Bacterial meningitis among children in Sana`a/Yemen

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BY

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ABSTRACT.

Three hundred children aged (postnatal-14 years old) admitted with clinical feature of meningitis in two major hospitals (Al-Sabaeen and Al-
according to the primary laboratory and clinically investigations, while 204 (68%) were excluded. When the accepted CSF specimens were cultured and serologically tested, bacteria were detected in 62 (64.6%) of CSF specimens. *Streptococcus pneumoniae* was the most commonly identified pathogen overall (38.7%), followed by *Haemophilus influenzae* (32.3%), *Neisseria meningitides* (27.4%) and Group B *streptococcs* (1.6%). Out of 62 cases with meningitis 51(82.3%) were aged below 4 years and 41 (66.1%)were aged below2 years. *Haemophilus influenzae* was the most common causative agent among children aged below 2 years. cefotaxim and ceftriaxon were the most antibiotics effective against the isolated microorganisms, followed by chloramphenicol. *Streptococcus pneumoniae* was the most isolated resistant to antibiotics, followed by *Haemophilus influenzae,* while *Neisseria meningitides* was the least.

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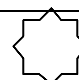
Finally I would like to thank WHO for their financial support.

Dedication

I sincerely dedicate this research to all members of my family, my parents, wife and sons.

Tawfique ALZubeiry
2001



Introduction.

Meningitis is the inflammation of meninges, the membranes that cover the brain and spinal cord (1). Cerebrospinal fluid (CSF) findings according to the causative agents are compared in the table below.

Table 1. Cerebrospinal fluid finding in meningitis.

Type of meningitis	White cells (cells/ ml)	Protein (g/l)	Glucose (mmol /L)
Acute bacterial	1000-60000 (neutrophils)	0.5-5	0.2-2.2
Mycobacterium	25-100 (lymphocytes /monocytes)	1-2	<2.5
Viral	5-200 (lymphocytes may be >1000)	Normal or slightly evaluation but < 1	Normal
Fungal	0-800 (lymphocytes)	0.2-5	Decreased (average 1.7)

ım Pollared AJ, Faust SN, Levin, M, 1998. Meningitis and meningococcal septicemia, *Journal of the Royal College of physicians of London* 32: 319-327.

Etiological agents of meningitis:

The most common etiological agents of bacterial meningitis in children are *H. influenzae* type b (Hib), *N. meningitides* (N.m), and *S. pneumoniae* (S.p), whereas group B *streptococcus, Escherichia coli* and *Listeria monocytogenes* are the most causative agents in neonatal (2). The uncommon pathogens are *Staphylococcus aureus,* and *Pseudomonas aeruginosa. Mycobacterium tuberculosis* is the less frequent of bacterial meningitis (3).

Many viruses have the ability of causing meningitis such as *Echovirus, Coxasackievirus* type A and B*, Herpes simplex virus* type 1 and 2, *Epstein-Barr* virus*, HIV, Vricella-Zoster* virus and *Cytomegalovirus (4).*

In addition, to bacterial and viral agents, fungi may also cause meningitis like *Cryptococcus neoformans and candida,* which are the most common fungi isolated from CSF (5). Parasites such as *Naegleria* has been identified as a causative agent of meningitis so far (3).

Meningitis has been characterized as:

Purulent meningitis (PM).

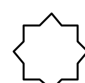
Acute form of the bacterial infection. The CSF is typically turbid due to the presence of large number of white blood cells (WBCs), most of them are polymorphs (6).

Aseptic meningitis.

Viruses are the principal agents of aseptic meningitis. The CSF is clear or slightly turbid in which moderate number of leukocytes is present, most of the cells are lymphocytes (6).

Modes of meninges infection

The different modes of meninges infection could be through contact with infected persons or carriers, and inhalation of infective aerosols in case of exogenous infection. On the other hand endogenous meningitis may result from spread of infection from otitis media, sinusitis, tonsillitis, bronchopneumonia mastoidetis, and head injury, especially among immuno-compromised patients (7).



Bacterial meningitis (BM) is the most common type of CNS infection in developing and developed countries in which the incidence of severe neurolological sequelae remains high despite modern anti microbial therapy. The early diagnosis and treatment are the corner stones of management of BM (8).

Several early studies revealed very varying pattern of BM with respect to age specificity, the incidence rate and endemicity, type of bacterial agents incriminated, morbidity, and mortality rates and other relevant epidemiological parameters which all vary from study to study and from country to country (9,10,11).

The age group with highest prevalence of meningitis is that of newborn with mortality rate as high as 20% (12). The high incidence rate of BM among this age group is due to the immature immune system of neonates, colonization of the organisms in the female genital tract and the increase of the permeability of the blood brain barrier of the newborn (12).
Franco et al, (13) reported that the neonatal meningitis caused by Gram-positive bacteria was twice of that caused by
Gram-negative bacteria, whereas the mortality rate due to Gram-negative microorganisms was almost three times higher than that of gram positive. It was reported that meningitis caused by *H. influezae* was the most common seen at the age 2-6 months and *N. meningitides* is predominant at the age 7-12 months, while group B *streptococci* occur in infants up to 6 months of age with the mortality rate of 25% (14). The high rate of mortality among these age groups is due to low birth weight, which is a significant risk factor in both neonates and post-neonatal infants (13).

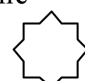
In developed countries the mortality rate is decreased gradually in the last 3 decades (15). However, significance
neuro-psychological sequelae still affect an average of 19% of the survivors (16). On the other hand, in developing countries the mortality rate remained higher, it was estimated that children die from acute BM under the age of 5 years (9).

H. infuenzae type b (Hib), *N. meningitidis* (N.m), and *S. pneumoniae* (S.p) cause about three quarters of cases of acute BM reported from Africa (10). In Sudanese children 38% of acute BM cases was due to *H. influenzae* and *N. meningitides*, followed by
S. pneumoniae, which account for 23% of cases, and the fatality rate was 29%. The proportion of causative organisms differs during endemic and post epidemic periods where the incidence of *N. meningitides* is higher in post epidemic (11). In Saudi Arabia the rate of incidence of BM in childhood (up to 11 years old) was recorded as follow, Hib 66%, S.p 24%, N.m 4%, Group B strept. 4% and 2% for *Staphylococcus aureus* (17). In 1978 it was reported in a study carried out in Egypt, that the rate of incidence of BM among children up to 14 years old, was 40% for
S. pneumoniae,, 23% for N. meningitidis 16 % for
H. infuenzae type b and the fatality rat was 39% (18). In contrast, a study carried out in Virginia, USA indicated that most cases of BM are caused by N.m 62%, followed by Hib and S.p 21% and 14% respectively (19).

In Britain, meningitides caused by meningococcus was the most common till 1980. It was found that 45% of cases were due to meningococcal infection and 33% were due to Hib (20).

N. meningitides.

Neisseria meningitides, which was first described in 1887, is the causative agent of the



meningitis and the major cause of mortality and morbidity in the world. It is still the major worldwide problem due to the limited effectiveness of the vaccine in the group at greatest risk for infection, where children younger than two-years old are at the greatest risk. An effective vaccine against serogroup B has not been developed (21).

Morphology.

Neisseria meningitides are Gram-negative diplococci, with flattened or concave opposing edges (kidney-shaped). Thy are non-motile, and typically seen in large number inside polymorphonuclear leukocytes (22).

Culture characteristics.

Neisseria meningitides are aerobic, but they are grow better in primary culture in an atmosphere containing 5-10% CO2. The optimum temperature for growth is 35-

without the addition of blood. Colonies on blood agar are 1-2mm in diameter, and appear as convex, gray and translucent. After 48haurs, colonies become larger with an opaque raised center and thin transparent margins, which may be creneated. Thy do not produce haemolysis on blood agar (23).

Biochemical reactions.

Quick positive oxidase reaction, utilize glucose and maltose with production of acid, but not lactose or sucrose. Catalase positive (22).

Sensitivity to physical and chemical agents.

ctants at their

correct use dilution.

Serogrouping.

The immuno chemical differences in the polysacchoride capsules are the basis for the principal system used in serological classification of

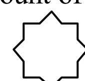
N. meningitides. Thirteen serogroups have been determined (24). Serotype classification of meningococci is based on capsular antigens in the outer membrane proteins and lipopolysaccharid, which become important in the epidemiological studies and development of new vaccines. The serogroups of meningococci known as; A, B, C, X, Y, Z, 29E and W135. Other scrogroups H, I, K and L their pathological significance is not yet clear. Group D was described but no capsular polysaccharide has been demonstrated for this group (24,21). Group A is common in Africa while group B is common in the European and American countries. The infectious groups C, Y and W135 are steadily rising worldwide. Epidemic meningitis is due to group A, while group B and C cause sporadic infections (25).

Transmission.

N. meningitids spread from person to person through the infective air borne droplet, direct contact, under crowded condition and poor hygiene. The nasopharynx is the portal of entry. The organisms attach to epithelial cells with the aid of pili; they may form part of the transient flora without producing symptoms (25).

Pathogencity.

The major risk factors of meningococcal infection are pathogenic organism, host susceptibility, influence of extrinsic environmental factor, or perhaps, most frequently combination of all. Epidemic meningitis becomes probable if the weather becomes dry and cool (26). The organism adheres to host epithelium by fimbriae or pili and colonizes the upper respiratory epithelium. The microorganism sheds large amount of



endotoxin, which is the essential part in the pathogenesis of meningococci (27). The capsular material (antigens) can bind host antibodies resulting in blocking them from serving as opsonins, it has been to inhibits serum bactericidal action by blocking Ig A antibody. Complement deficiency (C5, C6, C7 or C8) and absence of bactericidal anti body (IgG) leading to bacteremia (28).

