INTRODUCTION

The term otitis media denotes inflammation of the mucoperiosteal lining of the middle ear. Otitis media may be acute with suppurative middle ear infection of relatively sudden clinical onset or chronic. The term chronic otitis media encompasses several suppurative or non suppurative conditions of incidious clinical onset including otitis media with effusion (secretory otitis media). Complications of otitis media occur when there is extension of inflammation and infection beyond the mucoperiosteal lining of the middle ear (e.g mastoidits or epidural abscess). Acute otitis media is one of the most common infectious diseases of childhood and accounts for a large of both office and emergency visits. **(Teele et al, 1983).**

Teele et al (1989) reported that by 1 year of age 62 percent of children had at least one episode of acute otitis media and 17 persent had three or more episode. Also reported that children who are "otitis prone" (six or more episodes of acute otitis media) usually have two characteristics in common (1) An initial episode of otitis media during the first 6 months of life and (2) Initial infection with streptococcus pneumoniae.

Previous reports on bacteremia in children focused on hospitalized patients with positive blood culture. 50% of

whom had an underlying illness or were less than one month of age **(Johnston and Sell, 1964 and Hanninen 1971).** The causative organinsms most often were Staphylococcus aureus. **(Johnston and Sell, 1964).** 1.5% of children less than 2 years of age with otitis media and temperatures greater than 38.9°C were bacteremic **(Teele et al; 1975).** Bacteremia with otitis media was described also by **McCarthy et al;(1976)**in 5.8% of patients Meningitis developed in patients with otitis media accopained with pneumococcol bacteremia **(Bratton et al; 1977). Schwartz and Weintzeh (1982)** found bacteremia in 5.8% of febrile infants with otitis media. Bacteremia with otitis media may cause suppurative complications **(Dashefsky et al, 1983).** Meningitis developed in patients with otitis media accompained with Haemophilus influenzae bacteremia **(Anderson et al; 1987).**

AIM OF THE WORK

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This work is conducted on a group of febrile children with acute otitis media to determine :

1 - The occurence of associated bacteremia.

2 - The bacteriological agents associated with bacteremia

REVIEW OF LITERATURE

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Bacteremia:

One of the most serious and potentially life threatining infectious diseases in childhood is a bacteremic illness. Age between 7 and 24 months with temperatures between 39.4 and 40.6°C were considered criteria with increased specificity for the presence of bacteremia.**(McGown et al; 1973).**

Defintions

The spread of infection to the blood stream is known as bacteremia and if the organism multiply there, it is called septicemia, Further dissimination may result in "metastatic foci" of infection especially in bone, liver, brain or heart valves **(Macleod et al; 1987).**

Bacteremia-defined as a positive blood culture , is an expected finding in invasive or deep bacterial infections such as meningitis, sepsis, epiglottitis, septic artheritis, osteomyelitis and facial cellulitis, Bacteremia is less frequently found in well-appearing febrile children with no focus of infection, when bacteremia occurs in this setting it is termed occult. All socioeconomic groups are equally affected, children at greatest risk are those aged 3 to 24 months with temperatures of 38.9°C or more. Conversely occult bacteremia is very unlikely in a child who presents

with a fever of less than 38.9°C, has no clinical focus for the fever and does not appear toxic **(Hamilton et al; 1991).**

Microorganisms of bacteremia:

Bacteremia may be caused by a wide variety of gram-positive or gram negative microorganisms and it may or may not be associated with a specific focus of infection such as pneumonia or meningitis. The incidence of bacteremia in children has been studied in both hospital and ambulatory settings. In otherwise normal children, beyond the newborn age groups, S. Pneumoniae, H. influenzae type b, S. aureus, salmonella spp. and Neisseria spp are the most common micro-organisms causing bacteremia.**(McCarthy et al; 1977 and Winchester et al; 1977).**

The pathophysiology of bacteremia is highly variable and dependant upon the specific microorgansims isolated, and the nature of the immune status of the host. Highly encapsulated organisms such as H. influenzae type b. S. pneumoniae and N. meningitidis may normally reside in the nasopharynx. and for reasons that are poorly understood, are capable of invading beyond mucosal barriers into the blood stream, A preceeding viral upper respiratory infection may play some role in alterations in local host defense mechanisms that result in bacteremia **(Michaels et al; 1977 and Kaplan et al; 1981).**

Using human columnar nasopharyngeal tissue in organ cultures, **Stephens and Collegues (1983)** demonstrated that N. meningitidis organisms were ingested by columnar cells , then found within phagocytic vacules and later observed within subepithelial tissues which suggests that the meningococci had penetrated the epithelial layer. H. influenzae type b attach to nonciliated columnar epithelial cells and subsequently are found in the intercellular spaces in association with a preceeding disruption of the light junctions of epithelial cells **(Farley et al; 1986).**

Epidemiology of bacteremia:

The natural history of pneumococcal bacteremia is benign, provided that the spleen is functional compared to that of H.influenzae or N. meningitidis.In the majority of children with pneumococcal bacteremia, the organism is spontaneously cleared from the blood, the remainder continue with fever, and in 2% to 10% of all patients the illness progresses to meningitis. In contrast focal infection and sepsis develop in 50% to 80% of children with H. influenzae bacteremia. Although spontaneous clearing of N. meningitidis has been documented a high percentage of patients develop meningitis and sepsis **(Hamilton et al; 1991).**

Types of bacteremia:

1 - Bacteremia caused by Haemophilus influenzae:

Bacteremia is commonly demonstrable in patients with invasive infections (at least 80 percent of children with

meningitis, epiglottitis or cellulitis) caused by H. influenzae b. H. influenzae b is responsible for about 20 percent of cryptogenic bacteremia occuring in febrile child with mild non specific illness. Such patients are at considerable risk for subsequent serious localised infection (meningitis pneumonia epiglottitis). **(Dajani et al; 1979).**

2 - **Gram-negative bacteremia:**

Gram negative bacteremia gain access to the blood stream from foci of tissue infection or when host resistance is depressed from sites of heavy colonization and minor trauma. Although bacteremia creates the opportunity for metastatic infections, a more immediate and serious consequence of gram negative bacteremia is septic shock. **(Kreger et al; 1980).**

Rates of shock vary in different series from less than 20 percent to more than 50 percent. In comparable groups, shock is somewhat more frequent in gram-negative bacteremia than gram positive bacteremia or fungemia. **(Bone et al; 1989)**

3 - **Staphylococcal bacteremia:**

There are two varieties of S. aureus bacteremia primary and secondary, primary bacteremia exists when a patient presents with fever and chills but does not have an identifiable primary focus of infections and usually has unrecog-

nized endocarditis and should be treated accordingly. Secondary bacteremia is that associated with an obvious peripheral focus of infection. The diagnosis of staphylococcal bacteremia is made when S. aureus grows from several blood cultures obtained before an empiric course of antibiotics begun, some patients already have an obvious metastatic complication of the bacteremic episode when first examined **(Sheagren 1984)**.

