Title page

INCIDENCE OF BACTERIAL AND MYCOPLASMAL PNEUMONIA IN CHILDREN VISITINC THE JOS UNIVERSITY TEACHING HOSPITAL (JUTH), JOS NIGERIA

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DECLARATION

I hereby declare that the work reported in this thesis was done solely by me under the supervision of Dr. (Mrs) L.E. Odama and Dr. M.I. Agba (KSM).

No part of this thesis has been presented in any previous application for degree. All sources of information are acknowledged and referred accordingly.

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This thesis by Gimba Haruna meets the regulations governing the degree of Master of Science (M.Sc) of Ahmadu Bello University and is approved for its contribution to scientific knowledge and literary presentation.

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DEDICATION

This work is dedicated to my late grand mother - Iya Riyo. May her soul rest in peace. Amen.

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ABSTRACT

Nasopharyngeal aspirates (NPA) and sera specimen were taken from 112 children with pneumonia visiting the Jos University Teaching Hospital, Jos. Pleural effusions samples were also taken in 11 of the cases as positive control for bacteriology.

The NPA samples were cultured for Mycoplasmas and bacteria using standard procedures. Complement fixation and indirect haemagglutination tests were carried out on the sera samples to determine the *Mycoplasma pneumoniae* antibody titre.

Specific bacterial pneumonia was demonstrated in 45 cases (40.2%) of the patients with *S. aureus* dominating.

Mycoplasma pneumoniae was isolated in 12 cases (10.7%) based on growth inhibition test. The complement fixation test was positive in 44 cases (39.3%), while indirect haemagglutination test detected 54 positive cases (48.7%). Cumulative isolation and serology cases was found to be 55 (49.1%), and indirect haemagglutination test was found to be more sensitive compared to complement fixation test.

50 control patients were also included in the study. These are patients with no clinical symptoms of pneumonia. Non of their NPA samples yielded single bacterial isolate neither was M. *pneumoniae* isolated. Their sera samples also did not show diagnostic *M. pneumoniae* titre from either serological test.

CHAPTER ONE

INTRODUCTION

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1.0. INTRODUCTION

1.1. THE HUMAN RESPIRATORY TRACT

The human respiratory tract is anatomically divided into the upper and lower regions. The upper part consists of the mouth, nasopharynx, orthopharynx, the tonsils and the nose, while the lower region comprises the larynx, trachea, bronchi, bronchioles, alveoli and the accessory nasal sinuses (Coles, 1978).

1.2. RESPIRATORY TRACT INFECTIONS

A wide range of microbial pathogens are continuously being implicated in respiratory tract infections as a result of improved methods of laboratory diagnosis.

1.2.1 Classification of respiratory tract infections

The aetiology and symptomatology of respiratory tract infections vary *inter alia* with age, sex, season, the type of population at risk and socioeconomic class. These make it difficult to satisfactorily classify respiratory tract infections.

An anatomical classification is unsatisfactory since in most cases multiple portions of the tract are simultaneously affected. Aetiological classification is also unsuitable as one pathogen may give rise to a number of clinical and pathological affects and many illness produced by different pathogens are similar.

Most workers prefer the symptomatological classification. Hop-Simpson and Miller (1973), using this classification method classified respiratory tract infections into seven syndromes. These common cold, pharyngitis, primary otitis media, laryngitis, tracheitis, niae (in atypical pneumonia), *Rickettsia burneti* (in Q-fever), Mycobacteria and pathogenic fungi (Schudart and Huber, 1978).

1.2.3. Incidence of respiratory tract infections

Monto and Ullmann 1974, working in USA, reported an average number of respiratory illness to be 3.0/person/year, with the highest rate of 6.1/year in infants, while it is about 1.3/year in adults aged over 60 years. Similar results obtained by Jennings *et al.*, 1978, in New Zealand revealed that, in developed countries, the mortality rate was approximately 6.3% of all death. The highest death rate (300/1000 persons) was in the age group 65 years and above. In infants the death rate was about 50/1000. Morbidity and mortality rates are higher in developing countries compared with those reported in developed countries (Collee and Watt, 1990). studies conducted in Nigeria showed that respiratory tract infection are major causes of death in children (Obi, 1976; Adeyokunmu *et al.*, 1980; and Fagbule and Joiner, 1987).

1.2.4 Pathogenesis

Several factors innate in the invading microbial pathogen and the host play vital roles in the pathogenesis of respiratory tract infection. The predisposing factors in the host include congenital disease of the respiratory tract and pre-existing lung damage. The pathogenicity of the invading microbe depends on the nature of the bacteria involve. In order for an infection to be established; the causative agent must have the ability to overcome the defence system of the host. *H. influenzae* may actually depress ciliary activity by the production of specific cilio-inhibitory factors such as histamine (Collee and Watt, 1990). In addition to its possession of polysaccaride capsule which enables it to resist phagocytosis, pneumococcus possesses glycoconjugate receptors which specifically attach to human pharyngeal epithelial cells *in vitro* (Anderson, *et al.*, 1988). Pneumococcus also produce haemolysin (Pneumolysin) which destroys human lymphocytes, polymorphonuclear leucoytes and platelets. These properties were shown to enhance virulence of the organism (Kanclerski and Mullby, 1987).

Bacterial infections of respiratory tract may also be as a result of pathogenic synergy between viral and bacterial agents or even between different bacterial species. *S. aureus* causes pneumonia mostly as a result of prior influenzal infection due to the reduction in the host's resistance caused by the viral infection (Jawetz, 1985).

Antibiotic therapy may also predispose respiratory tract infections by opportunistic pathogens. Collee and Watt (1990), reported respiratory tract infectiouns caused by mixed flora of *E. coli, Proteus spp, Ps. aeruginosa* and yeast in patients given ampicillin.

1.3. TYPE OF PNEUMONIA

Pneumonia is an inflammatory process in the lung parenchyma mostly due to infection (Coles, 1978). There may be little or no destruction of the lining cells but the terminal air spaces are filled with fibrinous exudate containing swarms of the causative organisms. The affected part of the lung is said to be "consolidated" (Coles, 1978). Two anatomical forms of pneumonia are distinguishable; the lobar and the bronchopneumonia. The range of pathogens typically associated with hospital acquired pneumonia (HAP) differs from those associated with community acquired pneumonia (CAP), though there is an overlap (Coles, 1978).

1.3.1. Bacterial pneumonia

MacFarlane, *et al.*, (1982); and McNabb, *et al.*, (1984) found pneumonoccous as the predominant cause of community acquired pneumonia. This organism was found to be responsible for 34-70% of the cases. Other organisms implicated in community acquired pneumonia include *S. aureus, M. pneumoniae, H. influenzae, L. pneumophila* and *M. tuberculosis*. In hospital acquired pneumonia, the spectrum widens to include gram negative bacteria such as *K. aerogenosa, E. coli, Ps. aeruginosa* and *H. influenzae* (Collee and Watt, 1990).

1.3.1.1. Diagnosis of bacterial pneumonia

A major problem encountered in actual diagnosis of bacterial pneumonia is how to obtain good specimen. The nasal swab method is only 30% sensitive (Davidson *et al.*, 1976). Sputum specimen is not reliable as children are too young to produce it. Other techniques developed include transtracheal aspirate (Davidson *et al.*, 1976), lung puncture (Silverman *et al.*, 1977, Diakparomre and Obi; 1981; Adedoyin and Fagbule, 1987) and lung biopsy (Adamson and Bates, 1967). These techniques are 50-80% sensitive though not devoid of certain risks. Transtracheal aspirate technique is commonly associated with subcutaneous emphysema and transient haemoptyses. Complications due to lung biopsy technique is associated with unacceptable morbidity and mortality rates (Adamson and bates 1967). Thus these methods cannot be regarded as routine in normal management of uncomplicated cases of respiratory tract infections.

1.3.2. Primary lobar pneumonia

Primary lobar pneumonia a disease with grave prognosis is a severe pyrexial illness characterized by sudden onset, shaking chills, fever, chest pain and cough with rusty coloured sputum. A history of upper respiratory tract infection may be elicited (Jawetz 1985), consolidation involve part of or all of a single lobe of the lungs though more than one lobe may be involved and is said to be more in young adults. Untreated cases may recover after 7-10 days or die in the acute phase of the illness. During the initial phase of fatal cases of the

illness bacteremia may occur (George and Finegold, 1982).

Bacteriologically, 80-90% of cases of primary lobar pneumonia are caused by *Streptococcus pneumoniae* of which types 1-9 and 12 are commonly found in adults while types 6, 14, 19 and 23 are found in children. *St. pyogenes, S. aureus, H. influenzae* and *K. pneumoniae* cause the remaining cases of lobar pneumonia (Jawetz, 1985).

1.3.2.1 Characterization of *Streptococcus pneumoniae*

St. pneumoniae is a gram positive encapsulated lancet shaped diplococcus. The organism is best cultivated at 37°C on blood or serum enriched peptone infusion agar incubated in 5% CO_2. On blood agar pneumococcus tend to produce α-haemolysis, a greenish discoloration surrounding the colonies due to conversion of red blood cells to methemoglobin (Schudart and Huber, 1978). The organism's susceptibility to optichin and solubility in bile are exploited in simple laboratory identification.

1.3.2.2. Pathogenesis

Pneumococci present in the nasopharynx normally initiate an infection whenever the efficiency of the host's immune system is retarded. (Duguid, 1978). Virus infection and circumstances that warrant the aspiration of secretions from throat into the lungs may be the most significant factors.

The pneumococcus is transported in oedema fluid from the site of initial infection in air alveolus to adjacent alveoli, passing through the pores of kahn. Precipitation of the plasma fibrin in airsacs leads to consolidation of the area of

involvement. Inflammatory reaction progresses in the lung frequently accompanied by bacteraemia that may result to septicaemia. After 4-7 days in favourable cases antibodies may appear in blood circulation. The temperature may then fall by crisis and the organisms disappear in the circulation and clinically, the patient's condition improves, though lung abnormality may persist for more weeks (Collee and Watt, 1990).

1.3.2.3. Epidemiology

Lobar pneumonia caused by pneumococcus is an infectious disease though with unidentified aetiological factors.

Factors such as alcoholism, malnutrition, prior splenectomy and other debilitating conditions such as the Acquired Immune Deficiency Syndrome (AIDS), influence host resistance to the disease, (collee and Watt, 1990). There is a variation between serotypes in the degree of virulence depending on age, year and place. Austrian (1978), reported a mortality rate of 6% in cases of type 1, whereas upto 48% mortality rate was reported in type 3 cases. Primary lobar pneumonia is said to be caused by limited number of serotypes, whereas a wider range may be involved in carriage or with secondary infection (Duguid, 1978).

1.3.2.4 Diagnosis

The organisms are demonstrated in blood cultures in 15-25% of cases early in the disease, from the sputum by gram staining and by culture prior to antibiotic therapy. A typical sputum specimen from pneumococcal pneumonia

contains many red and polymorphonucleated (PMN) blood cells as well as many pneumococci (Jawetz, 1985). Where a good sputum specimen is not possible, the detection of pneumoccal antigen by counterimmunoelectrophoresis test is helpful (Miller, *et al.*, 1978). Methods such as enzyme-linked immunoabsorbent assay are more sensitive in detecting pneumococcal antigen and can be of potential value (Bertsson, *et al.*, 1978).

When typing of the isolates is required, the Neufield quelling reaction is best (Lennettee, *et al.*, 1980).

Rapid slide agglutination test as described by Kronvall 1973, is also useful for identification of pure serotypes.

1.3.2.5. Chemotherapy

Benzyl penicillin remains the drug of choice in proven pneumococcal pneumonia. The drug is administered intramuscularly and only after a definite response should oral penicillin V be given. Erythromycin or co-trimoxazole are alternative drugs in cases where the isolates are proven sensitive to these agents. In cases of isolates that are resistant to the alternative drugs, cefazolin is another drug of choice. Treatment should be continued for three days after defervescence (Jawetz, 1985).

1.3.2.6. Prophylaxis

General preventive measures such as good ventilation, avoidance of overcrowding, good coughing, sneezing and spitting manners are helpful.

Penicillin may be used for a short-term protection of immuno compromised patients (Collee and Watt, 1990).

Polyvalent vaccines tried in susceptible populations, such as miners, sickle cell patients, splenectomized patients and adults showed about 78-85% efficacy against the specific serotypes (Smith *et al.*, 1977; Amman, *et al.*, 1977; Rilley *et al.*, 1977).

Presently, a 23 pneumococcal capsular types polyvalent vaccine is available in the field for trial. Immune antibodies in some vaccinated individuals were shown to persist upto four years post vaccination (Borgono *et al.*, 1978).

1.3.2.7. Complications due to *Pneumococcal pneumonia*

Complications due to pneumococcal pneumonia which may arise include, pleural effusion, empyema, endocarditis, meningitis and pericarditis. Pneumococcal arthritis and lung abscess may also occur. In cases of pleural effusion, collections are aspirated and examined by smear and culture to enable early treatment (Jawetz, 1985).

