OCCURRENCE OF SOME IMPORTANT PATHOGGOS IN PATIENTS SUSPECTED OF RESPIRATIORY TRACT INFECTION IN MINNA

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CERTIFICATION

This Thesis entitled "Occurrence of some Important PATHOGENS in patients suspected of Respiratory tract infection in Minna" was examined and found to meet the regulations governing the award of the degree of Master of Technology of Federal University of Technology, Minna and is approved for its contribution to knowledge and literacy presentation.

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DEDICATION

This work is dedicated to my parents, wife, brothers and sisters for their support and encouragements that made my work possible: so also my institution Usmanu Danfodiyo University for the financial supports given to me.

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All praises are due to Allah who gave me the courage and power to carry out this work.

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ABSTRACT

A total of one hundred and sixty (160) patients with respiratory tract infections from four (4) centres in Minna town were screened. Sputa samples were obtained and analysed. Staining procedures employed are the gram stain, Ziel Neelson Stain and Giemsa Stain. A wet mount using potassium hydroxide K⁺H was also used. Three types of bacteria were encountered as the gram positive rod(s) (38), the gram positive cocci (92) and the gram negative rods (32). No gram negative cocci was encountered. Culture media employed are the Blood and chocolate agar. Confirmatory biochemical tests were carried out on the isolates. In all, six species of bacterial pathogens were encountered namely: Streptococcus pyogenes, Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aeruginosa, Corynebacterium diphtheriae and Bacillus anthracis. Their frequency of occurrence from the highest to the lowest are: Staphylococcus aureus (48.8%), Corynebacterium diphtheriae (20%), Klebsiella pneumoniae (11.3%), Streptococcus pyogenes (8.8%), Pseudomona aeruginosa (7.5%) and Bacillus anthracis (3.8%). The higher incidence rates and prevalence of these organisms suggest the need for proper control measures and improved diagnostic procedures and materials to conform with the standard procedures in our community health centres.

CHAPTER 1

INTRODUCTION

The human respiratory tract starts at the nose, and goes through the nasal cavity, the pharynx, the trachae and ends in the air sac or alveoli. This entire system is adapted to making air containing oxygen available to the circulatory system. Unfortunately, the respiratory tract is a frequent portal of entry for various microorganisms. Different organisms gain access to different levels in the system and this give rise to differences in the types of infections occurring at the upper and lower respiratory tract diseases. Many of the diseases are quite common and are among the most damaging of any disease that affects humans (Gaworzewska, 1988; and George, 1984).

Several infections of the respiratory tract are of great concern because of the ease with which they are transmitted and contracted and their diagnosis have always constituted complex problems in public health care. Some of the reports on the respiratory tract disease are of investigations carried out on the acute and chronic lower respiratory tract infections in the temperate and Monsoon regions of the world and very few in the tropics. Warrel (1975) was of the opinion that the respiratory infections are a major cause of morbidity and mortality in tropical countries, yet they are generally ignored in textbooks of tropical medicine (Osoagbaka and Njoku-Obi, 1982).

Certain diseases have not been proved but were assumed to be rare in Africa because no proper investigations are carried out due to lack of adequate facilities or because the infections are not reported. The need for further investigations in the tropics for microbial diseases in Africa, and particularly in Nigeria because of her high population is therefore, compelling.

In the northern parts of Nigeria the rate of these infections are high during the periods of harmattan when the south east trade winds are blowing into the country. Similar incidence is noticed during the raining season (Anon. 1994). This makes available spores on the ground to spread to other regions and affect animals and humans. This occurs more commonly in the pastoral districts in the north, and to a lesser extent in the central districts of southern Nigeria (WHO Memorandum, 1994).

Investigations carried out in Nigeria on the respiratory tract diseases are mostly conducted in the south eastern or western parts of the country among which are the works of Asoagbaka and Njoku-Obi (1982). Their work concentrated mostly on the lower parts of the respiratory tract diseases like the tuberculosis and pneumonia. There is the need therefore to investigate the occurrence of these pathogens both of the upper and lower respiratory tract in northern states of the country in order to facilitate treatment and control.

