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ISOLATION AND CHARACTERIZATION OF NOCARDIA SPECIES IN
LOWER RESPIRATORY TRACT INFECTIONS IN ENUGU STATE

BY

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A DISSERTATION PRESENTED IN PARTIAL FULFILMENT
OF THE COURSE REQUIREMENT OF A MASTER OF SCIENCE
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FEBRUARY 2008.
DEDICATION

This work is dedicated to my late mother Mrs. Monica Ukamaka Onyekwelum who encouraged me with all motherly love to enrol in this Masters programme but could not live to see my graduation.
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ABSTRACT

A study on the Isolation and characterization of Nocardia species in Lower respiratory tract infections in Enugu State was conducted on patients aged 2 years to 80 years between August 2006 and June 2007. Ethical Clearance was obtained from the Ethical Committee of University of Nigeria Teaching Hospital, Enugu. Consent Forms and Questionnaires were issued to patients. Sputum samples from 200 patients comprising 100 males and 100 females were collected in sterile containers and analysed by culturing for Nocardia species on Paraffin agar and staining by Ziehl-Neelsen method for acid fast bacilli [AFB]. A total of 25[12.5%] patients were positive for Nocardia species. Of the 25 patients infected with Nocardia species, 14[56%] were males while 11[44%] were females, the difference was not statistically significant [p> 0.05]. Furthermore, there was observation of co-infection of the patients with tuberculosis and / HIV. A total of 102 [51%] patients out of the entire population had tuberculosis and age group 18-25 and 26-33 years of age had the highest prevalence rate of 14% respectively, which was statistically significant [P < 0.05]. A total of 32 [16%] patients had HIV. There was a significant relationship between Nocardia infection and educational level of subjects (P<0.05). Highest rates were observed in subjects with secondary education level 12(48%). There was no significant relationship statistically between Nocardia infection and HIV infection (P>0.05) in subjects. Only 4(16%) patients out of the 25 had HIV infection. There was a significant relationship between Nocardia infection and tuberculosis infection of subjects (P<0.05). Of the subjects that had Nocardia infection, 21(84%) had tuberculosis. This strongly implies that tuberculosis infection can predispose a patient to Nocardia infection. Pathological studies were carried out on the representatives of Nocardia isolates using 4-weeks old mice. Histological studies on the various organs after 21 days showed evidence of abnormalities and damages in the kidneys, liver, spleen and skin suggesting some toxicity effects of the Nocardia isolates. Infection with Nocardia species appears to be more common than is generally appreciated hence a high index of suspicion for Nocardiosis should be monitored in hosts with pulmonary infections especially those with drug resistant tuberculosis.
CHAPTER ONE

INTRODUCTION

*Nocardia* is a Gram positive bacteria of the family actinomycetes. The nocardiae contain tuberculostearic acids but differ from the mycobacteria by possession of shorter chained (40 – 60 Carbon) mycolic acids. They have a type - IV cell wall characterized by a peptidoglycan made up of mesodiaminopimelic acid, arabinose and galactose (Saubolle and Den, 2003). *Nocardia* species are widespread in the environment (McNeil and Brown, 1994). *Nocardia* was named after Edmond Nocard (1850 – 1903) a French bacteriologist and Veterinary pathologist (Prescot et. al., 2005).

*Nocardia* are found extensively worldwide and are saprophytic, making an important component of the normal soil micro flora and often being associated with water. They may also be associated with decomposing plant material, dust and air (Brown and Neil, 2003). The *nocardiae* are Gram- positive bacillary, branching bacteria whose hyphae often fragment to coccobacillary forms (Pottumarty et. al., 2003).

The genus *Nocardia* has undergone a revolution in its taxonomy in recent years. Fewer than 10 years ago, only three species of pathogenic nocardia were defined based on the hydrolysis of Casein, tyrosine and Xantine substrates (Kiska et. al., 2002). Even at that time ,it was apparent that there was a great deal of heterogenicity within the most common species, *Nocardia asteroides* .Subsequent studies of antibiotic susceptibility patterns revealed six types within the asteroides complex (Wallace et. al., 1990).

Actinomycetes are true bacteria but they form long branching filaments that resemble the hyphae of fungi. They are Gram – positive but some (such as *Nocardia asteroides*) are also weakly acid fast (Levinson and Jawetz, 2002). Most actinomycetes are saprophytes that live in the soil, but members of this group are responsible for three main human infections: actinomycosis, nocardiosis and actinomycetoma (Brooks et. al., 2004).
Nocardia species are aerobes that will grow at 45°C as well as 35°C - 37°C and room temperature in about 3 – 14 days. They are acid fast with 1% Sulphuric acid as decolorizer. Resistance to Lysozyme differentiates Nocardia species from Streptomyces species (Ramchandran et. al., 2003). Nocardia asteroides is a Gram positive branching filamentous bacterium which is weakly acid- fast but not acid- alcohol fast that is; it resists decolorization with 1% but not 3% hydrochloric acid (Olson et.al., 1998). The organism grows well, but slowly on all laboratory media making their recovery difficult from clinical specimens. Moreover, the presence of Nocardia in such clinical specimens is often marred by the early growth of other flora (Ayyar et.al., 1992). These bacteria are Gram positive, catalase positive and partially acid fast bacilli. They produce urease and can digest paraffin. Nocardiae form extensive branching substrates and aerial filaments that fragment after formation, breaking into coccobacillary cells (Pottumarthy et.al., 2003).

Although a large number of species have been characterized both phenotypically and genotypically within the genus, the genotype remains greatly heterogenous and will continue to evolve (Roth et. al., 2003). It has been suggested that microscopic morphology can be suggestive enough to warrant empiric therapy for nocardiosis while awaiting culture results, especially in seriously ill patients and in those with impaired host defense (Subhash et. al., 2001).

Nocardia species cause nocardiosis. Nocardiosis is acute, sub acute or chronic infectious diseases that occur in cutaneous infection (cellulites or abscess), lymphocutaneous infection (sporotrichoid) or subcutaneous infection (actinomycetoma) (Hui et. al., 2003). Nocardiosis is initiated by inhalation of these bacteria and a variety of symptoms may occur, including fever, weight loss and chest pain. In most cases, the disease is an opportunistic infection associated with several risk factors (Brooks et. al., 2004).
Nocardiosis is an uncommon infection usually of a chronic nature, which occurs infrequently in children. The infection has a high affinity for the lungs from where it readily disseminates to other parts of the body and leads to a high mortality in cases which are not diagnosed at an early stage (Kim et. al., 1992).

Pulmonary nocardiosis, a chronic suppurative infection occurring in immunosuppressed patients can be self limited, transient or subclinical and it may progress to an acute, subacute or chronic illness mimicking tuberculosis, mycotic infection or a malignancy (Wadhwa et. al., 2006). Pulmonary nocardiosis is an infrequent infection whose incidence seems to be increasing due to a higher degree of clinical suspicion and the increasing number of immunosuppressive factors (Mari et. al., 2001).

The majority of primary cases present as pulmonary disease, although traumatically induced local abscess occur as well. Dissemination from the lungs may be manifested as bacteremia, empyema, brain abscess, pericarditis, synovitis and soft tissue infection. Typically, nocardiosis is characterized by an acute inflammatory response terminating in necrosis and abscess formation, granulomas are not normally formed (Saubolle and Sussland, 2003). Nocardiosis is usually an opportunistic infection and most commonly presents as pulmonary disease. The majority of patients with clinically recognized disease have underlying debilitating factors. It is not transmitted person to person (Neil and Brown, 1994). The clinical diagnosis of nocardiosis is difficult. Signs, symptoms and radiological studies may suggest the diagnosis but are not pathognomic. Serological tests are not available commercially and serologic diagnosis is unreliable.

*Nocardia asteroides* is the commonest species isolated from clinical specimens. Sixteen species have been implicated in human infections namely *Nocardia asteroides, Nocardia brasiliensis, Nocardia farcinica, Nocardia nova* (commonly associated with infections in humans) , *Nocardia*
otitidiscavarium, Nocardia pseudobrasiliensis (less commonly or infrequently associated with infections in humans), Nocardia abscessus, Nocardia Africana, Nocardia brevicatena, complex Nocardia cornea, Nocardia paucivorans, Nocardia transvalensis sensu stricto, Nocardia transvalensis new taxon 1, Nocardia transvalensis new taxon 11 and Nocardia veterana (rarely associated or prevalence not established). The geographical prevalence of each may change dramatically throughout the world, and some are uncommon (Saubolle and Sussland, 2003).

The lower respiratory tract comprises of the larynx, trachea, bronchi and the rest of the respiratory surfaces of the lungs (Grays, 1995). In health, the lower respiratory tract is sterile and is maintained by the mucocilliary escalator (Zeilher and Hornick, 1996).

Infections of the lower respiratory tract include inflammation of the bronchus (bronchitis), inflammation of the bronchioles (bronchiolitis) and inflammation of the lungs (pneumonia). These syndromes can be severe or fatal. Although viruses, bacteria and fungi can all cause lower respiratory tract infection, bacteria are the dominant pathogens accounting for much higher percentage of lower respiratory tract infections (Liu and Lowther, 1992). Lower respiratory tract infection is one of the major health problems in developing countries (Soependi et al., 1998).

Every year about 5 million people die of acute respiratory infections. Among these, pneumonia represents the most frequent cause of mortality, hospitalization and medical consultation. Several factors (age, underlying disease, and environment) influence mortality, morbidity and also microbial etiology (Bariffi et al., 1995).

Complications of the lower respiratory tract infections are empyema, meningitis, multiple lung abscess, bacteraemia, septicaemia, endocarditis, pericarditis and respiratory failure (Brooks et al., 2004).
Although nocardiosis resemble tuberculosis, first line anti-tuberculosis drugs have no role in its treatment (Lucas et al., 1994). In clinical practice, chronic cough, sputum production, weight loss and fever lead to a suspicion of pulmonary tuberculosis. If chest X-ray shows any feature of pulmonary tuberculosis, the patient is often started on anti-tuberculosis therapy given its prevalence in the environment. Even when sputum returns negative for acid fast bacilli, treatment is continued empirically. When response is poor or absent, the tendency is to doubt patient’s drug compliance, genuineness of drugs or suspect drug resistant pulmonary tuberculosis (Okeahialam and Asalu, 1998).

Pulmonary nocardiosis is a rare infection with high fatality rate (62%) (Presant and Serpick 1971). Nocardiosis presents at an advanced stage of HIV infection (Lucas et al., 1994) and can be fatal in advanced HIV infection (Uttamchandani et al., 1994).

In India, Ramchandran et al. (2003) during their study also observed that the clinical presentations of nocardiosis were similar to pulmonary tuberculosis. Secondly, that most laboratories discard bacterial cultures which are negative at 48 hours and tuberculosis laboratories do not process sputum samples in search of Nocardia species organism thereby documented that Nocardia infection was not a reportable disease.

Osoagbaka and Njoku-Obi (1985) working in Nigeria, observed an overall incidence of 5% for nocardiosis in patients suspected to be having pulmonary tuberculosis and bronchitis.

Since pulmonary nocardiosis mimic tuberculosis and anti-tuberculosis first line drugs do not eliminate Nocardia, and also knowing its high fatality rate in infected individuals it is therefore pertinent to delineate the exact role of Nocardia species in lower respiratory tract infections especially from smear negative and positive patients in order to control the devastating effects of lower respiratory tract infections in our environment.
There is however paucity of information on this infection in this part of the world. As also documented by Osoagbaka and Njoku-Obi (1982), the occurrence of nocardiosis in the tropics particularly Nigeria has not been adequately investigated and reported. Saubolle and Sussland (2003) documented that nocardiosis is usually an opportunistic infection with predisposing factors like underlying chronic lung disease, often in association with long term corticosteroid therapy, diabetes mellitus, haematological and other malignancies, transplantation and AIDS. This becomes vital in this time with a large population of immunocompromised HIV/AIDS candidates around and also with its consequent relationship with tuberculosis. The many assumed Multi-drug Resistant tuberculosis (MDR-TB) may be due to nocardiosis too, hence this work.

**AIMS AND OBJECTIVES**

1. To determine signs and symptoms associated with lower respiratory tract infections.
2. To determine the age and sex distributions of such patients.
3. To isolate and characterize *Nocardia* species from ZN smear negative and positive patients.
4. To determine antibiograms (sensitivity pattern) and pathological changes of *Nocardia* in Enugu State.
CHAPTER TWO

2.0 THE LOWER RESPIRATORY TRACT

Although the respiratory tract is continuous from the nose to the alveoli, it is convenient to distinguish between infections of the upper and lower respiratory tract, even though the same microorganisms might be implicated in infections of both. Lower respiratory tract infections can be broadly divided into acute and chronic (Mims et al, 2003).

Infectious agents gain access to the lower respiratory tract by the inhalation of aerosolized material, by aspiration of upper airway flora or by hematogenous seeding. Pneumonia occurs when lung defense mechanisms are diminished or overwhelmed. The major symptoms of pneumonia are cough, chest pain, fever, shortness of breath and sputum production. Patients are tachycardic, headache, confusion, abdominal pain, nausea, vomiting and diarrhoea may be present, depending on the age of the patient and the organisms involved (Scheld and Mandel, 1991, Baron et al, 1994).

2.1 ETIOLOGY OF LOWER RESPIRATORY TRACT INFECTIONS

Bacteria are the leading cause of pulmonary infections among all patients with immuno-compromising conditions, and most of these infections are due to common bacteria. Among the more unusual bacterial pathogens causing pulmonary disease in this group of patients are Legionella species, Nocardia species, and mycobacteria (Tenholder, 1991).

The etiological agents of community acquired pneumonia vary with the age and state of health of the patient. The most common etiological agent of
lower respiratory tract infection among adults younger than 30 years old is *Mycoplasma* pneumonia (Baron *et. al.*, 1994).

Lower respiratory tract infections in older patients are most commonly due to bacterial infection with *streptococcus pneumonia*. Aspiration pneumonia occurs in the community setting and involves primarily oral anaerobes and *viridans streptococi* but may also involve *staphylococcus aureus* or gram negative rods such as *Klebsiella pneumoniae*, other enterobacteriacea and *pseudomonas Species*, particularly in patients with recent hospital or nursing home experience (Parker, 1983, Marrie *et. al.*, 1987).

*Mycobacteria tuberculosis* is the most likely etiological agent of chronic lower respiratory tract infections but fungal infection and anaerobic pleuropulmonary infection may also run a subacute or chronic course (Baron *et. al.*, 1994).

Aspergillus species is now increasingly reported as a cause of invasive disease in compromised patients, usually in profoundly neutropenic patients or those receiving high dose corticosteroids (Mims *et. al.*, 2003).

There seems to be a small but increasing number of patients presenting with lower respiratory tract infections in which *pneumocystis carinii* is identified, prompting the diagnosis of unsuspected HIV infection. Recent studies show that pneumonia due to *Klebsiella pneumoniae* is much less common than older textbooks have suggested (Carpenter, 1990).

One hundred and sixty five invasive *streptococcus pneumoniae* strains were isolated from children under five at Dhaka Shishu (childrens) hospital in
Bangladesh during the period 1992 to 1995. More than 91% of the strains were isolated from patients aged 24 months or less (Saha et al., 1997).

Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract infection (LRTI) in infants throughout most of the world, but little is known about RSV infection in Africa where lower respiratory tract infections are among the leading causes of infant and childhood death. There is a substantial burden of disease attributable to RSV infection in this rural African setting, with the highest incidence and severity occurring in young infants (Loscertales et al., 2002).

Bacterial infections of the respiratory tract are public health and socio-economic problem of considerable magnitude in Nigeria. They pose serious threats to hospital patients and have attracted attention of many authors (Klan, et al., 1990, Akpala and Okeke 1996, Nwobu et al., 2004). This threat is even more in surgical patients who undergo general inhalational anaesthesia. The operations are invariably postponed in order to allow for treatment and resolution of the infection otherwise severe complications may follow. High population density, poor housing and inadequate environmental sanitation predispose to this infection. Often incriminated are microorganisms like streptococcus pneumoniae, staphylococcus aureus, streptococcus pyogenes, Klebsiella species and haemophilus influenzae (Akpala and Okeke 1996, Nwobu et al., 1996). The mechanism of spread is through dust borne particles, although direct spread can occur through kissing and use of infected cutlery items and cups (Thomas, 1979 and Klan et al., 1990). It is not surprising therefore that there is a higher incidence in people of low socio-economic class who are malnourished and live in over crowded houses in often polluted environment (Ibe, 1990, Nwobu et al., 2004).
2.2 GENERAL INCIDENCE OF LRTI

Epidemiological infections of the lower respiratory tract are responsible for 6% of all general practitioner consultations and form 4.4% of all hospital admission (Anderson et. al., 1993). They account for 3 – 5% of deaths in adults up to the age of 60 years. The best estimate from available data suggests that around 25 million prescriptions for antibiotics are written each year by general practitioners to treat respiratory infections (Hosker et. al., 1994).

Acute respiratory tract infections caused by pneumococci was screened in 318 Filipino children less than 5 years old. Nasopharyngeal samples were obtained from 292 children. Quantitative bacterial culture and detection of capsular polysaccharide antigens by coaglutination, counterimmuno-electrophoresis and latex agglutination, pneumococci were found in 160 (70%) of the 227 samples eligible for analysis. Culture was positive in 115 samples and antigen was positive in 140 samples (Lankinen et al., 1994).

Between 1991 and 1995, 2554 children less than 5 years old hospitalized with severe acute lower respiratory tract infections in Al-Sabe’en, Sana’a, and Yemen were studied. 47.7 percent (1218) were under 6 months of age 74.1 percent (1893) were in their first 12 months. Sixty-four percent (1633) were males. Of the 2554 cases, 221 died (Banajeh, 1998).

Respiratory infections are a major cause of morbidity and mortality in tropical countries yet they are generally ignored in textbooks of tropical medicine. The months of November and February which marked the end of the rainy season, the beginning of the dry season and the end of the harmattan season appeared to be the most favourable periods for these infections (Osoagbaka and Njoku-Obi, 1982).
The lower respiratory tract of malnourished children remains sterile when there is no clinical evidence of pneumoniae (Fagbule and Odewole, 1991). In a 9-month surveillance of the microbial agents causing acute lower respiratory infections in pre-school Nigerian children, 24 bacterial isolates were made from 22 (33%) out of 66 blood cultures. Of the 24 positive isolates, *staphylococcus aureus* accounted for 14 (58%), *klebsiella pneumoniae* for four (17%), *streptococcus pneumoniae* and *staphylococcus albus* for two (8%) each, and *Haemophilus influenzae* for only one case. (Johnson *et. al.*, 1993).

The work done by Oyejide and Osinusi (1991) in Nigeria on incidence of acute lower respiratory infections in a low socio economic community, found higher rates of acute lower respiratory infections in male children and those who had not received measles immunization.

Respiratory syncytial virus (RSV) is a major cause of lower respiratory tract infection in infants throughout most of the world (Saijo *et. al.*, 1998 and Loscertales *et. al.*, 2002).

In Nigeria, a total of 112 patients were enlisted in a study giving an incidence of 76 per 1000 out patient attendance. Out of this number, 80 (71.4%) had bacterial isolates in their sputa. The commonest bacteria isolated was *staphylococcus aureus* (38.7%) followed by *streptococcus pneumoniae*. The mean age was 47.0 years with a range of 16 – 74 years. The peak incidence was in the 7th decade of life. Male: female ratio was 1.2:1 (Nwobu *et. al.*, 2004).

### 2.3 LRTI CAUSED BY ACTINOMYCETES

Actinomycetes are aerobic, gram-positive bacteria that form branching filaments or hyphae and asexual spores. Most actinomycetes are non-motile. Actinomycete cell wall composition varies greatly among different groups and is of taxonomic importance. Four major cell wall types can be distinguished
according to three features of peptidoglycan composition and structure. (Prescott et al., 2005). Some actinomycetes (such as *Nocardia asteroides*) are also weakly acid fast (Levinson and Jawetz, 2002, Saubolle and Sussland, 2003).

Actinomycetes are also well known as agents of allergic alveolitis. Farmer’s lung, which occurs in Europe and North America, is caused by two sporoactinomycetes. These organisms have also been implicated in mushroom worker’s lung and humidifier fever (Collee et al., 1996).

Although possible causes of acute, community acquired lower respiratory tract infections, fungi and parasites are more commonly isolated from patients with chronic disease. *Actinomyces* and *Nocardia* may also be associated with gradual onset of symptoms. Actinomyces is usually associated with an infection of the pleural or chest-wall and *Nocardia* may be isolated along with an infection caused by *mycobacterium tuberculosis* (Baron et al., 1994).

### 2.4 THE GENUS NOCARDIA

The genera *mycobacterium* and *Nocardia* have been grouped into the family *mycobacteriacea* within the order actinomycetales based upon similarities in staining and motility, lack of spore formation and catalase production. These genera are charcterized by the presence of long chain fatty acids, called mycolic acids, which have the following general structures in their cell walls:

\[
\begin{align*}
B \quad \infty \\
R_1 \quad \text{CH} \quad \text{CH} \quad \text{COOH} \\
\text{OH} \quad R_2
\end{align*}
\]

The side chains (R₁ and R₂) vary on length according to the genus; C₆₀ to C₉₀ in mycobacterium, C₄₀ to C₅₀ in Nocardia. Several species produce disease in
humans (Goodfellow and Board 1980, Baron, 1996, Saubolle and Sussland 2003).

The family Nocardiaceae is composed of two genera, Nocardia and Rhodococcus. Because these and related genera resemble members of the genus Nocardia (named after Edmond Nocard (1850–1903), French bacteriologist and veterinary pathologist) they are collectively called nocardioforms. These bacteria develop a substrate mycelium that readily breaks into rods and coccoid elements. Several genera also form an aerial mycelium that rises above the stratum and many produce conidia. All genera have a high G + C content like other actinomycetes, and almost all are strict aerobes. Most species have peptidoglycan with mesodiaminopimelic acid and no peptide interbridge. The wall usually contains a carbohydrate composed of arabinose and galactose; mycolic acids are present in *Nocardia* (Nester *et. al.*, 1995, Prescott *et. al.*, 2005). *Nocardia* can cause both systemic and cutaneous disease (Poonwan *et al.*, 1995).

*Nocardia* species are aerobes and are found in the environment, particularly in the soil. In immunocompromised individuals, they can produce lung infection and many disseminate. In tissues, *Nocardia* species are thin, branching filaments that are gram positive on gram stain. Many isolates of *Nocardia asteroides* are weakly acid fast ie the staining process uses a weaker solution of hydrochloric acid than that used in the stain for *mycobacteria*. If the regular-strength of acid is used, they are not acid fast (Carriere *et al.*, 1999, Levinson and Jawetz, 2002).

Acid fast cells are not easily decolorized by an acid alcohol wash and hence remain red. This is due to the quite high lipid content of acid fast cell wall; in particular, mycolic acid – a group of branched chain hydroxyl lipids which appear responsible for acid fastness (Prescot *et al.*, 2005).
Mishra et al., (1980) have developed a taxonomic scheme that divides these genera on the basis of biochemical characteristics. Numerous species of *Nocardia* exist, not all of which have been recovered from clinical specimens. A simplified scheme for identification of the most frequently isolated species has been developed by McGinnis et al., (1982). For definitive identification of *Nocardia* – like organism, isolates should be sent to a reference laboratory for cell wall analysis by Gas-liquid chromatography (GLC) (Baron et al., 1994, Brown–Elliot et al., 2006).

The taxonomy of the genus has dramatically been revised during that last decade and at least 30 valid species have been reported, besides a number of unnamed genomspecies (Brown and McNeil 2003, Roth et al., 2003).

The recent explosion of newly described species of *Nocardia* results from the impact in the last decade of newer molecular technology, including PCR restriction enzyme analysis and 16S RNA sequencing. These molecular techniques have revolutionized the identification of the *nocardiae* by providing rapid and accurate identification of recognised *nocardiae* and at the same time, revealing new species and a number of yet to be described species. There are currently more than 30 species of *nocardiae* of human clinical significance (Brown–Elliot et al., 2006).

Over the course of several days to a week or more, *Nocardia* species develop heaped, irregular, waxy colonies. Strains vary in their pigmentation from white to orange to red. They are gram positive, catalase positive and partially acid fast. They produce urease and can digest paraffin. They form extensive branching substrates and aerial filaments that fragment after formation, breaking into coccobacillary cells (Brooks et al., 2004).
2.5 LRTI CAUSED BY NOCARDIA SPECIES

Infection with *Nocardia* species appears to be more common than is generally appreciated. A high index of suspicion for nocardiosis should be maintained in susceptible hosts with pulmonary infiltrates, particularly when there is evidence of metastatic infection (Georghiou and Blacklock, 1992).

*Nocardia asteroides* cause most nocardial pulmonary infections (80–90%), with *Nocardia brasiliensis* (5–6%) and *Nocardia caviae* (3%). The three species can be distinguished by their patterns of proteolytic hydrolysis or of acid fermentation of several substrates (Baron, 1996, Ramchandran *et. al.*, 2003).

*Nocardia brasiliensis* is the second most common clinically isolated actinomycete and is usually associated with localized cutaneous infections (Neil and Brown, 1994).

Studies done by Osoagbaka and Njoku –Obi (1985), at the Eastern Part of Nigeria observed the involvement of *Nocardia asteroides, Nocardia brasiliensis* and *Nocardia caviae* in human pulmonary infections. The work done by Idigbe *et. al.* (1992) in Lagos (Western Nigeria) observed only *Nocardia asteroides* and *Nocardia brasiliensis* from 320 randomly selected patients. In both studies, there were more patients infected with *Nocardia asteroides* than *Nocardia brasiliensis* as also observed in earlier works done by Hosty *et. al.* (1961) and Stevens (1983)

2.6 EPIDEMIOLOGY OF NOCARDIA SPECIES INFECTION

Although species of *Nocardia* occasionally may be found in healthy persons, they are not considered normal flora (Volk *et. al.*, 1996).

Nocardiosis is rare in normal persons. It usually occurs in recipients of organ transplants, in patients with Leukaemia, Lymphoma, humoral or Leukocyte defects or after prolonged steroid therapy (Baron, 1996).
Mortality in *nocardia* infection is related to the severity of the underlying disease, late diagnosis and an advanced or disseminated form of nocardial infection (Uttamchandani *et al.*, 1994, Kantoyianis *et al.*, 1994).

2.6.1 WORLDWIDE INCIDENCE

*Nocardiae* are found extensively worldwide and are saprophytic, making up an important component of the normal soil microflora and often being associated with water. They may also be associated with decomposing plant material, dust and air (Kerr *et al.*, 1992, Saubolle and Sussland 2003).

In India, the prevalence of nocardiosis as reported in 1973 was 4.6% among patients with suspected tuberculosis (Baily *et al.*, 1988). Also in India, the work done by Gaude *et al.*, (1999) and Verghese *et al.*, (1996) using non-HIV subjects found that more than two-thirds of the patients diagnosed to have pulmonary nocardiosis were initially diagnosed as having tuberculosis. About 5% of the patients with proven pulmonary tuberculosis were shown to have co-infection with *nocardia*.

Work done by Lucas *et al.*, (1994) on Nocardiosis in HIV Positive patients: an autopsy study in West Africa, 247 patients who died of HIV–related illnesses, 10 patients had nocardoidosis and among those with AIDS defining illnesses, 5% had this disease. Of the 10 patients diagnosed to have nocardiosis, 4 had been initially misdiagnosed as having pulmonary tuberculosis. The mean survival after establishing the diagnosis of nocardiosis was only five days.

In another autopsy study of HIV deaths, the cause of death could be attributed to pulmonary nocardiosis in 2 out of 11 patients treated for pulmonary tuberculosis (Greenberg *et al.*, 1995).
In the US: incidence of *nocardia* infection is 0.4 cases per 100,000 population. An estimated 500 – 1000 new cases occur per year in the United States. Internationally no reliable estimates are available (Menendez et al., 1997, Kageyama et al., 2001). As a specie, *Nocardia asteroides* sensu stricto type VI is distributed evenly throughout the United States. *Nocardia Farcinica* is also found evenly throughout United States, although it is less prevalent than *Nocardia asteroides*. The distribution of other species varies regionally. *Nocardia nova* is less commonly isolated in the South West (Saubolle and Sussland, 2003).

On the basis of the numbers of Nocardia strains referred to the National Reference Center for mycoses and anti-fungal agent (NRC), institute Pasteur, Paris, in the period from 1987 to 1990, it was estimated that between 150 and 250 cases of *Nocardiosis* are diagnosed in France each year (Boiron et al., 1992).

In Thailand, 37 strains of *Nocardia* were isolated and 25 strians belonged to the *Nocardia asteroides* group ie *Nocardia asteroides* sensu stricto and *Nocardia farcinica*. Three strains were identified as *Nocardia otitidiscavarium* and 2 strains were *Nocardia brasiliensis* while 7 were strains of rare pathogenic *Nocardia transvalensis* (Poonwan et al., 1995).

In Japan, between 1992 and 2001, up to 303 cases of nocardiosis were diagnosed. Speciation showed that 72 strains were *Nocardia asteriodes* indicating that most *nocardia* infections in Japan were caused by members of *Nocardia asteroides* (Kageyama et al., 2004).

Yildiz et al. (2005) found *Nocardia farcinica* to be the predominant species (60%) in Turkey rather than the *Nocardia asteroides* complex. They also observed an overall mortality of 33%.

In a retrospective survey of nocardiosis in 9 city hospitals in Northern Italy from 1982 to 1992, 30 patients had nocardiosis. Of the 30 isolates were *Nocardia*
asteroides (n =25) and Nocardia farcinica (n =5). Hence nocardiosis appears to be more common than generally realised by physicians in Northern Italy (Farina et. al., 1995).

Matulionyte et. al., (2004) in Geneva Switzerland, over a period of 15 years, isolated Nocardia species from 20 patients hospitalized at the Geneva University Hospitals. Sixteen patients have one or more underlying conditions. The median time between symptom onset and diagnosis was 30days. The most common species identified was Nocardia asteroides. A mortality rate of 15% was also observed.

One hundred and one patients with advanced symptomatic pulmonary infection were studied in the course of a twenty month period in Tehran. Forty-one patents had nocardia (Eshraghi et. al., 2005).

