

INTROCUCTION

Bacterial meningitis is an acute life threatening illness caused by invading bacteria that elicit inflammatory response in the leptomeninges (Pia matter and arachnoid of the brain and spinal cord). Early diagnosis and rapid institution of specific antibiotic therapy are essential steps to manage this problem (Hoffman, 1981). The incidence of meningitis varies greatly with the age but it is common in infancy and childhood. It occurs either as primary disease or secondary to disease in other parts of the body (Topely and Wilson 1975).

Braude (1981) classified leptomeningitis to the following types :-

- Purulent bacterial meningitis .
- Tuberculous meningitis.
- Viral meningitis .
- Cryptococcal meningitis .
- Coccidioidal meningitis.

Purulent bacterial meningitis is a syndrome that may be caused by different pathogenic organisms . According to Hoffman, (1981) the most common causes of acute bacterial meningitis in children were Nisseria meningitidis

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Haemophilus influenzae type b and Streptococcus pneumoniae, other uncommon causative agents were listeria monocytogenes, Staphylococcus, Beta haemolytic Streptococcus Pasturella multocida, E. Coli and other Gram negative Bacilli .

George (1976) explained the different routes of infection and revealed the following possible routes :-

- 1- Direct extension from the nose, ear and paranasal sinuses, through bone lymphatic, vascular channels or along the perineural sheath of olfactory nerves.
- 2- Haematogenous route during the course of septicemia is the most common route of infection. The primary foci of infection may be the nasopharynx, skin, lung heart, gastrointestinal or genitourinary tract, umbilical stump or elsewhere (Michael, 1983). Usually microorganisms present in the circulation experience a great difficulty in entering the C.N.S. This is largely because of the so called blood brain barrier (Wistreich and Leckman, 1980).
- 3- Penetrating injuries and primary infections involving the skull and spinal column.
- 4- Infection through lumbar puncture and neurosurgical procedures.

Diagnosis of acute bacterial meningitis :-

Michael, (1983) described the clinical picture of acute bacterial meningitis which is characterized by fever, headache, nuchal rigidity, signs of cerebral dysfunction, shaking chills, profuse sweats, weakness, anorexia, nausea, vomiting, myalgias of lower extremities or back (especially with meningococcal disease) and photophobia. Neck stiffness may be subtle or marked and accompanied by Brudzinski's and Kernig's signs. A rash develops in approximately 50 per cent of meningococcal infections.

The classic findings may be less apparent in several clinical cases. Neonates usually demonstrate neither nuchal rigidity nor elevation of temperature. The only clues are restlessness, high-pitched crying, fretfulness and refusal to feed.

Examination of the CSF is essential for definitive diagnosis of bacterial meningitis. Total and differential number of white blood cells should be encountered. Turbidity may result from presence of large number of leukocytes or bacteria and varies from slight opalescence, (typical in tuberculous meningitis) to grossly purulent appearance

in pyogenic meningitis (Kreig 1969). CSF are examined by Gram-stain for bacteria and keyoun stain for mycobacteria. It should be cultured on blood agar plate, chocolate agar plate and leventhal medium (Minear and Edman, 1978). The organisms are likely to be seen and recognized in over 70 % of CSF specimens by culture (Swartz and Dodge 1965) . Protein, glucose and chloride are estimated.

Quellung and agglutination reactions can provide immediate identification of various organisms if they are visible on smear and if appropriate type specific antisera are available. Countercurrent immuno electrophoresis has proved a useful technique for the rapid diagnosis of bacterial meningitis due to Haemophilus influenzae type b , Streptococcus pneumoniae and Neisseria meningitidis group A, C and D (Hoffman 1981).

AIM OF WORK

Bacterial meningitis in children is still encountered frequently despite the availability of chemotherapeutic agents. Although the number of reported fatalities from many infectious diseases has decreased by 10- 20 folds since 1932. The reported death from bacterial meningitis have decreased by only one half. (Nelson and Victor (1983)).

The aim of this present work is to find out the bacterial causes of acute meningitis in children who were admitted to Benha Fever Hospital and Benha University Hospital between September 1983 and February , 1984 . This study would bring knowledge about the organisms causing acute bacterial meningitis in relation to age , sex socioeconomic state and antibiotic sensitivity.

REVIEW OF LITURATURE

Historical Note

Acute bacterial meningitis has a World - Wide distribution . Because of its unique and specific clinical presentation this complex of infections has been recognized and described since the earliest days of medicine The first discription of cerebraspinal meningitis was given by Vissusseaux in 1805 who described an outbreak of the disease at Geneva . The disease in its classical form is characterized by well defined clinical symptoms which in most of the cases make the diagnosis easy However, the isolation of the meningococcus was performed eighty two years later by Weicheselbaum in 1887. He isolated the Meningococcus from the cerebrospinal fluid of patients diagnosed as cereboospinal meningitis. He described it as a Gram - negative diplococcus and gave it the name Diplococcus intracellularis meningitidis . Later on after the meningococcus was described by Weicheselbaum, it was found that the organism shared so many properties present in the Gonococcus which described by Neisser 1879 (Topely and Wilson 1975).

TAXONOMY

NEISSERIA MENINGITIDIS

Neisseria meningitidis is a Gram negative cocci found among the indigenous microbiota of the upper respiratory tract of man. It has been historically associated with world wide epidemic outbreaks of leptomenigeal inflammation for centuries (Paterson, and Hafeez, 1976).

Meningococcal meningitis has a peak incidence in children and young adults (Mandell et al., 1979). It occurs commonly between age of 3 months and 5 years (Topely and Wislon ,1975) . The disease occurs in adolescents and in adults at incidence rates that are substantially lower than those in children because of increase the incidence of antibody to various serogroups of Neisseria meningitidis (Hoffman ,1981) .

Morphology :-

Neisseria meningitidis is a Gram negative non motile non sporulating round or oval, small cocci, Paterson et al., 1975) . Individual cocci are kidney shape with the flat or concave sides adjacent (Duguid et al.1978) Capsulated organisms can be detected in young culture (Cruickshank et, al, 1982) Neisseria meningitidis is present chiefly intra polymorphonuclear leukocyte in the early stage as well as extra cellular (Duguid, et al., 1978).