UPPER RESPIRATORY TRACT INFECTIONS

Of all the infections known to occur in the pediatric age group, infections of the upper respiratory tract are the most common like rhinitis, common cold, nasopharyngitis and tonsillopharyngitis. Acute respiratory infections are a major cause of mortality among children in developing countries accounting for over four million deaths per year (Gwatkin 1980 and Leowski,1986). Mullholand (1992) reported that children experienced an average of 7-8 upper respiratory tract infections per year in the first four years of life whereas the number gradually declined to 3-4 per year in adolescent and adulthood. A number of host and environmental factors have been shown to affect the incidence of upper respiratory tract infections. Age is one of the factors because younger children have more upper respiratory tract infection than older children. Climatic changes also affect the incidence of the upper respiratory tract infections especially in Northern temperate climates (Schwartz et al, 1990).

The upper respiratory tract infections are caused by viruses, bacterial species and species of mycoplasma. The viruses include: picorna viruses like Rhinoviruses, Coxsackie viruses and Adenoviruses types 1-7, 14 and 21; Myxoviruses like influenza A,B and C; Mycoplasma pneumoniae Streptococcus pyogenes groups A,B,C and D and Neisseria gonorrheae (Cherry, 1973; Cleary, 1988). Many of these agents are more commonly associated with other infections but they are also commonly associated with upper respiratory tract infections (Cooney et al, 1975, Kohler, 1987).

MYCOPLASMA PNEUMONIA

Over the past 20 years, an annually increasing number of unusual and severe clinical pictures of M pneumoniae disease have been described, involving both the respiratory tract and other organ systems. Many of these symptoms are so common (Klaus, 1983). Complications of M pneumoniae disease such as unusual and severe courses of illness have been reported. They seem to occur predominantly in cases with the involvement of multiple organs systems, and the patients are generally older than those who have the classical pneumonia. Sequelae from the central nervous system and the heart belong to the most serious complications (Sterner, 1990). Complications may also arise within the respiratory tract, such as fulminant lobar pneumonia mimicking severe bacterial pneumonia, or such as acute diffuse alveolitis (Cockcroft, 1981).

Abscess and cavity formations have been described, as well as large pleural effusions. Also cases of fulminant evolution into diffuse interstitial fibrosis have been reported (Kawata, 1987). Underlying diseases such as cancer, diabetes, chronic heart disease and similar conditions may result in a complicated course of the M pneumoniae infection (Solanki, 1989).

Streptococcus pyogenes: In the acute stages of the upper respiratory tract infections, mucous membrances are hyperdemic and edematous. Serous and mucinous exudation tend to occur and the exudate is more purulent, tending to contain more cellular debris and greater numbers of inflamatory cells. Mucous membrances throughout the tract may be congested and thickened or signs of infection may be limited to one or two anatomical areas within the air way passages. In the nose, ciliary activity may be affected, accompanied by depressed nasal mucociliary flow rates (Cooney et al, 1975).

Epidemiologic investigations revealed that sore throat due to streptococcus is associated with aerosols and with contaminated milk products and water. The milk products may be contaminated by human handling or as a consequence of infection in the cows. Sore throat due to streptococcus is common in areas where pasteurization of milk is not practised. The epidemics may last from two to six weeks (George, 1984).

Staphylococcus aureus

Colonization of infants with S aureus is a predictable event occurring in almost 90% of infants during the neonatal period mainly in the nostril and on the umblical stump (Cerati, 1991). The nasal carrier rate drops during infancy and raises again beginning in childhood to about 40% in adult life. In the hospital environment, certain strains of Staphylococcus identifiable by bacteriophage typing are prevalent. The mode of spread by hand contact is more important than other means in hospital environment particularly in the newborn (Cerati, 1991). In the eastern parts of Nigeria, this organism was isolated from

a number of patients screened (Osoagbaka and Njoku-Obi, 1982) and ranked fourth position in the rate of occurrence of the pathogens isolated. In the works of Lynne and Bannard (1991) carried out from eight African countries including Nigeria, S aureus has the highest incidence rate.

Primary Staphylococcal pneumonia occurs most commonly in young infants preceded by a history of upper respiratory infections or staphylococcal skin lesion. The strain of Staphylococcus is likely to be hospital acquired. Staphylococcal pneumonia is a rapidly progressive disease with an abrupt onset. Tachycardia, grunting respirations, cyanosis, fretfulness, sternal and substernal retractions are common (Cerati, 1991).