Biscione et al., (2005) in their work on Nocardiosis in patients with human immunodeficiency virus infection, retrospectively analysed the clinical records of 27 HIV–positive patients with nocardiosis seen during the period of 1993 to 2004. Among the total, 81% were males and the median age was 30 years. There was an elevated percentage of alcoholism (89%), Smoking (80%) and intravenous drug use 82%. Plasma CD.4+T cell count at the time of diagnosis in 15 of 17 patients (88%) was below 50 cells/microl (median 15 cells/ microl). The most frequent clinical onset was pulmonary in 70%, followed by cutaneous in 11% and disseminated in 11%. The predominant pulmonary radiological pattern was alveolar infiltration (74%), followed by cavitations 32%. Nocardia asteriodes was isolated in 84% (n = 11).

Nocardia asteriodes is the most frequent cause of human disease in the United States; various species are dominant in other parts of the world. Nocardia species also cause infections in animals including bovine mastitis and sporotrichoid nocardiosis in horses (Filice, 2005).
2.6.2 INCIDENCE IN NIGERIA

In Nigeria, pulmonary nocardiosis is not an uncommon disease. At the Eastern Nigeria, one hundred specimens of purulent or mucopurulent sputum from patients clinically known to be suffering from a variety of broncho-pulmonary disease including chronic bronchitis, bronchiectasis, lobar pneumonia, bronchopneumonia and pulmonary tuberculosis were examined macroscopically, microscopically and by cultures. Five cases of nocardiosis due to *Nocardia asteroides* were detected giving an overall incidence of 5% in the whole study (Osoagbaka and Njoka-Obi, 1985).

Also within the Eastern Nigeria, 600 patients of various bronchopulmonary disorders were investigated by direct microscopy of sputum samples and cultures on appropriate media. Pathogenic *Nocardia* species were isolated from 25 patients, giving 4.1% incidence of infection (Gugnani et. al., 1991).

In Lagos, the Western Part of Nigeria, 320 randomly selected patients, clinically diagnosed as suffering from acute or chronic bronchopulmonary disorders were screened for Nocardia species infection. A total of 31(9.7%) were observed to be infected with Nocardia species (Idigbe et.al., (1992).

2.6.3 AGE AND SEX INCIDENCE

Nocardiosis is rare in childhood (Kim et. al., 1992). No racial predilection is evident of nocardiosis. The mean age at diagnosis is in the fourth decade of life. Nocardiosis occurs in male more frequently than in females in a ratio of 3:1 (Baron et. al., 1994, Matulionyte et. al., 2004). This is thought to be related to an exposure frequency differences rather than a sex difference in susceptibility to infection (Lerner, 1996, Matulionyte et. al., 2004).
Nocardial infection occurred more commonly in the elderly with most of the patients between the ages of 61 and 80 years of age. No significant difference regarding infectivity levels between the sexes was observed (Kageyama et. al., 2004).

In Nigeria, of the one hundred specimens from patients with lower respiratory tract infection, after analysis, all the isolates were from the 40–70 years age group of patients, (Osoagbaka and Njoku -Obi, 1985). There is no apparent geographical clustering of cases in the United States except for cutaneous infection with *nocardia brasiliensis*, which is more common in the South (Baron, 1996).

In Tehran, forty-one patients suspected for nocardiosis included 26 (63.4%) men and 15 (14.8%) women. The age of the patients varied from 70 – 80 years (Eshraghi et. al., 2005).

Retrospective analysis covering the period 1993 to 2004 documented among the total HIV positive patients that 81% were males and the mean age was 30 years. (Biscione et. al., 2005)

Lower respiratory tract infection is said to affect more commonly the very young and the aged while the middle age has the least incidence. All ages are affected (Nwobu et. al., 1996, Nwobu et. al., 2004).

Work done by Kim et. al., (1992) on pulmonary nocardiosis manifested as miliary nodules in a neonate, had a case of pulmonary nocardiosis in an 18-day old neonate. The baby was the first baby of twins and her delivery was without complications.
2.7 PATHOGENICITY OF NOCARDIA SPECIES

2.7.1 PREDISPOSING FACTORS

_Nocardia_ tends to attack patients with underlying (COPD) chronic obstructive pulmonary disease or immunodepressed patients treated with glucocorticoids or patients with HIV infection. Mortality is high in COPD and HIV patients (Kim et al., 1992, Menendez et al., 1997, Mari et al., 2001). Damage to host epithelial tissue by virus infection is known to predispose patients to secondary bacteria infection (Mills, 1984)

_Nocardia species_ infection has been reported in patients receiving cancer chemotherapy, corticosteroids, and post transplant on immunosuppressants and in profoundly immunosuppressed HIV positive patients (Poonwan et al., 1995, Ramchandran et al., 2003). _Nocardia_ species can infect immunocompetent individuals (Kim et al. 1992, Matulionyte et al., 2004).

Unlike tuberculosis, nocardiosis is not a reportable disease and its incidence among HIV negative and positive individuals is low (Javaly et al., 1992).

The most common factor that predisposed individuals to _nocardial_ infection was therapy by immunosuppressive agents, including SLE therapy followed by cancer, diabetes AIDS and organ transplant (Kageyama et al., 2004, Wauters et al., 2005).

Collagenous vascular diseases, chronic granulomatous diseases, dysgammaglobulinaemia, alcoholism and diabetes mellitus all enhance susceptibility to nocardiosis. Although some reports have indicated a surprisingly low incidence of nocardiosis among HIV infected patients, nocardiosis remains an important cause of morbidity and mortality in HIV positive patients with advanced infection particularly in those not receiving TMP/SMX prophylaxis (Javaly et al., 1992, Uttamchandani et al., 1994).
Most patients presenting with nocardiosis have a certain degree of immunodeficiency. Activated macrophages and T cells and humoral immunity do not appear as important in protecting the host. Impaired local pulmonary defences seen in chronic obstructive pulmonary disease or other chronic pulmonary diseases predispose to pulmonary nocardosis particularly in patients requiring long term corticosteroid treatment (Heffner, 1988, Lerner, 1996 and Eshraghi et. al., 2005).

Pre-existing lung disease and treatment with steroids and immunosuppression were risk factors for pulmonary and disseminated nocardosis. A history of inoculation in an outdoor setting was frequent in patients with cutaneous disease (Georghiou & Blacklock, 1992, Burgett, 1999).

In most cases, nocardosis is an opportunistic infection associated with several risk factors, most of which impair the cell mediated immune responses (Brooks et. al., 2004). In the United States, the over all number of Nocardia infections seems to be greatest in association with the dry warm climates of the Southwest. It may be that the dry, dusty, and often windy conditions in that region facilitate the aerosolization and dispersal of fragmental nocardial cells and enhance their acquisition via the respiratory route (Saubolle and Sussland, 2003).

In Paris, corticosteriod therapy represented a significant factor in mortality due to nocardosis (Boiron et. al., 1992).

With the increase in transplantation and the rising incidence of AIDS, the frequency of nocardosis is increasing, (Kim et. al., 1991). Diabetics and alcoholics have an increased incidence (Hasleton, 1996).

Nocardosis is an unusual infection among HIV–infected patients. The diagnosis should be considered in patients with CD4+Tcell counts below 50/MicroL and Lung or pericardial involvement (Biscione et al., 2005).
Many of the patients presenting themselves at the hospitals or clinics can rightly be said to be compromised because of the poor standard of living, the uncontrolled and irregular use of broad spectrum antibiotics, smoking and alcoholism. Consequently no organism isolated from sputum in reasonable number should be lightly dismissed as a non-pathogen (Osoagbaka and Njoku-Obi, 1982).

Work done by Biscione et. al., (2005) on Nocardiosis in patients with human immunodeficiency virus infection observed an elevated percentage of alcoholism (89%), smoking (80%) and intravenous drug use (82%).

2.7.2 MODE OF INFECTION

*Nocardia* cells have been isolated from soil and organic material throughout the world. Natural infections occur in domestic animals. Human infection is by inhalation of airborne bacilli from an environmental source (soil or organic material), the disease is not contagious. Skin lesions caused by *Nocardia brasiliensis* often result from direct inoculation (Kerr, et. al., 1992, Baron, 1996, Brown and McNeil 2003, Saubolle and Sussland, 2003).

Human infection is rare and contracted through inhalation. Infection is more common among immunocompromised patients especially those with impaired cell mediated immunity (Angeles and Sugar 1987, Tenholder, 1991, Ramchandran et. al., 2003).

A smaller number of infections are caused by traumatic introduction of organism percutaneously. Normally, primary infections with *Nocardia brasiliensis* and *Nocardia otitidiscavarium* in an immuno competent host is an empty card, *Nocardial* infections are not thought to be transmitted from person to person and are not usually acquired nosocomially (Brooks et. al., 2004).
The cutaneous, lymphocutaneous and subcutaneous forms of nocardiosis arise from local traumatic inoculation. Pleuro pulmonary disease presumably arises from inhalation exposure. Disseminated infection results from hematogenous dissemination, usually from a pulmonary focus. Most patients with disseminated nocardiosis have underlying immunocompromising disease or are receiving immunosuppressive therapy (Lerner, 1996).

*Nocardia* infect humans after inhalation of the organisms and primary establishment of growth in the lungs or by inoculation through breaks in the skin. The infection is usually chronic, but occasionally is fulminant and is usually seen in immunosuppressed patients (Baron *et. al.*, 1994).

### 2.7.3 MECHANISM OF ACTION

*Nocardiae* are involved in the degradation of hydrocarbons and waxes and can contribute to the biodeterioration of rubber joints in water and sewage pipes. Although most are free-living saprophytes, some species, particularly *Nocardia asteroides* are opportunistic pathogens that cause nocardiosis in human and other animals. People with low resistance due to other health problems are more at risk. The lungs are most often infected but the central nervous system and other organs may be invaded (Prescot *et. al.*, 2005).

Nocardiosis occurs frequently as a pulmonary disease in immunosuppressed or debilitated persons after the inhalation of the fragmented mycelium. In the course of events, one or more lung abscesses may develop and enlarge to form cavities similar to those seen in chronic tuberculosis. From the lung the organisms may spread by way of the blood stream, they have the potential to establish lesions in any area of the body, particularly the brain and kidneys. Unlike actinomycosis, bone destruction is rare, however, both *Nocardia asteroides* and *Nocardia brasiliensis* (and to a lesser extent *Nocardia caviae*) may
form mycetomas in which the abscesses extend by the destruction of soft tissues and bone to eventually erupt through the skin (Beneke & Rogers 1980, Volk et al., 1996, Brooks et al., 2004).

The infection has a high affinity for the lungs from where it readily disseminates to other parts of the body and this leads to a high mortality in cases which are not diagnosed at an early stage (Kim et al., 1992).

2.7.4 VIRULENCE FACTORS OF NOCARDIA SPECIES

*Nocardia* subverts antimicrobial mechanisms of phagocytes by inhibiting phagosome-lysosome fusion, causing abscess or rarely granuloma formation with hematogenous or lymphatic dissemination to the skin or central nervous system. Mortality is up to 45 percent even with therapy (Baron, 1996, Brown and McNeil, 2003).

Superoxide dismutase (SOD) combined with catalase had additive activity which completely protected the cells of nocardia strain. A mutant of *Nocardia asteroides* was found to be more virulent during the log phase than is the parental strain. This mutant contained at least seven times more catalase at this stage of growths than did the parent. These data indicate a role for both SOD and catalase in the resistance of *Nocardia species* to human neutrophils, and they represent at least two factors associated with virulence (Beaman et al., 1985).

The organism seems to be able to resist phagocytosis and therefore persists in the host. The filamentous stage of growth seems to be more virulent than the stationary coccoid phase (Lerner, 1996).
Nocardia brasiliensis is the main agent of actinomycetoma in Mexico, but little is known about its virulence and molecular pathogenic pathways. These facultative intracellular bacteria are able to survive and divide within the host phagocytic cells, in part by neutralizing the reactive oxygen intermediates. Superoxide dismutase (SOD) participates in the intracellular survival of several bacterial species and, in particular, constitutes one of Nocardia asteroides virulence factors (Revol et al., 2006).

Nocardia grow within macrophages and their virulence has been related to inhibition of phagosome and lysosome fusion, reduction in intracellular levels of lysosomal acid phosphatase, and the production of superoxide dismutase and catalase (Hasleton, 1996).

The organisms’ ability to survive the hosts’ inflammatory responses, infection is controlled by cell-mediated immunity, but this may be defective in immunocompromised patients (Mims et al., 2003).

2.8 IMMUNITY TO NOCARDOSIS

Host resistance to Nocardia species infection is thought to depend on functioning phagocytic cells. Neutrophils limit spread of infection in the early stage of tissue invasion (Fillice, 1985).

The natural resistance to infection is high in normal individuals and the disease is usually associated with cellular immune dysfunction, immunoglobulin deficiencies or leukocyte defects. Acquired resistance to nocardia is complex involving antibody – dependent phagocytosis by neutrophils, macrophage activation by the products of immune, T cells and the development of cytotoxic T-lymphocytes. Neutrophils ingest opsonized bacteria but may not kill them. Macrophage activation is associated with containment and clearance of Nocardia organisms from the lungs. In a murine model, resistance to nocardiosis can be
transferred with whole spleen cells or splenic T-cells from immune mice (Baron, 1996, Menendez et al., 1997).

2.9 CLINICAL FINDINGS/EFFECTS OF NOCARDIOSIS

The most common manifestations of nocardial infection is pneumonia: fever, weight loss, cough, pleuritic chest pain and dyspnea (Curry, 1980, Baron, 1994 and Hasleton 1996).

The clinical manifestations of nocardiosis are not distinctive and mimic tuberculosis and other infections. Pulmonary consolidations may develop, but granuloma formation and caseation are rare. The usual pathologic process is abscess formation. Spread from the lung often involves the central nervous system, where abscesses develop in the brain, leading to a variety of clinical presentation (Tenholder 1991, Brooks et al., 2004).

Nocardia species are most commonly acquired through the respiratory tract, with subsequent dissemination to extrapulmonary sites (Frazier et al., 1975, Adair et al., 1987).

Pulmonary involvement of Nocardia asteroides ranges from sub-clinical transient infection to prolonged severe illness mimicking lung abscess, tuberculosis, neoplasm or fungal infection. The diagnosis maybe overlooked because of the lack of pathognomonic features and difficulty in isolating the organism from contaminated specimens (Kerr et al., 1992, Kim et al., 1992, Torres et al., 2002, Subhash et al., 2001, Ramchandran et al., 2003).

Chest radiological findings of pulmonary nocardiosis in advanced HIV infection include alveolar infiltration, cavitation, pleural effusion and reticulonodular pattern (Feign, 1986, Uttamchandani et al., 1994, Subhash et al. 2001).
A study that evaluated the computed tomography finding of chest in patients with nocardiosis and AIDS showed multiple nodules in almost all patients and cavitation occurred in 80% of the patients (Buckley et al., 1995).

Bacteraemia due to this infection is very rare with few reports in immunosuppressed HIV negative and HIV positive patients (Burick et al., 1997). Some patients have sub-clinical lung involvement and present with brain lesions. Dissemination may also occur to the skin, kidney or else where. (Carriere et al., 1999 and Brooks et al., 2004).

