates. But, they are ototoxic (Jackson & Arceieri, 1971; Ryan & McGee, 1977; Ginsberg *et al.* 1980; Rudnick *et al., 1980; Huber* et al., 1980; Neu, 1981; Wright & Meyerhoff, 1988). This represents a major practical problem in the treatment of CSOM. Therefore, these antimicrobials are not recommended as first choice drugs. Care must be taken to use these antibiotics only when absolutely necessary. In fact, the risk of ototoxicity must be weighed against the risk of non-treatment when these antimicrobials are found to be the only effective or the best drug of choice.

VI. RECOMMENDATIONS

Based on this study, the following recommendations are made:

1. The study of the microbiology of otitis media should be continued. The samples we took from the two hospitals proved the poly-microbial etiology of this disease. Studies covering many parts of the country are important to see the real picture of the problem. The contribution of anaerobic organisms to otitis media should also be investigated.

2. The genetics of multiple drug resistance should be studied as this will help to know the mechanism of the acquisition of drug resistance. The kind of resistance and the plasmid profile should be investigated.

3. Present diagnostic capabilities should be supplemented with finer methods of typing serotyping, phage typing and bacteriocin typing.

4. The frequency of multiple drug resistance of the isolates is an indication of the wide misuse of antibiotics. The availability of antibiotics to the users without and/or by inappropriate prescription should be restricted. Although it may not be fair to suggest a restricted utilization of antimicrobials under the present limitations in all aspects of the health services, it should be recommended that a more wise utilization than has been followed in the past should now be followed.

5. Culture and sensitivity results are important to choose the antimicrobials with lower ototoxic effect and maximum antimicrobial effectivity as well as for cost-effective treatment. Therefore, the establishment of microbiology laboratories should be considered as this will lead us to formulating restricted antimicrobial policy.

6. The role of improved personal hygiene should be considered since the microbiology of CSOM is that of water and faecal contamination. Health education should be given to avoid manipulation of the ear canal with finger nails and contaminated devices such as feathers, sticks, metal bars etc. Patients with an open middle ear cavity could be advised to use ear-plugs during high risk activities such as showering, bathing, and swimming to control water born infection.

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