Antigenic structure :-

Craven et al.(1978) indicated that meningococci can be serogrouped immunologically and chemically on the basis of distinct capsular polysaccharides . Eight serogroups designated A,B,C,D,X,Y,Z, W 135 and 29 E are recognized of which serogroups A,B,C,Y, W 135 and 29 E account for most disease (Craven and Frasch,1979).The distribution of serotypes among strains of Neisseria meningitidis are responsible for a marked increase of meningitis cases in the Hamilton area (Ashton etl al,1980).Neisseria meningitidis serogroup B is currently responsible for a large proportion of meningococcal disease in the world (Bovre, et al., 1977, Varughese and Acres, 1980).

Wyle et al.,(1972) indicated that group A and C capsular polysaccharides are immunogenic in man while group B capsularpolysaccharide is non immunogenic . Two theories have been proposed by Paterson and Haffeez(1976) to explain the relative lack of immunogenicity of group B polysaccharide antigen . First the polysaccharide consists of neuraminic acid that is very similar or even identical to neuraminic acid residues on mammalian host cell membranes . The polysaccharide may therefore recognized as self rather than foreign by an infected host

that is immunologically unresponsive or tolerant of his own neuraminic acid determinants. The second possibility is that the neuraminidase enzyme concentration in host tissue fluids and cells lead to rapid degradation of group B polysaccharide. In this case breakdown of the antigen is so fast that critical antigenic determinants never impinge on critical receptor sites of immunocompetent lymphoid cells. The net result is failure to trigger antibody production.

Griffis and Brandt (1983) proved that the capsular polysaccharides of serogroups 29E and Z of Neisseria meningitidis were cross immunogenic and cross reactive. Also there was antigenic interaction between group W 135 and group Y strains (Craven, and Frasch, 1979).

Pathogenicity :-

The nasopharynx is the portal of entry of meningococcus. Organisms may form part of the transient flora without producing symptoms (Jawetz et al 1980). From nasopharynx organism may reach blood stream producing bacteraemia (Meningococcaemia). The route of spread of meningococcus from the nasopharynx to the meninges is either by direct spread or haematogenous route.

Cruickshank et al (1982) In case of haematogenous spread

blood culture is positive. Also organisms may be isolated from the purpuric rash (Duguid, et al., 1978).

Salle (1972), indicated that organisms are very easily killed outside the body and probably never reach the new individual except by direct contact.

Neisseria meningitidis invasion of the CSF is followed by an outpouring of polymorph leucocytes and fibrin into CSF. The meninges become inflamed swollen and covered by fibrinopurulent exudate (ForFor and Arnest , 1980).

Culture character :-

The organism is aerobic but primary culture grow better in atmosphere containing 5 -10 % CO_2 at optimum temperature of 35 - 37 °C . It grows on enriched media such as blood, chocolate and serum agar . After 24 hours incubation, colonies are small greyish transparent smooth disk 1-2 mm in diameter and non haemolytic .(Cr: uickshank et al., 1982) It grows best on Mueller Hinton and Thayer Martin media where the colonies are convex, glistening elevated mucoid of 1-2 mm diameter , translucent and non pigmented (Jawetz et al 1980) ~~Mueller~~ Hinton media with antibiotics (VCN Vancomycin, Colistin, Nystatine) favour the growth of Neisseriae and inhibits other bacteria.

Meningococci grow on specific media as modified Thayer Martin agar and NYC media, where colonies are grey and opaque (Morello & Bohnhoff 1980). They are destroyed by direct sunlight disinfectant but may survive freezing (Jawetz et al., 1980).

Biochemical reactions :

The organism ferments glucose and maltose with acid and no gas. It is necessary when testing their sugar reaction to add a small amount of serum (Duguid et al., 1982). It reduces nitrate with or without nitrogen production . Indole is not produced . The methyl red (MR) test is weakly positive or frankly negative, the Voges - Proskauer (V.P.) reaction is negative. Catalase is produced (Paterson, & Hafeez 1976) and oxidase is positive. Colonies of pathogenic Neisseria react positively to the oxidase test and develop a dark purplish colour within 30 seconds. Colonies of non pathogenic react more slowly (Duguid et al., 1978).

Serological reactions :

- Quelling reaction is positive in group A and C because they possess capsular antigen (Baily and Scott, 1974).

- Positive slide agglutination test (Ashton, et al., 1980) .
- Counterimmunoelectrophoresis (CIE). It is based on the migration of antigen, and antibodies in an electrical field under standardized condition of pH and ionic strength, when they are situated in a gel matrix they migrate toward each other, and form a visible precipitate in the gel (Strauss, 1979).
- Serogroup identification of meningococci by a modified antiserum agar method (ASA). It reduces the amount of group specific antisera required . In this technique, the antisera prepared in horses immunized with formaline inactivated meningococci of serogroup A,B,C,Y, W 135 and 29 E. Antisera were added at 50 °C to autoclaved flasks of tryptic soy broth and Noble agar. Using a 24 well Coster tissue culture dishes, 0.5 ml of ASA for each serogroup was pipetted into one of six vertical rows . Meningococci to be serogrouped taken directly from culture on brain heart infusion agar (Difco) containing 1 % normal horse serum were inoculated into well containing different ASA, incubate at 37 °C in 5 % Co₂ for 24 h and examined for group specific halos (Craven and Frasch, 1979).

- Precipitin test is positive following the addition of CSF on top of specific antisera in a capillary tube (Cruickshank et al., 1982).
- Agar gel double diffusion method using crude lithium acetate extract was used for serotyping Neisseria meningitidis strains (Ashton, et al. , 1980).

Vaccination :-

Gotschlich, et al., (1969) purified groups A, B and C polysaccharide antigens and described their immunochemical properties, the group A and C antigens have only negligible toxicity in humans while producing an excellent group specific bacterial response . Group B polysaccharide appears to be a poor immunogenic in human; there is a little response to these antigens with natural infections or when administered as purified high molecular weight preparations .

Artenstein et al., (1971) showed that the A and C polysaccharide vaccines could be administered effectively by injection . They were highly stable when lyophilized preparation were stored in the cold and would be breakdown in a few days when stored at 3 5 °C, The first proven effective meningococcal vaccines were purified capsular preparation their development and testing have been extensively

reviewed by Weiss and Hill, (1974) and Triau (1978).