1.3.3 Bronchopneumonia

Bronchopneumonia occurs as a diffuse lobar involvement of alveoli contagious to the larger bronchioles of the bronchial tree and often bilateral. In most case the onset is insidious. Bronchopneumonia is a primary disease in infants, but generally occurs secondary to bronchitis, fever, gastroenteritis, diabetes, nephritis, anaemia or carcinoma. Surgical operations of trachea,

abdomen which make coughing difficult may also dispose it. Foods and vomitus aspirated into the trachea may result in the disease. The illness could be sporadic or post influenzal epidemics. The frequency and case fatality rely on predisposing factors such as age, health status of patient and the nature of infecting bacteria (Collee and Watt, 1990).

1.3.3.1 Bacterial aetiology of bronchopneumonia

The aetiology of bronchopneumonia varies from one geographical region to another and from time to time.

Though Smillie and Dueschner (1947) in USA found pneumococcus and β-haemolytic streptococcus as the main aetiologic agents, Tanner *et al.*, (1969) in England showed that *E. coli* and *S. aureus* were mostly responsible.

Pneumococcus are said to be common primary causes of community acquired bronchopneumonia in both adults and infants. In bronchopneumonia secondary to acute fevers, pneumoccus; *S. aureus, H. influenza* are said to play vital roles in bacterial aetiology (Collee and Watt, 1990). The pneumococcus serotypes implicated in bronchopneumonia are believed not to belong to the pathogenic serotypes encountered in lobar pneumonia (Collee and Watt, 1990).

1.4 OTHER BACTERIA INVOLVED IN PNEUMONIA

Besides pneumococcus other bacteria are responsible for 25% of community acquired pneumonia and 80% of hospital acquired pneumonia. All of them may have similar physical and x-ray evidences of pulmonary infiltration of consolidation (Jawetz, 1985). The knowledge and identification of the

causative organisms is vital before treatment is initiated.

1.4.1 *Staphylococcus aureus* pneumonia

Staphylococcus aureus is mostly implicated in debilitated patients, hospitalized infants especially after antimicrobial treatment and as a post influenzal pneumonia in adolescent and young adults. It is always almost fatal within 24 hours of onset (Jawetz, 1985). Pleural effusion, empyema and pneumothorax are common complications. X-ray picture of pyopneumothorax and cavities with air fluid levels are indicative of S. *aureus* pneumonia (Jawetz, 1985).

Diagnosis is by demonstration of gram positive cocci mostly intracellular in sputum smear, and culture of the organisms from sputum, blood or pleural fluid. Treatment is by intravenous administration of nafcillin or vancomycin. In proven penicillin sensitive isolates, the drug may be given. Treatment is continued for several weeks after recovery. Drainage of empyema is essential in such complicated cases. Prognosis depends on efficiency of drug and patient's status (Jawetz, 1985).

1.4.2 *Klebsiella pneumoniae*, pneumonia

This is the so-called Friedlander's pneumonia caused by capsular serotypes 1-4 of *K. pneumoniae*. The onset is sudden with red mycoid, sticky sputum. Primary pneumonia due to this organism occurs in elderly people with history of debilitating disease. It also occurs as a superinfection in hospitalized patients.

The diagnosis is based on demonstrating predominant short encapsulated gram negative bacteria in sputum smears and Klebsiellae in blood and sputum cultures. Intensive treament is essential using cefotamine or any other cephalosporin administered intravenously in combination with aminoglycoside such as tobramycin which is given intramuscularly. The treatment is continued for two weeks to avoid relapses (Jawetz, 1985).

Complications may include necrosis and abscess formation of lung tissue. The fatality rate of untreated cases is 40-60%. This figure could be reduced to 30% with adequate treament, (Jawetz, 1985).

1.4.3 *Haemophilus influenzae* pneumonia

This is a rare cause of primary pneumonia in human adults. It occurs in patients with lung diseases and hypogammaglobulinemia.

sputum from such patients is bloody and gram stain smear may be confusing, so diagnosis rests on isolation of the organism from blood or sputum.

The drugs of choice are ampicillin and moxalactam which are given intravenously while chloramphenicol can be administered either orally or intravenously. Occasionally, penicillinase - producing *H. influenzae* are encountered in which case trimethoprim with sulfamethoxazole are preferable, (Stratton, 1980).

1.4.4. Streptococcus pneumonia

This occurs usually as a sequel to viral infections of respiratory tract especially influenza and measles or in pulmonary diseased patients (Jawetz, 1985).

Pleural effusion is seen early in streptococcoccal pneumonia and progresses to empyema in untreated cases.

Diagnosis is by evidence of large number of streptococcus in smear of sputum and cultivation of haemolytic streptococcus from blood or sputum.

Penicillin is the drug of choice and prognosis is good (Jawetz, 1985).

1.4.5. Pneumonia of non-fermenting rods

Pneumonia caused by gram negative bacilli and various non fermenting rods such as *E. coli, Proteus spp, Pseudomonas spp* and *Serratia spp.* occurs frequently in debilitated persons with lung and heart diseases. It also occurs as nosocomial infections in patients who required inhalation therapy or tracheal suction and received antibiotics. The hands of medical staff and contaminated equipments are vehicles of transmission (Jawetz, 1985).

The pneumonia presents early delirium, massive consolidation which proceeds to necrosis and abscess formation. It has a high fatality rate (Jawetz, 1985). Treatment is with aminoglycoside in combination with ticarullin or moxalactam, given intravenously. Prognosis is critical (Jawetz, 1985).

1.5. MYCOPLASMAL PNEUMONIA

1.5.1. General consideration

Mycoplasma is a genus belonging to the class Mollicutes (Mollis: soft; Cutis; skin), in the order of Mycoplasmatales (Freundt, 1983). Mycoplasmas are the smallest self replicating prokaryotes with a genome of about $5x10^8$ daltons (Edward and Freundt, 1967; Askaa *et al.*, 1973). They do not possess cell wall and have pleomorphic morphology (Razin, 1978).

Mycoplasma cell is enveloped by a triple layered plasma membrane which is composed of lipids, proteins and carbohydrates. The glycolipids portion of the membrane harbours the major antigenic determinants responsible for the production of metabolism, growth inhibiting, complement fixing and agglutinating antibodies. The membrane proteins are primarily involved in cell-host attachment (Razin *et al.*, 1972; Powel, *et al.*, 1976).

Inside the plasma membrane is the cytoplasm containing the chromosomes, macromolecular ribonucleic acid (RNA), plasmids and cytoplasmic granules (Razin, 1983).

The electron micrograph of the organism reveals an extracellular covering which chemical composition is similar to the capsules of other prokaryotes. It is this structure which probably endows the organism the property of resisting phagocytic cells of infected host (Razin, 1983).

1.5.2 Mycoplasma infections in man

The known human mycoplasmas and mycoplasma like-organisms are: *M. pneumoniae, M. salivarium, M. hominis, M. orale, M. primatum, M. fermentans, M. buccale, M. genitalium, M. faucium, M. arthritis, M. hyorhinis, M. lipophilum,* and *Ureaplasma urealyticum* (T-mycoplasma), (Taylor Robinson and Smith, 1990).

Majority of the human mycoplasmas are encountered in the orthopharynx but *M. pneumoniae* and probably *M. hominis* are the two, implicated in respiratory tract infections. (Chanock *et al.*, 1962; Organick and Worman, 1967; Purcell and Chanock, 1969; and Nagayama, *et al.*, 1988).

M. fermentans, M. genitallium, M. hominis, M. pneumoniae, M. primatum, M. slaivarium and *U. realyticum* (a T-mycoplasma) have been recovered from the human urogenital tract. Only *M. hominis, U. urealyticum* and *M. genitalium* have been implicated in the urogenital tract infection (Mardh and Westram, 1970; Capsi, 1971; 1972; Friberg and Gnarpe, 1973; Bowie, *et al.*, 1976; Adegbye, *et al.*, 1979 and Tully *et al.*, (1986).

M. hominis, M.pneumoniae, M. fermentans, M. arthritis M. hyorhinis and *U. Urealyticum* are encountered in the synovial fluid. *M. arthritis* is being implicated in the aetiology of human arthritis (Cole and Cassell, 1979).

1.5.2.1. Respiratory tract infections due to *M. pneumoniae*

Non-bacterial pneumonias were classified as "Primary atypical pneumonia" (PAP) to distinguish them from typical lobar pneumonia. In a variety of primary a typical pneumonia, cold agglutinins often develop and the

infecting agent the "Eaton agent" isolate. The isolate was cultivated on cell free agar medium, and it was named *Mycoplasma pneumoniae* after been implicated in respiratory tract infections (Chanock *et al.*, 1962). Infections by *M. pneumoniae* affect both the upper and lower respiratory tract. The spread of the disease is by continual or repeated close contact (Foy *et al.*, 1966). It is globally endemic and cyclic incidences occur at long intervals resulting in prolong epidemics every four years (Noah, 1974; Lind and Bentzon, 1976).

It was believed that *M. pneumoniae* rarely cause pneumonia before school age (Foy, *et al.*, 1970) and that infections in this age group are usually mild, (Fernald, *et al.*, 1975). However reports now indicate that any age group may be at high risk. (Stevens, *et al.*, 1978; White *et al.*, 1981).

The presence of highly sensitized T-dependent lymphocytes in previously *M. pneumoniae* infected humans, lead to the belief that cell mediated immunity probably play a predominant role in the host defence mechanism, (Leventhal, *et al.*, 1969). Fernald, (1969), further demonstrated that hamsters previously infected with *M. pneumoniae* showed low levels of antibodies yet they were found to be resistant to challenge with the organism.

1.5.2.2. Pathogenesis

Collier (1972), demonstrated the mechanism of infection by incubating *M. pneumoniae* into the human respiratory epithelia. The electron micrographs of the cells showed filamentous *M. pneumoniae* organisms among the cilia. Other features observed include progressive mitochondrial swelling, destruction of

cristae and protrusion of the apical portions of the cells into the lumen. Cytoplasmic vacuolization was observed and the cilia of the cells formed a "Sun burst" configuration and there was a destruction of the subcellular organelles.

A differentiated terminal structure seen only in virulent strains is believed to be vital in pathogenesis. The structure plays a role in the attachment of the organisms to epithelial ciliated cells. This close proximity to host cell membrane allows a build-up of enough toxins, such as hydrogen peroxide, produced by *M. pneumoniae* which interfere with ciliated cell motion. This results in epithelial cell injury and eventual sloughing (Collier, 1972).

1.5.2.3. Histopathological changes

The picture of histopathological changes due to *M. pneumoniae* infection was derived from experimental infection of small animals. The pneumonic infiltrates take the form of peribronchiolar and perivascular cuffing by thymus dependent lymphocytes. Cell mediated immunity was also demonstrated by positive lymphocyte transformation, macrophage migration inhibition and delayed type hypersensitivity skin tests (Fernald, 1979). The initial lymphocyte response is followed by a change in the character of bronchiolar exudate, with polymorphonuclear leukocytes and macrophage predominating. Such changes were shown to be more copious during reinfection with *M. pneumoniae* than in primary infection. Thus the resultant pneumonia is thought to be an immunopathological process (Fernald, 1979).

1.5.2.4. Clinical manifestations

Mycoplasmal pneumonia occurs more often in children than adults but more serious manifestations are seen with increase in age. Early definite diagnosis based on symptoms is difficult, just as with other non-bacterial pneumonia. This is because general symptoms such as lassitude, headache, myalgia with rising fever precede chest symptoms by about five days and physical signs and consolidation only appear after radiographic evidence of pneumonia has been obtained (Clyde, 1979).

Radiographs show patchy opacity, usually of one of the lower lobes, some show bilateral pneumonia, but pleurisity and pleural effusion are not common (Denny, *et al.*, 1971). The course of the illness is variable. Cough, abnormal chest signs and radiographic changes may last for several weeks. In children, it mimics whooping cough and in prolonged cases it could occur with paraxysmal cough and vomiting (Jawetz, 1985). In adults severe cases usually occur in immunocompromised patients (Foy, *et al.*, 1973), though death is rare.

1.5.3. Extrapulmonary manifestations due to *M. pneumoniae* infections

M. pneumoniae infections are usually limited to the respiratory tract but a number of extrapulmonary systems may be involved during or after respiratory tract infection. The most important ones include, Stevens-Johnson syndrome (Cherry, *et al.*, 1975), Pericarditis (Grayston, *et al.*, 1965), acute haemolytic anaemia (Barret-Connord, 1972), arthritis (Lambert, 1968), myocarditis (Friedli, *et al.*, 1977) hepatitis (Murray, *et al.*, 1975) and meningitis

and other neurological disorders Lerer and Kalvsky, 1973). Others are glomerulonephritis and tubo-ovarian abscess (Taylor-Rabinson and Smith, 1990).

1.6 LABORATORY DIAGNOSIS OF *M. PNEUMONIAE* INFECTIONS

Laboratory diagnosis of *M. pneumoniae* infections depends on culture and serological techniques or both.