DIPHTHERIA

Diphtheria is an acutely infectious communicable disease which has been known since ancient times; but its specific nature was only known in the nineteenth century (George, 1984). Toxin producing Corynebacterium diphtheiae produces severe inflammation of the throat. A tough membrane like structure, Pseudomembrane, forms on the tonsils and spreads to lower portions of the respiratory tract or upward into the nasal passages. The membrane may cause suffocation. The absorption of the toxin into the blood stain can cause complications such as paralysis and cardiac arrest (George, 1984). Most instance of diphtheria results from direct contact with droplets. C diphtheriae is a hardly organism which is able to withstand cold, heat and drying. And this makes it so rampant in the desert areas of the northern Nigeria.

Diphtheria occurs worldwide, mainly individuals older than six months but not past middle age. Carriers are believed to account for approximately one-fourth of the known cases. Predisposing factors associated with the disease include chilling, poor nutrition, overcrowded conditions, and operations involving the nose and throat. Diphtheria may result from the exposure of a non-infected individual to the pathogen or to a carrier of the pathogen.

CANDIDA INFECTION

Apart from the bacterial and viral agents of upper-respiratory tract infection, fungi such as candida species are also known to cause respiratory tract infection (Worthington, 1993). Candida pneumonia have been rarely documented. It is usually secondary to seeding of the lungs by hematogenous dissemination in an immusuppressed patient (HIV patient) (Rose, 1987 and Buff, 1982). A recent review mentioned only two previous cases of aspiration of candida pneumonia in a non-immunosuppressed adult (Rose, 1987).

Upper respiratory tract infections regardless of their etiology are manifested by a limited number of symptoms and signs of infections, in a part because there are a limited number of ways that mucous membrane lining the upper respiratory tract respond to and express infections. Tonsillar or pharyngeal exudate is a good example of a sign of upper respiratory tract infection (Hortal, 1991).

LOWER RESPIRATORY TRACT INFECTIONS

Most of the works carried out on the lower respiratory tract pathogens covered a limited range in Nigeria. Some of the few works carried out included the works of Fayinka (1977) and Aderela (1977). The few works carried out in the northern parts of the country covered much lesser range than those carried out in the south. And in some cases, are not documented.

TUBERCULOSIS

Tuberculosis has long been known to a be a major cause of morbidity and mortality throughout the world and has for past several decades been a neglected disease in both industrialized and developing countries. It is now attracting a renewed interest, and significant efforts to revive control activities are currently underway (Kochi, 1991). This is occurring largely because of the increased incidence of tuberculosis in many Human Immunodeficiency virus endemic countries (Bloch 1989 and Styble, 1990), the availability of and proven effectiveness of short course chemotherapy (Iseman, 1991), and the realization that tuberculosis control is one of the most cost effective health interventions in developing countries (Murray, 1991).

Infection with Mycobacterium tuberculosis occurs primarily through inhalation of droplet nuclei. Sputum, coughing sprays and droplets released by the sneezing of infected persons are common source of infection. So also contaminated dust during harmattan and raining seasons in Nigeria can spread the infection.

The overall tuberculosis situation in the world in 1990 and its recent trend as reviewed by the world health organization indicated that approximately one third of the world's population is infected with mycobacterium tuberculosis (Sudre et al. 1992).

Tuberculosis long known to be a major cause of morbidity and motality throughout the world has for the past decade been a neglected disease in both industrialized and developing countries. There is however efforts to revive control activities because of the increased incidence of tuberculosis in many Human immunodeficient virus (HIV) endemic countries, and also because tuberculosis is one of the most cost effective health interventions in developing countries (Styllo, 1990).

Of the one third of the world's populations infected with tuberculosis, the prevalence is highest in the western pacific region and lowest in the Eastern mediterranean region. The majority of infected individuals live in the South-east Asian region, China and Europe. The age distributions of tuberculosis of tuberculosis infection in the sub-saharan Africa and Western Europe have the highest figures, with Africa having about one hundred and seventy one million 171/million) infected persons (Sudre et al, 1992).

Patterns observed in some of the works carried out indicated that the developing countries has the highest infected individuals below 50 years of age. But with the industrialized countries, the prevalence of infection is very low among the same group. In general there were approximately 78000 deaths reported annually between 1984 and 1989 throughout the world (Bulla,

The world view on tuberculosis reflects a similar pattern in Nigeria (at least in some states). Studies carried out in some eastern and southern states of the country in the past decade indicated that the incidence of tuberculosis was high (19%) than other diseases (Osoagbaka and Njoku-Obi, 1982). Problems that are faced in Nigeria is the lack of proper data collection and notification of cases. Careful analysis of notification can provide a good insight into the tuberculosis control activities and their trends. When most of the population has access to health care services (Questionable in Nigeria) and when case reporting is made mandatory then such objectives could be achieved so that the exact situation in Nigeria can be ascertain.