Unless investigations like Gram Stain and culture for Nocardia are specifically done, most often the pulmonary infection is mistaken for tuberculosis (Javaly et al., 1992, Kim et al. 1992).

Clinical recognition of nocardial infection is difficult because of its relatively low incidence and a lack of pathognomonic symptoms. In some cases, the diagnosis has been established only after death (Farina et al., 1995, John et al., 2002).

Nocardia may occasionally cause a pulmonary mycetoma, a chronic fibrotic cavitating lesion usually found in the upper lobe of a lung containing a bell of necrotic debris with a tangled mycelium of the causative organism (Hasleton, 1996).

2.10 LABORATORY PROCEDURES ON ISOLATION OF NOCARDIA SPECIES.

Nocardia species are not fastidious organisms, and they grow well on a variety of media containing nitrogen and simple carbon source. The sub-optimal recovery rate is attributed to the slow growth of these organisms, which allows them to be over grown by other microbes. Bronchoalveolar Charcoal Yeast
Extract (BCYE) agar for a facilitated isolation of *nocardia asteroides* at 35°C in 5% CO₂ and growth is observable in about 5 days (Kerr, *et. al.*, 1992).

Although *Nocardia* species grow best on antibiotic free media, the frequency of their isolation may be increased through the use of selective techniques to prevent overgrowth by more rapidly growing organisms (Shawar *et. al.*, 1990, Coker *et. al.*, 1992).

Nocardioforms grow on mycobacterium media such as Lowenstein – Jensen medium but are often killed by routine decontamination procedures (Murray *et. al.*, 1987). Sabouraud dextrose agar with chloramphenicol has been suggested as a selective medium for the recovery of *nocardia* species from respiratory specimens although chloramphenicol inhibits the growth of many *nocardia* isolates (Gutmann *et. al.*, 1983, Baron *et. al.*, 1994).

When observed microscopically, either in gram stains of clinical specimens or cultures or when demonstrated histopathologically in tissues, *nocardia* are branching beaded, filamentous, gram-positive bacteria with a characteristic morphology to a trained observer. *Nocardia* usually are weakly acid-fast (Hasleton, 1996, Saubolle and Sussland, 2003).

A definitive diagnosis requires the visualization of long, branching, gram-positive filaments and fragmented bacillary bodies that are partially acid fast. The organisms are easily grown, and the classic wrinkled, frequently pigmented colonies of *nocardia* are easily recognised (Baron *et. al.*, 1994, Volk *et. al.*, 1996). Sputum culture is useful for patients with a productive cough. The presence of branching, weakly acid-fast organisms in histologic sections, pus or sputum suggests the clinical diagnosis (Baron *et. al.*, 1994, Mari *et. al.*, 2001).

*Nocardia* species grow on most laboratory media serologic tests are unreliable. Other specimens like pus, spinal fluid and biopsy material can be useful. The use of selective media and pretreatment of specimens improved
*nocardia* isolation. Isolation of *nocardia* by culture may take up to 2-4 weeks (Kerr *et al.*, 1992, Ramchandran *et al.*, 2003 and Brooks *et al.*, 2004).

Paraffin baiting is also reported to be more efficient than conventional culture techniques for the isolation of *Nocardia* from sputum (Singh *et al.*, 1987). Paraffin baiting techniques was invented by Gordon and Hogan in 1936 and further developed by McClung in 1960 and is based on the ability of *Nocardia species* to utilize paraffin as the sole source of carbon. A paraffin-coated glass rod is inserted into a carbon-free broth mixed with the sputum specimen. In positive cultures, growth appears on the paraffin coated rod just above the surface of the broth (Gordon and Hogan 1936, McClung, 1960).

Two selective agar media have also been described recently for the recovery of Nocardia species from respiratory specimens by using modified Thayer Martin Medium (Murray *et al.*, 1988). Paraffin agar was found to be an inexpensive and selective medium for isolation of *Nocardia species* when compared with modified Thayer Martin Medium and Paraffin bait techniques. With Paraffin agar, relatively lesser time is required for isolation of the organism (Ayyar *et al.*, 1992).

Blood cultures are positive in a minority of patients but always should be obtained when pulmonary or disseminated nocardosis is suspected (Saubolle and Sussland, 2003).

The routine identification of *Nocardia* strains at the species level is difficult in the laboratory. The isolation and identification is troublesome (Menendez *et al.*, 1997, Kageyama *et al.*, 2004).

Identification was based on microscopical morphology after Gram stain and modified Kinyoun stain, on strictly aerobic growth, and on physiological tests (casein, tyrosine, xanthine, starch, gelatin, Urea) susceptibility to lysozyme and growth at 45°C. Since 2001, species identification has been determined by the
amplification and sequencing of 16S rDNA (Ramchandran et al., 2003, Matulionyte et al. 2004).

Identification of *Nocardia* species by phenotypic characterization is often considered tedious and difficult and requires, at least some tests, long incubation times. Besides the decomposition of tyrosine, xanthine and hypoxanthine, it is mainly based on some conventional test, such as urea hydrolysis, growth at 45°C and assimilation of organic compounds on minimal media such as AUX medium used in the AP1 1D32C system (Muir and Pritchard 1997, Roth et al., 2003, Wauters et al., 2002).

One of the drawbacks of *Nocardia* identification is the difficulty to obtain a homogenous and/or standardized inoculum. Some authors use glass beads or other devices for this purpose (Kiska et al., 2002).

Molecular methods such as PCR-RFLP and 16S ribosomal DNA sequencing can accurately identify all medically relevant *Nocardia* isolates to the species level, however, not all laboratories have the capability to perform these tests. Also, biochemical characterization of species has been time consuming and problematic (Boiron and Provost, 1990, Biehl et al., 1996, Muir and Pritchard 1997).

Accurate identification often requires referral to a reference laboratory with molecular capabilities, as many newer species are genetically distinct from established species yet have few or no distinguishing phenotypic characteristics (Brown–Elliot et al., 2006).

Qualitative evaluation of mycolic acids can be achieved by the thin layer chromatography technique. Methanolysates of nearly all mycobacteria give a multi-spot mycolic acid pattern whereas those from nocardiae (and most other mycolic – acid containing actinomycetes) produce a single spot with an RF value
that reflects the chain length and structure of the mycolic acids (Collee et al., 1996)

Specimens may be taken from several sources – blood, bone tissue, bronchial washing, cerebrospinal fluid, Sinus discharge, urine, and biopsy and autopsy material for Nocardia diagnosis (Schaal and Lee 1992).

Sputum is the most readily available materials from cases of pulmonary nocardiosis (Colee et al., 1996). The isolation of nocardiae from sputum taken from patients with respiratory infections is highly indicative of pulmonary nocardiosis (Raich et al., 1961, Idigbe et al., 1999).

However for this study, sputum was the specimen used for investigation because in the Nigerian setting, as in many other developing countries the use of bronchoscropy is outside the reach of many hospitals. Also sputum collection is non-invasive.

2.11 ANTIBIOGRAM OF NOCARDIA SPECIES

Disk diffusion testing on Mueller Hinton agar is the best currently available clinical method (Wallace and Steele, 1988). Cotrimoxazole, the drug of choice for nocardiosis has excellent response against Nocardia brasiliensis, Nocardia asteroides and Nocardia transvalensis whereas Nocardia farcinica and Nocardia nova show 7% and 11% resistance respectively. It is not recommended for treatment of Nocardia otitidiscavarium infections due to high resistance among isolates (Lerner, 1996).

Work done by Wadhwa et. al., (2006) had an unusual report about Cotrimoxazole. The isolated strains of Nocardia brasiliensis were resistant to Cotrimoxazole, Ampicilline, Erythromycin, and Ciprofloxacin but sensitive to Amikacin, Gentamicin and Chloramphenicol.
In Paris, work done between 1987 and 1990 by Boiron et al. (1992) had isolates of *Nocardia asteroides* and *Nocardia farcinica* showing resistance to most antimicrobial agents. Only amoxicillin/clavulanic acid, imipenem, cefoxitin, kanamycin, amikacin, minocycline and vancomycin showed activity against both species.

In Italy, Farina et al., (1995) in their work had 29 isolates which showed resistance to several antimicrobial agents particularly erythromycin, fosfomycin, perfloxacin, sulfonamides and trimethoprim. Most strains tested were susceptible to amikacin and imipenem. Matulionyte et al., (2004) working in Geneva, Switzerland on secular trends of *nocardia* infection over 15 years in a tertiary care hospital, invitro susceptibility testing was performed on 14 of 20 strains, all strains were susceptible to imipenem and amikacin. Also 100% sensitivity to amikacin was observed by Menendez et al., 1997, Khan et al., 2007). Correct diagnosis is imperative because nocardiosis is susceptible to sulfonamide therapy but refractory to the current antibiotic treatments for tuberculosis, most bacterial and fungal infection (Kim et al., 1992). Within the *Nocardia asteroides* complex, *Nocardia farcinica* may be more pathogenic, as it shows a greater antibiotic resistance than *Nocardia asteroides* and also it is a significant clinical isolate found in disseminated disease and in AIDS patients (Boiron et al., 1992, Coarson and Hellyar, 1994).

Primary agents that have been used successfully are minocycline, amikacin, imipenem and linezolid. Combination therapy with a sulfa containing agent and one of the primary agents has been recommended for serious systemic disease. The use of amikacin in combination with imipenem has also
been suggested for serious infection (Burgett, 1999, Brown and Mcneil 2003, Saubolle and Sussland 2003).

2.12 ISOLATION STUDIES OF *NOCARDIA* SPECIES IN ANIMALS

The pathogenicity of *Nocardia* strains is highly variable and is influenced by factors that include the age of the culture of the pathogens, rate of growth, route of infection and by the immune status of the host (Beaman, 1985).

Several attributes such as acid fastness or tolerance to acid, alkali or malachite green, which have been used specifically for the diagnosis and isolation of *mycobacteria*, were found to be shared by pathogenic *Nocardia* such as *Nocardia asteriodes* and *Nocardia brasiliensis*. It is therefore necessary to have a sufficient knowledge of the pathogenicity of *Nocardia* not only for understanding “nocardiosis’ but also for distinguishing the disease from those caused by tubercle bacilli and other *mycobacteria* (Uesaka *et. al.,* 1971).

Work done by Gugnani *et al.,* (2004) on pathogenicity of *Nocardia transvalensis* for laboratory mice found it to be virulent for laboratory mice both by intraperitoneal and intravenous routes of inoculation. Cortisone administration was found to enhance the susceptibility of mice. They also observed that intravenous route of inoculation produced a much more progressive and disseminating infection than the intraperitoneal route as also documented by Beaman and Maslam (1980).

However, immunological studies have focussed on a small number of favoured models, notably the laboratory mouse. The latters’ cheapness, small size, short life span, ready availability, large number of inbred strains, plethora of anti-mouse regents and a relative lack of public sympathy for it, have all
contributed to its success in this regard. This has meant that the mouse is used as the main immunological yardstick for comparative work. (Turner, 1995).

Work done by Gugnani et. al., (1982) on mycetoma of thumb caused by Nocardia transvalensis observed in their pathogenicity test of the organism for laboratory mice that some animals died of the infection while some survived. In the animals dying of infection, multiple small miliary abscesses were observed on the liver, spleen and peritoneum. The surviving animals showed a few abscesses on the liver and spleen.

In an experimental murine model, the virulence of nocardia species is critically dependent upon the route of inoculation (Beaman et. al., 1980, Adair et. al., 1987)

Information on avian nocardiosis is limited, however, to a few reports on domestic fowl (Ainsworth and Austwick, 1973) a red- legged honey creeper (Bergmann et al., 1973), blue winged king parrots (Ehrsham and Hauser, 1979) and a purple–throated sunbird (Parnel et. al., 1982).

Work done by Okoye et. al., (1991) on experimental infection of chickens with Nocardia asteroides and Nocardia transvalensis, the chickens were infected by the oral or intraperitoneal routes. Grey nodules or foci were observed in the lungs, air sacs, liver and breast muscles.

2.13 CONTROL MEASURES AND MANAGEMENT OF NOCARDIA INFECTION

The treatment of choice is trimethoprim sulfamethoxazole. If patients fail to respond, a number of other antibiotics have been used with success, such as amikacin, imipenem and cefotaxime. Surgical drainage or resection may be
required. Nocardiosis is treated by prolonged (up to one year) therapy with trimethoprim – sulphonmethoxazole (Mims et al., 2003, Brooks et al., 2004).

The optimal duration of treatment is unknown hence prolonged course of medication is advised due to the relapsing nature of the infection (Tenholder 1991, Matulionyte et al., 2004).

Because of the wide spread occurrence of species of Nocardia, control is impossible. However, humans undoubtedly possess considerable resistance to infection by these forms, because most cases occur in debilitated or immunosuppressed patients. The drug of choice for treating nocardiosis is sulfadiazine, but the prognosis is poor, particularly if the organisms have metastasized to other organs of the body (Volk et al., 1996).

Surgical drainage may also be needed as occasional drug resistance occurs. No vaccine or prophylactic drug is available (Levinson and Jawetz, 2002).

Nocardiosis is an unusual infection among HIV-infected patients. The diagnosis should be considered in patients with CD4+ T cell counts below 50/μL and lung or pericardial involvement (Biscione et al., 2005).

Bacterial respiratory infection is a disease of the underprivileged. Effective control programme will involve the improvement of living condition and environmental sanitation of our people, a well organized health services and provision of portable water supply. Improvement on the literacy level will bring about health awareness. (Nwobu et al., 2004).
CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study area:

Enugu State is one of the 36 States in Nigeria. It is situated in the South Eastern Part of Nigeria. There are 17 Local Government areas in Enugu State. The main economic activities of the area are Civil Service, Trading and Farming. The state is well known for its rich coal reserve. Those dwellers within the municipality are described as urban dwellers, while those living within the outskirts or immediate vicinity of Enugu environs, though not exactly within the municipality but still have a slight influence of the township environment because of proximity (Semi-urban) while those living in remote village settlements (rural). Enugu State has numerous public and private health facilities. There are 366 Primary health care facilities, 41 Secondary health care facilities and 2 tertiary health care institutions.

University of Nigeria Teaching Hospital, Enugu is a tertiary Institution in the current Primary health care Scheme and provides a final point of reference for lower Sectors of the Scheme. The hospital provides in-patient and out-patient care, training of Medical, Laboratory and Nursing Personnel as well as Community health workers.

The Chest Unit has an out-patient Clinic as well as three wards for patients who might need in-patient care. It is also a recognized Directly Observed Treatment Short-Course (DOTS) Centre.

3.2 SAMPLING METHOD

Sputum samples were collected from patients attending the chest clinic at the University of Nigeria Teaching Hospital, Enugu and also from some patients on admission in the wards of the hospital. A total of 200 patients comprising of
100 males and 100 females between the ages of 2–80 years were included in this study. The survey was carried out between August 2006 and March 2007 within Enugu State.

3.2.1 COLLECTION OF SAMPLE

Informed consent forms were given to literate subjects while to the illiterate ones, reading and interpretation of the contents were done by the principal investigator. This was followed by giving questionnaires to the subjects that accepted to be enlisted. Vital informations were sourced through the questionnaires. These informations were age, sex, place of domicile, residential status, number of persons in an apartment, marital status, educational level attained by the subject, occupation, HIV status, Smoking habit and drinking habit. Sputum samples were collected from patients with signs and symptoms suggestive of lower respiratory tract infections e.g. cough, chest pain, dyspnea, fever, weight loss.