1.6.1. Culture technique

The Edward-type media is used for the isolation of *M. pneumoniae.* It consists of pleuropneumonia like organism (PPLO) broth, glucose, horse serum and yeast extract. Penicillin and Thallium acetate are included to cut down bacterial contaminants. Phenol red is used as indicator. The pH is adjusted to 7.6.

The broth is incubated with respiratory secretion such as throat swab, sputum, pharyngeal swab and is incubated at 37°C. Mycoplasmal growth is indicated by colour change (Pink to yellow) due to pH shift from 7.6. to 7.0 as a result of lactic acid production by glucose fermentation (Robert, *et al.*, 1967). The colour change may take 4-21 days and as soon as it is noticed the broth is subculture on pleuropneumonia-like organism agar medium for specific identification. The initial inoculum may be diluted to eliminate bacterial contamination. The plates on which the broth was incubated were then incubated at 37° in 5-10% carbon dioxide atmosphere. Cultures may take 20-30 days before considered negative for mycoplasmas (Jordan, 1983).

1.6.2. Purification of isolates

The colonies of mycoplasma exhibit specific morphological characteristic. They possess dense centre with downward growth into the agar. A clearer peripheral zone spreads out around the colony giving it a "fried-egg" shape (Smith, 1971). The colonies are purified and identified.

Cloning and filtration are two methods commonly used to purify mycoplasmas. A colony is cloned by cutting the agar block which supports it, and then transferring it to a tube of pleuropneumonia-like organism (PPLO) broth, from where two or more serial dilutions are made. (Anon, 1979). If the broth culture is contaminated, it is filtered through membrane discs of 0.45 or 0.22µm pore sizes, which allows mycoplasmas to pass but retain bacteria. Then dilutions of such filtrates are made and then incubated and observed for colour change (Tulley, 1983b).

1.6.3. Identification of pure mycoplasma isolates

The identification of *M. pneumoniae* is done biologically, biochemically and serologically.

1.6.3.1. Biological identification

β-haemolysis and haemadsorption tests are done.

(i) β-haemolysis test

A plate containing suspected colony is overlaid with a 4% suspension of guinea pig or sheep read blood cells in agar. The agar is allowed to solidify and the plate is incubated aerobically at 37°C. Beta-haemolysis observed 24 - 48

indicates *M. pneumoniae*. It is believed that *M. pneumoniae* produces enough hydrogen peroxide aerobically which diffuses and lyse the red blood cells to give the clear zone. It is only with prolong incubation that other human mycoplasmas cause incomplete or ∝ haemolysis (Clyde, 1964a, Cole, 1968).

(ii) Haemadsorption test

This was as described by Manchee and Taylor Robinson (1968). A plate containing the suspected colony is flooded with 0.5% suspension of guinea pig red blood cells. The plate is incubated at 37°C with the cells in contact with the colony for 30 minutes. The plate is then washed with phosphate buffer solution (PBS). The colony is then observed under X10 objective for adsorption. This phenomenum was exploited in *M. pneumoniae* identification in the belief that only this organism adsorb to red blood cells. However, recent report by Taylor-Robinson and Smith (1990) indicate that *M. ganitalium* also adsorb to red cells.

1.6.3.2 Biochemical characterization of *M. pneumoniae*

Two human mycoplasmas, *M. pneumoniae* and *M. fermentans* metabolize glucose and thus this phenomenum is utilized in the isolation and identification of *M. pneumoniae*. *M. pneumoniae* break the glucose to lactic acid which drops the pH of the medium and in the presence of phenol red as indicator, changes the colour of the medium from red to yellow, (Taylor-Robinson and Smith, 1990).

M. orale, M. salivaium and *M. hominis* are arginine metblozwrs (Purcell and Chanock, 1969), whereas *U. urealyticum* is an urea hydrolyze (Shpard and Luncedord, 1970).

Other provincial biochemical tests in mycoplasmology include, phosphatase activity, tetrazolium reduction, serum digestion and esculin hydrolysis (Kumar *et al.*, 1988).

Biochemical and Biological identification *M. pneumoniae* are not confirmatory as other closely mycoplasmas are known to exhibit similar characteristics. Serological methods provide more specific identification.

1.6.3.3. Serological techniques

Serological methods used in *M. pneumoniae* identification include; Growth inhibition, metabolic inhibition, immunofluorescence, complement fixation and indirect haemagglutination.

(i) Growth Inhibition test: This was as described by Clyde, 1964b. Filter papers impregnated with specific *M. pneumoniae* antiserum are placed on agar previously seeded with mycoplasma isolate. Zones of growth inhibition around the disc is confirmative of *M. pneumoniae.*

(ii) Metabolic inhibition test: Specific *M. pneumoniae* antiserum is added into broth culture containing suspected organisms. If the organism is *M. pneumoniae* growth is prevented. Thus glucose will not be fermented to produce lactic acid and the pH of the medium remains the same, resulting in no colour change of the medium (Purcell and Chanock, 1969).

(iii) Immunofluorescence test: The gammagloulin portion of specific *M. pneumoniae* antisera prepared in rabbits (Morton and Roberts, 1967) is conjugated with fluorescein isothiocynade (FITC). The FITC conjugated antiserum is then used to stain suspected colony directly on agar plate (Clarke, *et al.*, 1961). An antigen-antibody reaction can be visualized with fluorescence microscope. A yellow-green fluorescence of the colony is indicative of positivity.

iv. Complement fixation test: This is used in the detection of *M. pneumoniae* antibodies in the patient's serum. Such test is more sensitive to detect mycoplasmal infections than isolation of the organism.

M. pneumoniae antigen and patient's serum are allowed to interact in the presence of a measured amount of complement. If the patient's serum contains antibodies to the organism, antigen-antibody complex is formed onto which complement can get attached. however, in the absence of such antibodies, the complement remains in the solution. The free complement lyse the indicator system of sheep erythrocytes and haemoysin. Thus lysed red blood cells indicate negative result and intact red blood cells mean the presence of antibody (Clyde and Senterfied, 1983).

(v) Indirect haemagglutination test: Red blood cells sensitized with *M. pneumoniae* sonicate are allowed to react with patient's serum. In the presence of specific antibody, antigen-antibody reaction takes place and results in clumping or agglutination of the reactants, whereas in the absence of specific antibody the mixture remain uniform. (Kok, *et al.*, 1988a).

Promising rapid diagnostic methods to detect *M. pneumoniae* antigen in respiratory exudates have been reported. These include: enzyme immunoassay (Kok, *et al.*, 1988b), Immuno-blotting with monoclonal antibodies (Madsen, *et al.*, 1988) and DNA probes (Harris, *et al.*, 1988).

1.7. CHEMOTHERAPY AND IMMUNOPROPHYCAXIS OF *M. pneumoniae* INFECTIONS

1.7.1. Chemotherapy

Tetracycline and erythromycin were reported to be valuable in planned trails (Brunner and Laber, 1985), however, in practice the agents prove to be less effective. This might be due to the fact that the disease is often well established before treatment is commenced. The clinical recovery is not dramatic and the organisms may persists in the respiratory tract even after the illness. The presence of the organisms in the respiratory tract leads relapses and justifies prolonged antibiotic therapy.

The drugs are more efficacious if administered early in the course of the illness. Rapid serological methods are therefore more important in the diagnosis of *M. pneumoniae* infection than isolation methods.

1.7.2 Immunoprophylaxis

At present there is no effective vaccine against *M. pneumoniae* infections. Chanock, et al 1970 described a vaccine developed using a temperature sensitive mutant of *M. pneumoniae* which multiply at the temperature of the upper respiratory tract but not at the temperature of the upper respiratory tract. At the

upper respiratory region the mutant stimulate local immune response, capable of inducing a significant resistance to virulent stain. However, Barile, 1985 showed that such mutants may not be suitable in large scale vaccine production.

Two options are available in future vaccine development for mycoplasmas:

i) The use of adjutants to developed killed *M. pneumoniae* vaccine. Similar to the one developed by Carba and Terry (1985) using *M. mycoides* gladysdale stain preventing contagious boving pleuropneumonia.

ii) The development of purified component vaccine as suggested by Taylor-Robinson and Smith (1990).

Childhood pneumonia constitute a true global public health problem especially in developing country like Nigeria (Obi, 1976; Fagbule and Joiner, 1987). There is evidence now accumulating in the developing countries that indicate bacterial and mycoplasmal pathogens playing a great role as primary and secondary cause of pneumonia. (Shamn, *et al.*, 1984, Pio, 1986, Adedoyin and Fagbule, 1987; Nagayama, *et al.*, 1988, Mirza, 1989). The frequency and clinical expression of these agents remain obscure.

The aim and objective of the present study is to determine the incidence of bacterial and mycoplasmal pathogen associated with childhood pneumonia in patients visiting the Jos University Teaching Hospital (JUTH), Jos by (a) Isolation of pathogenic bacteria and mycoplasma from nasopharyngeal aspirate of pneumonic children. (b) Demonstration of the presence of antibodies to

Mycopasma pneumoniae using complement fixation and indirect haemagglutination tests from sera samples of the patients.

CHAPTER TWO

MATERIAL AND METHODS

CHAPTER TWO

2.0. MATERIAL AND METHODS

2.1 MATERIAL

2.1.1. Bacteriological media

Sodium Chloride (Nacl)	-	May and Baker Ltd., Dagenham England.
Potassium dihydrogen orthophosphate (KH_2P04)	-	Poole England
Phenol red	-	Difco laboratories, Detroit, USA.
Glycerol	-	Sigma chemical company St. Louis, USA.
Sodium hydroxide (NaOH)	-	Fison's PLC Loughboug, L.E. 116 Rg. England.
Trypticase soy agar	-	BBL Cockeysille Md.
Hydrochloric acid (Hcl)	-	May and Baker Ltd., Dagencha England.

2.1.2. Bacteriological identification

Citrated Human Plasma	-	Diagnostic Division, NVRI, Vom, Nigeria.
Opticin	-	Biotech Laboratories.
Hemin (X) and NAD (V) factors	-	Biotech Laboratories.
Bacitracin	-	Biotech Laboratories.

2.1.3. Media used in Mycoplasmology

Bacto pleuropneumonia like organism (PPLO) broth	-	Difco laboratories, Detroit, USA.
Bacto PPLO agar	-	Difco laboratories, Detroit, USA.
Donor Horse Serum	-	Flow laboratories Ltd., UK, Irine KA 8ND.
Glucose	-	AnaLaR BDH chemicals Ltd., Poole, England.
Yeast extract	-	Oxoid Ltd., England.
Thallium acetate	-	Fisher Scientific Co., New Jersey USA.
Benzylpenicillin	-	Rivopharm laboratories, 6928 Mannom Switzerland.

2.1.4. Material used for serological identification of *M. pneumoniae*

M. pneumoniae antisera	-	Mycoplasma Research Laboratory, NVRI, Vom, Nigeria.
M. salivarium antisera	-	Mycoplasma Research Laboratory, NVRI, Vom, Nigeria.

2.1.5. Materials used in the immunological studies for the detection of *M. pneumoniae* antibodies

Sera specimen from children with pneumonia and control (those without pneumonia)	-	JUTH, Jos
Sheep red blood cells (Srbc)	-	Experimental animal laboratory, NVRI, Vom, Nigeria.
Complement (C^1)	-	Burroug's Welcome England.
Haemolysin	-	Burrough's Welcome England.
M. pneumoniae antigen (titre 1/50)	-	Mycoplasma Research Laboratory, NVRI, Vom, Nigeria.
Veronal buffer diluent	-	Burrough's Welcome England.
Positive antisera	-	Mycoplasma Research Laboratory, NVRI, Vom, Nigeria.
Glutaraldehyde	-	Eastman Kodak, USA.
Tanic acid	-	BDH Chemicals Ltd., Poole, England.
M. pneumoniae (freeze-dried)	-	Mycoplasma Research Laboratory, NVRI, Vom, Nigeria.

Newborn calf serum	-	Flow laboratories U.K., Irvine KAR 8NB
Magnesium chloride (Mgcl)	-	AnaLaR BDH Chemicals Ltd., Poole, England.
Calcium chloride (Cacl)	-	AnaLaR BDH Chemicals Ltd., Poole, England.
Glucose	-	AnaLaR BDH Chemicals Ltd., Poole, England.
Sodium citrate	-	AnaLaR BDH Chemicals Ltd., Poole, England.
Barbituric acid	-	AnaLaR BDH Chemicals Ltd., Poole, England.
Sodium chloride	-	May and Baker Ltd., Dagenham, England.
Citric acid	-	AnaLaR BDH Chemicals Ltd., Poole, England.
Sodium bicarbonate	-	AnaLaR BDH Chemicals Ltd., Poole, England.
Sodium barbitone	-	AnaLaR BDH Chemicals Ltd., Poole, England.