ANTHRAX

From 1948 world health organization has been promoting activities on Anthrax control and research. In the last few decades, a number of countries, particularly Europe, have reduced morbidity and mortality due to Anthrax in both humans and livestock to a negligible levels through international co-operation and support (WHO Memorandum, 1994). However, many countries are still endemic today for anthrax in both human and animal populations including wild life, and the disease has a great public health, environmental and socioeconomic impact in Africa and Asia. In view of these reasons, the world health organization formed a working group experts for anthrax control and research comprising four sub-groups, epidemiology and information exchange, disinfection and decontamination, vaccines and vaccine development and consultative group for problems in developing countries (WHO memorandum, 1994). However, certain

problems are encountered as usual and this include among others (1) lack of co-operation over vaccination by farmers, (2) delays in diagnosis because regional laboratories lack facilities for confirming the diagnosis of anthrax and (3) finding problems for vaccination and diagnostic programmes (WHO Memorandum, 1994).

In Africa, many people have died of Anthrax, often after consuming infected meat or handling infected carcasses. Drying is a common method of meat of preservation in Africa, and in this form the meat can be readily transported over long distance and kept for a long time. This is however a potential means of spreading anthrax spores if the meat is delivered from an infected animal. In general however, Anthrax remain endemic in many parts of Africa despite efforts aimed at controlling it (WHO Memoranda, 1994).

Most of the works done in the respiratory tract disease are from the monsoon regions of the world and not in the topics. And some of the diseases were assumed (not proved) to be rare in Africa due to lack of proper investigations carried out and due to lack of adequate facilities. Sometimes the cases are not reported.

The aim of this research therefore is to further investigate the occurrence of these respiratory tract pathogens in Minna (one of the northern states) with the view to improving the diagnostic procedures in our hospitals which will inturn enhance the treatment pattern. This is because few cases have been documented in the north and the ease with which the organisms are transmitted and contracted calls for greater concern. Added to these is the fact that most of our hospitals have reverted to assumptive diagnosis due to economic constraints and lack of facilities.

CHAPTER TWO

MATERIALS AND METHOD

Methods

The methods employed for the collection and analysis of samples was in accordance to the procedures Fawole and Oso (1988) and Monica (1984).

1. SAMPLE COLLECTION

The samples collected was a Sputa from patients suffering from either the upper or lower respiratory tract infection in Minna town. The patients were instructed against washing their months before coughing out the sputum. Four Health Centres were visited and in each health centre, a number of patients were screened as 1-General Hospital Minna (100), 2-Taimako Clinics (30), Bisi Clinics (20) and the Clinics in the University (F.U.T. Minna) (10). A total of one hundred and sixty (160) patients were screened. The analysis of the samples was carried out in the Federal University of Technology Minna laboratory in the Department of Biological Sciences.

2. SAMPLE ANALYSIS

The sample analysis was carried out in accordance to the procedures of Monica (1984) as follows:

2.1 MACROSCOPY

This involves the physical examination of the sputa to show whether they are (1) purulent - i.e. green looking containing mostly pus, (2) mucopurulent - i.e. green looking containing pus and mucus, (3) Mucoid - i.e. mostly mucus and (4) mucosalivary - i.e. mucus with small amounts of saliva. When the sputum contains blood, it is reported as bloody.

2.2 MICROSCOPY

This involves the microscopic examination of the sputum on stained or wet preparations. Stained preparations done are the gram stain, the Ziel Neelsen stain and Giemsa stain preparations in accordance to the procedures of Fawole and Oso (1988) and Monica (1984).

2.3 ADDITIONAL PREPARATIONS

The additional preparation done was the potassium hydroxide solution (KOH) in order to detect any fungi present. This was carried out according to the procedures of Monica (1984).

3.0 CULTURE

3.1 Media Preparation

Blood and chocolate agar were prepared according to the directions of the manufacturers. Blood was obtained from the Blood bank in the hospital in Minna. The Blood agar was prepared by adding blood to the blood agar base medium after cooling from autoclaving to about 40°-45°C. The chocolate agar was prepared by further heating the blood agar to about 60°C.