Interaction of macrophages with endotoxin (lipo-oligosacchride or LOS) leads to release of cytokines, vaso active lipid (prostaglandins), free radicals which cause damage in the vascular endothelium resulting in deposition of platelets, and vasculitis leading to vascular disruption. Petchiae and ecchymoses are associated with meningococcol infection (21). Monocytes activation is accompanied by increase in disseminated intra vascular coagulation, and impaired pulmonary, cardiac, renal and cerebral function due to inadequate peripheral microcirculation that may cause death (29,21).

Clinical features.

N. meningitides cause meningitis and septicemia in which meningitis accounted for 30 to 50 % where the features septicemia are seen in 7-10% of cases, and 40 % of cases have mixed signs (30). The mortality from meningococcal meningitis and meningococcal septicemia is 5% and 20-40% respectively (31). The clinical features of meningococcal meningitis include headache, fever, vomiting, photophobia, lethargy, neck stiffness, conscious, neurological deficits, coma, raised intracranial pressure with Brady cardia, hypertension and often rash. The signs associated with meningococcal meningitis may be absent in infants, where fever, poor feeding, irritability, high pitched cry and a tense or bulging fontanelle are the most signs associated in infants with meningococcal meningitis. The features of meningococcal septicemia such as rash, vomiting, headach abdominal pain, myalgia, tachycardia, cool peripheries, hypotension and raising respiratory rate suggest the development of pulmonary odema or shock (32).

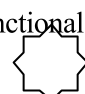
Epidemiology.

Meningococcal infections are common in both temperate and subtropical climates with sporadic cases throughout the year (33). Several studies have reported the occurrence of meningococcal epidemic caused by serogroup A in the Middle East, southern eastern Asia and in the sub-Saharan Africa (34,35,26). On the other hand, in developed countries most cases of meningococcal infection are caused by serogroup B. Meningococcal outbreak was reported in the northwestern European countries in the late 1970 (32). Intercontinental spread with outbreak in several countries of South America between 1980 and 1987 and in US in 1988 has been documented (34). Cases caused by serogroup B organisms began to decline slowly after 1988 where those due to group C organisms are increasing (36).

The second pandemic of meningococcal infection involves strain A: clone 111-1 which starts in China in 1983, spread to Nepal, north India, and other countries mostly by pilgrims. In August 1987 about 7000 cases were reported during pilgrim season (26).

Vaccination.

The capsular polysaccharide of the pathogenic strain of meningococcal is an antigenic material, which is used as a vaccine candidate. The currently licensed vaccine for serogroup A, C, J, W 135, and B is poor immunogenic because it is chemically and antigenically identical to human brain and fetal antigens, and its use might induce autoimmunity (37). Other bacterial components such as outer membrane proteins (OMP) are being sought as vaccine candidate (32). The menigococcal vaccine is recommended for persons with anatomic or functional



asplenia and those with terminal complement deficiencies. Although the need for revaccination has not been determined, antibody level decline over 2-3 years, and revaccination is recommended in
2-5years if risk continues (1).

Streptococcus pneumoniae.

S. pneumoniae is endemic throughout the world and it is more common in developing countries. The frequency of *S. pneumoniae* serotypes differs from country to another and from time to time (38).

S. pneumoniae is the main causative agent of lower and upper respiratory tract infection. It is part of naso and oropharynex normal flora, and carrier rate varies widely from time to time in the same population and in different nations (39).

Morphology.

Gram-positive cocci occurring in pair (diplococci), or short chain, 1mm in diameter, ovoid or lancet in shape with narrow distal ends,
non-motile, non-sporing and capsulated (12).

Culture characteristics.

Aerobic and facultative anaerobic, best growth in air containing 5-10 % CO_2 and grow in enriched media such as blood chocolate agar. The colonies on blood agar are small, smooth and transparent, aged colonies show central plateau with an elevated rim (draughtsman form), causes partial lysis of RBCs (alpha haemolysis). On repeated subculture, the capsulated smooth colonies (S-form) may give rise to non-capsulated, rough colonies (R-form) (12).

Sensitivity to physical agents.

course of days, and are highly sensitive to optochin (12). The organism is structurally delicate, and autolyze due to production of highly active autolytic enzymes (39).

Biochemical reactions.

Catalase negative, oxidase negative, form acid but not gas from glucose, lactose, and sucrose, soluble in bile or bile salt (40).

Antigenicity character.

Pneumococcal types are distinguished by type specific capsular polysaccharides antigen called soluble specific substance (SSS), which is immunologically distinct for each of more 83 serotypes (39).

Epidemiology.

Streptococcus pneumoniae causes sporadic infection. It is an endemic disease with a high incidence of carrier. The incidence of disease mostly occurs during cold months of year, where the carriage appears to be highest during coolest months of the year (fall, winter and early spring). The carrier rates of *S. pneumoniae* appear to be higher in children particularly those of a preschool age than in adults (41).

The oropharyngeal carriage duration ranges from 2 weeks to a year, the mean being 6 to 8 weeks, and reacquisition is commonly occurred (42).

S. pneumoniae is the most causative agent of BM in Egypt (18), while in Saudi Arabia; it is the 2nd of the most common of the BM agents (17), and in Sudan *S. pneumoniae* is the 3rd causative agent after *H*.

influenzae, and *N. meningitides* (14). Poverty, over crowding, inadequate clothing and exposure to climatic changes may play an important role in acquiring S. pneumonic infection (39).

Pathology.

In adults 75 % of pneumococal infection is caused by types 1-8, while in children types 6-14, 19 and 23 are frequent causes of infection in USA (40). While in UK, thirty per cent of healthy individuals carry *S. pneumonae* as commensal in the nasophraynx (43). Due to its ability to resist drying and other environmental condition, *S. pneumoniae* readily spread from the throat of one person to that of another by inhalation of infective air-borne respiratory tract secretion. The capsule is the main virulence factor of *S. pneumoniae*, which prevents or delays ingestion of pneumococcus by phagocytes. Respiratory tract infection is therefore facilitated by conditions such as primary viral infection that causes an excessive mucus secretion, which protects pneumococcus from surface phagocytosis and allows their free growth. Pneumococcal infection causes an outpouring of fibrinous edema fluid into alveoli with dense infiltration of polymorphonuclear leukocytes and increasing in vascular red blood cells. Infection may extend into the pleural space (in 5-10 % of patients) resulting in empyema or enter blood stream (in 15-20%) causing bacteremia, which may lead to meningitis (41,44).

Jadavji et al (45) reported in study carried out in Canada that 20 % of survivors from meningitis caused by *S. pneumoniae* had mild to severe handicap, and the sequelae (57%) was greater in children with pneumococcus meningitis, than those infected by *N. meningitides*. The sequelae include: hearing loss (12.9 %) development delay (5.3%), speech defect (4.7%), motor defect (3.0%), hydrocephalus (1.3%), and seizure disorder (1%).

The case fatality rate was greater (67%) in children with pneumococcal meningitis than other forms of meningitis (46).

Immunity.

The capsular polysaccharide is the antiphagocytic component of pneumococci and the principal protective antibody is the antibody specific for capsular polysaccharide. These antibodies can be detected in the blood 5-10 days after infection in non-immunized or untreated patients. Production of type specific anticapsular antibody is the main factor bringing about recovery. The anti capsular antibodies IgG antibodies together with complement enhance phagocytosis and intracellular killing by polymorphonuclear phagocytosis and alveolar macrophages. Susceptibility to infection is more common among patients with deficiencies in IgM, IgG, and secretory IgA than normal persons (47). The acquired immunity is specific for each of the 80 different types of pneumococci. Persons recovered from one type of pneumococi may at any time suffer a second attack of infection of different type. The majority of liver and splenic macrophages are more important for pneumococcal clearance from the blood rather than polymorphonuclear leukocytes, thus liver cirrhosis or splenectomy rather than neutropenia increase the risk for pneumococcal bacteremia, dissemination, and death. Many persons have not suffered pneumococcal infection nevertheless they posses antibodies to various types of the organism. They may have developed these antibodies either as a result of having carried the corresponding pneumococci for long period or by ingestion of cross-reacting antigenic polysaccharides in vegetable foodstuff (44,48).

Vaccination.

The pneumococcus vaccine contains 23 capsular polysaccharide antigens of pneumococcal serotypes that

can cause 90% of infection (49). The vaccine is poor immunogenic in the very elderly and can not induce immune response in the age groups below 18-24 months of age, it acts as thymus-independent antigen, so booster immunization is not effective. Revaccination of high-risk patients is needed at 3-5 years intervals. Vaccine should be offered to all patients at risk such as healthy adults of aged 65 years or more, patients with cardiac or pulmonary diseases, chronic liver diseases, spleen dysfunction, alcoholism, diabetes mellitus and CSF leaks (1,39). The great value of vaccination is to reduce bacteremia, dissemination, and mortality in-patients at risk (50). At present highly immunogenic vaccine (capsular polysaccharides conjugate to protein are under development (1)

<u>Sensitivity to antibiotics.</u>

The increasing prevalence of pneumococci resistant to penicillin, penicillin and chloramphinicol and multiresistant strains were reported in South Africa and Spain (51,52). In UK 2.9% of S. pneumoniae are penicillin resistant (53). In US most of the reports about *S.pneumoniae* resistance come from Alaska and the south of US, where in other states and Canada the pneumococcus resistance has been increased. The emerging of this resistance highlights the need for children vaccination (54).

Baraf et al, (55) have showed that resistance of pneumococci to penicillin is due to changes in affinity of penicillin-binding proteins for their beta-lactam substrates not by the presence of beta-lactmase, and using of beta-lactmase inhibitor is without value against pneumococcus.

Ampicillin or penicillin was used as the drug of choice for several years for treatment of pneumococcal meningitis, but the increase in the prevalence resistance of beta-lactamase producing a pneumococcal strain prompts using of chloramphinicol as alternative drug for succeful treatment of pneumococcal meningitis (56).

Cephalosporins are active against beta-lactmase producing microorganisms including *Haemophilus influenzae*. Vancomycine is generally active against pneumoniae but should not be used as a single agent due to its poor penetration into CSF (56).