The patients and/or their wards were instructed on how to collect samples appropriately in clean sterile universal containers. They were instructed to cough deeply in a bent position early in the morning before breakfast and after rinsing their mouth. All specimens found to be salivary were discarded and fresh specimens requested. Samples were processed within 24 hours of collection. Three sputum samples were collected from patients with just signs and symptoms of lower respiratory tract infections while two sputum samples were collected from patients who had previously been diagnosed as tuberculosis patients sometime in the past but have not recovered fully i.e. follow-up cases.
3.3 SAMPLE ANALYSIS

3.3.1 MACROSCOPIC EXAMINATION OF SPUTUM SAMPLES

This examination involved the visual examination for presence of mucus, blood, colour, opacity and consistency of the sputum sample.

3.3.2 CULTURAL PROCEDURE:

Sputum was homogenized by adding some quantity of (about 15) sterile glass beads as described by Kiska et. al., (2002) and vortexed for about 5 minutes.

Using a standard wire loop, a loopful of the homogenized Sputum was collected and inoculated unto chocolate agar plates, then incubated in CO₂ enriched atmosphere using an improvised candle jar for 24hrs at 37°C. Blood agar and MacConkey agar plates were inoculated and incubated aerobically for 24hrs at 37°C before cultures were examined for growth of organisms.

Paraffin agar plates first described by Shawar et.al., (1991) and modified by Ayyar et. al.,(1992) were inoculated as selective media for Nocardia Isolation and Sabouraud dextrose agar slants were also inoculated and incubated aerobically at 37°C for up to 3 weeks.

Paraffin baiting technique (McClung, 1960) was also employed as control. This was done using the McClung carbon free broth in the test tube with Paraffin coated glass rod inserted into it. To the carbon free broth, 1ml of the homogenized sputum was added using sterile syringe and stoppered with a sterile bunch of cotton wool and incubated at 37°C for up to 3 weeks.

Cultural growths on blood agar, chocolate agar, and MacConkey agar were examined after 24hrs incubation and organisms involved in lower respiratory tract infections other than Nocardia species were identified based on
colonial appearance, morphology, odour of growth, reaction with the media components and the environment. Also Gram stain, catalase test and Germ tube tests were performed.

Direct smears were made with the homogenized samples and stained by Gram Stain and Acid fast Bacilli stain.

Cultures on paraffin agar, Sabouraud agar slants and paraffin baiting were observed for growths on daily basis for up to 3 weeks. Observed growths were identified by gram staining, lactophenol cotton blue mounts, partial acid fast stain, and species were characterized to some extent using their reaction test result in urea hydrolysis test, nitrate reduction test, degradation of casein and also citrate utilization test.

Antibiotic sensitivity tests were performed on identified organisms using the disk diffusion technique on Mueller Hinton agar after subculturing the growths unto nutrient agar plate for discrete colony presentation (Wallace and Steele, 1998).

Prior to inoculation on Mueller Hinton agar a homogenous mixture of the organism is achieved by adding, a reasonable quantity of colony to a 2ml amount of sterile distilled water containing 10 glass beads and vortexing for five minutes.

3.4 MICROSCOPY TECHNIQUES

3.4.1 GRAM STAINING

**Principle:** Gram positive organisms tend to retain the primary stain while gram-negative organisms retain the counterstain colour.

**Method:** The method was used for both sputum samples and colonies of grown organisms.

For sputum samples, smears of the homogenized sputum were made on clean, grease free-labelled slides.
For colonies of grown organisms, a bit of the colony was emulsified in sterile distilled water on a clean grease free-labelled slide.

All were allowed to air dry under the class II Biosafety cabinet. The smears were fixed by passing them over the burner flame for about 3 times.

The fixed smears were flooded with crystal violet stain for 30 – 60 seconds. The stains were rapidly washed off with clean water.

Decolourization was done rapidly with acetone and washed immediately with clean water.

The smears were covered with neutral red stain for 2 minutes and then the stain washed off with clean water.

The back of the slides were wiped clean and placed on draining rack for the smears to air dry.

The smears were examined microscopically using oil immersion objective.

**Results:**

- Gram positive bacteria: dark purple
- Yeast cells: dark purple
- Gram negative bacteria: pale to dark red
- Nuclei of pus cells: red
- Epithelial cells: pale red.

### 3.4.2 ACID FAST BACILLI (AFB) STAINING, (WHO, 1998)

**Principle:** *Mycobacteria* retain the primary stain even after exposure to decolorising acid alcohol, hence the term acid fast.

**Method:** To a labelled, clean, grease free unscratched slide, a thin smear was made and allowed to air dry for about 15 minutes under the biological safety cabinet.
The smear was fixed by passing the slide over a Bunsen burner flame for about four times with the smear uppermost and allowed to cool.

The slide was flooded with Ziehl-Neelsen Carbol Fuchsin. With intermittent heat, the slide was maintained steaming for five minutes.

The stains were rinsed by gentle stream of running water until all free stain was washed away.

The slide was flooded with 3% acid-alcohol (decolorising) solution for three minutes and rinsed thoroughly with water.

Excess water was drained from the slide and flooded with the (counterstain) methylene blue for 60 seconds.

The slide was rinsed with water and excess water drained from the slide and allowed to air dry on a slide draining rack.

Slide was observed using 100x oil immersion objective.

The results were reported as follows:

- No AFB/100 fields reported as negative
- 1 -9AFB/100 fields exact figures reported
- 10 – 99AFB/100 fields reported as 1+
- 1 – 10 AFB per field reported as 2+
- More than 10 AFB per field reported as 3+

**3.4.3 PARTIAL AFB STAIN (KINYOON’S MODIFICATION) BENEKE AND ROGERS, (1980).**

**Principle:** The *Nocardiae*, because of unusual long chain fatty acids in their cell walls, can retain carbol fuchsin dye during mild acid decolorization, whereas other aerobic branching bacilli can not.

**Method:** To a labelled clean grease free slide, a thin smear of the organism was made and heat fixed by passing through a flame of Bunsen burner for about three times. Kinyoun’s Carbol Fuchs in was applied over the smear for 5
minutes at room temperature. The stain was rinsed off with distilled water and
decolorization was done using 1% H$_2$SO$_4$ until no red appears on slide. The slide
was rinsed with distilled water and the smear counterstained with methylene
blue for three minutes and then rinsed with distilled water.

The slide was air-dried and examined using 100X oil immersion objective.

Results were recorded as acid fast or non-acid fast. Positive and negative
controls are treated along side with the test sample.

3.4.4. LACTOPHENOL BLUE STAINING (BENEKE AND ROGERS 1980)

**Principle:** The microscopical appearances of the colonies of *Nocardia*
species are well observed when stained with lactophenol blue.

**Method:** A clean grease free slide was labelled.

Two straight teasing needles were sterilized by flaming, one was used to
lift a small quantity of the grown culture unto the clean grease free slide.

Two drops of lactophenol cotton blue solution was added and using the
two straight needles, the picked culture were teased and then covered with a
cover slip. The slide was left for about 15 minutes to enable penetration of
the dye then observed under the microscope using x 10 and x 40 objective.

*Nocardia* species show branched filamentous organisms

3.5 BIOCHEMICAL TESTS FOR IDENTIFICATION AND
CHARACTERIZATION

3.5.1 CATALASE TEST (CHEESEBROUGH, 2003)

**Principle:** Catalase acts as a catalyst in the breakdown of hydrogen
peroxide to oxygen and water.
**Method:** To a clean test tube, 2ml of hydrogen peroxide solution was poured.

Using a sterile wooden (broom) stick a good growth of the test organism was removed and immersed into the hydrogen peroxide solution. Immediate reaction was observed.

**Results:**

<table>
<thead>
<tr>
<th>Active bubbling</th>
<th>Positive Test (Catalase Produced)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No release of bubbles</td>
<td>Negative Test (No Catalase Produced)</td>
</tr>
</tbody>
</table>

### 3.5.2 GERM TUBE TEST (CHEESEBROUGH, 2003)

**Principle:** *Candida albicans* sprout within 3 hours when incubated at 35 – 37°C in human serum

**Method:** To a clean small test tube, 0.5ml of human serum was added. Using a sterile wire loop, the serum was inoculated with a colony of the yeast colony from the culture plate.

This was incubated at 37°C for 3 hours.

Using a Pasteur pipette, a drop of the serum-yeast culture was put on a slide and observed using 10 x and 40 x objective.

**Result:**

<table>
<thead>
<tr>
<th>Tube-like sprouting yeast cells</th>
<th>positive (<em>C. albicans</em>)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Non-sprouting yeast cell</td>
<td>Yeast other than <em>C. albicans</em></td>
</tr>
</tbody>
</table>

### 3.5.3 UREA HYDROLYSIS (WAUTERS ET AL. 2005)

**Principle:** Urease – producing organisms break down urea (by hydrolysis) to give ammonia and carbon dioxide. With the release of ammonia, the medium
becomes alkaline and this is shown by colour change of the incorporated indicator in the medium.

**Method:** Using Urea agar, a sterile *straight wire-loop* was used to pick some quantity of the suspected organisms and stabbed onto the urea agar slope in a test tube. The tube was incubated overnight and observed for urease production.

**Result:**
Yellow to redish pink colour  –  positive

### 3.5.4 CASEIN HYDROLYSIS (BENEKE AND ROGERS, 1980)

**Principle:** Some *Nocardia* species especially *Nocardia brasiliensis* hydrolyse casein.

**Method:** A heavy streak was inoculated on plates containing 10% (vol./vol.) skim milk in nutrient agar and incubated at 35°C for 7 days.

**Results:**
Clearing around the streak  Positive reaction
No clearing around the streak  Negative reaction

### 3.5.5 CITRATE UTILIZATION TEST (WAUTERS ET AL. 2005)

**Principle:** Some micro-organisms have the ability to use citrate as the sole carbon source. Utilization of citrate is shown by the reaction of the streak of growth of the organism with bromothymol- blue indicator in the media.

**Method:** Simmon’s citrate agar was used. The Simmons citrate agar was reconstituted according to manufacturer’s instruction. This was dispensed in 5ml amounts and autoclaved at 121°C for 15 minutes and allowed to set as slants in
McCartney bottles *Nocardia* organisms were subcultured on blood agar then growths were used to inoculate Simmons citrate agar slants. Cultures were incubated at 36.5°C and examined daily for 5 days checking for citrate utilization with alkali production.

**Results:**

<table>
<thead>
<tr>
<th>Colour of the Slant</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blue</td>
<td>Positive</td>
</tr>
<tr>
<td>Green</td>
<td>Negative</td>
</tr>
</tbody>
</table>

### 3.5.6 NITRATE REDUCTION TEST (WAUTERS ET AL. 2005)

**Principle:** Some *nocardia* species have the ability to reduce nitrate to nitrite after 4 hours incubation which is detected by adding sulphanilic acid reagent.

**Method:** To a 0.5 ml sterile nitrate broth a heavy growth of the test organism is inoculated and incubated at 36.5°C for 4 hours. After 4 hours a pinch of sulphanilic acid reagent and pinch of α-naphthylamine reagent were added and mixed by shaking.

**Results:**

<table>
<thead>
<tr>
<th>Colour</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>Red</td>
<td>Positive test (nitrate reduced)</td>
</tr>
<tr>
<td>No red</td>
<td>A pinch of zinc dust powder is added and observed for red colour.</td>
</tr>
<tr>
<td>No red</td>
<td>Positive test (nitrate reduced)</td>
</tr>
</tbody>
</table>

### 3.6 ANTIBIOTIC SENSITIVITY TEST

After identification and characterization of organisms with the biochemical tests, the organisms were subcultured onto sabouraud agar slants to obtain a pure growth.
To a 6ml-bijou bottle, 10 glass beads were put and autoclaved at 121°C for 30 minutes for sterility. After removal from the autoclave, the bottle was allowed to cool then 1ml of sterile distilled water was added. Some colonies of the test organism added and vortexed for 5 minutes to ensure a homogenous solution.

A loopful of the organism solution was inoculated unto Mueller Hinton agar and streaked smoothly to get an even and complete distribution of the organisms on the media surface.

With sterile forceps, multi disc antibiotics were collected and with gentle pressure, placed on the streaked Mueller Hinton agar plates. Also some single antibiotic discs were added and gentle pressures applied on them to ensure contact of the disc and the surface of the culture medium.

The plates were incubated aerobically for 5 days at 37°C. Zones of inhibition or clearing around each drug disc was observed and recorded as a measure of antibiotic sensitivity. Zones greater than 2mm as measured with a ruler were regarded sensitive while zones less than 2mm were regarded resistant.

The antibiotics used and their concentrations were Erythromycin 10mg, Ciprofloxacin 5mg, Clindamycin 10mg, Gentamicin 10mg, Cephalexin 30mg, Cotrimoxazole 25mg, Tetracycline 30mg, Augmentin 30mg, Amoxycillin 20mg, Chloramphenicol 10mg.

3.7: PATHOGENICITY STUDIES OF NOCARDIA SPECIES FOR MICE


3.7.1 INOCULUM PREPARATION

The organisms were subcultured unto Sabouraud agar slants and incubated at 37°C for 2 weeks.
To sterile McCartney bottles, scoups of the growths were put and 2ml of sterile distilled water and 10 sterile glass beads added.

The contents were put on a whirl mixer and vortexed for 5 minutes.

The McCartney bottles were allowed to stand for 5 minutes.

The supernatants were transferred to sterile screw capped tubes and centrifuged for 10 minutes at 3000 rpm.

The sediments were homogenized and the homogenate suspended in sterile normal saline and filtered through a thin layer of sterile cotton wool to remove the lumps.

The filtrate was centrifuged and the sediment so obtained was washed twice with sterile normal saline.

The sediment was resuspended in sterile normal saline.

3.7.2 ANIMAL PREPARATION

A total of 40 mice were used for the study. These were sourced from the veterinary medicine department of the University of Nigeria, Nsukka. They were brought at the age of 6 weeks and after one-week acclimatization period was observed. They were kept in plastic cages and provided with pellet diet and water ad libitum. The animals were divided into 4 groups whereby the first 3 groups were subdivided into 3 (A, B, C) subgroups and each sub-group contained 4 mice in a cage. The 4th group which was the control contained 4 mice in a cage i.e. the first 3 groups contained 12 mice per group while the 4th group was 4 mice only making a total of 40 mice for the study. They weighed 20 – 25g.
3.7.3 PATHOGENICITY TEST (GUGNANI ET. AL., 2004)

This was investigated by intraperitoneal inoculations of saline suspensions of the organism into separate groups of mice post cortisone administration. Cortisone administration was found to enhance the virulence of Nocardia species. Each animal received a total dose of 7.5mg hydrocortisone sodium succinate spread over 3 doses on alternate days, the schedule being completed in 6 days prior to inoculation with the organism. The organism was inoculated intraperitoneally at a dose of 1ml each to the same group of mice. Group 1 (A, B, C) was inoculated with *Nocardia asteroides*, Group 2 (A, B, C) was inoculated with *Nocardia brasiliensis*, Group 3 (A, B, C) was inoculated with *Nocardia Species* that were neither asteriodes nor *brasiliensis species*. The 4th groups were the control group. They were inoculated with sterile distilled water.