Laboratory diagnosis of bacteremia :

There is combination of laboratory tests and clinical observations that will rapidly differentiate the child with bacteremia from one with a self limited benign febrile illness. Diagnosis depends on positive results of blood cultures. A single blood culture is indicated in a child with a temperature of at least 39.5°C who has no focus of infection. The most common organisms isolated in positive blood cultures are S. pneumoniae, H. inluenzae, Neisseria species and salmonella species **(Hamilton et al; 1991).**

Trends in Bacteremia therapy:

In bacteremia Caused by H. influenzae. about 30 percent of strains of H. influenzae type b isolated, are ampicillin resistant and resistance to chloramphenicol also occurs regional variation in resistance patterns exists. In a pediatric hospital in Barcelona. Spain. 60% of meningeal isolates are ampicillin resistant and 66 percent are resis-

tant to chloramphenicol and 57 percent are resistant to both drugs. The resistance is due to B-lactam production which is the major cause of ampicillin resistance **(Wyngaarden et al; 1992).**

Otitis Media

Defintions :

Otitis media is an inflammation of the mucoperiosteal lining the middle ear cleft (eustachian tube, tympanic cavity, mastoid antrum, and mastoid air cell system). Extension of the inflammation beyond the mucoperiosteal lining represents a complication of the disease. **(Ralph and Gershon, 1981).**

Otitis media is one of the most common diseases of childhood By the age of six years 80-90% of children suffered from at least one episode of acute otitis media. Most children will experience only a single episode while others may be plagued by recurrent attacks with or without chronic perforation of tympanic membrane. Otitis media is a spectrum of middle ear pathology with differing clinical manifestations and sequalae. Various subdivisions include acute otitis media, serous otitis media and chronic otitis media **(Harold et al; 1983).**

The disease shows high incidence of recurrences especially during the first few years of life as the immunological defence mechanisms of a child mature relatively slowely during the first year of life leaving a young child prone to infections, but environmental factors such as population density and air pollution have also been shown to affect the occurence of otitis media **(Pukander et al; 1985).**

In childhood,acute otits media is a common and frequently recurring illness. It is one of the most common diagnosis made by physcians who provide health care to children **(Carlos - Gonzalez; 1986).**

Classifications of otitis mediae:

Stuart; 1974 Classified otitis media into:-i)suppurative acute and chronic otitis media ii)non suppurative acute and chronic otitis media. iii)Specific tuberculous and syphilitic otitis media. iv)Adhesive otitis media. v)Tympanosclerosis.

Another classification was produced by **Senturia et al; 1980** which classified otitis media into: i-Otitis media without effusion (or perforation) which may be acute, subacute or chronic. ii-Otitis media with effusion (without perforation) which may be: (a)acute serous or purulent (b)subacute serous, mucoid or purulent (c)chronic serous mucoid or purulent. iii)otitis media with perforation which may be : (a)Without discharage acute subacute or chronic (b)with discharge, acute serous and purulent or subacute serous, mucoid and purulent or chronic, serous mucoid and purulent otitis media.

Epidemiology of otitis media

Kessner et al (1973) estimated the incidence of otitis media to be 30 perecet from 6-months old to 3-year old age

in the United States **Howie et al (1975)** observed a high incidence of otitis media in Huntsville where 76 percent of the children had at least one attack of ear infection by 6 years of age.

Reliable data on the incidence of otitis media with effusion are not available because of its often asymptomatic nature **(Casselbrant et al; 1984).** Few studies were designed specifically to provide epidemiological information about middle ear disease in children. Reports of clinical experiences, therapeutic trials and microbiological studies of otitis media have provided some informations that can be used to calculate incidence and prevalence rates **(Lundgren, 1984).** Epidemiological studies of acute otitis media have been carried out in different parts of the world but in a selected groups of children e.g within a limited area in children visiting a private Ear, Nose and Throat or pediatric practice or in children belonging to different social or ethnic groups **(Kaplan et al; 1973).**

A survey of the office practice who provide medical care to children showed that otitis media was the most frequent diagnosis of illness **(Koch and Dennison, 1974).**

A number of studies in recent years have adressed the epidemiology and the natural history of otitis media and an excellent review was done by **Giebink (1984).** Several important factors appear to be closley related to the occuerence of otitis media:

1 - Age:

Otitis media is predominantly a disease of infants and young children, 45 percent of children have their first episode by age 1, and 62 percnet by age 2 **(Howie et al, 1975).**

A survey of the frequency of infectious diseases during the first year of life indicated that otitis media was the second only to the common cold as a cause of infectious illness **(Hoekelman, 1977).**

The incidence of otitis media decline with age after the first year of life except for a limited reversal of the downward trend between five and six years of age and the time of entrance into schools **(Sly et al, 1980).**

Otitis media is a disease of early infancy. Approximately 10% of children have an episode of otitis media by 3 months of age. The peak age-specific incidence is between 6 and 15 months in studies reported from the United States **(Bluestone and Klein; 1983).**

In the newborn infants otitis media may be an isolated infection or it may be associated with sepsis, pneumonia or meningitis. The incidence of otitis media in newborn is uncertain, but few studies suggest that the incidence is high in both normal infants and infants with underlying factors, such as prematurity that place them in intensive

care units. Approximately half of children at two months of age with otitis media with effusion were asymptomatic **(Marchant et al; 1984).**

Otitis media is very common in infants beyond the neonatal period (after 28 days of age) In the study of children in Boston, 9 percent had at least one episode of otitis media by 3 months of age, 25 percent had one or more episodes by six months of age, 65 percent experienced otitis media by 24 months of age. The highest age specific incidence for all episodes of acute otitis media (first and subsequent episodes) occured between 6 and 13 months of age **(Wright et al; 1985).**

Stangerup and Tos (1986) showed the incidence of acute otitis media to be about 22 percent in the first year 15 percent in the second year, 10 percent in the third and fourth years and 2 percent in the eight year. By the end of the third year of life, 50 percent of all children had had at least one episode of acute otitis media, by the age of nine year 15 percent had had one prevalence was about 25 percent during the first 5 years dropping to 7 percent during the eight and nine years. Most of attacks (80 percent) during the first 2 years were bilateral whereas after the sixth year 86 percent of children had experienced unilateral. A review of the causes to the ambulatory clinic of

the Boston city hospital in the fall of 1973 revealed that 19 percent of children between 4 and 24 months of age had otitis media **(Bluestone and Klein , 1988 d).**