2.1.6. Equipments, glassware and miscellaneous items used in the study

Universal bottle

Vials

Draining tubes

Microtitre (V bottom) trays

Microdiluter

37°C incubators (0_2 and Co_2)

Sorvall RB-SB-Ref super speed centrifuge

MSE sonicator

Filter paper discs

Stereomicroscope

bijou bottles

Water bath

Refrigerator 21°C and -20°C deep freezer

Pipettes 1ml, 5ml 10mls

2.2. METHODS

2.2.1 Methods for bacteriology

2.2.1.1. Transport media:

Stuart transport medium was used. A 200ml transport medium was prepared at a time by adding 0.82g sodium chloride, 0.20g sodium dihydorogen orthoposphate, 0.006g phenol red and 60.0ml glycerol into 140ml deionized water contained in a sterile conical flak. the mixture was stirred and heated

gently to dissolve. The medium adjusted pH 7.2 with IN Hydrochloric acid (Hcl) or IN sodium hydroxide (NaoH) and then dispensed in 10ml amounts in a universal bottle and autoclave at 121°C for 15 minutes.

2.2.1.2 Culture media:

Blood and chocolate agar plates as described by Finegold *et al.*, 1978 were used to cultivate bacteria.

(i) Preparation of blood agar (BA) Plates

Basal medium was dissolved in 500ml distilled deionized water and autoclave at 121°C for 15 minutes, then allowed to cool to 48-50°C before 20ml sterile fibrinated rabbit blood cells added ascetically. The contents were rotated to mix thoroughly and then poured into sterile petri dishes in approximately 20ml amounts and allowed to set. The plates were then inverted and incubated at 37° overnight to test for sterility.

(ii) Preparation of chocolate agar (CA) Plates

Chocolate agar plates were prepared by heating the rabbit blood to 56°C till the colour changed to brown prior to mixing with dissolved basal medium. The mixture was then poured as in (i) above.

2.2.2. Methods for mycoplasmology

2.2.2.1. Preparation of media for mycoplasmology

A 100ml broth and 100ml of agar media were prepared at a time as described below:

i Solid medium

3.5gm of pleuropneumonia like organism (PPLO) was added to 85mls of deionized water in a sterile 150ml bottle into which 1.0g yeast extract, 0.1ml glucose and 0.1ml of 10% phenol red solution were added, stirred and the pH adjusted to 7.6 using IN Hcl or IN NaoH. The agar was then autoclave at 121°C for 15 minutes. This was then stored at room temperature. On request 15ml sterile horse serum, 0.2ml thallium acetate (10% solution) and 1ml penicillin (10% of $1x10^6$i.u/ml) were ascetically added to molten agar at 40°C. The agar was then dispensed into sterile plastic petri dishes and allowed to set. The plates were then incubated at 37°C overnight to check for sterility. Sterile plates were stored in a petri dish can at 4°C till needed.

ii. Broth medium

2.1g of PPLO broth powder was added to 85mls of deionized water in a sterile conical flask into which 1.0g yeast extract, 0.1g glucose and 0.1g phenol red (10% solution) were added, stirred and the pH adjusted to 7.6 using IN NaoH or IN Hcl. The broth was then autoclave at 121°C for 15 minutes. It was then allowed to cool at 40°C water bath. 15ml horse serum, 0.2ml thallium acetate (10% solution) and 1ml penicillin (10% solution of $1x10^6$i.u) were then added into the broth, and well mixed. The broth was then dispensed in 4.5ml alliquots into a sterile universal bottle and incubated overnight at 37°c for sterility check.

2.3 METHODS OF PATIENTS SELECTION

122 paediatric patients with pneumonia comprising 54 males and 58 females, aged from 4 week to 12 years (mean 2.3 years) visiting the Jos University Teaching Hospital (JUTH), Jos who had no antibiotic therapy were selected for the study.

Diagnosis was initially based on clinical features as described by Weinberg, *et. al.*, 1989, i.e. tachypnea (respiration rate $\geq 50 min^{-1}$), chest indrawing (restrictions), stidor, cyanosis or wheezes or rales upon auscutation with or without fever or cough and confirmed by chest radiography for signs of lobar pneumonia or bronchopneumonia. The age of the subjects reflected that of total patients who visited the hospital.

50 non-pneumonic paediatric patients (25 males and 25 females) of similar age distribution with the patients were selected as the controls.

2.4 SAMPLE COLLECTION

Three types of specimen were collected from the patients studied. These are nasopharyngeal aspirate (NPA), pleural effusions (PE) and whole blood (WB) with the consent of at least a parent of the patients.

2.4.1 Nasopharyngeal aspirate (NPA)

NPA from all the cases were ascetically collected in a sterile container in the following manner: The outer surfaces of the nares were disinfected, then a sterile 'draining tube" attached to the container inserted upto the nasopharyngeal region. Using a second tube (also attached to the container)

the collector used mouth to draw in, hence draining the nasopharyngeal contents into the container. Half the volume of the specimen was then inoculated into 10ml Stuart transport medium and the other half into 4.5ml PPLO broth.

2.4.2. Pleural effusion (PE)

11 PE samples (10% of the total samples) were collected in the following manner: The skin of the chest over the area of maximum radiological evidence of effusion was disinfected with spirit and a 21G needle attached to a 10ml syringe used to collect the fluid. Half of the volume of the fluid was inoculated into 10ml transport medium and the other half into 4.5ml PPLO broth.

2.4.3 Whole blood

3mls of venous blood during acute phase was drawn from subjects using 21G needle attached to 10ml syringe which was then emptied into sterile 10ml vial during patients first visit to the hospital. Patients were also encouraged to return 10-14 days later when a convalescent blood samples were taken as per acute whole blood.

Only one blood sample was drawn from non-pneumonia patients as control.

2.5 SAMPLE TRANSPORTATION

The inoculated PPLO broth, transport medium and the whole blood were transported inside a colman flask containing ice-blocks to Vom

laboratory for processing.

2.6 SAMPLE PROCESSING

2.6.1. Bacterial Culture procedure

On reaching the laboratory the transport medium was centrifuged at 1,000 rpm for 15 minutes and the supernatant ascetically discarded. A loofful of the deposit was then directly inoculated onto a chocolate agar plate and on two blood agar plates. One blood and the chocolate agar plates were incubated at 37°C inside a 7% CO_2 incubator overnight. The other blood agar was incubated aerobically at 37°C overnight. Samples that showed no bacterial growth after 48 hours incubation were regarded as negative for bacterial growth. Whereas all bacterial isolates were appropriately identified as described in 2.7.

2.6.2. Mycoplasmal culture procedure

A series of 10 fold dilutions were prepared up to 1:1000 dilution by transferring 0.5ml aliquot of the inoculated broth to 4.5ml of PPLO broth. The four tubes were incubated at 37°C under aerobic conditions. When colour of the PPLO broth start to change from red to yellow, an aliquot of the broth was inoculated on to PPLO agar for identification. Broth cultures with no apparent colour change after 20 days incubation were blindly passage on to PPLO agar.

2.6.3 Whole blood

Whole blood samples were allowed to clot in the sterile 10ml vial

which was then kept at 4°C overnight. Sera samples were aspirated with sterile pipette and then centrifuged at 1,000 rpm for 30 minutes and stored in a sterile bijou bottle at -20°C till needed.

2.7. BACTERIAL IDENTIFICATION

All bacterial isolates were identified using standard microbiological methods (Finegold *et al.*, 1978).

2.7.1 *Staphylococcus aureus*

Colonies that exhibited *S. aureus* like cultural characteristics i.e. large, opaque, circular and smooth appearance that may produce golden yellow pigment were gram stained.

Coagulase test was carried out on fresh overnight nutrient broth cultures of gram positive clusters by inoculating a loopful of the culture into citrated rabbit plasma inside a small test-tube incubated at 35°C in a water bath. Cultures that showed coagulation of the plasma within 1-4 hours were identified as *S. aureus*. Coagulase negative cultures were identified as *Staphylococcus spp.*

2.7.2 *Streptococcus pneumoniae*

alpha-haemolytic mucoid colonies that appeared round, glittering, transparent on blood agar were gram stained and lancet shaped gram positive diplococcus were presumed to be *St. pneumoniae*. Confirmatory test was carried out as described by Bowers and Jefferies (1955). Single colony of isolate was heavily streaked on blood agar plate and optochin disc containing

5µg ethylhydrocupreine-Hcl was placed and the plate incubated at 37°C overnight in CO_2 incubator. Isolate that exhibited a zone of inhibition of at least 18mm in diameter were identified as *St. pneumoniae.*

2.7.3. *Haemphilus influenzae*

Small (1-2mm) colourless, transparent, most colonies with distinct "mousy odour" on chocolate or blood agar plates were suspected as *H. influenzae.* Such colonies were gram stained. All gram negative coccibacillus isolates were subjected to bacitracin sensitivity. Bacitracin resistent colonies were tested for dependency on hemin (X) and NAD(V) factors. Colonies that required both X and V factors for growth were identified as *H. influenzae.* Isolates that depend only on V factor were identified as *H. parainfluenza.*

2.7.4. *Klebsiella pneumoniae*

Colonies that appeared large, mucoid on blood agar plates and sting out on touch with wire loop were gram stained. Colonies that were gram negative fat rods were subjected to further biochemical tests according to Finegold *et al* , (1978), for *K. pneumoniae* identification.

2.7.5. β-haemolytic streptococcus

Bacitracin discs (0.4 unit) were placed ont thick inoculum of β-haemolytic colonies and incubated overnight at 37°C. Colonies with clear zone of inhibition were presumed to be β-haemoytic streptococcus.

2.7.6. Other bacteria

All other bacterial isolates were identified using standard microbiological tests as described by Finegold, *et al.*, (1978).

2.8. MYCOPLASMAL IDENTIFICATION

Inoculated PPLO agar plates were incubated at 37°C in a 7% CO_2 incubator and were examined every week for mycoplasma-like organism colonies using stereo-microscope at a magnification X10. Plates were incubated for 4 weeks before being considered negative for mycoplasmal growth.

2.8.1. Characterization of mycoplasma isolates

Characterization was based on standard methods as described by Tulley (1983) as follows:

A single colony was picked and incubated into PPLO broth. Serial dilution of the incubated broth upto 10^{-3} was carried out to avoid bacterial contamination. The culture was incubated for 3 days and subcultured onto PPLO agar plates and incubated in a 7% Co_2 incubator for 3 days. After incubation the typical mycoplasma colonies (fried egg shape) were studied for non reversion to bacterial form when grown on media free or bacterial inhibitors for three consecutive passages. Blood agar plates were also plated with broth culture isolates as purity check.

Serological identification as described by Clyde (1964) was carried out on pure mycoplasma isolates as follows:

The isolates were inoculated into PPLO broth and just prior to colour change 0.1ml of the culture was spread onto PPLO agar plate. The plate was allowed to dry at room temperature. Discs presaturated with *M. pneumoniae* and *M. salivarium* antisera were placed opposite each other on the surface of the agar plate. The plate was then incubated at 37°C in 7% CO_2 incubator. Colony growth inhibition due to antisera activity were examined after one week.

2.9 PREPARATION OF MATERIALS USED IN THE DETECTION OF *M. pneumoniae* ANTIBODIES

2.9.1. Alselver's solution (pH 6.1)

Alselver's solution was prepared by weighing 20.5g glucose, 8.0g sodium citrate, 4.2 sodium chloride, 0.55g citric acid and made up to 1.0 liter with distilled water. The solution was then autoclaved at 15lb pressure for 15 minutes.

2.9.2. Phosphate buffer solution (PBS)

83.8g sodium chloride, 2.52g sodium bicarbonate and 3.0g sodium barbitone were dissolved in 1400ml of distilled water as solution A. Then 4.6g, Barbituric acid, 1.0g magnesium chloride (Mgcl) and 0.2g calcium chloride (Cacl) were dissolved in 400ml hot water as solution B. Solutions A and B were then mixed together and made-up to 200ml with distilled water. Before use one part of the mixture was added to four parts of distilled water.

2.9.3. Sheep red blood cells (Srbc)

Blood was aseptically drawn from sheep in 75ml amounts into a bottle containing 125ml of sterile Alsever's solution. The cells were washed four times with phosphate buffer solution at pH 6.2 before use.

2.10. IMMUNOLOGICAL TECHNIQUES USED FOR THE DETECTION OF *M. pneumoniae* ANTIBODIES

Complement fixation and indirect haemagglutination tests were used in the detection of *M. pneumoniae* antibodies as described by Clyde and Senterfit, (1983) and Kok, *et al.*, 1988a) respectively.

2.10.1 Complement fixation test (CFT)

3% sheep red blood cells were sensitized with HD50 of the hemolysin. Complement (C^1), positive and negative antisera were prepared into their respective working dilutions, each time the test was done.

Sera samples were thawed and initially diluted 1:4 in CFT-phenol buffer (to inactive test sera complement) and the doubling dilutions were made in CFT buffer upto 1:256 inside bijou bottles.