Animals were monitored daily for 21 days and examined for vital signs and death.

3.7.4 AUTOPSY

Mice were autopsied at death. Portion of the liver, kidney and spleen were fixed in 10% formal saline for histological studies. Animals that survived after 21 days were sacrificed and a portion of their liver, kidney and spleen also fixed in 10% formal saline. Portions of the organ showing gross lesions were also minced into very small pieces and cultured on Sabouraud agar slants and paraffin agar plates to establish the presence of the organism.
HISTOLOGICAL ANALYSIS

The fixed portions of the liver, kidney, spleen and skin were prepared, stained with haematoxylin and eosin and studied under the light microscope.

The steps involved in tissue preparation (Gartner and Hiatt, 2001) are Fixation, dehydration and Clearing, embedding in a suitable medium, sectioning into slices to permit viewing by transillumination, mounting onto a surface for ease of handling, and staining so that the various tissue and cell components may be differentiated.

**Fixation** is treatment of the tissue with chemical agents that not only retard the alterations of tissue subsequent to death (or after removal from the body) but also maintain its normal architecture.

**Dehydration and Clearing:** Because a large fraction of the tissue is composed of water, a graded series of alcohol baths, beginning with 50% alcohol and progressing in graded steps to 100% alcohol are used to remove the water i.e. dehydration. For Clearing, the tissue is treated with xylene, a chemical that is miscible with melted paraffin. This makes the tissue transparent.

**Embedding:** This is to distinguish overlapping cells in a tissue and the extracellular matrix from one another. The usual embedding medium is paraffin. This is by placing the tissue in a suitable container of melted paraffin until the tissue is completely infiltrated. Once the tissue is impregnated with paraffin, it is placed into a small receptacle, covered with melted paraffin, and allowed to harden, forming a paraffin block containing the tissue.

**Sectioning:** After the blocks of tissue are trimmed of excess embedding material, they are mounted for sectioning using a microtome. For light microscope the thickness of each section is about 5-10 um.
**Mounting and Staining:** Paraffin sections are mounted (placed) on glass slides and then stained by water soluble stains (Haematoxylene and Eosin for this study) that permit differentiation of the various cellular components.

Haematoxylene is a base that preferentially colours the acidic components of the cell a bluish tint. Because the most acidic components are deoxyribonucleic acid (DNA) and ribonucleic acid (RNA), the nucleus and regions of the cytoplasm rich in ribosomes stain dark blue; these components are referred to as basophilic. Eosin is an acid that dyes the basic components of the cell pinkish colour. Many cytoplasmic constituents have a basic pH, regions of the cytoplasm stain pink, these elements are said to be acidophilic.

Stained slides were also photographed with photomicron camera, developed and printed to magnify abnormalities as may be seen in the portion of organs processed.

All data generated from the study were analysed using the Statistical Package for Social Sciences (SPSS) Version 10.
CHAPTER FOUR

RESULTS

Of 200 patients with signs and symptoms of lower respiratory tract infection (LRTI) were examined for *Nocardia* species infection, 100 (50%) were males and 100 (50%) were females. Out of the 200 subjects, total of 25 (12.5%) were positive for *Nocardia* species infection.

Table 4.1 shows the summary of age and sex distribution of patients with lower respiratory tract infections used for this study. The patients comprised 100 males and 100 females. Of the nine age groups, no patient in the study group was less than 2 years of age. Age group 26 – 33 years were the highest number 59 (29.5%) followed by age group 34-41 which had 41(20.5%) and 19.5% observed in age group 18-25years were available for the study. These observations were statistically significant in relation to age (P<0.05). Graphical representations are shown in figure 4.1 at the appendix.
### TABLE 4.1: AGE AND SEX DISTRIBUTION OF PATIENTS WITH LRTI USED IN THIS STUDY

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-9</td>
<td>3</td>
<td>1</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>10-17</td>
<td>6</td>
<td>7</td>
<td>13</td>
<td>6.5</td>
</tr>
<tr>
<td>18-25</td>
<td>14</td>
<td>25</td>
<td>39</td>
<td>19.5</td>
</tr>
<tr>
<td>26-33</td>
<td>27</td>
<td>32</td>
<td>59</td>
<td>29.5</td>
</tr>
<tr>
<td>34-41</td>
<td>23</td>
<td>18</td>
<td>41</td>
<td>20.5</td>
</tr>
<tr>
<td>42-49</td>
<td>11</td>
<td>8</td>
<td>19</td>
<td>9.5</td>
</tr>
<tr>
<td>50-57</td>
<td>9</td>
<td>6</td>
<td>15</td>
<td>7.5</td>
</tr>
<tr>
<td>58-65</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>1.5</td>
</tr>
<tr>
<td>above 65</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>3.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
<td><strong>100</strong></td>
<td><strong>200</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
The distribution of patients with *Nocardia* species infection according to age and sex is shown in Table 4.2 at the appendix. Of the 200 patients with lower respiratory tract infections, 25 (12.5%) had *Nocardia* species infections. Of the 25 patients, 14 (56.0%) were males while 11 (44.0%) were females. Though the number of infected males were higher but statistically it was not significant (P > 0.05).

There were no isolates of *Nocardia* species from patients within the age groups 2 – 9, 58 – 65 and above 65 years. The highest isolation of 6 (24.0%) occurred in patients within the age group 26 – 33. There was no significant relationship between the distribution of the Nocardia species and age (P > 0.05). The graphical representation is shown in figure 4.2.
FIGURE 4.2: DISTRIBUTION OF SUBJECTS WITH NOCARDIA SPECIES INFECTION ACCORDING TO AGE AND SEX
The prevalence of TB infection and HIV infection according to age and sex in patients with lower respiratory tract infection (LRTI) is shown on table 4.3 at the appendix.

For TB, out of the 200 patients with signs and symptoms of lower respiratory tract infections, 102 had tuberculosis. Of the 102 patients, 52 (50.98%) were males while 50 (49.02) were females. Patients within the age groups 18 – 25 and 26 – 33 years of age had the highest prevalence of 28 (27.45%) respectively. This percentage prevalence was found to be statistically significant (P < 0.05). Patients within the age group 2-9 years had 2(1.96%) followed by those in age group greater than 65 years with 1 (0.98%).

Of the 200 patients in the study group with signs and symptoms of lower respiratory tract infections 32 (16.0%) had HIV infection. Of the 32 patients, 12 (37.5%) were males while 20 (62.5%) were females. This difference was not statistically significant (P >0.05).

Patients within the age groups, 2 – 9, 10 – 17, 50 – 57, 58 – 65 and above 65 years of age had zero prevalence. However, the highest was with those within the age group 34 – 41 years, 31 (40.6%) followed by 12 (37.5%) in age group 26 – 33 years of age. Statistically, the age difference was not significant (P > 0.05). There was no variation according to sex (P<0.05) for TB and HIV infection. Graphical representations are on figures 4.3a and 4.3b.
FIGURE 4.3A: PREVALENCE OF TB INFECTION IN SUBJECTS WITH LRTI
FIGURE 4.3B: PREVALENCE OF HIV INFECTION IN SUBJECTS WITH LRTI
Table 4.4 shows the distribution of isolated *Nocardia* species according to age groups of infected subjects.

There were 25 isolates of *Nocardia* species from the study population comprising 13(52%) for *Nocardia asteriodes*, 7(28%) for *Nocardia brasiliensis* and 5(20%) for other species of *Nocardia*. Using the Chi-Square test of association, there was no significant relationship between the distribution of Nocardia species (P>0.05).

For *Nocardia asteriodes*, the highest number of isolates 5(38.5%) were from patients within the age groups 18 – 25 followed by those within the age group 34 – 41 years of age, 4(30.8%). The age group 26 – 33 years had 2 (15.4%) while age group 10-17 and 42-49 had 1(7.7%) respectively. Graphical representation is on figure 4.4a.

For *Nocardia brasiliensis*, age group 18 – 25 years had the highest number of isolates 3(42.9%) while for other species of *Nocardia*, the highest number of isolates 3 (60.0%) were from the age group 26 – 33 years. Graphical representations are on figures 4.4b and 4.4c.
TABLE 4.4: DISTRIBUTION OF ISOLATED *NOCARDIA* SPECIES ACCORDING TO AGE GROUPS OF INFECTED SUBJECTS

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th><em>N.asteroides</em></th>
<th><em>N.brasiiliensis</em></th>
<th>Other species</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-9</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10-17</td>
<td>1 (7.7%)</td>
<td>1 (14.3%)</td>
<td>0</td>
</tr>
<tr>
<td>18-25</td>
<td>5 (38.5%)</td>
<td>3 (42.9%)</td>
<td>1 (20.0%)</td>
</tr>
<tr>
<td>26-33</td>
<td>2 (15.2%)</td>
<td>1 (14.3%)</td>
<td>3 (60.0%)</td>
</tr>
<tr>
<td>34-41</td>
<td>4 (30.4%)</td>
<td>1 (14.3%)</td>
<td>0</td>
</tr>
<tr>
<td>42-49</td>
<td>1 (7.7%)</td>
<td>0</td>
<td>1 (20.0%)</td>
</tr>
<tr>
<td>50-57</td>
<td>0</td>
<td>1 (14.3%)</td>
<td>0</td>
</tr>
<tr>
<td>58-65</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>above 65</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>13 (52%)</strong></td>
<td><strong>7 (28%)</strong></td>
<td><strong>5 (20%)</strong></td>
</tr>
</tbody>
</table>
FIGURE 4.4A: PREVALENCE OF NOCARDIA ASTEROIDES ACCORDING TO AGE OF SUBJECTS
FIGURE 4.4B: PREVALENCE OF NOCARDIA BRASILIENSIS ACCORDING TO AGE OF SUBJECTS
FIGURE 4.4C: PREVALENCE OF OTHER SPECIES OF NOCARDIA ACCORDING TO AGE OF SUBJECTS
The Drug Sensitivity pattern of isolates is shown on table 4.5 at the appendix. The drugs used were ten types of antibiotics for the 25 isolates of *Nocardia* species. All the isolates showed a 100% resistance to Clindamycin and Ciprofloxacin. A total of 8(32%) isolates were sensitive to Gentamicin and Augumentin respectively which was the highest number, followed by 7(28%) isolates that showed sensitive to Cotrimoxazole. This observation was significant (P<0.05). Tetracycline was effective in elimination of only 5(20%) isolates while Erythromycin and Chloramphenicol were effective on 4(16%) isolates respectively and 2 isolates were sensitive to Cephalexin and Amoxycillin respectively. Graphical illustration is shown at figure 4.5.
FIGURE 4.5: DRUG SENSITIVITY PATTERN OF ISOLATES
The characterization tests done on the isolates are shown on table 4.6 at the appendix. All the \textit{Nocardia} species isolates were catalase positive, 21 isolates were positive for nitrate utilization tests, 20 isolates were positive for urea hydrolysis test, 9 isolates were positive for citrate utilization tests while only 7 were positive for caesin hydrolysis test. Graphical representation is shown at Figure 4.6. Colour Plates 4, 5 and 6 show the reaction of the organisms on different culture media.
FIGURE 4.6: CHARACTERIZATION TESTS

- Catalase Test
- Urea Hydrolysis
- Casein Hydrolysis
- Citrate Utilization
- Nitrate Utilization

Number of isolates

Biochemical Tests
Table 4.7 shows the socio-demographic data of subjects with *Nocardia* infection. This comprised their occupation, educational level, domicile, residential status and number of persons in an appartment.

For occupation, the highest number of respondents were traders/business 8(32.0%) followed by students 7(28.0%). Artisans were 6 (24.0%) and farmers only 1 (4.0%). None of the respondents was a housewife. The nature of an individual’s occupation may not be a predisposing factor to *Nocardia* infection (P>0.05). Graphical representation is at Figure 4.7a.

For educational level attained by the respondents, secondary had the highest 12 (48.0%) followed by primary 8 (32.0%) tertiary being 4 (16.0%) and the least being those with non formal education 1(4.0%). Statistically there was a significant relationship between the level of education and *Nocardia* infection hence, level of education may be a predisposing factor to *Nocardia* infection (P<0.05). Graphical representation is at Figure 4.7b.

For Domicile, residents from the semi urban were the highest respondents 13 (52.0%) followed by those in the urban 8 (32.0%) and the least from rural 4(16.0%). Statistically, location of an individual’s residence may not be a predisposing factor to *Nocardia* infection (P>0.05). Graphical representation is at Figure 4.7c.

For residential status, the number of respondents using one room or two rooms were equal in number and highest as well 9 (36.0%) respectively. This was followed by those residing in flats 4 (16.0%) with the least from those that reside in bungalow/duplex 3 (12.0%). Statistically residential status was not observed as a predisposing factor to *Nocardia* infection (P>0.05). Graphical representation is at Figure 4.7d.

Considering the number of persons in an appartment, respondents who were 6 – 8 persons in an appartment had the highest number 11 (44.0%)
followed by 1 – 2 persons in an apartment 9 (36.0%), then 3 – 5 persons in an apartment 5 (20.0%). There were no respondents who resided where 9 – 11 persons or above were in an apartment. There was no significant relationship between the number of persons in an apartment and Nocardia infection. Hence, the number of persons in an apartment were not observed in this study as a predisposing factor to *Nocardia* infection (P>0.05). Graphical representation is shown at Figure 4.7e.
FIG 4.7A: OCCUPATION OF SUBJECTS WITH *NOCARDIA* INFECTION
FIG 4.7B: EDUCATIONAL LEVEL OF SUBJECTS WITH NOCARDIA INFECTION
FIG 4.7C: DOMICILLE OF SUBJECTS WITH *NOCARDIA* INFECTION
FIG 4.7D: RESIDENTIAL STATUS OF SUBJECTS WITH *NOCARDIA* INFECTION
FIG 4.7E: ASSESSMENT OF CROWDY LIVING OF SUBJECTS WITH *NOCARDIA INFECTION*
Table 4.8 shows the Animal pathogenicity test. The group one mice which were injected intraperitoneally with solution of *Nocardia asteriodes* had an observable weight loss post treatment in the three sub-groups, non showed skin lesion caused by *Nocardia* before the 21 – days of treatment and observation.

The group two mice which were injected intraperitoneally with solution of *Nocardia brasiliensis* had observable weight loss in subgroups B and C, showed skin lesion in subgroups A and C, weakness and death occurred in subgroups B and C.

Group three were injected with other species of *Nocardia* organisms, showed weight loss in subgroups, A and B, showed skin lesion in subgroup, B, weakness was observed in subgrups, A and B while death was observed as group B only.

The fourth group (control group) showed no weight loss, skin lesion, weakness and death during the treatment period of 21 days.
**TABLE 4.8: ANIMAL PATHOGENICITY TEST**

<table>
<thead>
<tr>
<th></th>
<th>N.asteroides</th>
<th>N.brasiiliensis</th>
<th>Other Species</th>
<th>Control</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
<td>Group 3</td>
<td>Group 4</td>
</tr>
<tr>
<td>VIRULENCE</td>
<td>A</td>
<td>B</td>
<td>C</td>
<td>A</td>
</tr>
<tr>
<td>Weight Loss</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Skin Lesions</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Weakness</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Death</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

**KEY:**

1 = YES
0 = NO
Table 4.9 shows the histopathological observations in mice. The liver cells of the groups treated with *Nocardia* species organisms were affected. The organisms were observed in those affected organs. There were also inflammatory cells at the perivascular and periportal areas (1), and vacuolation of the liver cells (v) as also shown in Colour plate 8.