Age at first episodes is an important predictor of recurrent otitis media. Breast feeding during the first year of life is associated with decreased risk for recurrent otitis media **(Wald et al, 1988).**

2 - Sex :

Males have more myringotomies and tympanoplasties than do females. A fact suggesting that severe infections of the middle ear may be more common among males **(Solomon and Harris; 1976).**

In most studies the incidence of acute episodes of otitis media was not significantly different in boys and girls. However in the Boston study males had significantly more single and recurrent (three or more) episodes. Finnish males had significantly more episodes than did females in eight communities studies in a one-year period **(Pukander et al; 1982).**

Also most studies show a greater frequency of acute otitis media among males (61 to 70 percent with otitis media with effusion **(Giebink; 1984).**

3 - Race :

The severity of middle ear infection has been noted in African children where perforated ear drums are common **(Dugdal et al; 1982).**

Studies of American Indians and Alskan and Canadian Eskimo indicated that, there is an extraordinary incidence of infection of the middle ear and that the disease is severe in these groups. Otorrhoea is frequent but chronic otitis and persistant effusion are uncommon **(Dever et al; 1985).**

4 - Seasonal Variations :

The seasonal incidence of infections of the middle ear paralles the seasonal variation of the upper respiratory tract infections. It increases during outbreaks of bacterial infections of the respiratory tract in children. These are most likely to occur in the winter and spring seasons **(Henderson et al; 1982).**

5 - Social and economic conditions :

Acute otitis media is one of the most common bacterial infections of childhood and give rise to medical and socio-economic problems. There is a strong relationship between ear diseases and poor social conditions. The specific reasons for the high incidence and severity of the disease were not identified but predisposing factors include,

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crowded living conditions. poor sanitation and inadequate medical care **(Teele et al; 1980)**

6 - Family history :

The risk of chronic otitis media with effusion was reported to be two times higher for children with a positive family history than for those with negative family history these reports supports the notion of a genetic background that may be more susceptible to otitis media **(Kraemer et al; 1983).**

Children with positive sibiling or parent history of otitis media had a higher incidence of the disease than did children from families with no histories of otitis media **(Visscher et al; 1984).**

7 - Allergy :

Recent studies suggest that the allergic or atopic child is at increased risk of otitis media **(Visscher et al; 1984).**

8 - Other high-risk factors :

Children with cleft palate, Downs syndrome,kartagener's (or immobile cilia) syndrome are universally considered a high risk group. For cleft palate and Down's syndrom, the cause of the high inicidence is the anatomic defects of the craniofacial structures which affect tubal function. In Kartagener's syndrome, the defective motility of the cilia

in the tubotympanum deprives the ear of mucociliary protection resulting in easy bacterial invasion **(Paradise et al; 1969 and Sando and Harada; 1981).**

Pathogenesis of otitis media

Several important factors play a role in the pathogenesis of otitis media These factors include: a high incidence of nasopharyngeal bacterial colonization, upper respiratory viral infection and immunoglobulins which are considered predisposing factors in the pathogenesis of otitis media **(Bluestone and Klein; 1983 and Giebink, 1984).**

(1) Role of bateria (Nasopharyngeal flora) :

The normal tubotympanal mucosa is protected by the mucociliary transportation system and by surface mucosal immunoglobulins (SIgA), that inhibit bacterial adhesion to the mucosal surfaces, therefore, For otitis media to occur three conditions must be met :(1)bacterial adherence to the nasopharynx.(2)bacterial entry to the tubotympanum and (3)bacterial replication in the tympanum. There is ample evidence suggesting that there is a higher incidence of bacterial colonization (pathogens) in the nasopharynx among patients with histories of acute otitis media (90 peresent) as well as chronic otitis media with effusion (80 percent) compared with low incidence (2 percent) of nasopharyngeal colonization of normal patients**(Lim and DeMaria; 1982)**

(2) Role of chlamydia :

Chlamydia trachomatis is the etiologic agent of a mild but prolonged pneumonitis in infants. C. trachomatis could

induce acute otitis media as reported by **Dawson et al (1967).** Where 11 of 17 adult volunteers during experiments designed to induce experimental eye infections, developed otitis media.

Subsequently it was shown by **Schachter et al 1979 and Tipple et al; 1979** that children with chlamydial pneumonia also have otitis media.

(3) Role of viral infection :

The upper respiratory viral infection has been associated with the pathogenesis of otitis media and it was found a close association between repiratory viruses (e.g respiratory syncytial virus influenza A and adenovirus) and acute otitis media **(Henderson et al; 1982).**

Furtheremore, **Yamagiuchi et al (1984)** demonstrated positive secretory viral antibodies (SIgA) to respiratory viruses in 24 percent of the chronic middle ear effusions studied indicating that these resiratory viruses may have an immediate or well as a long term effect on the pathogenesis of otitis media. Experimental evidence supporting the role of influenza A virus in the pathogenesis of acute otitis media was presented when the nasopharynges of chinchillas were instilled with Strep. pneumonia together with influenza A virus.the incidence was 67 percent. The fcllow-

up investigation documented that influenza A virus caused immediate destruction of the tubal mucosal lining, therefore, it appears that an individual whose tubotympanun suffers from influenza A infection becomes highly vulnerable to a secondary bacterial infection from the nasopharynx **(Giebink et al; 1987).**

(4) Role of immunoglobulins :

An immune response reflected in a rise in specific serum antibody occurs in some children after acute infection of the middle ear **(Howie et al; 1973).**

(a) Immunoglobulin A (IgA) :

Secretory component of IgA is a nonimmune glycoprotein formed by the local epithelial cells that exists either in a bound state with immunoglobulin A or in a free state in effusion fluids. The production of (SIgA) begins when an antigen is presented to immunocompetent cells in the mucosa. IgA is a predominant immunoglobulin in middle ear effusions **(Magi et al, 1973).**

Specific IgA can interfere with adhesion of bacteria to mucous membrane and can neutralize viruses **(Meurman et al, 1980).**

(b) Immunoglobulin M (IgM) :

IgM is produced in response to primary exposure to a microbial antigen and is present in the middle ear effusions

of patients with both acute and chronic otitis media with effusion but concentrations are lower than in serum **(Bluestone and Klein, 1988 g).**

(c) Immunoglobulin G (IgG) :

It is present in the effusions of the patients with both acute & chronic otitis media in a concentration suggesting that its local development occurs in the middle ear **(Freijd and Coworkers; 1985).**