Immunity require at least five days to develop after vaccination (Petersdorf, et al (1975) The peak antibody titre was reached 2 to 8 weeks after immunization and the antibody present for more than a year (Arntstein et al. , 1971).

Meningococcal polysaccharide vaccine is currently available only against serogroup A and C meningococci (Finley 1976).

Frasch (1979) tried to use noncapsular cell surface antigen as potential vaccines against meningitis due to group B meningococci. With the development of vaccines against meningococcal serogroups other than A and C, the formulation of a polyvalent meningococcal vaccine has become feasible (Zollinger, 1979 Griffiss et al., 1981).

The group 29E capsular polysaccharide induce antibody which lysed group Z strains. Its inclusion in polyvalent preparation should provide protection against the later serogroup (Griffiss and Brandt, 1983).

Green wood et al (1981) proved that in malaria endemic area the antibody response to vaccination can be enhanced by administration of chloroquin at the time of vaccination.

Epidemiology :-

Sero group A, B and C cause most meningococcal disease. group A strains historically have been responsible for world wide epidemic outbreaks involving large numbers of individuals and occurring with cyclic periodicity. Therefore group A Neisseria meningitidis was considered for decades to be an epidemic serotype. In contrast Group B and C Neisseria meningitidis tended to cause solitary cases of sporadic disease during the interepidemic periods, when Group A strain were not prevalent. Group B and C strains were designated endemic serotypes of Neisseria meningitidis (Lapeyssonie 1969, Paterson, et al., 1975).

Since world war II a progressive shifts in the relative importance of these three serotypes has occurred. In 1963, Group B strains began to replace those of group A as the cause of most cases of meningococcal disease. Since 1967, Group C strains have been responsible for proportionally more cases than Group B strains and are currently replacing Group B as the most important serotypes (Souzar de et al., 1974) . Group A disease still occurs in various parts of the world such as Canada , Brazil , England and Africa (El Akkad 1969; Ronald, et al., 1972 Anonymous 1976 Makela et al., (1977)).

Meningococcal disease occurs in all age groups , young children are generally the most susceptible because they have not yet developed immunity to different serogroups at different ages . It is noticed that the age distribution of cases caused by different serogroups is varies (Devine, et al., 1972); most Groups B disease occurs in very young children wherease the highest incidence for A and C is in older children and young adults , and in group Y is young adults and adults .

Meningococcal carriers are adult (over 21 years of age) who harbour the organisms in the nasopharynx for months (Feigin and Cherry 1991). When the carrier rate reach 95 % the incidence of systemic disease is less than 1 % (Goldschneider et al., 1969). The carrier rate may be as high as 25 % without epidemic and 50 % or more during epidemic (Salle 1972). The decline of the epidemic takes place where fewer foci remaining till finally the endemic state is regained . (Dopter 1921).

Dopter (1921) observed that the real disease which the meningococcus gives rise is rhinopharyngitis, the meningitis which occasionally develops is to be regarded not as the main disease but as a complication . Meningococcus does not act as a pure commensal because antibodies

are sometimes formed as a result of its presence in the nasopharynx. Most persons who contract meningococcal disease have acquired the organism from a carrier, not from a patient. Persons who are carriers appear to be at lower risk of illness than those who are not (Topely and Wilson 1975 , Jacobson and Fraser, 1976).

Hablas (1975) recorded that 25 % of both the outpatients of Abassia Fever Hospital as well as their contacts harbour Neisseria meningitidis.

Bosmans 1976 found that the carrier rate in Rawonda in Central Africal vary between 10 to 16 per cent .

Bassyouni (1983) reported that in Assiut carrier rate was 1.96 per cent among 916 pharyngeal swabs taken from individuals whose ages ranged from 5 - 20 years old. He attributed the decrease of the carrier rate recorded than that recorded in other investigations to the previous massive treatment with ampicillin and chloramphenicol and to previous mass vaccination .

Prophylaxis against meningococcal infection :

Jawetz et al., (1980) found that a rise in the number of cases is always preceded by an increased number of respiratory carriers. In the past (Finley, 1976), found that widespread chemoprophylaxis with sulphonamide was successful in preventing meningococcal disease caused by sulphonamide sensitive strains but not effective against sulphonamide resistant strains .

Treatment with penicillin does not eradicate the carrier state . Rifampin 600 mg twice daily by mouth for two days (or minocycline 100 mg every 12h) can often eradicate the carrier state and serve as chemoprophylaxis for household and other close contacts (Jawetz et al.,1980).

Alman et al., (1975) proved that the use of Minocycline may not be advisable in children because of problems related to dental staining and vestibular toxicity . On the emergence of resistance strains of meningococci to this agent may be relatively rapid (Munford et al., 1974).

The second line for prophylaxis is the administration of meningococcal polysaccharide vaccines. The vaccines are administered as single (50 ug) injections . It cause only

mild local reaction. The vaccines may be not effective in children less than one year of age and should not be used in this age group unless newdata establish their efficacy (Jacobson, Fraser, 1976).

HAEMOPHILUS INFLUENZAE

Koch (1883) was the first bacteriologist who observed bacteria of genus Haemophilus during his examination of conjunctivitis exudates in Egypt . Influenzal meningitis was first described by Slawyk (1899).

Haemophilus influenzae type b is the strain which causes meningitis(Forfer and Arrest(1980); Haemophilus meningitis is common between six months and two years of age . It is often preceded by mild upper respiratory tract infection with the same organism. It is rare under two months or over six years of age (Forthergill and Wright 1933) ;The aggregated incidence of Haemophilus influenzae meningitis decreases with each succeeding year after one year and become negligible after childhood (Hoffman , 1981) .

Haemophilus influenzae is a small Gram negative bacillus showing considerable pleomorphism. They are non motile and non sporeforming . In sample of pathologic material such as CSF sometimes simulat diplococci . In older culture or when it grows under unfavourable condition the rod become elongated (Kilian, 1981) .