0.025ml of each dilution was added to microtire trays well and 0.025ml of *M. pneumoniae* antigen and 0.025ml of complement were added. The last three wells were used in each tray for serum, antigen and positive antiserum controls respectively. Serum control well consists of serum, complement, buffer but no antigen. Antigen control well consists of buffer complement and antigen but no serum. While positive antiserum control consists of

antisera of known titre, complement and antigens all at 0.025ml volumes.

The trays were covered and incubated at 37°C for 30 minutes, shaking at 10 minutes intervals. to all the wells 0.025ml of sensitized sheep red blood cells (Srbc) were added and shaken, the trays were covered and re-incubated at 37°C for an hour with shaking at 10 minutes intervals. The tests were read after storing at 4°C overnight. Titration end points are represented by wells showing 75% complete cell buttons.

2.10.2 Indirect haemagglutination test (IHAT)

2.10.2.1 Fixation, tanning and sensitization of sheep red blood cell (Srbc)

First the sheep red blood cells (Srbc were fixed, tanned and sensitized in the following way:

Fresh Srbc were fixed in 1% (v/v) glutaraldehyde and treated with equal volume of freshly prepared tannic acid to a final concentration of 1:80,000 (w/v).

Freeze-dried *M. pneumoniae* whole organisms were suspended in 2.0ml phosphated buffer solution (PBS) pH 7.2 and sonicated using MSE sonicator for 10 minutes with cooling intervals. Equal volume of *M. pneumoniae* sonicate 2% (v/v) newborn calf serum in PBS. After 2 days at 4°C the cells were resuspended in a fresh volume of diluent buffer and then used in the indirect haemagglutination assays.

2.10.2.2 Indirect haemagglutination assay for the measurement of *M. pneumoniae* antibody

An initial dilution of 1:10 of test sera was made, the doubling dilution upto 1:640 made in a microtitre (V-bottom) trays. An equal volume of 0.4% (v/v) *M. pneumoniae* sensitized Srbc (Srbc-MP) was added to each well, to obtain a final cell concentration of 0.2% (v/v) in each well. The first serum dilution in each case was 1:20 and the last was 1:1280. The trays were read after 2-3 hours at room temperature and after overnight incubation at 4°C. An agglutination mat of cells covering the bottom of the well indicated the presence of specific *M. pneumoniae* antibodies while a bottom of cells at the bottom of the well indicated a negative result.

CHAPTER THREE

RESULTS

CHAPTER THREE

3.0 RESULTS

3.1 RESULTS OF BACTERIOLOGICAL STUDIES

Table 3.1: INCIDENCE OF SINGLE BACTERIAL PATHOGENS FROM THE NASOPHARYNGEAL AND PLEURAL EFFUSION OF 112 PNEUMONIC CHILDREN:

BACTERIA	PLEURAL EFFUSION		NASOPHARYNGEAL ASPIRATE	
	No. of patients positive for each pathogen	% of single bacteria out of 11 samples	No. of patients positive for each pathogen	% of single bacteria out of 112 samples
Staphylococcus aureus	4	36.4	18	16.1
Haemophilus influenzae	2	18.2	10	8.9
Klebsiella pneumoniae	2	18.2	9	8.0
Streptococcus pneumoniae	1	9.1	5	4.5
β-haemolytic Streptococcus	0	0	3	2.7
No bacterial growth	2	18.2	21	18.8
Mixed bacterial growth	0	0	46	41.1

Of the nasopharyngeal aspirate (NPA) samples, 45/112 or 40.2% and 9/11 or 81.8% of the pleural effusion (PE) samples yielded single bacterial growth. From the 11 PE samples 36.4% yielded pure *S. aureus* isolates. *H. influenzae* and *K. pneumoniae* were isolated in 18.2% of the PE samples each. *St. pneumoniae* was also isolated in 9.1% of all the PE samples. No. β-haemolytic streptococcus was isolated in the PE samples. 18.2% were bacteriologically barren and no mixed bacterial growth was isolated in any of the PE sample. From the NPA samples the percentages of *S. aureus, H. influenzae, K. pneumoniae, St. pneumoniae* and *B.* influenzae streptococcus were 16.1, 8.9, 8.0, 4.5 and 2.7 respectively. 41% of the NPA samples yielded mixed bacterial growth and 18.8% yielded no bacterial growth. The rates of single bacterial isolates from the 45 positive Nasopharyngeal aspirate positive for *S. aureus, H. influenzae, K. pneumoniae, St. pneumoniae* and β-haemolytic streptococcus were 40.0%, 22.2% 20.0%, 11.1% and 6.7% respectively. These rates were closely reflected in pleural effusion sample results which were *S. aureus* (36.0%), *H. influenzae* (18.2%), *K. pneumoniae* (18.2%), *St. pneumoniae* (9.1%) though no β-haemolytic streptococcus was isolated.

The similarity of incidence and rates of single bacterial isolates between the two types of specimen most probably indicates aetiology.

46/112 (33.0%) of the NPA samples yielded 2 of more bacterial isolates mostly commensals (Appendix 1). Also 21/112 (19.8%) of the NPA samples and 2/11 (18.2%) of the PE samples recorded no bacterial growth. All the 50

control subjects fall within these latter two groups. Multiple isolates were encountered in 21 control cases (Appendix II). 29 control cases yielded no bacterial growth. Thus making it impassible to relate the multiple organism isolated with aetiology.

Table 3.2: INCIDENCE OF SINGLE BACTERIAL PATHOGEN ISOLATED IN RELATION TO AGE:

ORGANISM	AGE/YEARS				
	0 - 2	3 - 6	7 - 10	Over 10	Total
Staphylococcus aureus	12	5	1	0	18
Haemophilus influenzae	8	1	1	0	10
Klebsiella pneumoniae	7	2	0	0	9
Streptococcus pneumoniae	3	0	0	2	5
β-haemolytic streptococcus	3	0	0	0	3
Total %	33(73.3)	9(16.8)	2(4.4)	2(4.4)	45(100%)

The table contrasts the frequency of single bacterial isolates in relation to age. 73.3% of these pathogens isolated were from patients 2 years and below of which *S. aureus* accounted for 26.7%, *H. influenzae* (17.8%), *K. pneumoniae* 15.6%, *St. pneumoniae 6.7% and* β-haemolytic strepotococus 6.7%.

17.8% of the single pathogens were isolated from patients 3-6 years of age. *S. aureus, H. influenzae* and *K. pneumoniae* accounted for 11.1%, 2.2% and 4.4% respectively. *S. aureus* and *H. influenzae* were the only single pathogens from 7-10 years age group each accounting for 2.2%. In patients more than 10 years old, the only organism isolated in pure culture was *St. pneumoniae* which accounted for 4.4%.

Table 3.3: INCIDENCE OF SINGLE BACTERIAL PATHOGEN IN RELATION TO CLINICAL MANIFESTATION:

ORGANISM	LOBAR PNEUMONIA	BRONCHPNEUMONIA
Staphylococcus aureus	5	13
Haemophilus influenzae	3	7
Klebsiella pneumoniae	7	2
Streptococcus pneumonia	2	3
β-haemolytic streptococcus	-	3
Total (%)	17(33.7)	28(62.2)

The table shows single bacterial pathogens isolated in relation to clinical and radiological diagnosis.

Cases diagnosed as bronchopneumonia were made in 71(63.4%) and lobar pneumonia in 41(36.6%) as confirmed by radiology. 17/41 (41.5%) and 28/71(39.4%) cases yielded single bacterial isolates in lobar and bronchopneumonia respectively. *K. pneumoniae* and *S. aureus* were the predominant single isolates in lobar and bronchopneumonia cases respectively. β-haemolytic streptococcus was never encountered in lobar pneumonia cases studied.

Table 3.4: INCIDENCE OF SINGLE BACTERIAL PATHOGEN IN RELATION TO SEX:

ORGANISM	MALE	FEMAle	TOTAL
Staphylococcus aureus	12	6	18
Haemophilus influenzae	4	6	10
Klebsiella pneumoniae	3	6	9
Streptococcus pneumoniae	3	2	5
β-haemolytic streptococcus	3	0	3
Total (%)	25/(55.5)	20(44.5)	45(100)

The table illustrates the incidence of single bacterial isolates in relation to sex. 25/45 (55.5%) of the single pathogens were encountered in males, whereas 20/45(44.5%) were isolated from female patients.

In the males, *S. aureus* was clearly the predominant single pathogen isolated with 48.0%, but in females *S. aureus, H. influenzae* and *K. pneumoniae* equally accounted for 30% each. No female case yielded β-haemolytic streptococcus.

3.2 RESULTS OF MYCOPLASMOLOGY

Table 3.5: SEROLOGICAL IDENTIFICATION OF *M. pneumoniae* USING GROWTH INHIBITION TEST

NUMBER OF ISOLATE	ANTISERUM	
	M. pneumoniae	*M. salivarium*
001	+	-
019	+	-
022	+	-
025	+	-
030	+	-
035	+	-
049	+	-
053	+	-
066	+	-
069	+	-
077	+	-
086	+	-

(+) Zone of inhibition, (-) No zone of inhibition.

M. pneumoniae was isolated from 12 out of 112 pneumonic patients, a 10.7% culture positive. Isolates were confirmed using growth inhibition test as in Table 3.5 above. *M. pneumoniae* was not isolated from any of the 50 control subjects.

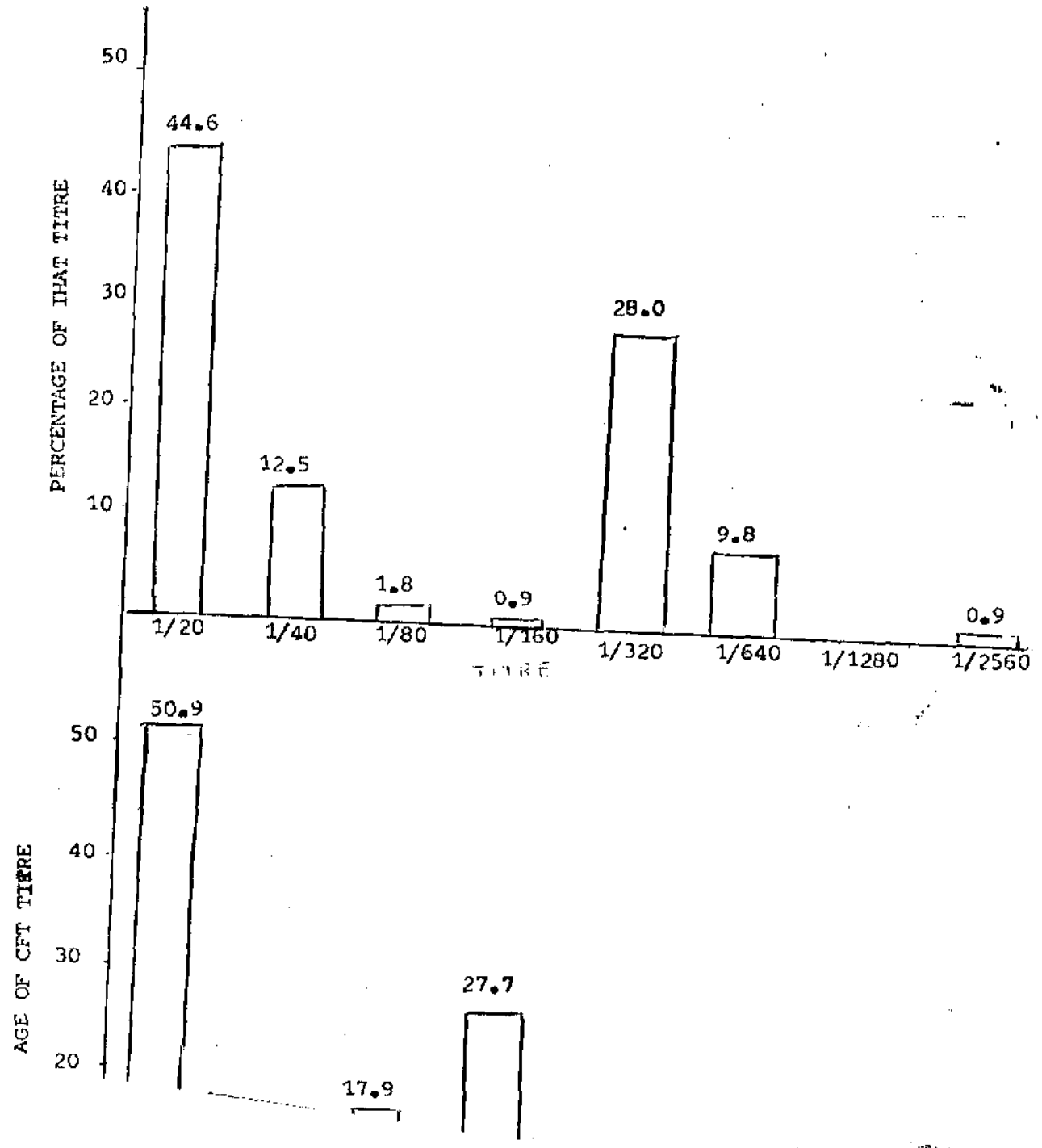
Table 3.6: SIGNIFICANT *M. pneumoniae* antibody TITRE DEMONSTRATED USING CFT AND IHAT IN PNEUMONIC CHILDREN:

NUMBER OF SAMPLE	RECIPROCAL CFT TITRE		RECIPROCAL IHAT TITRE	
	ACUTE PHASE	CONVALESCENT PHASE	ACUTE PHASE	CONVALESCENT PHASE
001+	≤8	64	≤20	640
009	64	ND	320	ND
010xx	≤8	≤8	320	320
012	64	128	80	1280
015	64	ND	320	ND
O16	64	ND	320	ND
017	64	ND	320	ND
019+	≤8	64	320	640
020	32	64	40	640
021	≤8	64	80	320
022+	64	128	640	640
025	64	ND	320	ND
026	64	64	320	320
028	128	ND	2560	ND
O30+	64	ND	320	ND
033	64	ND	640	ND
035+xx	≤8	ND	320	ND
037	64	64	640	320
039	64	ND	640	ND
042	64	ND	320	ND
049+	64	ND	320	ND
053+	128	ND	640	ND
055	64	ND	640	ND
058	64	ND	320	ND
060xx	≤8	8	640	40
061	≤8	64	640	1280

65xx	32	ND	320	ND
066+	64	ND	320	ND
067	64	ND	640	ND
068	64	ND	320	ND
071	≤8	64	320	80
072	≤8	64	≤20	320
074	64	32	320	80
075	≤8	64	40	320
076xx	32	32	160	640
077+	≤8	64	≤20	320
081	≤8	128	40	320
083xx	≤8	ND	320	ND
085	64	ND	320	ND
086+	64	ND	640	ND
087	≤8	64	≤20	320
090	64	≤8	320	40
O91	64	ND	320	ND
092	64	ND	640	ND
093xx	32	ND	320	ND
094	64	ND	320	ND
097	64	ND	320	ND
099	64	ND	320	ND
100xx	32	ND	320	ND
102xx	≤8	ND	320	ND
104	64	ND	640	ND
105	64	ND	320	ND
109xx	32	ND	320	ND
111	64	ND	320	ND

ND = Not Done
xx = Positive by IHAT only
\+ = Isolation positive

Figure 3.1: HISTOGRAM OF PERCENTAGE OF VARIOUS CFT AND IHAT TITRES OBTAINED IN PNEUMONIC CHILDREN VISITING JUTH, JOS.



3.2.1: Presence of *M. pneumoniae* CF and IHA antibodies in relation to isolation of the organism

Of the 112 samples cultured 12 were positive by isolation whereas 42 patients were additionally confirmed to be infected with *M. pneumonia* serologically as shown in Appendix III.

Of the 12 culture positive samples examined for CF and IHA tests the results were as follows: 9 had a diagnostic titre of both antibodies, 2 had diagnostic IHAT antibody titre alone. One culture positive case was missed by CF and IHA tests. Of the 54 samples that were serologically positive. 44 had both diagnostic CFT and IHAT antibody titres. The other 10 were positive for IHAT only. No CFT positive sample was IHAT negative.

3.2.2. Isolation - positive versus isolation negative case:

Table 3.7: COMPARISM OF ISOLATION POSITIVE AND ISOLATION NEGATIVE CASES IN RELATION TO THE SEROLOGICAL PICTURE:

	IHAT TITER		CFT TITER	
	1:40	1:320	1:8	1:64
Isolation positive case	27.3% (3/11)	27.3%(3/11)	45.5% (5/11)	36.4%
Isolation negative cases	9.5% (4/43)	81.6%(34/42)	21.9% (7/32)	84.4% (27/32)
Px	N.S	0.01	N.S	0.01

By X2
NS not significant

The rate at which *M. pneumoniae* was isolated varied considerably in relation to phase of the infection. Among the sera samples collected simultaneously with the nasopharyngeal aspirates samples for *M. pneumoniae* culture, 11 were positive both by culture and by serology. 42 were positive by IHAT but negative by culture. Only one was positive or culture but negative by serology.

The number of patients with acute IHAT titres ≤1/40 was 3(27.3%) of the 11 culture positive, whereas it was 4(9.5%) of the 42 for those who were culture negative. On the other hand an IHAT tire of ≥1:320 was observed in 3(27.3%) of 11 patients who were isolation positive whereas such a high titre was observed in 34(81.0%) of 42 patients who were isolation negative. The isolation rate of *M. pneumoniae* was therefore 3/7 (42.9%) of patients who were negatives by IHAT (≤1:40) and 8.1% (31/37) among patients with IHAT ≥1:320.

Thus *M. pneumoniae* was isolated more frequently in the early stage of illness when antibody titre was not significant than when antibody titre rose to ≥1:320 (P>0.01).

This result was reflected also between CF titre and results of *M. pneumoniae* isolation.

FIGURE 3.2: HISTOGRAM OF PERCENTAGE OF POSITIVE CF and IHA TITRES IN RELATION TO AGE:

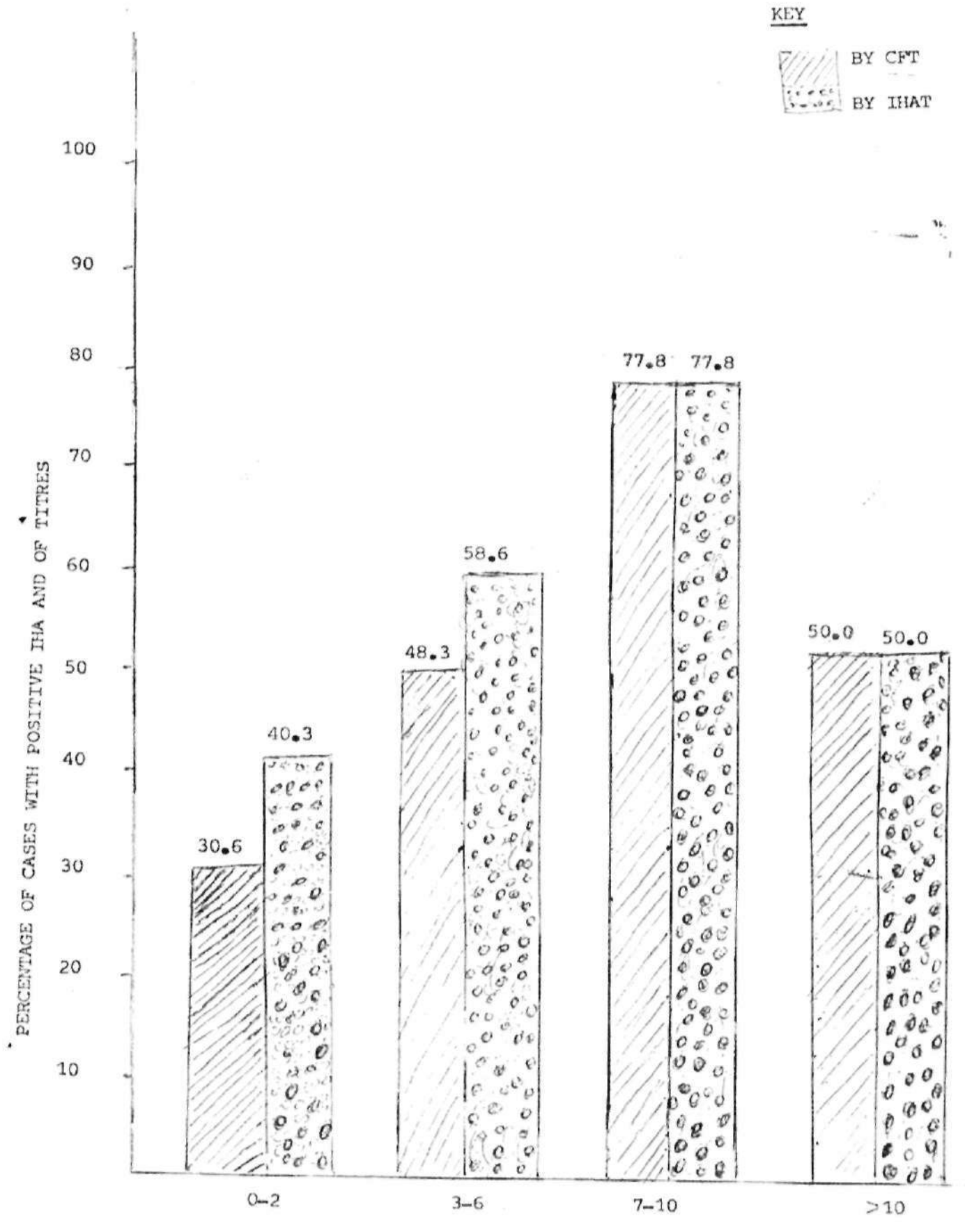


Table 3.8: INCIDENCE OF *M. pneumoniae* INFECTION IN VARIOUS AGE GROUPS BY SEROLOGY:

AGE (YEARS)	TOTAL CASE	TEST	
		CFT (%)	IHAT (%)
0 - 2	72	22(30.6)	29(40.3)
3 - 6	29	14(48.3)	17(58.6)
7 - 10	9	7(77.8)	7(77.8)
Over 10	2	1(50.0)	1(50.0)
TOTAL	112	44(39.7)	54(48.2)

Serologically the highest incidence was in 0-2 year age group but the highest rate remained in the 7-10 year age group as indicated in Table 3.8 and Figure 3.2 However by culture method the highest incidence was in the 3-6 year and least in the 0-2 year age group.

Table 3.9: INCIDENCE OF *M. pneumoniae* INFECTION IN RELATION TO CLINICAL MANIFESTATION BY SEROLOGY:

MANIFESTATION	TOTAL	TEST	
		CFT (%)	IHAT (%)
LOBAR PNEUMONIA	41	19(46.3)	20(48.8)
BRONCHOPNEUMONIA	71	25(35.2)	34(47.9)

47.9 and 48.8% of cases from the bronchopneumonia and lobar pneumonia respectively had diagnostic IHA titer indicative of *M. pneumoniae* infection. Similarly, 46.3% lobar pneumonia cases and 35.2% Bronchopneumonia cases showed diagnostic CFT titre which indicated infection by *M. pneumoniae* as shown in Table 3.9.

Table 3.10: INCIDENCE OF *M. pneumoniae* INFECTION IN RELATION TO SEX BY SEROLOGY:

SEX	TOTAL NUMBER	TEST	
		CFT (%)	IHAT (%)
MALE	54	17(31.5)	23(42.6)
FEMALE	58	27(46.6)	31(53.4)

Serologically 42.6% of the males and 53.4% of the females had diagnostic IHA antibody titres. This result was also reflected CFT with 31.5% of the male and 46.6% of the females as having diagnostic CF titrers.

3.3 CONCURRENT *M. pneumoniae* AND BACTERIAL INFECTION

Table 3.11: CONCURRENT PATHOGENIC BACTERIAL AND *M. pneumoniae* INFECTION WITH RELATION TO AGE:

M. pneumoniae Infection +	AGE/YEARS			
	0 -2	3 - 6	7 - 10	>10
St. pneumoniae	0	0	0	1
H. influenzae	7	1	1	0
K. pneumoniae	3	1	0	0
S. aureus	3	1	0	0
β-haemolytic	0	0	0	0
TOTAL	13	3	1	1

M. pneumoniae isolated together with only *H. influenzae* in two cases, however serological evidence of *M. pneumoniae* infection was seen to frequently occur concurrently with bacterial pathogens. *M. pneumoniae* infection was seen to occur nine times *H. influenzae,* four times with *K. pneumoniae* and *S. aureus* each and once with *St. pneumoniae.* No concurrent *M. pneumoniae* infection occurred with β-haemolytic streptococcus.

Dual *M. pneumoniae* and bacterial infection were noticed to decrease with increase in age, whereas 13 cases were noticed in 0-2 year age group, 3 concurrent infections were noticed in 3-6 year age group and only one each in 7-10 and more than 10 year age group.

Table 3.12: CLINICAL FEATURES IN CHILDREN WITH BACTERIAL AND MYCOPLASMAL PNEUMONIA:

FEATURE	PERCENTAGE OF PATIENTS WITH BACTERIAL PNEUMONIA	PERCENTAGE OF PATIENTS WITH MYCOPLASMAL PNEUMONIA	p**
SYMPTOMS			
Cough	100	73.0	0.05
Chest pain	74.0	8.1	0.01
Malaise	7.4	64.9	0.01
Antecedent upper respiratory tract infection	3.7	48.7	0.01
Gastrointestinal symptoms (vomiting and Diarrhoea)	7.4	40.5	0.01
Headache	3.7	24.3	0.01
Skin rashes	11.1	10.8	NS
Conjuctivities	7.4	2.7	NS
PHYSICAL SIGNS			
Fever (38.00°C	100	46.0	0.01
Tachyponia	100	18.9	0.01
Tachycardia	66.7	24.3	0.01
Dyspnoea	33.3	18.9	NS
Bronchial breathing	22.2	16.2	NS
Kwashiorkor	7.4	2.7	NS

NS Not significant
By X2 test

Special signs and symptoms of bacterial and mycoplasmal pneumonia were seen to differ. Cough, fever and tachypnia were always noticed in bacterial pneumonia, but only atimes in mycoplasmal pneumonia.