The normal flora of the upper respiratory tract :

The upper part of the respiratory tract includes the anterior and posterior nares and the nasopharynx. The bacterial flora of the nasal-passges differ in several respects from that of the nasopharynx. Diphtheriod bacill and staphylococci-both staph - aureus and albus are far more frequent in the nose than in the nasopharynx whereas strept viridans, indifferent streptococci and gram-negative cocci of the N. subflava type are far less frequent. These non-haemolytic-streptococci and gram-negative cocci appear to constitute the basal of the nasopharynx in most communities **(Noble et al; 1928).**

In the nasopharynx. Pneumococci and to a slight extent H. influenzae were more frequently in cold, damp seasons than in the dry hot periods. In the summer months the organisms were confined largely to the nasopharynx. but in late winter and early spring they tend to colonize downwards into the trachea and forward into the nasal-cavity **(Box et al; 1961).**

Studies showed that one person may carry one or more given types of pneumococcus in the nasopharynx over a period of months and years **(Smillie et al; 1943).**

The nasopharynx is virtually sterile at birth. **Gundel and Schwartz(1932)** record colonization in the first 2 days

of life by strept. viridans gram-negative cocci of the subflava type and occasionally diphtheroid and coliform. bacili. Pneumococci and haemophilus bacilli appeared by the 2nd or 3rd day and appear to establish themselves more frequently than streptococci in the infant's upper respiratory tract **(Box et al; 1961).**

The microbiology of otitis media :

Abd-Elrehim et al; 1988 showed that the microbiological study of otitis media for some children in Benha Faculty of Medicine was presented in the following table:

Organism.	Percentage
- Staphylococcus aureus	32%
- Pneumococcus	20%
- Proteus species	16%
- E. Coli	16%
- Strept. Pyogenes	8%
- Anaerobic Streptococci	8%
- Diphtheroids	8%
- Bacteroides fragilis	4%
- Klebsiella	4%
- Staph epidermis	4%

S. aureus is the commonest cause of acute otitis media (32%), followed by S. pneumoniae (20%), and other organisms as show in the previous table.

The results of bacteriological studies of otitis media in children from Sweden, Finland and United States during the period 1952 to 1987 showed that S. pneumoniae and H. influenzae are the most frequent agents in all age groups. B. catarrhalis, group A streptococci. S. aureus and gram-negative enteric bacilli are less frequent causes of otitis media **(Bluestone and Klein; 1988 e).**

(I) Acute otitis media :

The most common bacterial pathogens recovered from patients with acute otits media and may be isolated from middle ear fluid according to **Shurin et al; 1983 are :**

i- Streptococcus Pneumoniae :

The pneumococcus is a part of the normal flora of the upper respiratory tract **(Loda et al; 1975 and Klein; 1981).** It is one of the major causes of acute otitis media. among all age groups **(Riding et al; 1978).** S. pneumoniae Serotypes 19, 24, 6 14, 3, 18, 4, 15, 9, 7 and 1 are responsible for about 25 percent of otitis media among children **(Gray et al; 1979).**

Gray et al; (1979) have correlated the occurence of acute otitis media with nasopharyngeal acquistion of new serotypes of S. pneumoniae. A review of several series by **Schwartz; 1981** showed that the incidence of pneumococcal otitis media may range from 20 to 45 percent of all cases.

The bacteria Cultured from middle ear effusions in children with acute otitis media have been shown to be the same found in the nasopharynx and S. pneumoniae was cultured from approximately 30 percent of the effusions and is the most common causative agent in all age groups **(Rohn et al; 1985).**

ii- Branhamella catarrhalis :

B-catarrhalis formerly named Neisseria catarrhalis is a common commensal organism of the human oral cavity **(Catlin; 1970).** Although the clinical significance of B-catarrhalis has been quistioned in the aetiology of acute otitis media suffecient evidence exists to implicate it as a primary pathogen in 5-10 percent of cases. **(Howie et al;1970).**

In a separate report by **Kovatch et al (1983)** this incidence was found to be 22 percent of 146 infants and children from one private pediatric practic in suburban Pittsburgh.

Shurin et al; (1983) also reported the incidence of this bacterium to be 27 percent in Cleveland between 1980-1982 while was significantly greater than the 6 percent recovered the year before. However the results were shown by **Van Hare et al (1987)** which suggest an increasing rate of isolation of B-cattarrhalis approaching 30 percent of cases of acute otitis media.

III- Haemophilus Influenzae:

The incedence of H-influenzae causing acute otitis media varies but appears to be responsible for 20-30 percent of overall infection **(Halsted et al; 1968 and Klein 1980).**

Most H. influenzae isolated from middle ear fluid are nontypable and some investigators have reported as few as 9 percent type b. H. influenzae isolates from patients with acute otitis media **(Kamme et al; 1971).**

About 25 percent of children with otitis media due to H. influenzae type b have concomitant bacteremia or meningitis **(Harding et al; 1973).**

Previously thought to be limited to preschool children. H. influenzae now is known to cause otitis media in older children and adolescents **(Schwartz and Rodriguez; 1981).**

Long et al; (1983) described a significant assocaition between the recovery of abundant H. influenzae(> 50 percent total colony count) from the nasopharynx and bacteriologically confirmed otitis media .Thirty percent or more of H. influenzae isolated from middle ear fluid produce beta-lactamase which has important implications in the therapy of acute otitis media **(Bluestone; 1988).**

The occurence of purulent conjuntivitis in association with acute otitis media (conjunctivitis-otitis syndrome) ususally attributable to nontypable H influenzae **(Bodor; 1989).**

iv- Staphylococcus aureus :

S. aureus is an inhabitant of the normal skin and may be isolated as a contaminant from the middle ear if the external canal is not properly cleaned., With proper preparation of the external canal, staph. aureus has been implicated as a pathogen in acute otitis media in 2-5 percent of cases. **Brook (1979)** showed 79 isolates of staph. aureus from the ear in 186 patients with acute otitis media. Also **(Brook and Schwartz 1981)** showed 15 isolates of staph. aureus from the ear in 28 patients with acute otitis media.