Organism has a capsule in young culture which is rapidly dissolved by autolytic enzymes and there for it is poorly seen in old cultures (Jawetz, et al. 1980). The capsule composed of polysaccharide poly-ribitol phosphate , which is specific antigen for type b strains of Haemophilus influenzae (Hoffman, 1981) . Vaccine was prepared from purified polyribophate . It gives antibody response in adults and children over 2 years only (Anderson et al- 1972).

Haemophilus influenzae is divided into capsulated and non capsulated strains the capsulated strains could be differentiated serologically into six types from a to f some of which cross react with pneumococcal capsular antigen (Pittman, 1931) . Non capsulated strains of Haemophilus influenzae are antigenetically heteroginous (Cruickshank, et al., 1982)The Capsuler, material of all six types are polysaccharide and all except those of type d and e contain phosphorus (Kilian , 1981) .

(1981)

Feigin and Cherry xfound that noncapsulated strains of Haemophilus influenzae may be found in the throat or nasopharynx of up to 80 % of children or adult at various period of time . The encapsulated form especially type b produced suppurative respiratory

infection and meningitis in young children (Duguid et al., (1978).

Haematogenous spread from the upper respiratory tract is the principle route by which the organisms produce meningitis. Also direct spread from the nasopharynx and infected head trauma may cause meningitis (Hoffman 1981).

Kilian(1981); found that the organism is aerobe, and facultative anaerobe, its optimum temperature is 37 °C and needs 5 - 10 % Co₂. It grows very poorly on ordinary laboratory media. It needs enriched media in which the two growth factors heat stable X factor and heat labile V factor, are found. The X and V factors are present in chocolate agar so that it gives the best growth of Haemophilus influenzae. Colonies of capsulated strains are large (1 - 2 mm) more opaque and mucoid (Duguid et al. 1978) Colonies of Haemophilus influenzae grow in the neighbourhood of certain other bacterial colonies e.g of Staphylococci which secrete V factor, . This phenomenon is called satellitism (Kilian 1981). Growth appears as small colonies 1-2 mm in diameter , colourless, transparent moist with musty odour (Baily and Scott., 1974).

Levinthals medium give best growth and used if clear medium is required, it give distinct colonies (Cruickshank , et al, 1982).

Biochemically the members of the Haemophilus group reduce nitrate to nitrite (Difco 1968) and ferment glucose and xylose with production of acid only (Kilian 1981). It produces indole ornithine decarboxylase and catalase (Kilian 1976 , Lenette et. al., 1980).

The organisms may be detected by a direct capsular swelling procedure (quellung tests), it may be carried out by using type b rabbit antiserum added to spinal fluid containing type b Hemophilus influenzae . (Baily and Scott 1974). Haemagglutination test and precipitin test were used to detect specific polysaccharide antigen in the spinal fluid. (Young 1973). Strauss, (1979) used counterimmunoelectrophoresis for detection Haemophilus antigen in the CSF but it was less sensitive than coagulation and not considered a specific test .

Epidemiology of Haemophilus influenzae meningitis :

Turk and May (1965) reported that the

frequency of Haemophilus influenzae meningitis had increased in the decades since 1930 at Boston Children's Hospital ; Los Angeles children's Hospital and Oxford Children's Hospital. Michaels (1971) recorded that a ten - fold increase in the number of admissions to Children's Hospital of Pittsburgh for Haemophilus influenzae meningitis occurred since 1945, whereas the total number of admission increased less than two fold during the same period. Minear & Edman (1978) recorded that the ratio for Haemophilus influenzae meningitis was low in relation to the ratio of Meningococcal meningitis, it reached 2.6%. While meningococcal meningitis reached 52% in children admitted to Abbasia Fever Hospital in Cairo from 1971 to 1975 .

The incidence was greater in rural area than in urban area (Feigin & Cherry 1981). In U.S.A the incidence increased in blacks than in whites but this distribution appeared to be related to poverty than to races (Fraser et al., 1973).

Meningitis due to Haemophilus influenzae type b occurs as a complication of inadequate treatment of otitis media (Nelson & Victor 1983). This infectious

organisms spread from healthy carrier, but case to case spread is very rare (Kilian 1981). The carriage rate of capsulated strain seldom exceed 5 % although it may be higher in closed communities of children (Kilian, 1976).

STREPTOCOCCUS PNEUMONIAE

Pneumococcal meningitis is most common in adults and is frequently associated with other suppurative foci as pneumonia, otitis media, mastoiditis sinusitis, endocarditis and previous head trauma. It is usually recurrent in about 10 % of cases .(Michael., 1983) . It occurs in children from two months and over (Grossman & Delmer, 1978).

Fraser et al(1973)reported that in U.S.A. the risk of pneumococcal meningitis is 5.5 fold greater in black than in white independent of income or population density .It is endemic with high risk of carrier, the healthy carrier is more important in disseminating infection than the sick patient. (Jawetz et al 1978) .

The organism is gram positive diplococcus 0.8 - 1.0 μ m, ovoid with chains in liquid media it is, non motile, and non spore forming (Cruickshank, et al 1982) In infective material the pneumococcus shows a well defined capsule surrounding the diplococci but in culture the capsule can be demonstrated only by the

use of special methods e.g in a wet film with indian ink (Duguid, et al., 1978) .

Typing of pneumococci is based on variation of the chemical composition of the capsular polysaccharide which is immunogenic to man (Ginsburg 1981) and it is differentiated into 83 serotypes (Feigin & Cherry 1981). The most common serotypes causing meningitis are 1 , 5; 6, 2, 23, 3, 13. Serotype 1 is predominant in adults where serotypes 2, 5, 6, and 23 are most common below the age of two years (Cadoz et al., 1981).

Haematogenous spread from the upper respiratory tract is the principal route of infection (Hoffman 1981). Also direct invasion of the meninges from contiguous focus of infection may occur (Nelson , 1983). The main pathogenicity of the organisms is attributed to its capsule which is a virulence factor . It prevent phagocytosis by both polymorphnuclear leukocytes and macrophages (Ginsburg 1981). Pneumococcal meningitis most common in patient suffuring from sickle cell disease, splenectomy, alcoholism and head trauma (Hoffman 1981).

Thorn et. al., (1977) found that the three

common meningeal pathogens (Haemophilus influenzae, Neisseria meningitidis Diplococcus pneumoniae) are invasive and depend upon antiphagocytic capsular or surface antigens for survival in the tissues of the infected host. The three organisms are inhabitants of the nasopharynx.