<u>aemophilus influenzae.</u>

Eighty percent of healthy individuals carry non-capsulated *H. influcnzae* in the nasopharynex as normal flora, while the capsulated organism is carried by approximately 1% of children less than 5 years (57). H. influenzae is the leading organism cause of invasive bacterial disease in children less than 5 years. It can infect upper respiratory tract, epiglottis, middle ear, lung, sinuses, pericardium, joint, blood stream, and meninges (58).

Morphology.

H. influcnzae are small Gram-negative bacilli (coccobacilli) but in old culture show pleomorphic, filamentous and swollen forms, non-motile and non-sporing(12).

Culture characteristics.

H. influcnzae are grow aerobically and poorly anaeorbically, few strains require 5-10 % CO2. The range of temperature for growth is

20- -

factor X is heat-stable, which is chemically protoporphyrin IX, haemin or some other iron containing substances. The factor V is heat-labile, and can be replaced by nicotinamide adenine nucleotide (NAD). The colonies of

capsulated organism are 1-3mm in diameter, high convex, mucoid and blue shades which alter with the angle of observation, and non haemolytic on blood agar. The colonies of non-capsulated strains are 0.5-1mm in diameter, circular, low convex, smooth, pale gray and transparent. The non-pathogenic beta-haemolytic strains are classified as
H. haemolyticus. Heated blood agar plus 0.2-0.5 units/ml of penicillin or 5-19 units/ml bacitracin plus 5mg /l of cloxacillin are used as a selective media. Filled agar is moderately selective for haemolyticus (59,60).

Biochemical reactions.

Catalase and oxidase positive, ferments glucose and galactose producing acid but not gas, some strains also ferment fructose, maltose or xylose, do not ferment lactose, sucrose or manitol (60).

Subtypes of H. influenzae.

Of three types of surface antigens called capsular polysaccharide, lipopolysaccharide, and outer membrane proteins (61). On the basis of biochemical reactions (indole production, urease activity and ornithine decarboxylase), *H. influenzae* is differetiated into eight biotypes. Three biotypes (I, II, and III) are clinically important. More than 90 % of invasive type b strains are of biotype I (60). According to the antigenic types, the encapsulated H. influenzae contain capsular poly saccharides of one of 6 types (a, b, c, d, e and f). The capsular antigen of type b is
a polyribose-ribitol phosphat (PRP) (40)

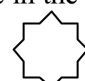
Epidemiology.

H. influenzae is endemic throughout the world and usually occurs as sporadic cases. Nasopharangeal carriage of *H. influenzae* is more common in developing countries and occurs in younger children (62). The incidence of Hib meningitis is greater in developing countries than in developed countries and the disease occurs in younger children with cases occurring before 12 months of age and almost half before the age of 6 months (63). 20-40% of patients die in sub-Saharan Africa after admission to hospitals (64).

In developed world as the US, the mortality rate due to invasive Hib in children less than 5 years ranged between 3% to 6% but the permanent neurological sequelae affect 20-30 % of meningitis survivors (65). Some studies have suggested a potential genetic susceptibility to Hib disease. This evidence includes secondary spread of *H. influenzae* disease in twins, impaired immune response in siblings of patients with
H. influenzae disease (66). High incidence in several Native American population (67) and increase risk for black has been documented (68). Black race children 2 to 5 months of age have low risk for *H. influenzae* disease than white children; however, the risk increases for black children at the age of 12 to 35 months. The low relative risk among black race children 2 to 5 months may be due to the role of maternal antibodies during the first 6 months of age, and the higher incidence of *H. influezae* disease in black than white attributed to genetic markers (67).

Pathogencity.

1. The mode of acquiring *H. influenzae* is similar to that of meningococcal. The endotoxin, capsules and other virulence factors play a major role in colonization and pathogencity of *H. influenzae* (69). The virulence factors include.
2. The capsule that inhibits opsonizaton, clearance and intra cellular killing.
3. Fimbriae, which enhance the adherence of *H. influenzae* to mucosal surface.
4. Lipo-oligosaccharides (LOSs), which facilitate *H. influezae* survival on mucosal surface in the



nasopharynex and initiate blood stream invasion.

5. Outer membrane proteins (OMPs), which act either as a pure protein or associated with iron binding.

The central nervous system is the primary site invaded via choroid plexus. Endotoxin (LOSs) is important in the initiation of inflammatory process (pyogenic meningitis) involving release of various cytokines and local accumulation of leukocytes at the site of infection. Uses of antibiotics that liberate LOSs from organisms accelerate the inflammatory process (70). Endotoxin stimulates endothelial cells, osterocytes, and microglia cells in central nervous system to release cytokines such as interleukin (IL), and tumor necrosis factor alpha (TNF), which induce meningitis either alone or together. The risk of neurologic complication is increased in patients with high concentration of endotoxine in CSF at the time of diagnosis (71).

Vaccines.

Hib vaccines, which consist of capsular polysaccaride protein conjugates, have been introduced into routine vaccination programs in several countries (72).

The vaccines against Hib are highly effective in reducing Hib disease and carriage in developed countries. In developing and new industrialized countries, *H. influenzae* infections are still an important cause of morbidity and long-term sequela e, and mortality in infants and young children (73).

Hib vaccines consist of capsular polysaccharide, polyribosyl-ribitol phosphate (PRP), or oligosaccharide coupling to a protein carrier. The PRP-D vaccine is composed of capsular polysaccharide conjugated to diphtheria toxoid via an adipic acid linker, while in the PRP-T vaccine polysaccharide is conjugated to tetanus toxoid (74). HbOC containing oligosaccharide (OSs) directly coupled to a nontoxic variant diphtheria toxin. The PRP-OMP vaccine, where the polysaccharide polyribosyl-ribitol phosphate is coupled by bigeneric linker to an outer membrane protein complex (OMP) of the meningococcal. PRP-D, PRP-T, and HbOC are highly immunogenic (75).

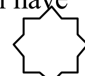
In 1985, Hib vaccine was licensed for use in the United States (76). This vaccine consists of purified PRP antigens, which elicits thymus independent B-cells response, generates B-cell but fail to induce a response in neonates and infants because polysaccharides are T-cell independent antigens. These antigens stimulate B-Lymphocytes without the help of T-cells, and the B-cells of young infants are not sufficiently mature to respond to polysaccharide antigens (77). To overcome this problem, conjugate vaccines were introduced (T.cell-dependent antigens), which require T. helper cells that stimulate B. Lymphocytes for synthesis of antibodies. The conjugate vaccines are more immunogenic than purified capsular polysaccharide alone (78). The infants at 2 to 6 months of age are recommended for immunization (79).

Sensitivity to antibiotics.

Comps et al, (80) had reported in a study carried out in Spain that 60 % of *H. influenzae* type b was resistant to ampicilline, 65.7 % were resistant to chloramphinicol and 57 % to both with high prevalence of these multiple resistant strains in carriers. They advised the using of new cephalosporins such as cefotaxim, moxalactam, and ceftriaxon as alternative treatment for meningitis caused by multiple resistant Hib.

<u>Laboratory methods for diagnosis of bacterial meningitis:</u>

Routine methods such as direct examination of CSF microscobically (Gram stain), cultural methods for isolation of causative agent in CSF, cell count and determination of glucose and protein concentration have



been accepted as standard methods in the laboratory for diagnosis meningitis infection (81).

CSF glucose and CSF-blood Ratio.

When the CSF glucose is less than 1.9 mmol/L or the ratio of CSF-blood glucose is below 0.23 g/L both values are used as predictors of BM (82).

CSF protein.

The value more than 2.2g/L is a predictor of BM, and acute B.M should not be excluded by low protein. 10 % of cases of acute BM were in the normal level (below 0.4 g/L) (82).

CSF leukocyte count.

More than 2000/cmm of leukocyte count is a predictor of acute BM, 45% of cases the CSF leukocyte count is less than 1000/cmm, and 21% below 250/cmm. In the cases of acute viral meningitis the polymorphonculear (PMN) cells are predominated in 40 % of cases in the first spinal sample collected in the first day of hospitalization (82).

Cultural methods.

Cultural method is need at least 18h for isolation and identification of microorganism (83,84). Previous antibiotic treatment before sample collection may yield sterile culture (85). In ordinary conditions culture yield 33.9 % (10). In addition, cultural methods require facilities available only in large hospitals in developing countries (85).

Coagglutination test (COA) on CSF.

This test is used to detect antigens of *N. meningitides, H. influenzae* type b, *S. pneumoniae* and group B *streptococcus* in CSF. The reagent is
a suspension of killed *Staphylococcus aureus* (Cwon 1strain) coated with type-specific antibodies (against these microorganisms mentioned above), bound by their Fc portion to the protein A of Staphylococcal cell wall leaving the Fab (antigen binding site) free to bind its corresponding antigen (86).

The sensitivity of COA range between 48-100%, which varies with bacterial species; it is highest for *H. influenzae* 78-100%, followed for
S. pneumoniae and *N. meningitides* 50-77% and 48-93% respectively (87).

Coagglutination reaction is highly specific, but may not be as sensitive for detecting small quantities of antigen in CSF as Latex agglutination (88).

Latex agglutination test (LAT).

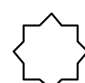
It consists of polystyrene latex particles sensitized (coated) with
globulin. Since the number of antibody molecules bound to each latex particle is large, the potential number of antigen binding sites is also large. In the presence of corresponding antigen, in solution being tested, the antigen will bind to the combining sites of the antibody exposed on the surface of the latex beads, forming cross-linked aggregates of latex beads and antigen (89).

False positive reaction may occur due to many factors such as rheumatoid factor or cooled-reactive immunoglobuline (IgM) antibodies. To counteract this problem, it is recommended that all specimens be pretreated with ethylene diamine tetra acetic acid (EDTA) to extract the antigen before testing (12).

Direct immuno fluorescence (IFL).

Specific antisera IgG conjugate with fluorescein is used to detect antigen. The antigen antibody complexes are visualized by fluorescence microscope (90).