On the other hand malaise (64.9%), antecedent upper respiratory tract infection(48.9%) were in most case features of mycoplasmal pneumonia. Occurrence of cryptations, bronchial breathing, kwashiorkor, skin rashes and conjuctivities were seen not to differ significantly in the two type of pneumonia.

CHAPTER FOUR

DISCUSSION AND CONCLUSION

CHAPTER FOUR

DISCUSSION AND CONCLUSION

4.0 DISCUSSION AND CONCLUSION

4.1 DISCUSSION

This study reports the incidence of bacterial and mycoplasmal pathogens associated with the respiratory tract of paediatric patients with pneumonia visiting the Jos University Teaching Hospital (JUTH), Jos. JUTH, Jos serves a cross-section of the population. Though not all patients who visited the hospital with the illness were sampled, the age, sex and the socio-economic class distribution of the patients reflected the total.

A simple and acceptable nasopharyngeal aspirate (NPA) technique was used for culture. Complement fixation and indirect haemagglutination tests were also used for measuring *Mycoplasma pneumoniae* antibodies in the patient's sera.

Since bacterial pathogen involved in pathological process are indicative of aetiology of respiratory disease (Loda, *et al.*, 1968, Lennette, *et al.*, 1980), the value of nasopharyngeal aspirate technique was evaluated by concurrently processing the aspirate pleural effusion sample where possible. The results from bacteriology were categorized into three groups. In group 1, single bacterial pathogens were obtained in 45 of the 112 cases (40.2%) probably playing a role in the aetiology, as identical organism with similar incidences were obtained, wherever both nasopharyngeal aspirate and pleural effusion samples were

analyzed. This was further confirmed as no control case yielded any single bacterial pathogen.

In group 2 cases 46/112 (41.1%) multiple bacterial isolates were obtained from the nasopharyngeal aspirate sample making any meaningful interpretation very difficult. Whereas it is tempting to suggest "mixed bacterial infection", as reported in adults, (Gohar and Abduod, 1933; Abdel-Kalik, *et al.*, 1938; Mimica, *et al.*, 1977; Veghese, and Berk 1983), it is safer to assume nasal contamination as described by Davidson, *et al.*, (1976) where tracheal contamination disrupted specific diagnosis of pneumonia. This is more so as most of the isolates were commensals, and no pleural effusion sample yielded multiple bacteria. These commensals may however be opportunistic pathogens as a result of the low immune status of the subjects.

Group 3 cases 21/112 (18.8%) showed no bacterial growth indicative of non bacterial aetiology. Few pleural effusion samples (2/11) fall into this group. However, bacterial pneumonia cannot be ruled out even with this negative results. This is because it is possible to obtain false negative results even using lung aspirate samples. (Sappington Favorite, 1936).

The nasopharyngeal aspirate technique is therefore useful in the diagnosis of childhood bacterial pneumonia. This is in agreement with Shamn *et al.*, 1984, who isolated *H. influenzae* and *St. pneumoniae* from the nasopharynx region in more than 80% of pneumonic children with lung aspirate or blood positive for these organisms. In this study the nasopharyngeal aspirate technique yielded

specific single bacterial pathogen in 40.2% of the patients who had no previous antimicrobial therapy. This finding is more favourable to that of throat swab and tracheal aspirate techniques with 10-30% success (Mimica, *et al.*, 1977). Lung aspirate and lung biopsy techniques yield higher success rate (54 - 83%) but require expertise and expense, yet may not even be necessary for appropriate management of the illness (Davidson, *et al.*, 1976; Shamn, *et al.*, 1984).

The commonest bacterial pathogen in this study was *S. aureus* (40.0%), this agrees with the finding of Diakparome and Obi (1981) in Benin and that of Adedoyin and Fagbule (1987) in Ilorin, though in variance to a finding in Zaria, (Silverman *et al.*, 1977) and that of Shamn *et al.*, (1984) in Papua New Guinea who demonstrated the predominance of *Streptococcus pyogenes* and *H. influenzae* respectively. *S. aureus* pneumonia in adolescents and young adults is almost always a fatal post-influenzal illness especially after antimicrobial therapy, (Jawetz, 1985). The significance of this finding is probably that self medication is rampant in the area which gives rise to *S. aureus* pneumonia, an organism known to be resistant to the conventional therapy of penicillin. (Jawetz, 1985). It is therefore suggested that broad spectrum antibiotics such as Vancomycin of Nafalin should be the initial drug of choice prior to laboratory finding in this locality.

The highest incidence of bacterial pathogens was recorded in patients of

2 years and below. This may be due to the fact that patients in this group are more prone to various infections due to their underdeveloped immune system. This study could not ascertain if bacterial infection is a primary cause of pneumonia or is secondary invasion sequel to viral infection, considering the fact that viruses are the major primary causes of respiratory infections in 0-2 year age group (Chancock and Parott 1965; Loda *et al.*, 1968, Monto and Cavallaro 1971; and Fagbule *et al.*, 1987).

St. pneumoniae was the only single pathogen isolated form case of 10 years and above age group suggesting it's significance in older children. This finding agrees with a finding by Sham *et al.*, 1984). *St. pneumoniae* may therefore be said to play an important role in bacterial pneumonia in this age group. However, there was no significant relationship between age and the incidence of bacterial pneumonia ($P>0.05$) in agreement to a previous finding by Loda *et al.*, 1968.

The predominant bacterial pathogen isolated in bronchopneumonia case was *S. aureus* (13/28) whereas *K. pneumonia* (7/17) dominated in lobar pneumonia. This finding contradicts the view that *St. pneumoniae* dominates the aetiology of lobar pneumonia (Jawetz, 1985) but in agreement with a finding in Britain that *S. aureus* is predominant in the aetiology of bronchopneumonia, (Tanner, *et al.*, 1969). The bacteriology of the two types of pneumonia could then be said to vary from place to place.

The present study showed that at ($P>0.05$) there was no significant difference in the incidence of bacterial pathogen in relation to either type of

pneumonia holding the view that radiological findings may therefore be non-diagnostic.

S. aureus accounted for 12/24 in females whereas *S. aureus, K. pneumoniae* and *H. influenzae* accounted for 6/20 each in males. It could not be explained from, this study if any anatomical, physiological or clinical differences is responsible to this difference.

However, it was observed that the male/female bacterial attack rage was found to be 1:1.2. Statistically at (P.>05) there was no relation between sex and the incidence of bacterial pneumonia, this is in agreement with a finding by Loda *et al.*, (1968). This finding suggests that identical defense mechanisms may exist for the two sexes in bacterial pneumonia infection and one prophalyxis method suffice. This is unlike other bacterial infections such as meningitis (Washburn *et al.*, 1965), Pertussis (Bradford, 1965) and diphtheria (Bradford, 1959).

The laboratory diagnosis of *M. pneumoniae* respiratory tract infection is based on the isolation of the causative organism from respiratory tract secretion (Tulley, 1981). Non pulmonary sites such as pleural fluid (Nagayama *et al.*, 1987) and on adequate serological analysis of patient's serum for specific antibodies to *M. pneumoniae* (Nagayama *et al* 1988; Kok, *et al.*, 1988a).

M. pneumoniae has been well established as a major cause of childhood respiratory illness worldwide (Chanock, 1965; Grayston *et al.*, 1965; Lehtomaek *et al.*, 1987; Mutson *et al.*, 1970; Noah 1974; Gupta *et al.*, 1986; Ali *et al.*, 1986).

From this study *M. pneumoniae* was isolated from 10.7% of the patients and the diagnostic antibody titre to *M. pneumoniae* was demonstrated in 39.3% and 48.7% cases based on Complement Fixation Test (CFT) and Indirect Haemagglutination Test (IHAT) respectively. With the prevalence of 49.1% it was shown that *M. pneumoniae* was involved in childhood pneumonia in Jos, Nigeria. This result was comparable to those reported from other parts of the world, 49.0% in Omaha, USA (Saliba *et al.*, 1967), 52.0% in New South Wales (Reid and Murphy, 1970). However, others reported a lower prevalence of *M. pneumoniae* infections, 1.5% in seattle, USA (Alexander *et al.*, 1966), 10.05 Bathesda, USA. (Macfarlance *et al.*, 1979), 22.0% in Tokyo Japan (Nagayama *et al.*, 1988), 23.05 in Chapel Hill, USA (Glazen *et al.*, 1971) 33.05 in Zaria, Nigeria (Mirza, 1989) 33.05 in Linkoping, Sweden, (Lindstorm and Stahalfurenhed 1981) and 38.0% in Wisconsin, USA (Broome *et al.*, 1980). Three reasons could be offered as to the higher prevalence reported in this study. Firstly both isolation and serological tests were carried out while most other studies use only serology. This confirms a previous finding by Taylor-Robins, *et al.*, 1966 that use of two three techniques increases the percentage of positive children. Secondly *M. pneumoniae* infection is higher during early winter seasons (Foy, *et al.*, 1973). Jos being a cold environment probably explain the higher prevalence. Thirdly, and most importantly, non-hospitalized patients were included in this study, while most other surveys focused only on hospitalized patients where infection rate is low (Loda *et al.*, 1968).

M. pneumoniae was isolated from all age groups except from 10 years and above. The highest isolation incidence was 3-6 year age group (6/12) and lowest in 0-2 year age group (2/12. However, the rate of isolation was highest in 7-10 years age group (2.8%). This is in agreement with a finding by Alexander *et al.*, 1966, Denny *et al.*, 1988. Infection due to *M. pneumoniae* was also demonstrated serologically in all age groups with highest incidence in 0-2 year age group and lowest in more than 10 year age group. However, it should be noted that the number of patients examined in ages 10 and above are statistically insignificant for any analysis. This survey however, demonstrated the frequent occurrence of *M. pneumoniae* infection from patients 0-2 years based on isolation and serology. The report contradicts previous findings by Grayston *et al.*, 1965; Alexander *et al.*, 1966, who suggested that *M. pneumoniae* infection is only frequent in 5-9 year age group and that of Foy *et al.*, 1966; Fernald *et al.*, 1975 which said even if infected younger children were totally asymptomatic. However, the report agrees with findings by Biberfeld and Sterner, 1969, who found that clinical expression of infection in both young and old children was similar.

In ages 7-10 years, mycoplasmal pneumonia was demonstrated in 77.8% of cases which may suggest that probably most cases of pneumonia in this age group is mycoplasmal especially in Jos.

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In ages 7-10 years, mycoplasmal pneumonia was demonstrated in 77.8% of cases which may suggest that probably most cases of pneumonia in this age group is mycoplasmal especially in Jos.

The chances of successful mycoplasmal isolation was shown to be determined by the phase of the infection (Table 3.7). Using both CFT and IHAT it was shown that whereas isolation rate was highest when infection was just starting as indicated from low antibody titres, it was significantly reduced ($P>0.01$) when the antibody titre was very high i.e. when the infection persisted. This finding is significant as it indicates the role of humoral factors involved in protection or at least in neutralizing the already invading organism. This contradicts the general belief that protection is mainly due to local and cell mediated immunity (Collier, 1972; Brunner, *et al.*, *1973*, Fernald, 1979), but in agreement with a finding by Smith *et al.*, 1967 that antibodies are also involved in resistance. However, what cannot be ascertained is whether resistance is due to serum antibodies or a manifestation of a relationship of serum antibody to another immune system such as local respiratory tract antibody or cell mediated immunity.

It is however safer to conclude that both humoral and cell mediated immunity play a vital role in resistance and protection of *M. pneumoniae* respiratory tract infection.

There was a small female (53.4%) preponderance of *M. pneumoniae* infection over males (44.4%) based on serology and isolation. This difference

is statistically insignificant (P>0.05). This result is in agreement to a finding by Foy, *et al.*, 1979). However, Stevens, *et al.*, 1978 reported male predominance in children with *M. pneumoniae* infection. *M. pneumoniae* can therefore be said to common in both sexes and therefore might have similar defence mechanisms.

Bronchopneumonia due to *M. pneumoniae* was found to be 8.5% as against 14.6% in lobar pneumonia by isolation. Also 47.9% of bronchopneumonia cases and 48.8% of lobar pneumonia cases were serologically positive by IHAT. A finding reflected in CFT also. Based on serology and isolation there was no significant difference between the incidence of *M. pneumoniae* infection in lobar and bronchopneumonia. This is in agreement with a report by Jawetz, 1985, that X-ray pattern is variable and not of diagnostic significance in *M. pneumoniae* infection.

All the control cases were negative by isolation and serological methods. CFT and IHAT were not able to detect any specific *M. pneumoniae* antibodies in 68.0% and 36.0% of the samples analyzed respectively. This clearly demonstrates the higher specificity of CFT over IHAT.