3.2 Inoculation

The sputa samples were inoculated using the procedures of Monica (1984). The blood agar plates was incubated aerobically and the chocolate agar plates were incubated in an atmosphere with 10% carbon dioxide (CO_2). The plates were incubated for 24 hours at 37°C. When there was no growth, the plates are further allowed to remain in the incubator for another 24 hours after which they were considered no growth and then discarded.

Actively growing cultures (24 hours old) of the bacteria on Blood agar and chocolate agar media were screened for colonies

that could be any of the suspected pathogens in accordance to the procedures of Osogbaka and Njoku-Obi, (1992) and Monica (1984). These were again gram stained and isolated for purification.

4.0 CHARACTERIZATION OF THE BACTERIA

The purified colories were put on slants for Biochemical reactions in order to know the particular species of the suspected colonies. For the Gram negative rods suspected to be members of the entrobacteriacease, the following tests according to cruickshank et al, (1957) were carried out: Aesculin hydrolysis, citrate utilization, indole test, methyl red test, motility test, oxidase test, Triple sugar (TSI) and Urease test.

For the gram positive cocci, catalase, coagulase, DNase mannitol, Hemolysis and Gelatin test were carried out. For the gram positive rods, test carried out included motility, Hemolysis, morphology, glucose, lactose and sucrose utilization; Cruickshank et al; (1957), Monica (1984) and Norris and Ribbons (1979).

4.1 Aesculin hydrolysis

The test organism was grown in aesculin agar for 24 to 48 hours. A positive test is indicated by the development of a dark to black colouration of the whole medium. A negative test is indicated by lack of colour change.

4.2 Citrate utilization

A simon citrate agar slants was inoculated with the test organism in a b'jou bottle for 24 to 72 hours at 37°C. The development of a deep blue colour indicates a positive test.

4.3 Indole test

The test organism was grown in 5ml peptone water for 24 hours. Kovacs reagent was added dropwise. This was then agitated gently. A positive reaction is indicated by the development of a red colour in the reagent layer above the broth within on minute. A negative test is indicated by the retention of the yellow colour of the indole reagent.

4.4 Methyl red test

5 ml of methyl red broth was inoculated with the test organism and incubated for 48-72 hours at 37°C. 1 ml of the broth was transfered to a small serological tube. 2 to 3 drops of methyl red solution was added to this small quantity of broth. A red colour on the addition of the indicator signifies a positive methyl red test. A yellow colour signifies a negative test.

4.5 Motility test

The motility medium was inoculated by making a fine stab with a straight wire to a depth of about 1-2 cm short of the bottom of the tube. This was incubated at 37°C for 24-48 hours. If the organism is non motile, the line of inoculation would be sharply defined and the rest of the medium would look clowdly.

4.6 Oxidase

A piece of filter paper was soaked in oxidase reagent. A colony of the test organism was smeared on the soaked paper. If the organism is oxidase producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour. No change in

the colour of the filter paper indicates a negative test.

4.7 Triple sugar Iron agar (TSI)

This is a composite media, hence several reactions can be read after 24 hours of incubation at 37°C. It is usually done to test the ability of the organism to ferment Glucose, lactose, and sucrose or all the three sugars. It can also be used to detect for Gas (H_2S).

A straight wire was used to obtain growth from either a culture plate or broth. The bottom of the slant was stabbed 2 to 3 times. The surface of the slant was streaked. This was capped loosely and incubated at 37°C for 24 hours.

4.8 Gas detection:

This was determined by the appearance of one or several bubbles in the butt. Vigorous gas formation was indicated by cracks. Formation of hydrogen sulphide (H_2S) was determined by the blackening of the whole butt or a ring of blackening at the slant-butt junction.

4.9 Sugar fermentation:

This was indicated by the butt becoming yellow if only Glucose is fermented and no other sugar. The reaction is read as K/A (alkaline/Acid). If Gas was produced than the reaction is read as K/AG.

If either sugars are fermented in addition to glucose i.e. lactose or sucrose or both, the butt and the slant would become yellow i.e. acidic. The reaction is red as A/A (Acid/Acid).

5.0 Urease test

Urea agar slant was inoculated with the test organism in a b'jou bottle and incubated for 24 to 72 hours. The development of a bright pink coloration or red colour indicates a positive test.