Radio immunoassay (RIA).



The radio actively labeled antigen binding to a limited fixed amount of antibody can be partially inhibited by addition of unlabelled antigen, the more unlabeled (patient) antigen that is added, the less the labeled antigen will be bound to the antibody, and the exert of this inhibition can be used as a measure of the unlabeled material added (90). RIA techniques are highly specific and sensitive, but they require very expensive instruments and highly skilled technologist. Additionally, the institution must be licensed and willing to deal with the use of radioactive substances (89).

Polymerase chain reaction (PCR).

Kristiansen et al, (91) used two oligonucleotides flanking the dihydropteroat synthase gene (dhps) as primers, which were isolated from meningococcal strain for detection of meningococcal DNA in CSF. In brief, 250uL of CSF mixed with 250uL of TE buffer (Tri-HCL and EDTA), centrifuged, and the pellet was resuspended

PCR. Amplification was done with Taq polymerase, dNTP, primers at 40 cycles. The sample was then applied for electrophoresis and bands were visualized with UV light after staining by ethidium bromide.

A negative control was PCR reagents without adding DNA and positive controls were applied.

Counter current immuno electrophoresis (CIE).

Most bacterial antigens are negatively charged, while antibodies are neutral. The solution containing antibodies and body fluid (CSF) to be tested are placed in small wells of agarose in tow opposite sides. The side of the agarose, which contains antigen wells, is connected to cathode chamber containing buffer, and the other side of antibody wells is connected to the anode buffer chamber. When an electric current is applied through the buffer, the negatively charged antigen molecules migrate toward the anode. The neutrally charged antibodies are carried toward the cathode by the flow of slightly alkaline buffer. Antigen-antibody complexes form a visible precipitin band at some point between the wells (92).

The procedure usually takes about one hour. The sensitivity is about the same of coagglutination, 0.01-0.05mg/ml of antigen can be detected. Bands are often difficult to be seen, so removing nonspecific precipitin reaction may require over night washing of agarose in distilled water. CIE is more expensive than la tex particle agglutination or Coagglutination (89).

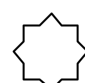
Enzyme Linked Immunosorbent Assay (ELISA).

Consist of a set of three plastic tubes coated with antibodies against *N. meningitides* (group A, B, and C), *S. pneumoniae* (25 selected types) and *H. influenzae* type b. CSF to be tested is placed to each of the three tubes and incubated for 30 minutes, if bacterial antigen is present in CSF it will bind to the antibody. Then an enzyme-lapelled specific antibody is added which binds to the antigen antibody complex. After washing the substrate is added and incubated for 10 minutes at room temperature. Finally 2N HCL is added to stop the reaction. The development of a yellow-orange color, which can be seen by the naked eye, is considered a positive reaction.

ELISA procedure is simple, takes less than 1h and can be read by the naked eye (83).

ELISA is highly sensitive that detects even minute amounts of antigen (<1ng antigen /ml) (12). However, ELISA technique is expensive and is not the system of choice when extremely rapid results are required, as for specific diagnosis of meningitis (93).

Aims of the study:



hospitals.

** To evaluate the sensitivity and specificity of the Gram stain and cultural techniques in comparison to the coagglutination technique.*

**** To study the effect of antibiotics pretreatment on cerebrospinal fluid results.***

Materials.

Media & Reagents.

The bacteriological culture media used in this study were:

Mueller-Hinton agar, MacConkey agar, Clumbia agar, and Blood agar. They were commercially available. They were prepared according to the instructions of the manufacturer.

Chocolate agar:

To the basal medium prepared 5% of defibrinated sheep and human blood was added separatly, the mixture

petridishes (94).

Sheep and human blood agar:

To the basal medium prepared 5% of defibrinated sheep, and 5-10 % of human blood were added separatly (12,94).

Carbohydrate medium for biochemical identification of isolates:

Rabid carbohydrate utilization test (RCUT) for identification N.meningitides.

This test is a non-growth dependent method for carbohydrate fermentation studies. In this method, small volume (one drop) of a dense suspension of the tested organism in buffer-salt solution containig 1% aqueous phenol red was added to 2% filter sterilized sugar (through

a syringe containing a membrane filter with pores 0.2 um in diameter) containig tubes. The tubes were shaked, and

A yellow colour or yellow orange is positive; red is negative (23).

Bile solubility test:

For detection of autolytic enzyme of S. pneumoniae .The reagents contain sodium desoxycholate 10 %. 2drops of this reagent was added to 2ml turbid suspension of tested organism in physiological saline. If the suspension

pneumpniae (94).

Oxidase reagent:

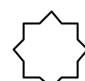
Contain 1% Dimethyl-p-phenyl diamine hydrochloride in distilled water (94).

Optochin sensitivity:

The disc contains 5ug of optochin (ethylhydrocuprin). The disc was placed on the plate of blood agar inoculated with suspected pneumococcus-like colonies from primary diagnostic plate. The plate -10%

CO_2 A growth of pneumococcus will be inhibited for at less 5mm from the margin of the disc (12).

Glacial acetic acid (diluting fluid for CSF cell counting) (95).



Gram staining reagent:

It contains: - Violet dye.

- Gram iodine.

- Decolorize: Acetone

-Counter stain: Safranine (0.5%) in distilled water (94).

Catalase reagent: The reagent contains H_2O_2 10 vol (95).

Glucose reagent:

For determination of glucse concentration in CSF. Commercially available (Randox Laboratories, UK).

Trichloroacitic acid 5%:

For determination of protein concentration in CSF (94).

Coagglutination reagent:

Phadebact CSF test 20 (Boule Diagnostic AB Hodding, Sweden), was used as a confirmatory test for detection of bacterial antigens (capsular) of *S. pneumoniae* (83 stereotypes), *N. meningitides* group A, B, C, Y, and W135, *H. influenzae* type b, and group B *streptococcus*. Each Phadbact CSF test package contains reagents sufficient for 20 determinations. The reagents are coloured blue (methylene blue) to facilitate interpretation of results.

Reactive ingredients.

-CSF Pneumococcal reagent.

-CSF H. influenzae type b reagent.

-CSF Strep. B reagent.

-CSF Meningococcal reagent.

Antibiotics disks:

cefotaxime, cefotriaxon, penicillin, penicillin G, chloramphincol, ampicilline, and amoxicilline plus clavulinic acid (augmentin).

Patients and methods.

Patients.

Case definition.

Suspected case, based on clinical diagnosis only, with no laboratory diagnosis. While the probable case, based on clinical diagnosis, laboratory test suggestive but not conformed. And the conformed case, based on clinical diagnosis and conformed laboratory investigation.

The cases investigated in this study were patients with bacterial meningitis, based on clinical diagnosis, and detection of causative agents by culture or coagglutination techniques.

Cases of bacterial meningitis due to head injury, CSF shunt or neurosurgery, were excluded.

Target population.

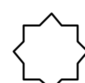
Based on our case definition, infants and children up to 14 years of age seeking medical care in AL-Thawra and AL-

Ethics and consent.

The nature of the study was explained briefly to the clinic ians and the relatives of the patients. Verbal approval was taken.

The relatives of the patients had the right to withdrow from the study without giving any reason.

Study design.



This study describes the causative agent of bacterial meningitis among 96 children admitted in Al-Thawra and Al-

Method of sampling.

The standard sampling method was used to achieve the aims of the study. The use of random methods for collection of specimens was not possible and not practical.

Throughout the study period from April to December 1999 a total of 300 CSF specimens were collected from children of target group (postnatal-14years) clinically diagnosed by pediatricians and physicians as suspected cases of meninges infection in pediatric emergency units or pediatric word of Al-Sabaeen and Al- hesamples of CSF were collected under complete aseptic condition by lumber puncture in screw caped sterile containers and transferred directly to the laboratory for investigation. Each specimen was accompanied by a questionnaire containing relevant information as age, sex, clinical diagnosis, preadmission treatment and number of persons occupied per room.

Specimens of CSF were designated normal on initial examination if no organisms were seen on Gram stained film, the white blood cells count was less than 5 per cubic milliliter, the glucose concentration was more than 3 mmol/L and the protein concentration was less than 0.45g/L (82).

The diagnosis was established when significant inflammatory changes were detected upon CSF examination by Gram stain film, increase white blood cells count, increase protein concentration over the value mentioned above and decrease in glucose concentration. Only 96 (32%) CSF specimenes were accepted according to the primary investigation, and then cultured for isolation and identification of microorganisms. Coagglutination test was applied on all accepted CSF specimens as a confirmatory test.

METHODS

Sample collection and examination.

The sample of the CSF was collected under aseptic condition into two containers for.

A-Cell counting and biochemical testing.

B-Bacteriological and serological investigation.

CSF was examined by the naked eye for.

1- Appearance: turbid or clear.
2- Color: blood stained. (94)

Cell count:

The leukocytes were counted by counting chamber. Clear or slightly turbid CSF were examined undiluted, but highly turbid CSF was diluted for cell counting by using Glacial acetic acid as diluting fluid (96).

The CSF specimens contaminated with blood, the leucocytes and erythrocytes were counted separatly. The increase in leukocytes counted of 1 per 1000 erythrocytes suggests the presence of meningitis (96).

The normal range of leukocytes in CSF is 0-to 5-leukocytes/ ml, whereas in neonates it ranges up to 30/ml (95).

Gram staining.

ıe film of the deposit was performed on all CSF sediments. A drop of the sediment was allowed to air dry, heated or methanol fixed and stained by Gram stained procedure (96).

Protein and glucose determination.

The CSF glucose and protein were determined by using Synchron CX-5 machine, Beckman, U.S.A, by application of CSF sample type program according to the method described by the manufacturer. In addition, CSF glucose and protein were measured colorimetrically using a glucose oxidase method for measurment of CSF glucose, and trichloroacetic acid total protein method for measurment of CSF protein (97).

Cultivation.