Role of viruses :

A virus was isolated from only 29 of 663 (4.4 percent) specimens obtained by tympanocentesis **(Klein and teele; 1976).**

Recent studies have focused on the role of viruses, Mycoplasma and chlamydia in acute otitis media. Epidemiologic data support an association between viral respiratory infection and the occurence of acute otitis media infection with respiratory syncytial virus, influenza viruses and adenoviruses was associated with a great risk of otitis media than was infection with other viruses. In contrast to this epidemiologic association is the low viral isolation rate from middle ear fluid in patients with otitis media **(Henderson et al; 1988).**

Isolates have included respiratory syncytial virus, influenza viruises adenoviruses, parainfluenza viruses, enteroviruses and rhinoviruses,Concomitant isolation of viral and bacterial pathogens from middle ear fluid appears to be common **(Chonmaitree et al., 1986).**

II- **Otitis media with effusion :**

Otitis media with effusion has been assumed to be sterile since several reports describe unsuccesful attempts to culture bacteria. However a study was conducted in Pittsburgh by **Riding et al. (1978)** of 179 children aged 1 to 16 years who had chronic middle ear effusions, bacteria were cultured from 86 (48 percent) chronic middle ear effusions. Bacteria were present in serous and mucoid effusions as well as the purulent effusions. The research centre of Pittsiburgh from 1980 - 1985 in a study on children suffering from chronic otitis media with effusion found that from approximately 4500 middle ear aspirates, about two-thirds had bacteria isolated, of the one third that were considered to be pathogens, the most common bacteria were H. influenzae, B-cattarrhalis and S. pneumoniae, which are the common pathogens found in middle ear aspirates from children with acute otitis media. In addition Staph. epidermidis was cultured from many middle ear effusions.

III- Chronic suppurative otitis media :

Chronic suppurative otitis media develops from a chronic bacterial infection. However the bacteria that caused the initial episode of acute otitis media with perforation are usually not those isolated from the chronic discharge when there is chronic infection in the middle ear and mastoid. The bacteriology of chronic suppurative otitis media associated with cholesteatoma has been reported by **Jokipii et al. (1977)** and by **Brook (1981)** which illustrated that the most common aerobic organisms isolated were P. aeruginosa and S. aureus and the most frequent ananaerobic organisms were bacteriodes species, Peptostreptococcus species and Peptococcus species.

IV- Otitis media in the newborn infants :

the unexplained bacteriology of otitis media in the newborn infants are available from aspiration of middle ear fluids of 169 neonates with otitis media. S. pneumoniae and H. influenzae are the bacteria isolated most frequently in the very young as in older infants and children. However, organisms associated with local and septic infection in the newborn infant are group B-streptococci; S. aureus and gram-negative enteric bacilli. These organisms are the important pathogens in the newborn infant within a weak after birth or from older

infants who have remained in the nursery because of risk features (low birth weight or prematures) or disease (respiratory distress syndrome **(Bland, 1972; Tetzlaff et al., 1977; Berman et al., 1978 and Shurin et al., 1978).**

Fungus infection of the ear :

Otitis externa is a disorder that can affect the external auditory meatus, as well as the auricle of the ear. The condition can be due to idiopathic dermatitis, trauma, allergy or infection by bacteria, fungi or viruses mycotic otitis externa, otomycosis can be a localized or generalized infection, localized otomycosis are confined to the external auditory canal and may occur as a primary infection or secondary to a bacterial infection or other cause of otitis externa **(Senturia and Lucente, 1980).** This form of otomycosis is most frequently caused by saprophytic fungi especially Aspergillus spp. and Candida spp. **(Maher et al., 1988).**

Generalized infections affect not only the auditory canal but also adjacent areas. These infections are most commonly an extension of an infection of the adjacent skin by dermatophytic fungi such as Trichophyton spp. or Microsporum spp. but it also be an extension of either tinea versicolor caused by Malassezia furfur or cutaneous candidiasis. Primary infections of the inner or middle

ear have not been reported but not occaisions, extension from an adjacent serious infection such as orbital-rhino-cerebral zygomycosis or haematogenous spread of a disseminated systemic fungal infection such as blastomycosis or cryptococcosis have occured **(Meyerhoff et al., 1979).**

BACTEREMIC COMPLICATION OF OTITIS MEDIA

The majority of children with otitis media and bacteremia due to H. influenzae type b were unimproved or caused a new focus of infection. **Harding et al. (1972)** studied 300 children with acute otitis media due to H. influenzae. 28 were due to type b and the remainder were due to non typable strains. Four children with acute otitis media due to type b H. influenza had sepsis or meningitis. Thus the child with acute otitis media due to type b is at increased risk for more severe disease.

In a study of febrile children less than 2 years of age. **Teele et al. (1975)** found that an age between 7 and 18 months, a temperature > 38.8°C and diagnosis of pneumonia or upper respiratory infection or fever of unknown origin, had increased specificity for bacteremia especially pneumococcal bacteremia. In studies of bacteremia in patients with otitis media in urban emergency departments in 1975 **Teele et al.** also reported that 1.5% of children less than 8 years of age with otitis media and temperatures greater than 38.9°C were bacteremic and in 1976 **McCarthy et al.** described an incidence of 5.8%.

In 1977 Bratton et al. reported 1 case of meningitis among 16 patients with otitis media and pneumococcal bacteremia which found that the outcome of unsuspected

bacteremia due to H. influenza differed in some respects from outcome of unsuspected pneumococcaemia previously reported from this institution.

In an office setting in 1982 **Schwartz and Weintzen** found a 5.8% incidence of bacteremia in febrile infants with otitis media. Otitis media is the most common focal bacterial infection of febrile young patients and accounts for a large percentage of both office and emergency visits **(Teele et al., 1983).**

Bacteremia associated with otitis media may cause suppurative complications. **In (1983) Dashefsky et al.** reported that meningitis occured in 1 of 16 patients with otitis media and unsuspected meningococcaemia. **In 1987 Anderson et al.** reported that 4 of 20 children with otitis media and H. influenzae bacteremia subsequently developed meninigitis.

MICROBIAL DIAGNOSIS OF OTITIS MEDIA

The correlation between results of bacterial cultures of the nasopharynx or the oropharynx and those of cultures of middle ear fluids is poor. The poor correlation occurs because of the frequency of colonization of the upper respiratory tract with organisms of known pathogenicity for the middle ear and less commonly because of abscence in cultures of the oropharynx or nasopharynx, of the pathogen responsible for infection of the middle ear. Thus cultures of the upper respiratory tract are of limited value in specific bacteriologic diagnosis of otitis media. Specific microbiologic diagnosis is acheived by culture of middle ear fluid obtained by needle aspiration through the intact tympanic membrane. If the patient is toxic or has a localized infection elsewhere, culture of the blood or the focus of infection should be performed. Bacteremia is rarely associated with otitis media due to nontypable strains of H. influenzae, uncommonly associated with otitis media due to S. pneumoniae, but frequently associated with otitis media due to type b strains of H. influenzae **(Harding, et al., 1973).**

Diagnostic aspiration of the middle ear :