Other tests for the non-enterobacteriaceae are:

5.1 Coagulase test

Slide test - A drop of physiological saline was placed on each ends of the slide. A colony of the test organism was emulsified in each drop. A drop of human plasma was added to one of the suspension and was mixed gently. The clumping of the cells within ten (10) seconds was observed.

5.2 Bile solubility:

A thick bacterial suspension was made in 1 ml physiological saline contained in a clean serological tube. 0.5 ml of 10% sodium deoxycholate solution was added to the tube and incubated at 37°C. A positive test was indicated by the clearing of the suspension within 30 minutes. No clearing indicates a negative test.

5.3 DNAse test

The Deoxyribonuclease (DNAse) agar plate was divided into four sections. Using a small portion of growth from solid medium, a short streak was made in the middle of one section. The plate was incubated at 37°C for 24 to 48 hours. The plate was flooded with 10% Hydrochloric acid HCl). A positive test is indicated by a distinct zone of clearing around the streak. No clearing indicates a negative test (Norris and Ribbons, 1979).

5.4 Mannitol utilization

The suspected organisms were subcultured on mannitol salt agar and incubated at 37°C for 24 to 48 hours. Organisms capable of utilizing mannitol under high level of salt concentration have been observed to grow.

5.5 Gelatin liquefaction

The Nutrient gelatin medium tubes kept in the refrigerator were inculated with an 18-24 hours old culture using a straight wire to a depth of One (1) inch. A control tube having no organism was used. This was incubated at 37°C for 24 hours to 14 days. To read the tubes, they were first placed in a refrigerator for 2 hours before tilting to check for lignefaction. (Norris and Ribbons, 1979).

The following organisms were used as reference in all the test except few. These are:-

Staphylococens aureus, sheptococcus pyogenes, Coryrebacte rium diphtheriae, Klebsiella preumoniae and Psendomonas aeruginosa. They were obtained from the Department of Microbiology in the Federal University of Technology, Minna.

CHAPTER III

RESULTS

A total of one hundred and sixty (160) patients from four health centres in Minna town were screened for any pathogen responsible for the respiratory tract infections. The number of males and females obtained from each health centre is given on table 1 below:

TABLE 1: Male and Female cases surveyed from different Health Centres.

Hospital/Clinic	Male	Female	Total
General	62	38	100
Taimako	22	08	30
Bisi	08	12	20
Campus	07	03	10
Total Overall	99	61	160

The highest number of patients screened was from the General Hospital (100), followed by Taimako Clinics (30) and Bisi Clinics (20). The smallest number of patients screened was from the clinics in the campus (10) of the Federal University of Technology Minna.

MACROSCOPY

The physical examination of the sputa samples obtained fall under the following categories: Purulent (28), mucopurulent (40), mucoid (52), mucosalivary (35) and Bloody (5). The results as obtained from the different health centres are shown on table II.

TABLE II: Categories of Sputum from the four Health Centres.

Characteristic of Sputum	General Hospital	Taimako	Bisi	Campus
Purulent	18	06	4	0
Musopurulent	27	2	10	1
Mucoid	33	13	2	4
Mucosalivary	20	7	3	5
Bloody	2	2	1	0

MICROSCOPY

Stained preparation included the Gram stain, Giemsa stain and Ziehl Neelsen stain preparations. An additional wet preparation of potassium hydroxide (KOH) to detect for fungi was done also. of all the stained preparations, only Gram staining preparations yielded results. There was no Acid fast bacilli seen, so also no any protozoa or fungi was observed. The results of the Gram staining preparations is shown on table III.

TABLE III: Gram Reaction of the Isolated Pathogens.

Cell Shape/Gram Reaction	Gram Positive	Gram Negative
Rods	38	30
Cocci	92	0
Total	130	30

Out of the one hundred and sixty (160) isolates gram stained, one hundred and thirty (130) were gram positive and the remaining thirty (3) were gram negative.

CULTURE

The pattern of growth of the bacteria in the two media used

(Blood and chocolate agar) was the same. The following are the suspected organisms encountered: Staphyloccus aureus, Klebsiella pneumoinae, Corynebacterium diphtheriae, Streptococcus pyogenes, Bacillus anthracis, Pseudomonas aeruginosa, Serratia spp and S Pneumoniae.

CHARACTERIZATION

In all, three categories of bacteria were obtained as gram positive rods, gram positive cocci. The results of the Biochemical reaction and the confirmed organisms are given on table IV.