CSF specimens were centrifuged and the sediments were vortexed and several drops of sediment were inoculated heavily onto chocolate agar, blood agar, Mueller-Hinton agar and MacConky agar in safety cabinat. The plates were incubated in humid atmosphere containing

5-10% CO_2

Identification of the isolates.

The colonies of bacterial growth on different inoculated media were examined macroscopically, microscopically, and biochmically.

The suspected organism was identified as follows:

N. meningitides:

The suspected colonies of organism on Mueller-Hinton agar plate were identified by Gram stain, oxidas test, sugar fermentation (95), coagglutination (23,92).

S. pneumoniae:

The suspected colonies on blood and chocolate agar were identified by Gram stain, Catalase test, Coagglutination, Sensitivity to optochin disc, and Bile solubility (12).

H. influenzae:

The suspected colonies on chocolate agar were identified by

Gram stain, satellitism tests (94,99), and Coagglutination (92).

Coagglutination test for identification of the isolated microorganisms.

The COA test is performed as a simple slide test with suspension of the presumptive homologous antigen from bacterial culture or, alternatively, the test can be used directly for detecting the presence of bacterial antigens in clinical specimens, CSF, serum, and urine (24,92). Finegold & Baron (12), reported that the organisms once isolated, can also be rapidly identified with the reagent which is used for detection of bacterial antigen in CSF.

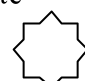
Identification of the isolated microorganisms by coagglutination mehtod.

Rapid extraction bacterial antigens procedure (REAP).

One part of bacterial cell suspenssion in sterile normal saline (0.9%) was added to centrifuge tube containing three part of 0.1 M ethylenediaminetetraacetic acid (EDTA), the tube was tightly closed, vertoxed, and heated for 3min

pipette, and one drop of this solution was mixed with one drop of the reagent on slide. The drops were mixed gently with

a disposable loop. Then the slide was rocked, and the result was read within one minute



(12).

Antibiogram testing.

The identified colonies were subcultured onto an appropriate medium for sensitivity testing of isolated organism to antibiotics by using disc diffusion method (For each isolated pathogen 7 antibiotic disks were used). The antibiotic disks used in this study include:

Cefotaxime, Cefotriaxon, Penicillin, Penicillin G, Chloramphincol, Ampicilline, and Amoxicilline plus Clavulinic acid (100).

According to the size of the zone of inhibition measurement, the organism was determined as sensitive or resistant to the antibiotics by comparing the results of the tested antibiotic inhibition zone with standard zone of antibiotic disc in millimeter (80).

Coagglutination test for detection of bacterial antigens in CSF.

Phade bact CSF test 20 commercially available (Boule Diagnostic AB Hodding, Sweden) was used as a confirmatory test. It is intended for direct identification of capsular antigens of the following organisms: *S. pneumoniae, H. influenzae* type b, *N. meningiidis* groups A, B, C, Y, W135, and streptococcus agalactiae (Strep B) in CSF.

Principle of the test.

Antibodies, raised in rabbit, specific against *Streptococcus pneumonae, Neisseria meningitides, Haemophilus influenzae* type b and *Streptococcus agalactiae* are bound to Protein A on the surface of non-viable staphylococci. When a CSF specimen containing microorganisms belonging to one of these groups is mixed with reagents, the specific antigens on the surface of the microorganisms bind to the corresponding specific antibodies. A coagglutination lattice is formed, which is visible to the naked eye (101, 102).
In brief, the CSF (supernatant) sample was heated in water bath at
allowed to cool, one drop of each reagent was placed in a separate oval on the slide, and one drop of preheated sample was added to each drop of reagent on the slide. The drops were mixed gently with a disposable loop. The slide was then rocked, and the result can be read within one minute.

The specificity of the coagglutination test is 99%, where its sensitivity is as follow: *H. influenzae* type b 87%, *S. agalactiae* 82%, *S. pneumoniae* 84%, and *N. meningitides* 60% as reported by the manufacturer.

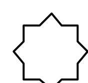
Evaluation of the sensitivity and specificity of the Gram stain and cultural tests results in comparison to the coagglutination test.

Gram stain and cultural techniques were conducted to the96 CSF specimens for evaluation in comparison to coagglutination technique

The sensitivity, and specificity of the test were calculated as follows:

$$\text{Sensivity of the test} = \frac{\text{True positive}}{\text{True positive + false negative}} \times 100$$

$$\text{Specifcity of the test} = \frac{\text{True negative}}{\text{True negative + false positive}} \times 100$$



Where the true positive (TP) was defined as the results which were positive by both techniques coagglutination and Gram stain or coagglutination with culture, while the true negative (TN) was defined as the results which were negative by both techniques coagglutination and Gram stain or coagglutination with culture. False positve was defined as the results which were positive by either Gram stain or culture for one microorganism and negative by coagglutination for that microorganism, while false negative results were defined as the results which were negative by Gram stain or culture and positive by coagglutination (103).

Analysis of data.

The data were analyzed using the Epi Info program version 6.04.

Results:

A total of 300 CSF specimens were collected from children (target group) admitted to Al-Sabaeen, and Al-Thawra hospitals, clinically diagnosed (suspected case) to have meningitis. Lumbar punctures were done on all of them, and CSF was analysed bacteriologically and serologically for common causative organisms.
Of these, 96 (32%) CSF specimens were accepted according to the primary laboratory and clinically investigations, while 204 (68%) were excluded.
When the accepted CSF specimens were cultured and serologically tested, bacteria were detected in 62 (64.6%) of CSF specimens.
As seen in table 2, *S. pneumoniae* was the predominant organism of acute BM, with a percentage of 38.7%, followed by *H. influenzae* with 32.3%, *N. meningitides* with 27.4% and Group B *streptococcs* with 1.6%. It was seen that *H. influenzae* type b was the most common agent of acute BM in children less than 2years of age (46.3%) as shown in table 2 & 5.

Age-sex, and bacterial meningitis relationship.

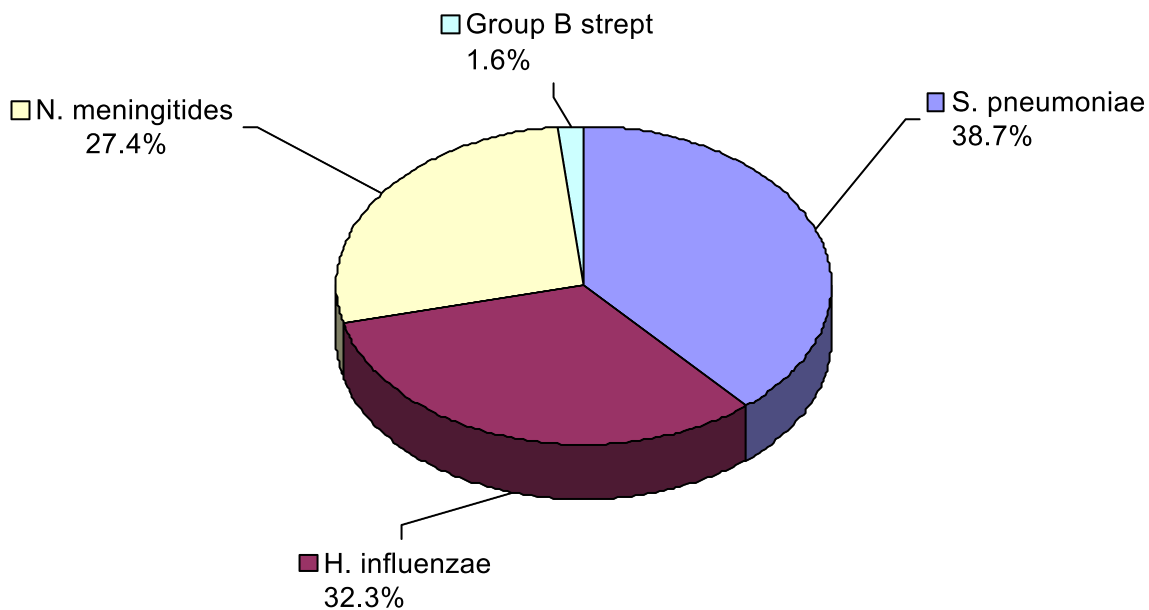
The number of infected males were 41 (66.1%), and females were 21 (33.9%). The males : females ratio among all age groups was 1.95:1, while the ratio of males : females up to 2 years was 1.4: 1 (table 3).
The frequency of BM in relation to sex shows a significant association between males and the incidence of BM, ($p < 0.015$), especially in those with age of 2 years or more. It was seen that there is no significant differences between males and females less than two years of age and the incidence of BM, $p > 0.28$ (table 3). On the other hand, there is no significant association between the genus of microorganism and the sex of infected children up to 14 years of age, $p > 0.8$ (table4).
According to the age of children, it was seen that 82.2 % of acute BM occurs among children with less than 4 years of age, and the majority of these cases (66.1%) occurs in children less than 2 years of age. The age group up to 2 years of age was found to be more susceptible to acquire infection, $p < 0.015$ (table 2).

Age/yr*	No. **	%	**S. pneumoniae**		N. meningitidis		H. influenzae type b		GroupB Streptococci	
			No.	**%**	No	%	No.	%	No.	%
0- 2	41	66.1	13	31.7	8	19.5	19	46.3	1	2.4

-4	10	16.1	6	60.0	3	30.0	1	10.0	0	0
-6	3	4.8	1	33.3	2	66.7	0	0	0	0
-10	4	6.5	2	50.0	2	50.0	0	0	0	0
10-14	4	6.5	2	50.0	2	50.0	0	0	0	0
Total	62	100	24	38.7	17	27.4	20	32.3	1	1.6

Statistical association of BM with age group up to 2 years of age
$p < 0.015$ & Chi Sq = 5.9

* Age/ years.
** Number of cases.