It is aerobic, facultative anaerobic grows at 37°C and may need 5 - 10 % Co₂. On blood agar, colonies are small about 1mm in diameter smooth surface later develop raised rim (Draughtsman colony), and surrounded by alpha haemolysis (Duguid, et al., 1978).

Pneumococci is liquified by bile and can be distinguished from alpha haemolytic streptococcus since the later is not liquified by bile (Moffet , 1975). The growth of pneumococci is inhibited on solid media around a disk of optochin (ethylhydrocupreine hydrochloride) (Jawetz, et al 1980. Inuline is fermented by pneumococci (Baily and scott, 1974) and the organism ferment lactose, sucrose, trehalose, raffinose with production of acid only (Ginsburg , 1981).

Quellung reaction is the most accurate, reliable,

and specific test for identification of pneumococcus directly from clinical material. Capsular swelling with specific antibody can be detected (Baily and Scott, 1974).

Strauss (1979) applied counterimmunoelectrophoresis (CIE) : to detect capsular polysaccharide in CSF . An omnivalent serum which contain antibodies to all 82 capsular polysaccharide has been employed to identify the organisms in clinical material (Ginsburg 1981).

Typing of pneumococci in CSF by coagulation of killed Staphylococcus aureus technique was explained by Kronvall (1973). The procedure of this technique is based on the ability of protein A which is found on the outer surface of some strains of Staphylococcus aureus to combine with FC fragments of IgG leaving free Fab fragments (Forogren, & Sjoquist 1966 , Kronvall, 1973). Coagulation occurs when this coated antibody combine with specific homologous antigen (Strauss, 1979).

UNCOMMON CAUSES OF PURULENT BACTERIAL MENINGITIS :

TUBERCULOUS MENINGITIS :

Tuberculous meningitis can occur at any age except in neonates (Crousaz, 1981). Mycobacterium bovis causes up to 40 % of tuberculous meningitis in children but it is now absent in many countries due to pasteurization of milk. Sporadic cases of M.bovis infection still occur (Jones, & Silva 1982). Pediatric cases have virtually disappeared, and two thirds of cases occur in those aged 20 to 40 years with a hint of shift toward older age groups (Hoas et al., 1977).

Haematogenous route is responsible for all cases of meningial tuberculosis (Rich and Mc-Cordock, 1933). Release of tubercle bacilli from acute infection into leptomeninges occur in disturbance of patients resistance such as immunosuppression and malnutrition (Pathok, & Tandon 1973). Direct spread from tuberculous otitis media and skull osteitis is unusual (Crousaz, 1981).

Mycobacteria are slender, rod shaped show thick cell wall (high content of lipid) acid fast, alcohol fast non sporing and non motile (Smith, 1981, Kim and Barksdale 1977). It is aerobic, grows slowly at 37°C, the

doubling time 10 - 14 hours (Smith, 1981). Growth occurs on complex media such as lowenstein Jensen media and Transparent media e.g Middle brook and Cohn:

In young children a positive tuberculin reaction may be taken as indicative of infection. Reactivity appears one month after infection as indurated area more than 5 mm and persist for many years . A positive test may reveal previous infection with various mycobacteria . It has limited diagnostic value. It may be reported negative in 15 - 37 percent of cases due to malnutrition and to the frequency of recent measles (Osuntoken et al., 1977),

LISTERIA MONOCYTOGENOUS :

Listeriosis is a Zoonotic disease which could be transmitted to man as a result of contact with infected rats and domestic animals (Michael 1983) .

Over 80 % of Listeria monocytogenes meningitis occurred in neonates or in adult over 40 years of age (Gray and Killinger, 1966) . It occurs also in immunosuppressed patients (Lavetter, et al. 1971) debilitated patients (Louria, et al 1970) and renal transplant patients (Michael, 1983) .

It is small Gram positive rods, non capsulated non - sporing (Murray, et al 1926) At 20 °C. the cell shows peritrichous flagellation, at 37 °C only one polar flagellum is apparent so motility is best around 20°C (Seelinger, 1981). The organism is Aerobic to microaerophilic grows on ordinary media at pH 7.2 at 37 °C. On nutrient media colonies are small, whitish flat , smooth and shiny while on blood agar the colonies are small, translucent grey to white surrounded by beta haemolysis , in broth it produces turbidity and slimy deposits (Baily and Scott, 1974, and Pyatkin, 1980).

It has two antigens somatic O antigen and flagellar H antigen. The somatic O antigen contains four thermostable types I, II, IV, V and one variable-

antigen III. The H antigen contains antigens A,B,C and D which are destroyed by exposure to formalin (Pyatkin, 1980).

The newborn may be infected during delivery either by infected vaginal secretion or by swallowing infected amniotic fluid. (Allbritton et al 1976) Trans-placental infection lead to abortion or stillbirth baby (Sellinger, 1981). Placenta play a major role in transmission of the disease (Sonnenwirth and Jarett, 1980). Recently listeriosis increases in patient with severe underlying disease as alcoholics, diabetic, lymphoma, aplastic anaemia, and in patients received immunosuppressive treatment (Seeliger, 1981). Listeria produces an exotoxin in the form of haemolysin (Duguid, et al., 1978).

Biochemical activities of the organism is indicated by fermentation of glucose and maltose with acid and no gas. It produces no indol and hydrogen sulphide and don't reduce nitrates, It not liquify gelatin or ferment manitole. (Pyatkin 1980). Voges proskauer test, Methyl red test and Catalase test are positive (Killinger, 1973).

BETA HAEMOLYTIC STREPTOCOCCI GROUP B:

Beta haemolytic streptococci group B causes meningitis and pneumonia during the first two months of life (Patterson & Hafeez 1976) Streptococcus meningitis is un common in adults and usually associated with previous neurological damage (Stringer, et al 1981).

Group B streptococci contain as a major constituent of the cell wall, a group - specific carbohydrate (G-substance) composed of rhamnose, N. acetylglucosamine and galactose. L-rhamnose is the significant component of the antigenic determinant. The capsule antigen (S-substance) can serve to classify further the group into four serologic separate and distinct types, Ia Ib, II and III. A fifth type, Ic , contains the Ia carbohydrate antigen plus a protein antigen (Ginsburg 1981). Serotype III strain is associated with most infection (Franciosi et al 1973).