When the diagnosis of acute otitis media is doubt or when determination of the etiologic agent is desirable,

aspiration of the middle ear should be performed. Indications for tympanocentesis or myringotomy include :

1- Otitis media in patients who are seriouly ill or appear toxic.
2- Unsatisfactory response to antimicrobial therapy.
3- Onset of otitis media in a patient who is receiving antimicrobial agents.
4- Prescence of suppurative complications.
5- Otitis media in the newborn, the very young infant or in the immunologically defecient patient, in each of whom an unusual organism be suspected. Following tympanocentesis the effusion caught in the syringe or collection trap is sent to the laboratory for culture and sensetivity **(Feingold et al., 1966).**

Nasopharyngeal culture :

In an attempt to identify the causative organism in a child with acute otitis media, the results of a nasopharyngeal culture could be less traumatic than a tympanocentesis or myringotomy. The concept is an attractive one. Since the bacteria found in middle ear aspirates are the same type found in the nasopharynx of children with acute otitis media. However the correlation between the organisms found in the middle ear and the nasopharynx has proven by **Schwartz et al. (1979)** which reported a technique

that improved correlation of organisms isolated by the nasopharyngeal culture with bacteria identified by culture of middle ear fluid. The method used involved immediate planting of the nasopharyngeal swab on solid media and a semiquantitative estimation of colonies growing on culture plates.

Antibiotic resistance :

Factors that determine the susceptibility and resistance of micro organisms to antimicrobial agents :

There are multiple factors that determine the relative antimicrobial activity of a drug against a specific micro-organism. For an antibiotic to be effective it must bind to target sites of action on or in the bacterial cell. Bacteria can develop resistance to specific antimicrobial agents by preventing their access to these sites **(Vaudaux, 1981).**

Some bacteria produce enzymes that reside at or within the cell surface that inactivate the drug, others possess impermeable cell membranes that prevent influx of the drug. Hydrophilic antibiotics traverse the outer membrane of microbial cells via aquous channels (Pores) comprised of specific proteins (Porins). Bacteria defecient in these channels can be resistant to such drugs **(Jaffe et al., 1983).**

Since many antibiotics are organic acids. Their penetration may be pH dependant **(Bryant, 1987).**

Acquired resistance to antimicrobial agents :

The development of resistance to antibiotics usually involves a stable genetic change, heritable from generation to generation. Any of the mechanisms that result in

alteration of bacterial genetic composition can operate. While mutation is frequently the cause, resistance to antimicrobial agents may be acquired through transfer of genetic material from one bacterium to another by transduction, transformation or conjugation **(Datta and Nugent, 1984).**

The mechanism of drug resistance varies from micro-organism to micro-organism and from drug to drug. For example over 80% of both hospital and community acquired strains of S. aureus that were resitant to penicillin G appeared shortly after this antibiotic was introduced, This resistance was the result of elaboration by the bacteria of beta-lactamase, an enzyme that hydrolyzes and inactivates penicillin G. In recent years other strains of S. aureus have appeared that are highly resistant to all beta-lactam antibiotics. These is called methicillin resistant organisms are prominent in hospitals especially in intensive care units where antibiotic use is great **(Chambers, 1988).**

Otitis media and antimicrobial agent

Choice of antimicrobial agent :

Amoxicillin or ampicillin is the currently preferred drug for initial empiric treatment of otitis media, since both are active both in vivo and in vitro against S. pneumoniae, H. influenzae and B. catarrhalis. Other regimens that are satisfactory include amoxicillin-clavulanate trimethoprim-sulphamethoxazole, cefaclor and combinations of a sulphonamide with benzathine penicillin G (adminstered by the intramuscular route as a single injection) oral penicillin G or V. or erythromycin. For the child who is allergic to penicillin, trimethoprim-sulphamethoxazole and erythromycin or clindamycin combined with a sulphonamide provide equivalent antimicrobial coverage. If the child has acute otitis media with otorrhoea or tympanocentesis was initially performed. Gram's stain, culture and susceptibility studies of the causative organism will provide more precise selection of an antimicrobial agent **(Bluestone and Klein, 1988 i).**

Duration of therapy :

Physicians must relay on empirically derived schedules of therapy to plan drug regimens that lead to rapid and complete resolution of disease with minimal risk of clinical or microbiological faliure or drug toxicity. The dosage schedules for a 10-day course on the basis of

currently available data. Other dosage schedules, longer or shorter than the traditional 10 to 14-day course may be as good, if not better. Studies compared the results of 3 and 10-day courses of amoxicillin **(Chaput de Saintonge et al., 1982)**, treatment with penicillin V adminstered for 5 or 10 days **(Ingvarsson, and Lundgren, 1982)** and with penicillin V for two or seven days.**(Meilstrup-Larsen et al. 1983)**. In each study, the shorter course was similar in clinical results to the longer course. Methodological problems including absence of microbiological diagnosis limit the validity of the results.

Specific recommendation :

In formulating specific recommendations, particular attension should be focused on studies that have assessed antibiotic activity within the middle ear. Antibiotics have been measured within purulent material obtained by needle tympanocentesis from children receiving varying doses of different antibiotics. The concentrations of various penicillins within the middle ear have been assessed. In general, oral penicillin G and V acheive concentrations within the middle ear sufficient to inhibit most strains of S. pneumoniae, S. pyogenes and penicillin sensitive S. aureus. Concentrations of oral penicillin however exceed the minimum inhibitory concentration (MIC) of H. influenzae in only about 50 percent of cases.Although

administration of aqueous penicillin and even procaine penicillin, produces middle ear concentrations of penicillin sufficient to inhibit gram-positive organisms, administration of benzathine penicillin (Bicillin) does not. Thus clinical studies of comparisons of single dose benzathine penicillin with other modes of therapy have been flowed at their inception by use of a form of penicillin that is inappropriate for therapy of otitis media **(Silverstein et al., 1966).**

Administration of ampicillin at 50 to 75 mg/kg/24 hr. has been followed by middle ear concentrations of 1.6 to 12 ug/ml. These levels exceed the MIC of the usual gram positive organisms, other than penicillin resistant, S. aureus and equal or exceed the MIC of most strains of H. influenzae. Amoxicillin, a congener of ampicillin has been shown to acheive high levels in middle ear fluid following a single oral dose of 15 mg/kg. Erythromycin administration also is followed by adequate levels of antibiotic within the middle ear for treatment of S. pneumoniae, S. pyogenes and S. aureus infection but may be inadequate for many cases of H. influenzae infection **(Krause et al., 1982).**

Penetration of sulpha into the middle ear fluid was studied by **Krause and associates (1982)** and concentrations were generally found to exceed the MIC of strains of