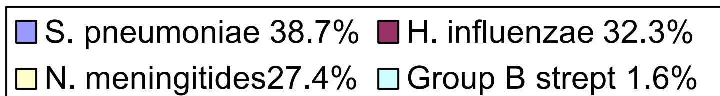
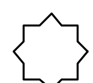


Table 3: The age groups and sex distribution of 62 infected children with bacterial meningitis admitted in Al-Thawra & Al-



Age group In years	Males		Females		Total		Chi Sq	P*
	N0.	%	No.	%	No.	%		
0-2	24	58.5	17	41.5	41	66.13	1.17	>0.28
-4	8	80.0	2	20.0	10	16.13	---	---
-6	2	66.7	1	33.3	3	4.84	---	---
6-14	7	87.5	1	12.5	8	12.90	---	---
Total	41	66.1	21	33.9	62	100.0	5.93	<0.015

The relationship between the sex and bacterial meningitis was statistically significant, (P < 0.015).

P*: P value.

Table 4: Sex distribution of patients with the causative agents of bacterial meningitis.

Sex	Distribution of cases		S. pneumoniae		H. influenzae		N.meningitidis		GrouipB strept	
	N0.	%	No.	%	No.	%	No.	%	No	%
Males	41	66.1	16	39.0	13	31.7	11	26.8	1	2.4
Females	21	33.9	8	38.1	7	33.3	6	28.6	0	0
Total	62	100.0	24	38.7	20	32.3	17	27.4	1	1.6

Statistically no significant deference between the sex and the causative agents of bacterial meningitis, (Chi sq = 0.4 & p > 0.82).

Table 5: Distribution of bacterial meningitis in children up to 2years of age.

*** Age/mo.**	**** No.**	**%**	**S. pneumoniae**		**N. meningitidis**		**H. influenzae b**		**Group.B. Strept.**	
			No.	**%**	**No.**	**%**	**No.**	**%**	**No**	**%**
0 - 6	12	29.3	2	16.7	1	8.3	8	66.7	1	8.3
-12	19	46.3	5	26.3	3	15.8	11	57.9	0	0

-18	6	14.6	4	66.7	2	33.3	0	0	0	0
18-24	4	9.8	2	50.0	2	50.0	0	0	0	0
Total	41	100	13	31.7	8	19.5	19	46.3	1	2.4

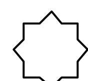
*Age/months.

**Number of cases.

Out of 52
sitive cases, 21 (40.4%) were resided in household containing more than 5 persons per room, 14 (26.9%) of infected ildren were resided in household containing 3-5 persons per room, and 17 (32.7%) were resided in household containing 3 persons per room. This study shows no statistical significant association between the increase in number of persons per om in these groups and BM infection (p >0.84 and Chi sq = 0.34).

Evaluation of the sensitivity and specificity of the Gram stain and cultural test results in comparison to the coagglutination test.

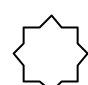
The CSF results are shown in table 5,6,7, and figure 3. Out of 96, thirty of cases (31.3%) had positive Gram stain, coagglutination was positive in 62 (64.6 %), and culture was positive in 18 (18.8%). The difference between the results yielded by all of these techniques found to be staistically significnnt, p < 0.00014, (table 6).



The difference in percentage between the results of Gram staining
(31.3%) in comparison with coagglutination rsults (64.6 %) found to be staistically significnnt ($p < 0.009$), and the results of culture (18.8 %) in comparesion with coagglutination found to be staistically significnnt
($p < 0.0003$).

The sensitivity and specificity of Gram stain were 48.4% and 73.5% respectively compared to coagglutination results, while the sensitivity and specificity of culture results were 29 % and 97% respectively compared to coagglutination results (figure 4). The sensitivity and specificity results of coagglutination were 78% and 99% respectively, were previously evaluated in comparison to other immunoassays (104).

Table 6. The percentage of microbiological results on 96 cereprospinal



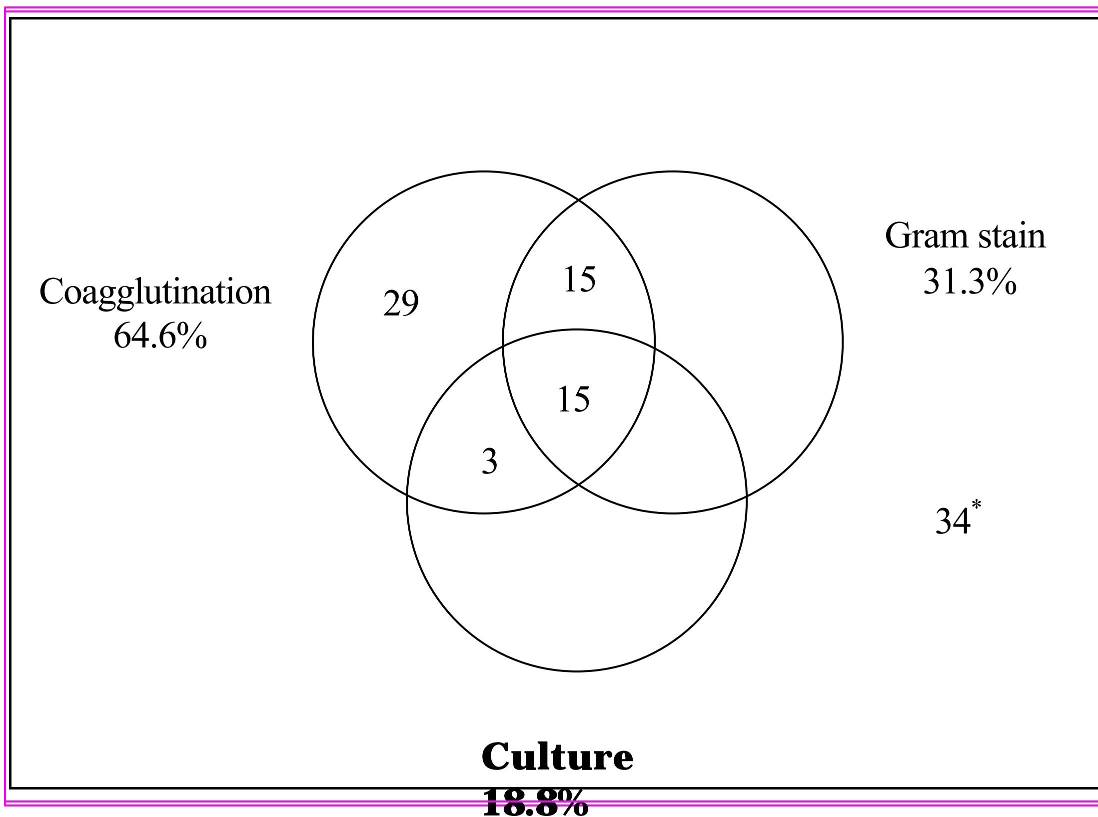
Methods	Results			
	Positive		Negative	
	No	%	No	%
Coagglutination	62	64.6	34	35.4
Culture	18	18.8	78	81.3
Gram stain	30	31.3	66	68.8

Chi Sq =17.75 $p < 0.00014$ for positive results

Figure 3.

Venn diagram showing the results of 96 CSF specimens using coagglutination test, culture and Gram stain. Figures within a circle represent positive.

*Samples negative in all tests



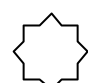


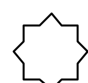
Table7: The results of 96 CSF specimens tested by coagglutination and Gram stain.

Gram stain	**Coagglutination**		**Total**
	+ve	**-ve**	
+ve	30	9	39
-ve	32	25	57
Total	62	34	96

Table 8: The results of 96 CSF specimens tested by coagglutination and culture.

Culture results	**Coagglutination results**		**Total**
	+ve	**-ve**	
+ve	18	1	19
-ve	44	33	77
Total	62	34	96

: 4, Comparison between sensitivity and specificity of Gram stain, culture and coagglutination in diagnosis of bacterial meningitis.



100%
90%
80%
70%
60%
%
50%
40%
30%
20%
10%
0%

78%
29%
48.4%
99%
97%
73.5%

Sensitivity
Specificity

Coagglutination Culture Gram stain

<u>The effect of antibiotics pretreatment on cerebrospinal fluid results.</u>

The results of this study show that the culture yielded small number of isolates. This low number of isolates is due to pretreatment of patients, where most of cases (61.4%) received antibiotics before coming to hospitals or before sample collection (table 9).

Out of 37 CSF specimens which were collected from children before antibiotics treatment, the percentage of culture 14 (37.8%) and Gram stain 16 (43.2%), statistically no significant different between the results of both culture and Gram stain. On the other hand the results of the culture on specimens which were collected from cases with antibiotics pretreatment (6.8%) (table 9) shows that the antibiotics highly affected the outcome of the cultural results compared with Gram stain, 23.7% ($p<0.01$) and coagglutination results 64.4% ($p<0.0000001$).

The difference between pretreated and non-treated cultural and Gram stain results were found to be statistically significant $p<0.00015$ and <0.044 for culture and Gram stain resppectively, while the coagglutination results shows no significant difference between pretreated and
non-treated results ($p>0.96$).

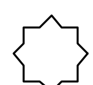


Table 9: The effect of pretreatment on cerprospinal fluid cultural results.

Duration of pretreatment	Culture result				Total of specimens	
	+Ve*		-Ve**			
	No.	%	No.	%	No.	%
Zero time.	14	37.8	23	62.2	37	38.5
1-12 hr.	3	10.7	25	89.3	28	29.2
13-24 hr and more.	1	3.2	30	96.8	31	32.3
Total.	18	18.8	78	81.2	96	100

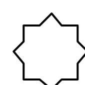
+Ve*: Positive results (growth).
-Ve**: Negative results (no growth).

Table 10. The effect of pretreatment on cerprospinal fluid Gram stain results.

Duration of pretreatment	Gram stain results				Total of specimens	
	+Ve		-Ve			
	No.	**%**	**No.**	**%**	**No.**	**%**
Zero time.	16	43.2	21	56.8	37	38.5
1-12 hr.	11	39.3	17	60.7	28	29.2
13-24 hr and more.	3	9.7	28	90.3	31	32.3
Total.	30	31.3	66	68.7	96	100

Table 11. The effect of pretreatment on cerprospinal fluid coagglutination results.