The organism is Gram positive non motile cocci arranged in chains of varying length (Topely and Wilson , 1975) it is non sporing, and may be capsulated. The capsule consists of hyaluronic acid (Stewart, 1974).

This organism occurs as a normal flora of female genital tract (Jawetz, et al. 1980). So meningeal infection acquired during the process of birth through a cervix or vaginal canal which may harbour the organisms (Feigin & Cherry 1981).

It is aerobic, facultative anaerobe, optimum temperature at 37°C (Lennette, 1980). On blood agar, colonies are small, soft translucent, surrounded by small haemolytic zone (Baily and Scott, 1974). It may produce orange pigments (Jawetz, et al. 1980). Group B streptococci are resistant to 0.04 unit bacitracine disk (Ginsburg, 1981). It is catalase negative (Gallis, 1976).

STAPHYLOCOCCUS :

Staphylococcus meningitis occurs in newly born babies, surgical patients, malnourished persons and patients of chronic disease (Duguid, et al 1978 as a result of suppurative process elsewhere in the body (Hoffman, 1981).

It is non motile, non spore forming Gram positive cocci and after ingestion by phagocyte or in old culture it appears Gram negative (Stephen, 1981). It

presents in irregular aggregates form (Tzagaloff and Novick, 1977). Staphylococcus aureus strains posses a distinct capsule demonstrated by the indian ink method or by quelling reaction with specific capsular antiserum (Morse , 1978).

Two species of staphylococcus : Staphylococcus aureus (pathogenic) and Staphylococcus albus (non pathogenic) (Duguid,etal,1978), according to Stephen, (1981). There are three types of antigens :

- 1- Specific antigen : Cell wall techoic antigen in Staphylococcus aureus is ribitol techoic acid. In Staphylococcus albus is a glycerol techoic acid .
- 2- Cellular antigen; as capsule,protein A, bound coagulase .
- 3- Extra cellular antigen as coagulase, leucocidine.

Staphylococci are member of the normal flora of the human skin and of respiratory and gastrointestinal tract. Its pathogenicity is mainly due to exotoxin production and the invasive properties of the strains (Jawetz,et al 1980).

Infection occurs directly from patient with Staphylococal lesion draining pus externally, also

direct contact via the hand is the most important route of transmission. Carriers are conceded to be a main source of infection. Staphylococci present in number of body sites but the anterior nares is the major reservoir of infection (Willett, 1976). Staphylococcus aureus meningitis may occur after open head trauma, neurosurgical procedure or in association with endocarditis (Michael, 1983). Staphylococcus epidermidis are non pathogenic and present on the skin and nostril as a commensal organisms (Duguid et al. , 1978). It causes meningitis in case of infected cerebrospinal fluid shunts (Michael, 1983) as ventriculo-cardiac shunts used for the treatment of hydrocephalus (Forfor, & Arnest 1981).

ESCHERICHIA COLI :

Egihly per cent of meningitis in neonate period (most frequent under 2 months age) are caused by E. coli (Michael, 1983) Group K1 capsular polysaccharide antigen is responsible for 84 % of E. coli infection (Robbins & et al., 1974).

It is Gram negative bacilli non spore forming motile, (Baily and Scott, 1974). Few strains are

capsulated (Duguid, 1951 , 1964). Most strains are fimbriated .

Three types of surface antigens are conceded for serotyping of E.coli :

Somatic antigen (O) which is a lipopolysaccharide and contains more than 140 groups (Kauffmann, 1969 , Edwards and Ewing, 1972). It is heat stable . Capsular polysaccharide antigen (K) is a heat labile and differentiate into more than 100 groups . Flageller (H) antigen is protein in nature and contains 50 different groups (Jawetz, et al., 1980).

It is facultative aerobe, sometime grows aerobically (Zwadyk, 1980), optimum temperature at 37 °C.

On MacConkey's medium colonies are smooth, glossy translucent and rose pink in colour (Topely and Wilson , 1975). Colonies on blood agar are surrounded by zone of haemolysis (Ewing and Edwards, 1962).

Escherichia coli form part of the normal flora of the intestinal tracts and it becomes pathogenic when, it reaches tissues outside the intestinal tract (Jawetz, et al 1980) Infection usually acquired at birth

PSEUDOMONAS AERUGINOSA :

Pseudomonads are Gram - negative bacilli which live in soil and water and are rarely pathogenic for man. However they may produce disease, particularly in newborn infants and children with impaired host defense , such as those with cystic fibrosis immunodeficiency disease, malignancies, other chronic diseases, burns, or malnutrition and those receiving immunosuppressive therapy. (Nelson 1983).

The organism is motile, most strains possess single polar flagellum and fine fimbria (Nelson, 1983).

It is aerobic optimum temperature 37°C require moisture, grows on nutrient broth, producing uniform turbidity . On nutrient agar, colonies are large, low convex rough, oval have a distinctive musty smell and form a soluble blue green pigments (Pyocyanin) (Phillips, 1969). The organism produces on blood agar Beta haemolysis (Hugh, 1973). On gelatine stab incubated at 20°C revealed liquifaction from the surface and pigmentation (Duguid, et al., 1982).

It ferment glucose but not lactose with production of acid and no gas (Baily and Scott, 1974). Oxidase is positive. Catalase, citrate and nitrate are positive (Hugh, 1973).

PASTEURELLA MULTOCIDA (P. SEPTICA):

It is Penicillin - susceptible organism found in the mouth of domestic animals (Hoffman, 1981). Any age may be susceptible .

It is Gram negative bacilli or coccobacilli , bipolar staining, may be capsulated, non sporing and non motile (Chen and Elberg, 1981).

The species is not antigenically homogenous . Five types have been determined (Hudson, 1959). The human strains fall into group A and D (Carter and Byrns, 1953).

It may be found in the throat of healthy humans (Smith, 1959). As a result of contact with animal and under condition of stress or debilitation of the host, the parasite previously benign may penetrate beneath the mucous membrane and result in frank disease. It may produce systemic infections as meningitides. Human infections are acquired from animal bites, organisms may remain in the host for a long time and regain its pathogenic properties when resistance to infection diminishes (Francis et al., 1975).