Whereas in 42 cases *M. pneumoniae* infection was only detected immunologically, it was in only one case that the organism was isolated whose serum analysis failed to show diagnostic titre. This demonstrates that serology is a more sensitive method in the diagnosis of *M. pneumoniae* infections. Isolation of *M. pneumoniae* with no corresponding antibody in the

sera may mean that at the time the patient's blood was collected the infection was on going (Mirza, 1989). Alternatively, as *M. pneumoniae* survives in the respiratory tract 1-2 months after illness, isolating the organism may not mean current infection (Foy, *et al.*, 1966).

The serological results obtained illustrated a greater sensitivity of IHAT over CFT as noticed by the greater number of patients with very high IHAT (7, 1/640) titres. Although CFT was more specific to *M. pneumoniae* antibodies (as most control patients had minimal CF antibody on titre of ≤1:8, it was noticed that IHAT also retained high specificity as well, considering the high number of control patients having low antibody IHAT titres of ≤1/80 as shown in Appendix IV.

This high sensitivity of IHAT could be explained in two ways. Firstly, sonicated or disrupted suspension of *M. pneumoniae* was used to sensitized the erythrocytes in IHAT, which contains both internal and surface mycoplasma antigens involved in IHAT reaction but CFT only measures surface antigens. Secondly, it is probably due to the greater efficiency of haemagglutination assay in measuring IgM class antibodies while the *M. pneumoniae* CF test antigen used in the test measures antibodies predominantly in the IgG class. The decline of IgM antibody to *M. pneumoniae* was reported to be satisfactory (Kok, *et al.*, 1988) and that only 2% of positive cases remain so one year after the illness as reported by Coombs, *et al* (1988). Thus IHAT appears to be a more useful marker of current

infection than CF test and this is in agreement with previous findings by Taylor-Robinson *et al.*, (1966).

This study shows that concurrent pathogenic bacterial and *M. pneumoniae* infection is more serious in ages 0-2 years followed by ages 3-6 years after which there is a drastic decrease in the concurrent occurrence of bacterial and mycoplasma pneumonia. This leads to the conclusion that *M. pneumoniae* is a primary pathogen in older children (in agreement with its epidemiology). In younger children other bacterial infections especially *H. influenzae* probably provoke sufficient host stress to activate latent *M. pneumoniae*. Similar phenomenum was observed by Alder (1965) in a variety of animal disease.

This study also showed that in ages 0-2 year the most commonly found bacterial pathogens which may complicate *M. pneumoniae* infection are *H. influezae, K. pneumoniae* and *S. aureus*. These three organisms are also found to occur in mycoplasmal pneumonia but in lower frequency in ages 3-6 year. Another important thing to note is the fact that *St. pneumoniae* is the only bacterial pathogen found in ages 10 year and above but not in any other age group. This could serve as indicator for possible mycoplasmal pneumonia in that age group.

Although all the clinical signs and symptoms associated with pneumonia had been recorded for both types of pneumonia. Table 3.12 shows that there are signs and symptoms that are more characteristic of one

type than the other.

For bacterial pneumonia such signs and symptoms like cough, fever, tachypnia, chest pain and tachycardia occur more frequently than found in mycoplasmal pneumonia.

However, in mycoplasmal pneumonia the most common features are signs and symptoms like cough, malaise, vomiting and diarrhoea, fever and anticedent upper respiratory tract infection.

Invariably, one may be about 90% correct if pneumonia accompanied by such signs and symptoms like malaise, anticedent upper respiratory tract infection and vomiting/diarrhoea is grouped as mycoplasmal pneumonia. This is because cases with these symptoms listed above represented 64.9%, 48.7% and 40.5% respectively in mycoplasmal pneumonia, as against 7.4%, 3.7% and 7.4% in the case of bacterial pneumonia.

It was also shown in the same table that virtually all cases of bacterial pneumonia are accompanied with cough, fever, tachypnia and chest pain. The table also shows that any pneumonia case that does not have any record of chest pain, but have malaise, vomiting and diarrhoea and especially antecedent upper respiratory tract infection is likely a pointer to mycoplasmal involvement.

4.2. CONCLUSION

The study clearly demonstrates that bacterial and mycoplasmal pneumonia are of great public health concern in children of this part of the

country and that a simple and acceptable Nasopharyngeal aspirate technique is of value for diagnosis.

Specific bacterial pneumonia was demonstrated in 40.2% of the patients with *S. aureus* demoninating. Since *S. aureus* pneumonia is known to be as a result of prior antibiotic therapy, this may imply that self medication is rampant in the society and only when this fails patients are brought to the hospital. Thus broad spectrum antibiotics such as Nafcillin or Vancomycin should be in the mind of paedriticans when dealing with untreatable pneumonia.

Mycoplasma pneumoniae infection was also demonstrated in 49.1% with highest rate in older children and probably as a secondary infection in younger children. In this light physicians are advised to seek for this agent in pneumonic children especially at the epidemic age group.

This study demonstrated the superiority of IHAT over CFT and isolation techniques in routine laboratory work.

There is no doubt that a lot needs to be done if the complexity of acute respiratory tract infections is to be demystified. The complex nature of disease makes it impossible to fully exhaust any single aspect of the disease at a time. However, it is advocated that this type of study should be carried out in future to elucidate bacterial, mycoplasmal and viral agents involved concurrently in the aetiology of the disease. Also of future interest is the seasonal influence on the aetiology of the disease.

APPENDIX I: INCIDENCE OF MULTIPLE BACTERIAL ISOLATES FROM 46/112 NASOPHARYNGEAL ASPIRATES:

BACTERIA	INCIDENCE
Aeromonas spp	15
Micrococcus spp	14
Staphylococcus spp	11
Corynebacterium spp	10
Klebsiella spp	7
Coliforms	4
Candida spp	2
Pseudomonas spp	1
Staphylococcus aureus	1
Total	65

APPENDIX II: INCIDENCE OF MULTIPLE BACTERIAL ISOLATES FROM NASOPHARYNGEAL ASPIRATE OF 21/50 NON-PNEUMONIC CHILDREN

BACTERIA	INCIDENCE
Aerominas spp	16
Micrococcus spp	14
Corynebacterium spp	11
Staphylococcus spp	6
Coliforms	3
Klebsiella spp	2
Proteus	2
Total	54

APPENDIX III *M. pneumoniae* ANTIBODY TITRE DEMONSTRATED USING CFT AND IHAT IN PNEUMONIC CHILDREN

NUMBER OF SAMPLES	TEST			
	RECIPROCAL CFT TITRE		RECIPROCAL IHAT TITRE	
	ACUTE PHASE	CONV ALESC ENT PHASE	ACUTE PHASE	CONV ALESC ENT PHASE
001+*	≤8	640	≤20	640
002	32	ND	≤20	ND
003	≤8	ND	≤20	ND
004	≤8	≤8	≤20	≤20
005	≤8	16	≤20	≤20
006	≤8	ND	40	ND
007	≤8	ND	≤20	ND
008	≤8	16	≤20	≤20
009*	64	ND	320	ND
010**	≤8	≤8	320	320
O11	32	ND	≤20	ND
012*	64	128	80	1280
013	≤8	≤8	≤20	≤20
014	32	ND	≤20	ND
015*	64	ND	320	ND
016*	64	ND	320	ND
O17*	64	ND	320	ND
018	32	ND	≤20	ND
019+*	≤8	64	320	640
020*	32	64	40	640
021+	≤8	64	80	320
022+*	64	128	640	640
023	≤8	≤8	≤20	≤20
024	32	ND	≤20	ND

APPENDIX III:

NUMBER OF SAMPLES	TEST			
	RECIPROCAL CFT TITRE		RECIPROCAL IHAT TITRE	
	ACUTE PHASE	CONV ALESC ENT PHASE	ACUTE PHASE	CONV ALESC ENT PHASE
025+*	64	ND	320	ND
026*	64	64	320	320
027	32	ND	≤20	ND
028*	128	ND	2560	ND
029	32	ND	≤20	ND
030+*	64	ND	320	ND
031	≤8	ND	≤20	ND
032	32	ND	≤20	ND
033*	64	ND	640	ND
034	32	ND	≤20	ND
035+*	≤8	ND	320	ND
036	32	ND	40	ND
037*	64	64	640	320
038	≤8	ND	≤20	ND
039*	64	ND	640	ND
040	32	ND	40	ND
041	≤8	ND	≤20	ND
042*	64	ND	320	ND
043	32	ND	≤20	ND
044	≤8	ND	≤20	ND
045	32	ND	40	ND
046	ND	ND	ND	ND
047	ND	≤8	ND	≤20
048	≤8	ND	≤20	ND
049+*	64	ND	320	ND
050	≤8	≤8	≤20	≤20
051	≤8	ND	≤20	ND
052	≤8	ND	≤20	ND

APPENDIX III:

NUMBER OF SAMPLES	TEST			
	RECIPROCAL CFT TITRE		RECIPROCAL IHAT TITRE	
	ACUTE PHASE	CONV ALESC ENT PHASE	ACUTE PHASE	CONV ALESC ENT PHASE
053+*	128	ND	640	ND
054	32	ND	≤20	ND
055*	64	ND	640	ND
056	≤8	ND	≤20	ND
057	32	ND	40	ND
058*	64	ND	320	ND
059	≤8	ND	≤20	ND
060	≤8	≤8	640	40
061*	≤8	64	640	1280
062	≤8	≤8	40	40
063	≤8	≤8	40	40
064	≤8	16	≤20	20
065**	32	ND	320	ND
066+*	64	ND	320	ND
067*	64	ND	640	ND
068*	64	ND	320	ND
069+	≤8	ND	≤20	ND
070	≤8	ND	≤20	ND
071*	≤8	64	320	80
072*	≤8	64	≤20	320
073	≤8	≤8	≤20	<20
074*	64	32	320	≤80
075*	≤8	64	40	320
076**	32	32	160	640
077+*	≤8	64	≤20	320
078	≤8	≤8	≤20	≤20

NUMBER OF SAMPLES	TEST			
	RECIPROCAL CFT TITRE		RECIPROCAL IHAT TITRE	
	ACUTE PHASE	CONVAL ESCENT PHASE	ACUTE PHASE	CONVA LESCEN T PHASE
079	≤8	32	≤20	40
080	≤8	≤8	≤20	≤20
081*	≤8	128	40	320
082	≤8	≤20	≤20	≤20
083**	≤8	ND	320	ND
084	≤8	ND	40	ND
085*	64	ND	320	ND
086+*	≤8	64	≤20	320
087	≤8	64	≤20	320
088	≤8	ND	40	ND
089	≤8	ND	≤20	ND
090*	64	≤8	320	40
091*	64	ND	320	ND
092*	64	ND	640	ND
093**	32	ND	320	ND
094*	64	ND	320	ND
095	≤8	ND	≤20	ND
096	≤8	ND	≤20	ND
097*	64	ND	320	ND
098	≤8	ND	≤20	ND
099*	64	ND	320	ND
100**	32	ND	320	ND
101	≤8	ND	≤20	ND
102**	≤8	ND	320	ND
103	≤8	ND	40	ND
104*	64	ND	640	ND
105*	64	ND	320	ND
106*	64	ND	320	ND
107	≤8	ND	≤20	ND
108	≤8	ND	≤20	ND
109**	32	ND	320	ND
110	≤8	ND	≤20	ND
111*	64	ND	320	ND
112	≤8	ND	≤20	ND

ND = Not Done
* = Positive by CFT and IHAT
** = Positive by IHAT only
+ = Isolation positive

APPENDIX IV: *M. pneumoniae* ANTIBODY TITRE DEMONSTRATED USING CFT AND IHAT IN 50 CONTROL CHILDREN (NON-PNEUMONIC):

NUMBER OF SAMPLE	RECIPROCAL CFT TITRE	RECIPROCAL IHAT TITRE
C01	≤8	≤20
C02	16	80
C03	≤8	40
CO4	32	80
C05	16	40
C06	≤8	≤20
C07	≤8	80
C08	16	≤20
C09	≤8	≤20
C10	≤8	40
C11	≤8	≤20
C12	≤8	80
C13	≤8	≤20
C14	≤8	40
C15	≤8	80
C16	≤8	40
C17	≤8	≤20
C18	≤8	40
C19	≤8	40
C20	16	40
C21	32	160
C22	16	≤20
C23	≤8	40
C24	≤8	≤20
C25	≤8	40

C26	≤8	≤20
C27	≤8	40
C28	≤8	**40**
C29	32	**40**
C30	≤8	**40**
C31	≤8	**40**
C32	16	**40**
C33	≤8	≤20
C34	16	≤20
C35	≤8	**80**
C36	32	**80**
C37	32	≤20
C38	≤8	**80**
C39	≤8	40
C40	≤8	≤20
C41	16	40
C42	16	≤20
C43	≤8	**40**
C44	16	**40**
C45	16	**40**
C46	≤8	≤20
C47	≤8	≤20
C48	≤8	80
C49	≤8	≤20
C50	≤8	40

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