Duration of pretreatment	Coagglutination results				Total of specimens	
	+Ve		-Ve			
	No.	**%**	**No.**	**%**	**No.**	**%**
Zero time.	24	64.9	13	35.1	37	38.5
1-12 hr.	18	64.3	10	35.7	28	29.2
13-24 hr and more.	20	64.5	11	35.5	31	32.3
Total.	62	64.6	34	35.4	96	100



ure 5.Frequency coagglutination, Gram stain, and cultural results according to the duration of pretreatment with antibiotics.

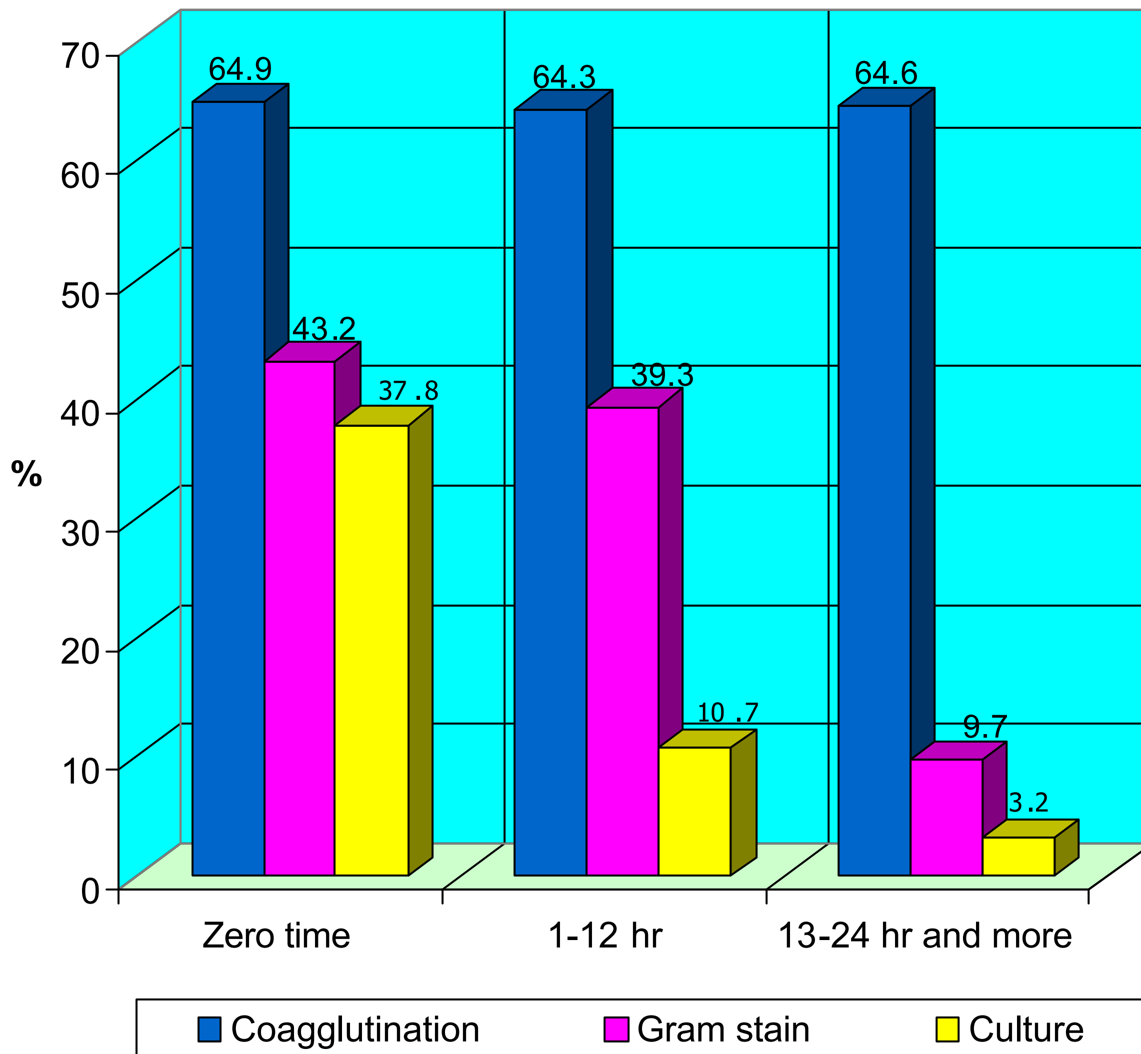
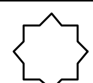


Table 12. Statistical comparison between non-treated and antibiotics pretreated cerebrospinal fluid results.

<table>
<tr><th rowspan="2">Methods</th><th colspan="4">Results</th><th rowspan="2">P Value</th><th rowspan="2">Comment</th></tr>
<tr><th colspan="2">Non treated
N=37 (%)</th><th colspan="2">Pretreated
N=59 (%)</th></tr>
<tr><td>Coagglutination</td><td colspan="2">24/37 (64.9)</td><td colspan="2">38/59 (64.4)</td><td>0.96</td><td>Antibiotics pre-treatment no affect COA results.</td></tr>
<tr><td>Cultural</td><td>14/37(37.8)</td><td rowspan="2">P Value 0.5</td><td>4/59(6.8)</td><td rowspan="2">P Value 0.01</td><td>0.00015</td><td>Antibiotics pre-treatment affects cultural results.</td></tr>
<tr><td>Gram stain</td><td>16/37 (43.2)</td><td>14/59(32.2)</td><td>0.044</td><td>Antibiotics pre-treatment affect Gram stain results.</td></tr>
</table>



Conclusion	**Antibiotics pretreatment affect the out come of both Gram stain & cultural results, but the effect is greater on cultural results**

Antibiotics sensitivity test results:

As seen in table 5, culture was yielding a positive result on 18 (18.8%) of the total 96 accepted CSF smaples. The antibiotic sensitivity test was applied on all isolated microorganisms using commercially available disks.

As indicated in table 13 & 14, out of the 18 isolates 16 (88.9%) were sensitive to one or more of the antibiotics used. All 16 isolates were sensitive to the new cephalosporins (cefotaxim and ceftriaxon), 14 (77.8%), were sensitive to chloramphenicol, 11 (61.1%) were sensitive to amoxicillin plus clavulanic acid, and 10 (55.6%), 9 (50.0%) and 8 (44.4%) were sensitive to penicillin G, penicillin, and ampicillin respectively. Tow cases caused by S. *pneumoniae* and *H. influenzae* type b were found to be completely resistant to all tested antibiotics.

According to the causative microorganism, it was seen that *S. pneumoniae* was the most causative agent resistant to antibiotic followed by *H. influenzae* type b and *N. meningitids*. *N. meningitides* was the least causative agent resistant to antibiotics, table 14.

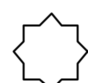


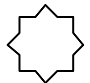
Table 13: Antibiotics susceptibility of the isolated bacteria, in Sanaa 1999.

The isolated Pathogen	No.	%	ANTI BIOTICS													
			#Cef		Ceft		C		Am+Cla		PG		P		Am	
			S' (%)	R" (%)	S	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)	S (%)	R (%)
Hib *	7	38.9	6 (85.7)	1 (14.3)	6 (85.7)	1 (14.3)	5 (71.4)	2 (28.6)	4 (57.1)	3 (42.9)	4 (57.1)	3 (42.9)	4 (57.1)	3 (42.9)	3 (42.9)	4 (57.1)
S.p**	6	33.3	5 (83.3)	1 (16.7)	5 (83.3)	1 (16.7)	4 (66.7)	2 (33.3)	3 (50)	3 (50)	2 (66.7)	4 (33.3	2 (66.7)	4 (33.3)	2 (33.3)	4 (66.7)
N.m***	5	27.8	5 (100)	0 0	5 (100)	0 0	5 (100)	0 0	4 (80)	1 (20)	4 (80)	1 (20)	3 (60)	2 (40)	3 (60)	2 (40)
Total	18	100	16 (88.9)	2 (11.1)	16 (88.9)	2 (11.1)	14 (77.2)	4 (22.8)	11 (66.1)	7 (33.9)	10 (55.6)	8 (44.4)	9 (50)	9 (50)	8 (44.4)	10 (66.6)

Hib*: Haemophilus influenzae type b. .p**: Streptococcus pneumpniae. N.m***: Neisseria meningitidis .

#Cef: Cefotaxim , Ceft: Ceftriaxon, C: Chlormphenicol, Am+Cla: Amoxcillin+ Clavulanic Acid, PG: Penicillin G, P: Penicillin, Am: Ampicillin

S': Sensitive. R": Resistance.



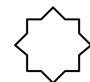


Table 14: Frequency of susceptibility of isolated bacteria to antibiotics.

The isolated Pathogen	No.of isolates	%	Frequency of Susceptibility of bacteria to antibiotics.				Total of disks
			Sensitive	%	Resistance	%	
Hib*	7	38.9	32	65.3	17	34.7	49
S.p**	6	33.3	23	54.8	19	45.2	42
N.m***	5	27.8	29	82.9	06	17.1	35
Total	18	100.0	84	66.7	42	33.3	126

Hib*: *Haemophilus influenzae* type b.
S.p**: *Streptococcus pneumpniae.*
N.m***: *Neisseria meningitidis*.

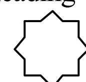
requency of antibiotics activity on isolated microorganisms

Discussion.

There is a scarcity of information about childhood bacterial meningitis and the impact of this problem in this age group of Yemeni population. This is due to the absence of a nationwide register, hospital studies or reports which remain the important source of information regarding the trend of disease. No studies about mortality, disability, or complication of bacterial meningitis among Yemeni children. In addition, in several areas of Yemen, health institutions are rare or poorly staffed and resorces for health care are often inadequate. Therefore, we have designed this study to achieve modest goals regarding meningitis.