It is aerobe, may be facultative anaerobe the optimum temperature is 35 °C.

The growth is better on blood agar. Colonies are smooth convex of butyrous consistency. There is no lysis of blood cells but may be greenish discoloration of blood agar with musty odour (Weaver and Hollis, 1980). No growth could be obtained on MacConkey's medium (Duguid, et al 1978) Broth culture may be turbid or foliculare with granular deposite (Chen and Elberg, 1981).

The organism is oxidase positive, indol positive as will as it is catalase positive (Duguid, et al 1982). It reduces nitrate to nitrite (Baily and Scott, 1974).

RARE CAUSES OF BACTERIAL MENINGITIS :

1- Citrobacter diversus :

During a two years period five infants born at the same hospital developed meningitis due to citrobacter diversus four of the infants developed brain abscesses. The causative organisms belonged to C.diversus serogroup O₂ (Graham,et al1981) Mac Conky agar containing kanamycin (10mg/ml) was found to be a satisfactory selective medium (Andreson et al., 1981)., for its isolation .

2- Mima Polymorpha (acinetobacter calcoecticus)

As a cause of meningitis (Waite and Kline, 1959) A disease simulating meningococcal meningitis both clinically and bacteriologically, may be produced by Gram negative coccobacillus Mima polymorpha (Olaffson et al., 1958). This organism appears coccoid on solid media and shows both bacillary and coccal forms in liquid media, shows bipolar staining nonmotile and does not reduce nitrates and oxidase positive (Waite and Kline, 1959).

3- Flavobacterium species as a cause of meningitis :

Flavobacterium species are Gram negative bacilli whose natural habitat is the soil and water. A report by Brody et al., (1958) recorded two outbreaks of meningitis among new born infants in hospital nurseries in which a Gram negative bacillus was isolated from the spinal fluid. The organisms are Gram negative rods, thin non motile, proteolytic and nitrate negative, producing small amount of indol. On blood agar after incubation 24h at 36 °C a lavender-green discolouration is seen .

4- Meningitis causes by Edwardsiella :

Sonnenwirth and Kallus, (1968) reported the first case of meningitis due to Edwardsiella tarda. The organism is characterized by its marked production of hydrogen sulfide negative urease and beta - galactosidase , the organism usually ferment glucose and maltose but not lactose or manitol (Edwards, & Ewing 1972).

MATERIALS

1- Patients :-

This study included meningitic patients who were admitted to the Benha Fever Hospital and Benha University Hospital between Sept., 1983 and Feb, 1984 . These patients were children below 16 years old of both sex with symptoms and signs of meningeal irritation . The age and sex of the studied cases were shown in Table (1)

Table (1) illustrate the number of collected specimens according to age and sex.

Age group/years	Males	Females	Total Number
< 1	4	3	7
1 - 5	7	5	12
5 - 9	7	6	13
> 9	5	4	9
	23	18	41

2- Cerebro - Spinal fluid :-

Cerebro-spinal fluid was drawn by Lum-
ber puncture from patients with suspected meningitis
under complete aseptic precaution to be taken direc-
tly to the laboratory for prompt bacteriological exa-
mination .

3- Stains :-

Gram stain : It is used for direct staining
of the centrifuged CSF samples and staining
the organisms from the colonies produced.

Ziehl - Neelsen stain for direct staining
of the centrifuged CSF samples in suspect-
ed tuberculous cases.

4- Media :-

All the dehydrated media used in this study
were commercially available . They were prepared
and sterilized according to the manufacturer instruc-
tions ; They were products of B.B.L., Difco and Oxoid
laboratories.

a) Mueller - Hinton Agar : (Mueller and Minton, 1941)

The ingredients were made from :-

Beef infusion	300 ml.
Casein hydrolysate	17.5 gm
Starch	1.5 gm
Agar	10 gm

Distilled water used to dissolve the above ingredients and complete it to one liter.

pH was adjusted at 7.4 and the medium was autoclaved at 121°C for 15 minutes, cooled and poured into disposable or ordinary sterile plates.

b) Carbohydrate Fermentation Medium :-

This was the basic media used to test the biochemical reactions of Neisseria and other organisms which were isolated .

Peptone water (1% peptone, 0.5 % Na cl per one liter of water). 950 ml.

Andrade's indicator 10 ml

Test compound (10 per cent solution, sterile) 50ml

c) Blood Agar :-

The medium was prepared by adding sterile blood 10 % to sterile nutrient agar that has been

melted and cooled to 50 °C . This medium was used for growth of H-influenzae, Staphylococci, Streptococci and other organisms which were isolated .

d) MacConkey's Agar :-

This medium was used for cultivation of Gram negative bacilli. It is composed of :

Peptone	20 gm
Sodium taurocholate commercial	5 gm
Agar	20 gm
Neutral red sol.2 percent in 50 percent	
Ethanol	3.5 ml
Lactose 10 percent aqueous solution	100 ml

The above ingredients dissolved in water
pH adjusted at 7.5 and sterilized by autoclaving
at 121 for 15 M.

E) Lowenstein - Jensen medium :(Jensen 1955) :

The ingredients were composed of

Mineral salt solution	600 ml
Malachite green solution	20 ml

Beaten fresh egg (20 -22 egges).

All utensils used must be sterile, This media was used for isolation of tubercle bacilli.

5 - Oxidase test (Steel 1961):

The oxidase test is originally devised to identify all Neisseria spp. and Pseudomonas . It is based on the bacterial production of the oxidase enzyme. 1% tetramethyl-p-phenylenediamine dihydrochloride was dissolved in distilled water, and warmed gently . Kovac's (1956) indirect paper procedure was used in which a 6 Cm piece of Whatman No. 1 filter paper was placed in a petri dish. 2 to 3 drops of the reagent were added to the center of the paper . A loopful of the suspected colony was smeared into the reagent impregnated paper . A positive colour reaction would occur within 5 to 10 seconds where the faint purple colour of the reagent would turn into a deep purple tint.

6- Motility test:-

Used to detect motile from nonmotile Gram negative bacilli.