S. pneumoniae and S. pyogenes and ampicillin-sensetive and ampicillin resistant strain of H. influenzae. The aminopenicillins (ampicillin, amoxicillin, cyclocillin and bacampicillin) have been used widely in the therapy of acute otitis media. These agents are active in vitro against most of the usual pathogenes with the exception of B-lactamase-producing strains of H. influenzae and B-catarrhalis. After equivalent oral doses, mean peak serum concentrations and bioavailability of amoxicillin, cyclocillin and bacampicillin are superior to that of ampicillin.The addition of B-lactamase inhibitor (Potassium clavulanate) to amoxicillin expands the drug's spectrum of activity to include the B-lactamase-producing strains of H. influenzae, B-catarrhalis and S. aureus without altering its pharmacokinetic properties. All these agents have been found effective and safe in the treatment of acute otitis media **(McCracken, 1985).**

Clinical course :

With appropriate antimicrobial therapy, most children with acute bacterial otitis media are significantly improved within 48 to 72 hours. The physician should be in contact with the patient to acertain that improvement has occured, children whose course has become worse should be re-examined by the clinician, since a suppurative complication may have developed. At this stage a tympanocentesis for

microbiological diagnosis and possibly myringotomy for drainage is the most effective management option. If there is persistance or recurrence of otalgia or fever or both then the child should also be re-examined before the completion of the antibiotic course children may be re-examined at the end of the course of antibiotic therapy after 10 to 14 days **(Bluestone and Klein, 1988 i).**

MATERIALS AND METHODS

MATERIALS AND METHODS

Patients :

This work was done from September 1992 to January 1993 75 cases were selected after diagnosis by Ear, Nose and Throat specialists of Al-Ahrar Outpatient Clinic of Zagazig General Hospital. 75 children were included aged from 2 to 12 years from both sexes with temperatures of 39°C or greater. The clinical diagnosis revealed cases of acute suppurative otitis media or acute otitis media on top of chronic. No chest, heart nor throat symptoms or signs of infectious diseases were revealed.

Materials :

Flexible throat absorbent sterile cotton swabs (Dansu A/S) Diphasic media for collection of blood samples. These media were prepared by pouring about 30 ml sterile melted nutrient agar under aseptic precautions into sterile glass bottle with a flat side (Casdanida bootle). Covered by sterile rubber stopper and left to solidify in a slope position, then about 30 ml of sterile nutrient broth were added under complete aseptic precausions.

- Blood agar (Nutrient agar with 5-10% sheep RBCs).
- Nutrient agar (Difco).
- Chocolate agar (Heated blood agar) **(Cruickshank, 1989).**
- Gram's stain

blood containing 0.33% trisodium citrate discarded from the blood transfusion service.

- Optochin sensitivity discs (Oxoid).
- 10% sodium deoxycholate solution (Sigma).
- 1% solution of tetramyethylene-P-phenylene-diamine dihydrochloride-filter paper strips were soaked with a freshly prepared solution and freeze .
- 5-10% of aquous solution of glucose, maltose, lactose and sucrose.
- Filter paper strips soaked with penicillin 25% dilution and also soaked with pH indicator dye **(Cruickshank, 1989).**
- Antibiotic discs : (Oxoid) as shown in the following table :

Antibiotic zone inhibition measurement (BBL)

Antibiotic	Concentration	Zone diameter		
		Resistant	Intermediate	Sensitive
1- Rifampicin	30	11	12-13	19
2- Chloramphenicol	30	12	13-17	18
3- Penicillin G	10	11	12-21	22
4- Velosef	30	14	15-17	18
5- Ampicillin	10	20	21-28	29
6- Erythromycin	15	13	14-17	18
7- Sulphonamides	250-300	12	13-16	17

- Whatman No. 1 filter paper discs (Sigma).
- Chloramphenicol sensitive E. coli obtained by culturing

a sample of stool . The resulting E. coli that give a high sensitivity to chloramphenicol antibiotic sensitivity discs were used (Slack et al., 1977).

- Chloramphenicol vial (Hoechst).
- Ampicillin vial (Hoechst).
- Nutrient broth (Oxoid).
- Tryptcase soya broth (Oxoid).

Methods

(I) Sample collection :

Iwo samples were collected from every patient.

1- Nasopharyngeal swab sample :

The child sit in front of a good light opening his mouth, the flexible swab is introduced and rubbed against the nasopharyngeal wall. The swab was withdrawn carefuly without touching the tongue. The nasopharyngeal swab samples were cultivated on chocolate agar plates which were incubated at 37°C in 5-10% Co2 in candel jar for 24 hours, also the samples were cultivated on blood agar.

2- Blood samples :

3-8 ml of blood were withdrawn under complete aseptic precautions then the blood samples were incubated in diphasic media and incubated for 24-48 hrs. at 37°C for up to 2 weeks then the resulting colonies were cultivated on chocolate agar plates which were incubated at 37°C in 5-10% Co_2 in Candel jar for 24 hours and also the samples were cultivated on blood agar plates.

(II) Films :

Stained with Gram's were prepared from the growing colonies on diphasic media and nasopharyngeal swab plates.

(III) Biochemical reactions :

1- Identification of suspected staphylococci by coagulase test which was done by two methods :

a- The slide method :

- A drop of saline solution or water was put on a clean microscope slide. One or two colonies were emulsified it must give a smooth milky suspension.
- A drop of concentrated plasma-warmed to room temperature was put on a drop of bacterial suspension on the slide.

  Positive reaction gives coarse clumbing visible to the naked eye within 5-10 seconds **(Cruickshank, 1989).**

b- Tube method :

- 1 in 10 dilution of the plasma in saline solution was prepared and 0.5 ml of the diluted plasma was placed in a small tube.
- 0.1 ml of 18-24 hrs. broth culture of the organism was inoculated into the diluted plasma.
- The tube was incubated at 37°C in a waterbath and examined for coagulation at 1,3 and 6 h. and again if still negative after standing overnight at room temperature.

2- Sugar fermentation test :

Gram positive cocci were subjected to sugar fermentation of glucose, lactose maltose, mannite and sucrose.