The kidney cells were affected in group 1 sub-groups A and C, Group 2 subgroups A and B and in group 2 subgroup B. The renal corpuscles were intact (r) but there were mild tubular damage as shown in Colour plate 10.

The spleen cells were also affected in group 1 sub group A, B, C, group 2 subgroup A an C and also in group 3 subgroup A and B.

Skins were affected only in groups 2 and 3. The skin showed the presence of inflammatory cells around the hair follicle (i) as shown in Colour plate 9.
### TABLE 4.9: HISTOPATHOLOGICAL OBSERVATION IN MICE

<table>
<thead>
<tr>
<th></th>
<th>N. asteroides</th>
<th>N. brasiliensis</th>
<th>Other Species</th>
<th>CONTROL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kidney</td>
<td>A 1 B 0 C 1</td>
<td>A 1 B 1 C 0</td>
<td>A 0 B 1 C 0</td>
<td>0</td>
</tr>
<tr>
<td>Liver</td>
<td>1 1 1 1</td>
<td>1 1 1 1</td>
<td>1 1 1 1</td>
<td>0</td>
</tr>
<tr>
<td>Spleen</td>
<td>1 1 1 0</td>
<td>1 1 1 0</td>
<td>1 1 1 0</td>
<td>0</td>
</tr>
</tbody>
</table>

**KEY:**
- 1 = AFFECTED
- 0 = NOT AFFECTED
Table 4.10: Show the prevalence of co-infection of TB, HIV and \textit{Nocardia} species in subjects. Of the 25 subjects, 21 (84.0\%) had \textit{Nocardia}/TB co-infection while only 4(16.0\%) had \textit{Nocardia}/HIV co-infection. Ages 10–57 years had TB/\textit{Nocardia} co-infection while ages 18–33 years had \textit{Nocardia}/HIV co-infection. Graphical representations are at appendix 3 and appendix 4.
### TABLE 4.10: PREVALENCE OF CO-INFECTION OF TB, HIV AND NOCARDIA SPECIES IN SUBJECTS

<table>
<thead>
<tr>
<th>Age[ Years ]</th>
<th>Nocardia /HIV</th>
<th>Nocardia / TB</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10-17</td>
<td>0</td>
<td>4(19.1%)</td>
</tr>
<tr>
<td>18-25</td>
<td>1(25.0%)</td>
<td>5(23.8%)</td>
</tr>
<tr>
<td>26-33</td>
<td>3(75.0%)</td>
<td>5(23.8%)</td>
</tr>
<tr>
<td>34-41</td>
<td>0</td>
<td>3(14.3%)</td>
</tr>
<tr>
<td>42-49</td>
<td>0</td>
<td>2(9.5%)</td>
</tr>
<tr>
<td>50-57</td>
<td>0</td>
<td>2(9.5%)</td>
</tr>
<tr>
<td>58-65</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>above 65</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>4(16.0%)</td>
<td>21(84.0%)</td>
</tr>
</tbody>
</table>
PLATE 1

SPUTUM SAMPLES READY FOR CULTURE IN THE BIOLOGICAL SAFETY CABINET
PLATE 2

SABOURAUD AGAR SLANTS SHOWING PURE GROWTHS OF NOCARDIA SPECIES

PARAFFIN AGAR PLATES SHOWING GROWTHS OF NOCARDIA SPECIES
PLATE 3

MICROSCOPY SLIDE

LACTOPHENOL COTTON BLUE DEMONSTRATION:

FILAMENTOUS MESH WORK OF NOCARDIA SPECIES (F)
PLATE 4

UREA HYDROLYSIS

UREA AGAR SLANTS

NUMBERS 1-4 SHOW UREA HYDROLYSIS

NUMBERS 5 AND 6 SHOW NO HYDROLYSIS
CASEIN AGAR
NOCARDIA BRASILIENSIS SHOWING CASEIN HYDROLYSIS
BY CLEARING AROUND THE STREAK OF THE ORGANISM AT
THE CENTER
PLATE 6

CITRATE UTILIZATION

SIMMONS CITRATE AGAR

1, 2, 3, 4 – NEGATIVE

5, 6 - POSITIVE
PLATE 7

MICE UNDERGOING DISSECTION
PLATE 8
HISTOPATHOLOGY OF LIVER CELLS

Features
- Inflammatory cells at
- Perivascular and periportal areas (I).
- Vacuolation (v)
- Presence of Nocardia (spp)

Features
- Normal Liver parenchyma (P)
- Normal central vein (v)
- Absence of Inflammatory cell
Features (Skin):
- Presence of inflammatory cells around the hair follicle (i)
- Hair follicle (H)
Features (Kidney)
- Intact renal Corpuscles (r)
- mild tubular damage (t)
CHAPTER FIVE

DISCUSSION

Pulmonary diseases and their diagnosis have been documented to constitute complex problems in public health care (Osoagbaka and Njoku Obi, 1982, Idigbe et al., 1992). In this study, out of the 200 patients in this study, 25(12.5%) had nocardia species infection. This value is greater than the value 5.0% obtained by Osoagbaka and Njoku Obi (1985), and also 4.1% obtained by Gugnani et al. (1991), both from parts of Eastern Nigeria. This higher value obtained in this study maybe due to increasing incidence of HIV infection in this part of the world. This is in agreement with the findings by Giorghiou and Blacklock, (1992) after their review work on 102 clinical isolates in Queens Land, that infection with Nocardia species appears to be more common than is generally appreciated and also Farina et al., (1995) after their study on Human nocardiosis in Northern Italy.

More males 14(56.0%) were infected by the Nocardia species than females 11(44.0%). This was statistically not significant (P < 0.05). Hence, more males than females being infected is related to differences in frequency of exposure rather than a sex difference in susceptibility to infection as also supported by Eshraghi et al. (2005) in Shareati Training Hospital Teran and Matulionyte et al. (2004) in Geneva, Switzerland and Kageyama et al. (2004) in Japan.

None of the patients in the study group was less than two years of age. The work done by Nwobu et al. (1996, 2004) found that lower respiratory tract infections affect more commonly the very young which is not in agreement with this finding on Nocardia species infection. Kim et al. (1992) reported that
nocardiosis though a rare childhood disease was isolated from a neonate as military nodules in an autopsy.

The highest number of nocardia species isolates 6(24.0%) were from age group 26-33 years. On the whole, it was observed that infection occurred in patients aged 10 – 57 years. This observation was not in agreement with the work done by Kageyama et. al. (2004), in Japan, who found Nocardia infection in patients between the ages of 61-80 years of age. The work done by Osoagbaka and Njoku-Obi (1985) had isolates from patients 40 -70 years which were still above the observed age in this study. Since most patients presenting with nocardiosis as observed in this study have a certain degree of immunodeficiency as was supported by the work done by Heffner (1988), Eschraghi et. al. (2005). Pre-existing lung disease for example tuberculosis as risk factor may have been the reason for a mild drop of age at which patients were infected as observed in this study.

Furthermore, 102 patients out of the study population had tuberculosis. This gives a 51.0% infection rate which is quite high. This observation agrees with the report by Baron et.al. (1994), that Mycobacteria tuberculosis is the most likely etiological agent of chronic lower respiratory tract infections. Of the examined 102 patients, there were more males 52(50.98%) than females 50 (49.02%) but the difference was not statistically significant (P> 0.05). This observation agreed with the work done by Samb et.al. , (1999) at Dakar, Senegal. In their work, 450 patients were diagnosed with pulmonary tuberculosis; there were more males (71.3%) than females (28.7%).

Work done by Reider (1999) comparing infection and disease rates suggest that propensity to develop disease after infection with Mycobacteria tuberculosis (progression rate) may be greater among women of reproductive
age than among men of same age. At older ages men have a higher rate of progression and this report is in agreement with the finding in this study.

In this study, age groups 18 - 25 and 26 - 33 years had the highest rate 28(27.4%) respectively and this was statistically significant (P< 0.05). This finding is not in agreement with the report of Orjioke et. al. (1998), that individuals between 15 - 35 years of age were affected.

From this study, none of the patients were less than two years of age, this may be due to the type of specimen (sputum) used.

Out of the 200 patients sampled, 32 (16.0%) had HIV infection and out of which 12(37.5%) were males while 20(62.5%) were females. This difference was not statistically significant (P> 0.05). Nwokedi and Azeez –Akande (2007) after their seven year study on the trend of HIV in Kano, Nigeria, reported that there were higher seroprevalence rates in men in the remaining two years. Females are freer in the South Eastern Nigeria than in the Northern Nigeria which is a socio religious reason. Further more, age groups 18 - 25, 26 - 33, 34 - 41, 42 - 49, ie 18 - 49 years were the ages involved. This finding is higher than the value obtained by CDC (1998) in 3-year HIV and AIDS surveillance in United States where age group 13 - 24 years was involved. This may also be due to social reasons.

There were 25 isolates of *nocardia* species and 13(52.0%) were *nocardia asteroides*, 7(28.0%) were *Nocardia brasiliensis* and the other 5 (20.0%) were other species of *Nocardia*. This finding is in agreement with the work done by Baron (1996) and Ramchandran, et. al. (2003) which in their work observed the highest percentage of the pulmonary nocardial infection being caused by *Nocardia asteroides* then followed by *Nocardia brasiliensis* and then *Nocardia caviae*. Also in Geneva, Switzerland, *Nocardia asteroides* was identified as the most common species over a period of 15 years in their University Hospital.
(Matulionyte et al., 2004) but Yildiz et al., (2005) in Turkey found Nocardia farcinica to be the predominant species rather than Nocardia asteroides.

There are over ten species of Nocardia. In this study, Nocardia brasiliensis ranked second and this is supported by the work done by Neil and Brown (1994) which reported that Nocardia brasiliensis is the second most common clinically isolated actinomycete.

There was high level of resistance of the isolates to several antimicrobials as also observed in Italy by Farina et al. (1995). As observed in this study, Cotrimoxazole was second most effective drug. This observation is neither supported by Brooks et al., (2004) nor Lerner (1996) because they documented that Cotrimoxazole was the drug of choice though to be administered for a long duration. There was a supported observation by Wadhwa et al., (2006) where the supposed Cotrimoxazole, the recommended drug of choice failed in sensitivity to isolates. Augumentin was observed to show high sensitivity as also observed by Boiron et al. (1992) in Paris.

Many patients presenting themselves at the hospitals or clinics can be said to be immunocompromised due to poor standard of living (Osoagbaka and Njoku –Obi, 1982). Bacterial infections of the respiratory tract are public health and socio economic problems in Nigeria (Nwobu et al. 2004, Akpala and Okeke, 1996). Occupation and educational level attained has some level of effect on an individual’s financial status and exposure frequency to certain “hazardous” environment. As observed in this study, traders/business inclined individuals 8(32.0%) were the highest respondents amidst the patients infected with Nocardia species organisms. This was then followed by students 7(28.0%) and artisans 6(24.0%). This finding was not statistically significant (P>0.05) hence nature of occupation may not be a predisposing factor to Nocardia species infection.
Since *Nocardia* species infection involving the lower respiratory tract (pulmonary nocardiosis) is by inhalation of the bacteria. Reider (1999) documents that volume of shared air space and length of exposure determine the risk of becoming infected with bacteria like *Mycobacteria tuberculosis*, this may also be applicable to *Nocardia* species organisms. CDC (1994) also wrote that crowded living is an enhancement to being infected with some organisms that cause respiratory tract infections. As observed in this study, patients that resided in either one or two rooms apartment 9(36.0%) respectively were the most infected likewise those that were about 6 – 8 persons in an apartment 11(44.0%), were the highest respondents. This observation is in agreement with the observations made by Reider (1999), CDC (1994), Deibert and Demke (2000).

The histopathological findings made in the organs (liver, kidney, spleen and skin) of the mice were as also observed by Gugnani *et. al.* (1982).

There was also observable concomitant occurrence of pulmonary nocardiosis with pulmonary tuberculosis in some of the patients. This was also observed by McQuown, (1955), Hosty *et. al.* (1961) and Idigbe *et.al.* (1992). The lung is a major target of attack in HIV – Positive and AIDS patients because the Human immunodeficiency virus damage the host epithelial tissue and predispose patients to secondary bacterial infection (Mills, 1994).

Lucas *et. al.* (1994) in their work reported that 5% of their subjects had concomitant occurrence of nocardiosis and HIV while this study recorded a higher rate of 16%. This higher rate may have been caused by socio-economic factors affecting HIV patients like poor standard of living as was also observed in this study. It was noted from this study that most patients had HIV infection or tuberculosis as a predisposing factor as also documented by Heffner (1988) and Eshraghi *et. al.* (2005).
CONCLUSION

From this study it can be concluded that nocardiosis is important in our environment with 12.5% prevalence rate. All cases of nocardiosis encountered in the study were associated with other underlying illnesses e.g. HIV, TB, or both, hence it may likely be considered an opportunistic infection.

Sex didn’t seem to play any major role in Nocardia infections as observed in the study where both sexes did not differ significantly.

The age distribution didn’t seem to show significant variation except that the two age extremities 2 – 9 and 58 - >65 years age groups recorded no positives, while the age groups 18 – 25 years had an overall highest rate being followed by the age groups 26 – 33 and 34 – 41 years.

It can also be concluded that in this environment, Nocardia asteroides is the most implicated in pulmonary nocardiosis followed by Nocardia brasiliensis.

Augumentin and Gentamicin gave the highest Sensitivity rate, while Ciprofloxacin and Clindamycin were the least and the two earlier drugs may be the most potent treatment for nocardiosis in this area.

The use of Paraffin agar for Nocardia species isolation from contaminated specimens is of great importance as a selective media and for fast growth of the organisms.

Area of domicile, type of residence with number of persons in the apartment, and occupation didn’t seem to play any role in predisposing infections.
RECOMMENDATIONS

It is probable that in developing countries, other pulmonary infections may be misdiagnosed and treated as pulmonary tuberculosis. Moreover, some of the bacteria and fungi causing these pulmonary infections do not respond adequately to most Chemotherapeutic agents used in the treatment of classical tuberculosis. Sequel to this, it has become very important that clinical symptoms and radiological features be backed-up with laboratory investigations into the differential diagnosis of infection of the respiratory tract.

It is therefore recommended that immunosuppressed patients and/or with any underlying chronic lung disease should be screened routinely for isolation of *Nocardia* species organisms and treated accordingly.

If sputum repeatedly tests negative for acid fast bacilli in the setting of radiological suspicion of tuberculosis, or if the patient’s condition worsens despite anti-tuberculosis therapy, testing for *Nocardia* species should be considered.

Isolation and identification of *Nocardia* though troublesome, the use of Paraffin agar as a Selective Media is recommended and cultures should be maintained for at least three weeks before being discarded as negative.

Differential diagnosis often delays the time for diagnosis which worsens the situation. New diagnostic tools such as Polymerase chain reaction (PCR) could provide more rapid and reliable results.