Match was dipped in petroleum jelly, a ring was outlined round the concavity on a slide with a wire loop, drop of broth suspension of organisms was placed on a coverslip. The slide was inverted

over the coverslips and then quickly turned upside down so the coverslip becomes uppermost. The slide was examined by the low and high power after down the condenser.

7- Grouping sera :-

The following grouping sera were produced by Difco laboratories, Detroit Michigan, U;S.A included

Neisseria	meningitidis	Antiserum	poly.
Neisseria	meningitidis	Antiserum	poly 2 .
Neisseria	meningitidis	Antiserum	Group A
Neisseria	Meningitidis	Antiserum	Group C
Neisseria	Meningitidis	Antiserum	Group X
Neisseria	Meningitidis	Antiserum	Group Y
Neisseeria	Meningitidis	Antiserum	Group Z

8- IMViC Tests :

Used for identification of Gram negative bacilli

Indole test :

Medium composed of peptone (2% peptone and 0.5 Na cl in distilled water), Kovac's reagent, the medium was inoculated with the suspected colonies and incubated at 37 °C for 48 h then 0.5 ml of Kovac's reagent was added and shaken, red ring developed in positive cases.

Methyl red test :-

It is composed of glucose phosphate, peptone water medium and methyl red indicator, suspected colonies were inoculated on the glucose phosphate media incubated at 37 °C for about 48 h . Then we added about five drops of methyl red reagent . A positive tests gave bright red colour while negative result was Yellow.

Voges Proskauer test :-

Composed of glucose phosphate, peptone water and O'Meara reagent. Colonies were inoculated on the glucose phosphate media incubated at 37 °C for about 48 h, then 0.5ml of O'Meara reagent was added . Tubes were placed in water bath at 37 °C for 4h . Positive reaction was denoted by the development of eosin - pink colour .

Citrate utilization test :-

Drop from saline suspension of the organism was inoculated on koser's liquid citrate medium, and incubated at 37 °C for 96 hrs . Positive test shows turbidity in the tube (growth) while negative test shows no turbidity.

9- Bile solubility test :-

One part of 10 % sodium Taurocholate added to 10 parts of unifromly turbid broth culture, after 15 minutes incubation at 33 °C complete clearance occurs in positive test.

10- Chemical Analysis of Cerebrospinal Fluid :-

Protein turbidimetric methods were used for the estimation of total protein (Baker, 1976). Principle: proteins in CSF are precipitated by either dilute trichloroacetic acid or dilute sulphosalicylic acid in sodium sulphate solution, and the turbidity of the resultant uniform suspension is measured spectrophotometrically against a standard solution similarly treated.

Chloride : Mohr's method: (Baker, 1976) Principle : Chloride is estimated as sodium chloride by titration against silver nitrate using potassium chromate as indicator. Silver nitrate was added until all the chloride ions present in the CSF have combined with the silver ions. Any further silver nitrate added was no free to combine with the potassium chromate indicator to yield a red precipitate of silver chromate. The solution at this point suddenly changes from pale yellow to faint bright- red colour.

Antibiotic sensitivity :

The disk diffusion method is used for antibiotic sensitivity tests for all isolated organisms . Standard bacteriosensitivity discs for antibiotic were obtained from BBL , Difco laboratories, CTBA - GEIGY and Detroit Michigan. It contains Garamycine (30 m cg/disk), Penicillin 30 Unite / disk), Ampiclox (ampicillin 25 mcg and cloxacillin 5 mcg / disk), Erythrocyin (15 mcg / disk), Rifampicin (30 mcg / disk) and topramycin (30 mcg / disk).

METHODS

The bacteriological investigation was based on the isolation and identification of organisms presents in CSF of the individuals examined .

1- Collection of specimens and culture .

Specimens of CSF were collected under complete aseptic precaution

a)- Staining methods:

The collected sample was concentrated by centrifugation and examined at first by Gram stain and Ziehl - Neelsen stain. Samples which gave positive stain for T.B.were inoculated on Lowenstein Jensen medium to be examined for presence of colonies after 10 - 14 days .

b)- Culture media :

Negative sample for T.B. by stain inoculated on Mueller Hinton plates, blood agar plates, incubated for 18-24 hours at 37°C in an atomsphere of 5-10 % Co₂ .

Media used, for culturing meningococci were

prewarmed to 25°C before inoculated by exposure to ambient temperature kept to a minimum.

2- Isolation and identification of Neisseria :

The plates after incubation at 37°C were carefully examined for suspicious Neisseria colonies. From each colony an oxidase test was done using Kovac's indirect filter paper method described before. From the oxidase - positive colony a film was prepared , stained with Gram stain and examined microscopically to demonstrate the classical morphology of Neisseria being Gram - negative diplococci. Subcultures of the separate oxidase-positive, Gram negative diplococci colonies of Neisseria from the primary culture were done on glucose, maltose, sucrose and lactose sugar media enriched with serum. All the inoculated tubes were allowed to grow at 35 °c .

3- Serological grouping :

A drop of rehydrated meningococcus antiserum polyvalent was placed on a clean slide.

A loopful of the isolated colony was transferred to the drop of polyvalent antiserum and rubbed into the serum to give a uniform suspension .

The slide was rocked for 1 minute to ensure

complete contact between the antiserum and micro-organisms.

A positive reaction was manifested by the appearance of clumping or agglutination of the bacteria seen by the naked eye .

If the reaction was positive with the polyvalent antiserum, the individual groups can be identified by the same steps using the individual Meningococcus antiserum groups .

When the reaction was negative and no agglutination occurred, the same steps were repeated using Meningococcus antiserum poly 2 .

4- Isolation and identification of Gram negative bacilli:

From primary culture, colonies which gave Gram negative bacilli by Gram staining. Oxidase test, motility test, carbohydrates fermentation tests, subculture on Mac-Conkey 's medium and IMViG test were done .

5- Antibiotic sensitivity tests were applied for all the isolated organisms .

6- Chemical analysis of cerebrospinal fluid : From the supernatant fluid of centrifuge CSF sugar protein and chloride were estimated by methods discussed before .

N.B. identification of the different isolates were performed according to the methods described by Cruickshank, et al (1982) Baily and Scott, (1974) Toply and Wilson (1975).