A loopful of the colony was transferred to 5% of aquous solution of sugars and 0.005% andrad's indicator and inverted Durham's tubes and incubate for 24 hours at 37°C **(Cruickshank, 1989).**

3- <u>Identification of suspected S. pneumonia by :</u>

a- Optochin sensitivity test :

- A paper disc-containing 5 ug of optochin (ethyl-hydrocuprein) was placed on an area of blood agar plate which has been spread confluentyl with material from the growth or a light broth suspension of pneumococcus like colonies from the primary diagnostic plate.
- Incubation at 37°C was done in air with 5-10% Co 2 for 24 hours where a growth of pneumococcus will be inhibited in a zone extending radially for at least 5 mm from the margin of the disc **(Cruickshank, 1989).**

b- Bile solubility test :

It was done by touching a suspected pneumococcal colony with a loopful of 2% sodium deoxycholate solution at pH 7 and incubate the plate for 30 minutes at 37°C where colonies of pneumococcus disappear leaving an area of alpha-haemolysis on blood agar **(Cruickshank, 1989).**

4- Identification of suspected B-catarrhalis by :

Oxidase test : Using a platinum bacteriological loop, a portion of non haemolytic white or greyish convex colony was transferred to one strip of the filter paper impregnated with the oxidase reagent. An immediate purple colour (within 30 seconds indicate Neisseria oxidase-positive) **(Cruickshank, 1989).**

(IV) Antibiotic sensitivity testing :

1- Broth cultures were prepared from the resulting colonies after 18 hours incubation.
2- The young broth cultures were adjusted by McFerland turbidity tubes to be No. 5.
3- 0.5 ml of broth culture was poured on the plate.
4- The plate was left to dry for 5 minutes and the antibiotic discs were distributed at a distance of 2 cm apart.
5- The sensitivity was recorded according to the reference table **(Cruickshank, 1989).**

(VI) Chloramphenicol resistant strains testing (Picture 1) :

1- A plate of nutrient agar was floaded with a barely turbid broth culture of chloramphenicol-sensitive E. coli and left to dry for 5 minutes.
2- Three discs of Whatman No. 1 were placed on the plate.

3- A heavy inoculum of the tested organism was applied over the surface of one of the discs.

4- The two other discs were similarly inoculated with a stock strain of S. aureus that was chloramphenicol sensitive.

5- Stock strain is prepared by subculturing the isolated strains of S. aureus and the resulting colonies were tested for chloramphenicol sensitivity and those giving a high sensitivity were used as a stock strain.

6- After 5 minutes chloramphenicol discs (Oxoid) were placed centrally on the disc inoculated with the test organism and on the disc inoculated with the control organism.

N.B :

The chloramphenicol thus has to pass through the filter paper disc to reach the culture medium, it will fail to do so in an active state if the filter paper disc has been inoculated with a strain that is producing chloramphenicol acetyl transferase **(Slack et al., 1977).**

7- The plate was incubated at 37°C for 4 hours where a visible growth of E. coli with a zone of inhibition on the disc inoculated with the chloramphenicol sensitive strain was obtained, and the zone of inhibition is much reduced or absent around a chloramphenicol disc that has been placed on the filter paper disc

inoculated with a strain that is inactivating chloramphenicol.

(VII) Determination of minimum inhibitory and bactericidal concentrations :

1- The ampicillin and chloramphenicol antibiotics were dissolved in sterile saline to give working solution containing 100 ug/ml.

2- A ten clear sterile cotton plugged test tubes of small size were selected and marked from 1 to 10.

3- Under aseptic technique, a ten fold serial dilution method was done **(Cruickshank, 1989)** and 1 ml of sterile nutrient or tryptcase soya broth was pipette into each tube from the second through the tenth of the series.

4- Into the first and second tubes 1 ml of the working solution of the antibiotic was pipette and contents of the second tube were mixed well and 1 ml was transferred to tube No. 3. Mixed well and 1 ml was transferred to tube No. 4. This procedure was continued to tube No. 9 and 1 ml of tube No. 9 was discarded and tube No. 10 was not received antibiotic and serves as the control.

5- To all tubes is, then, added 0.1 ml of an inoculum prepared by making 1/100 dilution in broth of an overnight broth culture of the organism to be tested.

6- The series is incubated at 37°C and is examined macroscopically for evidence of growth. The tubes should be incubated as long as it is necessary for the control tube to show turbid growth usually 18 to 24 hours is the optimal time. The lowest concentration of the antibiotic which prevents growth (manifested by turbidity) is taken as the minimal inhibitory concentration (MIC) of the antibiotic and is expressed as micrograms per milliliter**(Cruickshank, 1989).**

7- The test can be extended to measure the minimum bactericidal concentration (MBC) of the antibiotic by preparing subcultures from each test culture and noteing the smallest concentration of the antibiotic from which no growth is obtained in the subculture **(Cruickshank, 1989).**

(VIII) **Determination of kinetic killing curve (Picture 2):**

1- An overnight culture for each isolated type of organisms was prepared and this represents 100% concentration of the organism containing 10^7 of organisms per ml then take 1 ml of this bacterial concentration in a test tube and add 9 ml broth to make 10% dilution and continue this procedure to reach 0.001 dilution of the organisms representing 10^2 number of organisms per ml.

2- Make a reference plate subculture by using chocolate

agar plate for reading the test by culturing each above concentration of the tested organism on a straigt line on this plate then incubated for 24 hours at 37 °C and the resulting number of colonies in each line represents the standard number of organism in each dilution i.e. from 100% concentration to 0.001 concentration of organisms.

3- Add 1 ml of other 50 ug/ml of ampicillin or 100 ug/ml of chloramphenicol each by itself then both are combined to 1 ml of and overnight broth culture of each isolated type of organisms and incubate for 2 hours then subculture and incubated at 37 °C for 24 hours. This procedure is done for the incubated organism with the antibiotic at 4 hrs, 6 hrs. and 48 hrs. incubation and these subcultures then read by the reference plate.

4- A curve was done for each test strain by plotting time against concentration of the organism **(Thornsberry et al., 1983).**

STATISTICS

The data were presented and analysed according to standerdized statistical methods of presentation **(Armitage, 1983)**. Different methods were used as follows :

(I) Graphical presentation method :

As line graphs and Bar graphs which include multiple bar graphs or charts which include more than one group of data, each bar of each group is identified by shading or colouring.

(II) Tests of significance : for qualitative variables :

1- Chi Square test :

Compute $X^2 = \sum \frac{(O-E)^2}{E}$

where O is observed figures.

E, Expected : $\frac{\text{raw total X column total}}{\text{Grand total}}$

obtain the significant X^2 from table of X^2 according to the degree of freedom (df) **(Armitage, 1983).**

2- Z - test :

$$Z = \frac{P_1 - P_2}{\sqrt{\frac{P_1\,(100-P_1)}{n_1} + \frac{P_2(100-P_2)}{n_2}}}$$

P_1 = % of variable I.
P_2 = % of variable II.
n_1 = Variable I
n_2 = Variable II **(Armitage, 1983).**