A prolonged course of medication is recommended for treatment of nocardiosis due to the relapsing nature of the infection.

Due to the poor economic situation obtainable in Nigeria now, there is an increase in poor standard of living and also uncontrolled and irregular use of
broad spectrum antibiotics. It is therefore recommended that no organism isolated from sputum in reasonable number should be dismissed as a non-pathogen.

Improvement on the Literacy level of people will bring about health awareness. Effective control Programme will involve the improvement on the environmental sanitation of our people and also their living conditions.

Finally, it is highly recommended that proper Laboratory diagnosis should be the gold standard for diagnosis of *Nocardia* before tagging a patient to be Multi-Drug Resistant Tuberculosis (MDR-TB) patient.
REFERENCES


APPENDIX 1

PREPARATION OF REAGENTS/MEDIA USED

CASEIN AGAR

SOLUTION A:
Skimmed milk (dehydrated) 10g
Distilled water 100ml

SOLUTION B:
Distilled water 100ml
Agar 2g

Autoclave both solutions separately at 121°C for 15 minutes. Cool and mix together. Pour into Petri dishes and allow to set.

BLOOD AGAR

Blood agar formulation g/l
Beef extract 10.0
Balanced peptone No 1 10.0
Sodium chloride 5.0
Agar No 2 12.0

Dispense 37g in 1L of deionized water. Soak for 10 minutes. Swirl to mix and sterilize by autoclaving for 15 minutes at 121°C. Cool to 47°C and add 5-7% of defibrinated blood.

Mix before pouring into Petri dishes and allow to gel then dry the agar surface prior to use.

LACTOPHENOL COTTON BLUE

Phenol 10.0g
Cotton blue 0.04g
Glycerol 20ml
Distilled water 10ml

Procedure:
Weigh the cotton blue and dissolve in the water. Warming the water will help the stain to dissolve quickly.

Weigh the phenol in a beaker and add the stain solution. Stir to dissolve the phenol. Transfer to a clean brown bottle. Add the lactic acid and glycerol and mix well.

Label the bottle and mark it corrosive. Store in a cool dark place.

**MC CLUNG’S CARBON FREE BROTH (Mishra and Randhawa, 1969).**

**Formula:**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaNO₃</td>
<td>2.0 g</td>
</tr>
<tr>
<td>K₂HPO₄</td>
<td>0.8</td>
</tr>
<tr>
<td>MgSO₄ .7H₂O</td>
<td>0.5 g</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>10 mg</td>
</tr>
<tr>
<td>MnCl₂.4H₂O</td>
<td>8 mg</td>
</tr>
<tr>
<td>ZnSO₄</td>
<td>2 mg</td>
</tr>
<tr>
<td>Distilled water</td>
<td>1 L</td>
</tr>
</tbody>
</table>

Other items: Glass rods, test tubes, paraffin wax, and Cotton wool.

**PROCEDURE:**

Mix reagents in distilled water and sterilize by autoclaving at 121°c for 15 minutes. Sterilize test tubes with cotton wool stopper in hot air oven at 160°c for 1 hour and allow to cool. Dispense the sterile broth into the test tubes (about 10ml) and immerse a paraffin coated glass rod (previously immersed for 10-12 hours in 95% alcohol after the alcohol has drained off) and stopper with the sterilized cotton wool.

**MUHELLER HINTON AGAR**

**Formulation:**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Beef infusion</td>
<td>2.0 g</td>
</tr>
<tr>
<td>Acid hydrolyzed Casein</td>
<td>17.5 g</td>
</tr>
<tr>
<td>Starch</td>
<td>1.5 g</td>
</tr>
<tr>
<td>Agar No 1</td>
<td>17.0 g</td>
</tr>
</tbody>
</table>
Calcium ions                               5 – 100mg/l
Magnesium ions                          20 -35mg/l

**Preparation:**

Weigh 38grams of powder and disperse into 1L of deionised water. Allow to soak for 10 minutes, Swirl to mix, then sterilize by autoclaving at 121°C for 15 minutes. Cool to 47°C, pour into Petri dishes and allow to solidify.

**NITRATE BROTH**

Nitrate broth is a biochemical medium available in dehydrated form, alternately nitrate broth can be prepared

**Formula:**

<table>
<thead>
<tr>
<th>Component</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Potassium nitrate</td>
<td>0.1g</td>
</tr>
<tr>
<td>Peptone water</td>
<td>100ml</td>
</tr>
</tbody>
</table>

**Preparation:**

Dispense in 0.5ml amounts in small tubes. Sterilize by autoclaving (with loose caps) at 121°C for 15 minutes. Allow the medium has cooled, stopper tightly.

**PARAFFIN AGAR (Ayyar et. al., 1992).**

<table>
<thead>
<tr>
<th>Component</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>McClungs Carbon Free broth</td>
<td>1000ml</td>
</tr>
<tr>
<td>Nutrient agar</td>
<td>20g</td>
</tr>
<tr>
<td>Paraffin wax (melted)</td>
<td>100ml</td>
</tr>
</tbody>
</table>

**PROCEDURE:**

Sterilize McClungs Carbon free broth by autoclaving at 121°C for 15 minutes. Allow to cool to about 60 - 65°C then add the melted paraffin wax and mix. Then pour into Petri dishes in 20ml amount ensuring an even distribution of the paraffin wax. Allow to set.

**SABOURAUD DEXTROSE AGAR SLANTS**

<table>
<thead>
<tr>
<th>Component</th>
<th>Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plupeptone</td>
<td>10.0g</td>
</tr>
<tr>
<td>Glucose</td>
<td>40.0g</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>0.05g</td>
</tr>
<tr>
<td>Agar</td>
<td>15.0g</td>
</tr>
</tbody>
</table>
Preparation:
Suspend 65g of the powder in 1Litre of distilled water. Heat the suspension to boil in order to dissolve completely. Sterilize for 15 minutes at 121°C. Care must be taken not to overheat the medium so that the dextrose present will not be forced to produce undesirable polymeric compounds.
Pour into test tubes and stopper with sterile cotton wool then keep in a slanting position to solidify.

SIMMONS CITRATE AGAR

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ammonium dihydrogen phosphate</td>
<td>1.0g</td>
</tr>
<tr>
<td>Magnesium sulphate</td>
<td>0.2g</td>
</tr>
<tr>
<td>Dipotassium phosphate</td>
<td>1.0g</td>
</tr>
<tr>
<td>Sodium Citrate</td>
<td>2.0g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5.0g</td>
</tr>
<tr>
<td>Bromothymol blue</td>
<td>0.01g</td>
</tr>
<tr>
<td>Agar No 2</td>
<td>15.0g</td>
</tr>
</tbody>
</table>

Preparation:
Disperse 24g in 1L of deionised water, soak for 10minutes, Swirl to mix and bring to the boil. Dispense into tubes and sterilize by autoclaving for 15minutes at 121°C. Let the medium set as a slope, ensuring that the slant is over a buff about 3cm deep.

UREA AGAR

Formula:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peptone from meat</td>
<td>1.0g</td>
</tr>
<tr>
<td>Glucose</td>
<td>1.0g</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>5.0g</td>
</tr>
<tr>
<td>Potassium dihydrogen phosphate</td>
<td>2.0g</td>
</tr>
<tr>
<td>Phenol red</td>
<td>0.012g</td>
</tr>
<tr>
<td>Agar agar</td>
<td>12.0g</td>
</tr>
</tbody>
</table>
**Preparation:**
Dissolve 21g/L in demineralized water by heating in a boiling water bath or in a current of steam: autoclave at 121°C for 15 minutes. Cool to 45 – 55°C and add 50ml/L of a filter sterilized 40% Urea solution. Dispense in tubes and allow to set as slants.

**CARBOL FUCHSIN SOLUTION**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basic fuchsin</td>
<td>0.3g</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>10ml</td>
</tr>
<tr>
<td>Phenol crystals</td>
<td>5g</td>
</tr>
<tr>
<td>Distilled water</td>
<td>100ml</td>
</tr>
</tbody>
</table>

**Procedure:**
1. Dissolve basic fuchsin in Ethanol to make solution 1.
2. Dissolve phenol crystals in distilled water using a gentle heat to make solution 2.
3. Mix 10ml of solution 1 with 90ml of solution 2.
4. Filter before use.
5. Store in an amber bottle for 6 – 12 months at room temperature.

**3% ACID ALCOHOL**

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Conc Hydrochloric acid</td>
<td>3ml</td>
</tr>
<tr>
<td>95% Ethanol</td>
<td>97ml</td>
</tr>
</tbody>
</table>

**Procedure:**
1. Add the concentrated HCL to the ethanol carefully.
2. Mixture will heat up.
3. Allow to cool, then store in an amber bottle for 6 – 12 months at room temperature.
**METHYLENE BLUE SOLUTION (COUNTERSTAIN)**

**Reagents:**

Methylene blue Chloride 0.3g  
Distilled water 100ml

**Procedure:**

1. Dissolve the dye in water.  
2. Store in an amber bottle for 6 – 12months at room temperature.  
3. Filter before use.
FIGURE 4.1: DISTRIBUTION OF SUBJECTS ACCORDING TO AGE AND SEX
TABLE 4.2: DISTRIBUTION OF PATIENTS WITH *NOCARDIA* SPECIES INFECTION ACCORDING TO SEX AND AGE.

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10-17</td>
<td>1</td>
<td>4</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>18-25</td>
<td>3</td>
<td>2</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>26-33</td>
<td>2</td>
<td>4</td>
<td>6</td>
<td>24</td>
</tr>
<tr>
<td>34-41</td>
<td>4</td>
<td>0</td>
<td>5</td>
<td>20</td>
</tr>
<tr>
<td>42-49</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>50-57</td>
<td>2</td>
<td>0</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>58-65</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>above 65</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>14</strong></td>
<td><strong>11</strong></td>
<td><strong>25</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>
TABLE 4.3: PREVALENCE OF TB INFECTION AND HIV INFECTION ACCORDING TO AGE SEX IN PATIENTS WITH LOWER RESPIRATORY TRACT INFECTIONS.

<table>
<thead>
<tr>
<th>Age[Years]</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
<th>%</th>
<th>Males</th>
<th>Females</th>
<th>Total</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-9</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>10-17</td>
<td>2</td>
<td>3</td>
<td>5</td>
<td>5.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>18-25</td>
<td>10</td>
<td>18</td>
<td>28</td>
<td>27.5</td>
<td>0</td>
<td>4</td>
<td>4</td>
<td>12.5</td>
</tr>
<tr>
<td>26-33</td>
<td>12</td>
<td>16</td>
<td>28</td>
<td>27.5</td>
<td>2</td>
<td>10</td>
<td>12</td>
<td>37.5</td>
</tr>
<tr>
<td>34-41</td>
<td>15</td>
<td>8</td>
<td>23</td>
<td>22.6</td>
<td>9</td>
<td>4</td>
<td>13</td>
<td>40.6</td>
</tr>
<tr>
<td>42-49</td>
<td>5</td>
<td>2</td>
<td>7</td>
<td>6.9</td>
<td>1</td>
<td>2</td>
<td>3</td>
<td>9.4</td>
</tr>
<tr>
<td>50-57</td>
<td>4</td>
<td>1</td>
<td>5</td>
<td>4.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>58-65</td>
<td>2</td>
<td>1</td>
<td>3</td>
<td>2.9</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>above 65</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>52</td>
<td>50</td>
<td>102</td>
<td>100</td>
<td>12</td>
<td>20</td>
<td>32</td>
<td>100</td>
</tr>
<tr>
<td>ANTIBIOTICS</td>
<td>NUMBER OF SENSITIVE ISOLATES</td>
<td>%</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-----------------------------</td>
<td>------------------------------</td>
<td>----</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin 10mcg</td>
<td>8</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalexin 30mcg</td>
<td>2</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotrimoxazole 25mcg</td>
<td>7</td>
<td>28</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Erythromycin 10mcg</td>
<td>4</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Augmentin 30mcg</td>
<td>8</td>
<td>32</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxycillin 20mcg</td>
<td>2</td>
<td>8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline 30mcg</td>
<td>5</td>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol 10mcg</td>
<td>4</td>
<td>16</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clindamycin 10mcg</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ciprofloxacin 5mcg</td>
<td>0</td>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
**TABLE 4.6: CHARACTERIZATION TESTS ON ISOLATES**

<table>
<thead>
<tr>
<th>TYPE OF TEST</th>
<th>NUMBER OF POSITIVE ISOLATES</th>
</tr>
</thead>
<tbody>
<tr>
<td>Catalase Test</td>
<td>25 (100.0%)</td>
</tr>
<tr>
<td>Urea Hydrolysis</td>
<td>20 (80.0%)</td>
</tr>
<tr>
<td>Caesin Hydrolysis</td>
<td>7 (28.0%)</td>
</tr>
<tr>
<td>Citrate Utilization</td>
<td>9 (36.0%)</td>
</tr>
<tr>
<td>Nitrate Utilization</td>
<td>21 (84.0%)</td>
</tr>
</tbody>
</table>
### TABLE 4.7: SOCIO DEMOGRAPHIC DATA OF SUBJECTS WITH NOCARDIA INFECTION.

<table>
<thead>
<tr>
<th>OCCUPATION</th>
<th>NUMBER OF RESPONDENTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artisan</td>
<td>6</td>
</tr>
<tr>
<td>Students</td>
<td>7</td>
</tr>
<tr>
<td>Farmer</td>
<td>1</td>
</tr>
<tr>
<td>Housewife</td>
<td>0</td>
</tr>
<tr>
<td>Trader /Business</td>
<td>8</td>
</tr>
<tr>
<td>Professional</td>
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<table>
<thead>
<tr>
<th>EDUCATIONAL LEVEL</th>
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<tr>
<td>Non Formal</td>
<td>1</td>
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<tr>
<td>Primary</td>
<td>8</td>
</tr>
<tr>
<td>Secondary</td>
<td>12</td>
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<tr>
<td>Tertiary</td>
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<table>
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<tr>
<th>DOMICILE</th>
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<tbody>
<tr>
<td>Rural</td>
<td>4</td>
</tr>
<tr>
<td>Semi Urban</td>
<td>13</td>
</tr>
<tr>
<td>Urban</td>
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<table>
<thead>
<tr>
<th>RESIDENTIAL STATUS</th>
<th>NUMBER OF RESPONDENTS</th>
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<tr>
<td>1 Room</td>
<td>9</td>
</tr>
<tr>
<td>2 Rooms</td>
<td>9</td>
</tr>
<tr>
<td>Flat</td>
<td>4</td>
</tr>
<tr>
<td>Bungalow/Duplex</td>
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</table>

<table>
<thead>
<tr>
<th>NUMBER IN APARTMENT</th>
<th>NUMBER OF RESPONDENTS</th>
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</thead>
<tbody>
<tr>
<td>1 -2 Persons</td>
<td>9</td>
</tr>
<tr>
<td>3 -5 Persons</td>
<td>5</td>
</tr>
<tr>
<td>6 -8 Persons</td>
<td>11</td>
</tr>
<tr>
<td>9 -11 Persons</td>
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</tr>
<tr>
<td>&gt;12 Persons</td>
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FIG 4.12 CO-INFECTION OF TB, HIV AND NOCARDIA IN SUBJECTS