This study revealed that the *S. pneumoniae* accounts for (38.7%) of meningitis cases, this finding is in agreement with some studies conducted in several countries, such as Egypt, (18), Korea, (105), and US, (106), where 40.6 %, 35.0 %, and 32.3 %, of infections were due to *S. pneumoniae* respectively. Duma, (41) showed the increase in the rate of *S. pneumoniae* carriers among population and the socioeconomic status of population play a major role in spreading of pneumococcus infection, and carrier rates of *S. pneumoniae* are higher in preschool age children than adults. Bruyn et al, (48) reported that *S. pneumoniae* is sub typed into more than 80 strains and the acquiring immunity against one strain did not give protection against other strains indicating that the acquired immunity is specific for each of the 80 different types. Due to the ability of *S. pneumoniae* to resist drying and other environmental conditions, it can readily spread among individuals and acquiring of *S. pneumoniae* infection is enhanced by exposure to climatic changes, inadequate clothing, poverty, and over crowding (41,39).

In this study *H. influenzae* is the 2nd causative agent of bacterial meningitis in children, and it is the leading



microorganism causing meninges infection among children with less than one year of age, wich is in agreement with that foundation by Bijlmer, (107) who reported that the most cases of Hib occurring before 12 months of age. Similarly, Ahmed et al, (11), in Sudan, Al moneef et al, (17), in Saudi Arabia, reported that the Hib is the main causative of BM in children less than 2yrs of age.

Kritsnsen et al, (108) showed that the failing of the immune system to protect the body with other factors such as inheritance, socioeconomic status, and inadequat breast feeding, influence the risk of acquiring *H. influenzae* type b infection.

The prevalence of *N. meningitides* also differs from one country to another, it is the leading causative agent in countries of the meningitis belt, or the nearest (49, 11, 9). In this study, although *N. meningitides* was not the most common of causative agent of BM in children, it was responsible for 27.4 % of the total meningitis detected among children.

The distribution of BM among males and females in most studies was similar. No significant association between the sex of children and BM infection. In this study the difference between the sex and the age among children up to 2 years of age, was not statistically significant (P >0.28). In contrast, in overall cases of age groups, the defference (41 males and 21 females) of males : females ratio (1.95:1) was statistically significant, P < 0.015. This could be explained by the fact that boys older than 2 years mingle and play in poor hygienic areas, hence exposed to infection more than girls. Garages et al, (18) reported in a study carried out in Egypt, that the number of infected males were (200) and females were (150) among children with 10-14 yrs of age. Salih et al,(9) reported in a study carried out in Sudan, male/female=1.8, and 57% of cases were < 1 year.

The age-bacterial meningitis relationship was proved in our study, like others, where BM was higher in children less than 2 years of age (66.1%) than other age groups. The difference in occurrence of BM among this age group in comparision to others, was statistically significant (p<0.015). It appears that this age group at great risk, where most of cases occur in the first year of life. Nearly all reports in agreement at this point with some differences in the rate of incidence per population (9,11,13,17,62,63,68).

The decline in the percentage of cases with increasing in age of children, may be attributed to the development of protective antibodies due to the frequent exposure to causitive agents in the environment.

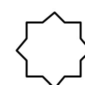
The results of crowding in this study shows that the difference in the numbers of persons resided per room was not staistically significant (p>0.84). this can be explained by presnce of many others factors which may contributed in aquiring of infection.

Cochi et al, (68) showed that the magnitude of risk associated with crowding increased as the extent of crowding increased. However, in our study, we did not find significant association between crowding and the incidence of meningitis.

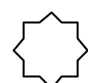
The definitive diagnosis was made by coagglutination, culture and Gram stain with coagglutination or with culture, which were positive in 64.6% of 96 CSF specimens. Coagglutination was useful in identifying organisms in cases that pretreated with antibiotics. Coagglutination is a rapid method for early diagnosis of bacterial meningitis.

Our results show that coagglutination technique is more likely to be genuine rather than false negative of culture and Gram stain techniques.

In this study, coagglutination was the only positive test in 29 (47%) of cases when culture and Gram stain results were negative.



The percentage of coagglutination results in this study seems to have valuable potential for rapid presumptive diagnosis of BM in conjunction with Gram stain, it also provide to be aparticular help in situations where pretreatment with antibiotics was likely.
In other studies the percentage of coagglutination was 90% (109), 63% (81), and 55% (110), while, the sensitivity was 61-82% (111).
Microorganisms were detected in CSF by Gram stain in 30 (31.3%) out of 96 specimens. In comparison, the results of Gram stain reported by Saleh et al (83) and Deuren et al, (84) was 30.4% and 62.0% respectively. Polared et al, (32) show that the CSF Gram stain is positive in 40- 60% of acute bacterial meningitis cases even after initial antibiotic treatment, but may be negative ifantibiotics have been given in the community. In this study, the difference between the results of Gram stain and cultural results on pretreated specimens found to be staistically significant $p < 0.01$.
The results of this study show that culture yielded small number of isolates (18.8%) compared to other studies, where the culture yielded 33.9 % (83), and 70% (84). This low number of isolates is due to pretreatment of patients, since many physicians relay on clinical diagnosis. Polared et al, (32) show that following antibiotics CSF cultures are positive in fewer than 50%.
The sensitivity and specificity of Gram stain were 48.4% and 73.5% respectively, while the sensitivity and specificity of culture results were 29 %, and 97.0% respectively.
For comparison, Monica Cheesbrough (94) reported that the sensitivity of the Gram stain, and culture results were 78.8% - 81.7%, and 65.5% -81.2% respectively.
As seen in table 8, a low recovery of BM organisms from pretreatment CSF specimens by culture was increased with increasing in the duration of antibiotics pretreatment, it was seen that only two microorganisms were isolated from specimens of two patients prior therapy with antibiotics, one of them treated for more than 24hr and the other for less than 12hr, where the isolated microorganisms were found resistant to all tested antibiotics. In this study, we could determine the antibiotics pretreatment interfere with the culture rsults where the recovery rate of microorganismes was 18.8% by culture, and 31.3% by Gram stain in 96 CSF specimens of which 62 were positive cases. A low recovery of BM organisms from pretreatment CSF specimens by culture and Gram stain has been rported. In one study, culture was positve in forty-two (40%) of 106 CSF specimens collected from pretreatment children with antibiotics (112). In other study, Gram stain yielded 28% of CSF specimens taken on admission from patients with pre-admission intake of antibiotics(110).
Finally, this study show that the results of coagglutination technique were not affected by the duration of pretreatment or pre-antibiotics intake (figure 5). This finding is in agreement with several studies, (32,109,83,110)
The results of sensitivity testing show that the third generation of cephalosporins (Cefotaxim and ceftriaxon) were the most antibiotics effective against B M. The 2nd antibiotic expressed high activity against the isolated microorganisms was chloramphincol, which is widely used for treatment of B M in our country due to its low cost and high activity. Most cases treated with chloramphincol show recovery especially when used in combination with other types of antibiotics, but it is used mostly as 2nd or 3rd choice and rarely used as the first choice line for treatment of suspected cases of B M. Several studies (104, 111, 58, 51) reported that the cefotaxim and ceftriaxon are safe and active against lactam producing organisms and most useful antibiotics in treating B M, where the emerging of multiple resistance microorganisms to other antibiotics is increasing.



Comps et al, (80) reported that 60% of isolated Hib strains were resistant to ampicillin compared to 57.1% in this study, and 65.7% were resistant to chloramphenicol compared to 28.6 % in this study.

In this study, *S. pneumoniae* was the most microorganisms resistant to antibiotics (45.2%), which is in agreement with data obtained by Pecoul et al (51) & Baraf et al, (113) who all show the increase in the resistant of *S. pneumoniae* to antibiotics, so the emerging of this resistance highlights the need for children vaccination.

H. influenzae type b was resistant to 34.7% of tested antibiotics and *N. meningitides* was resistant to17.1%. *N. meningitides* was the least causative agent resistant to antibiotics in this study. Galimand et al, (36) reported that *N. meningitides* is one of the least problematic in terms of antibiotic resistance, and the sensitivity of this organism to penicillins is due to an alteration in penicillin-binding protein-2.

The isolated microorganisms were more resistant to ampicillin and penicillin than other antibiotics, which could be related to the random wide use of these types of antibiotics for several infectious diseases.

During this study no recovery was seen among patients infected by BM who were treated by either penicillin or ampicillin alone; however, ampicillin is widely used as first choice in treatment of suspected cases of B M or other infectious diseases.

Conclusion:

S. pneumoniae is the most common causative agent of BM among children, while *Haemophilus influenzae* type b is the predominant among children less than one year.

*Children with 2years or less are more susceptible to BM than other age groups.

*Coaglutination technique is the best methods for rapid diagnosis of BM especially in pretreated cases.

*Pre-admission of antibiotics therapy or subsequent CSF culture following pretreatment with antibiotics accounted for the low percentage of culture results.

*Third generation cephalosporins (cefotaxim & ceftriaxon) and chloramphenicol were the most effective antibiotics against BM, whereas penicillin and ampicillin were the least active against BM.

S. pneumoniae was the most causative agent resistant to antibiotics tested.

Recommendations.

* More studies need to be done to provide comprehensive picture about the disease and including:

Serotyping and genotyping of the causative agents.

Environmental factors which influence the occurrence of the disease.

* The Introduction of meningitis vaccine in M.o.H. Plan.

Questionnaire for research study.

Name: ..Age ..Sex

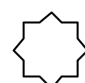
Clinical diagnosis ..

Previous treatment (Antibiotics)

Yes (). Duration: .

No ()

Family size: .



Number of children occupied per room:

1/R 2/R 3/R 4/R 5/R >5/R

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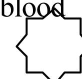

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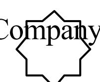

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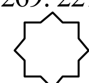

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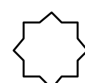

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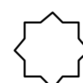

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