In general six (6) pathogens have been isolated as: Streptococcus pyogenes, Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphlococcus aureus, Bacillus anthracis and Corynebalerium diphtheriae. The incidence of the most common pathogen from the four centres visited is shown on table V. The order of occurrence in decending order is S aureus 48.8% (78) and C diphtheriae 20% (32) predominated. Others rankwise are K pneumoniae 11.3% (18), St pyogenes 8.8% (14), pseudomonas aeruginosa 7.5% (12) and B anthracis 3.8% (6).

Plate 1. Staphylococcus aureus growth on mannitol salt agar after 24 hours of incubation at 37°c.

Plate 1.1 Staphylococcus species showing hemolysis on blood agar after 24 hours of incubation at 37°C.

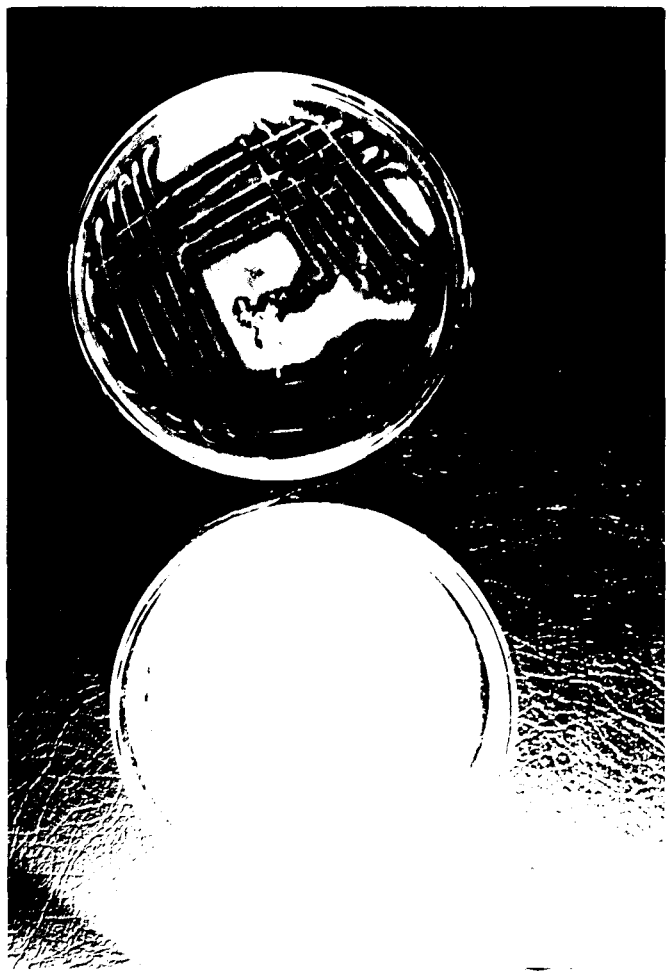

TABLE IV. Confirmatory Biochemical Tests for the Isolated Pathogens.

Test	1	2	3	4	5	6
Morphology	Cocci	Rod	Rod	Cocci	Rod	Rod [r]
Gram reaction	+	-	-	+	+	+ [c]
Oxidase	-	-	+	NA	-	+
Acid from Glucose	+	+	-	NA	+	NA
Gas from Glucose	+	-	NA	+	-	"
Acid from Sucrose	+	NA	"	+	-	"
H_2S	-	-	"	-	-	"
Acid from lactose	+	+	-	-	-	-
Motility	-	+	-	-	-	-
Indole	-	+	NA	NA	NA	NA
Citrate	NA	+	+	"	"	"
Aesculin	"	+	-	d	-	"
Urease	+	+	±			
Methyl red	NA	-	-	NA	-	NA
Gelatin	"	±	+	"	-	"
Catalase	+	NA	NA	-	+	"
Bile solubility	NA	NA	NA	NA	NA	"
Coagulase	+	"	"	"	"	"
Mannitol	+	"	"	"	"	"
Hemolysis	B	"	"	"	"	"
DNASe	NA	"	"	"	"	"

d-differential
c- Capsule
r=colony is rough and flat with many comma shaped outgrowth.

1 = S aureus
2 = K Pneumoiae
3 = P aeruginosa
4 = Str Pyogenes
5 = C diphtherine
6 = B anthracis

+ = Positve
- = Negative
± = Pos/Neg.
NA = Not applicable.

Plate 2.0 Reactions of some isolates on Triple sugar iron agar media after 24 hours of incubation at 37°C.

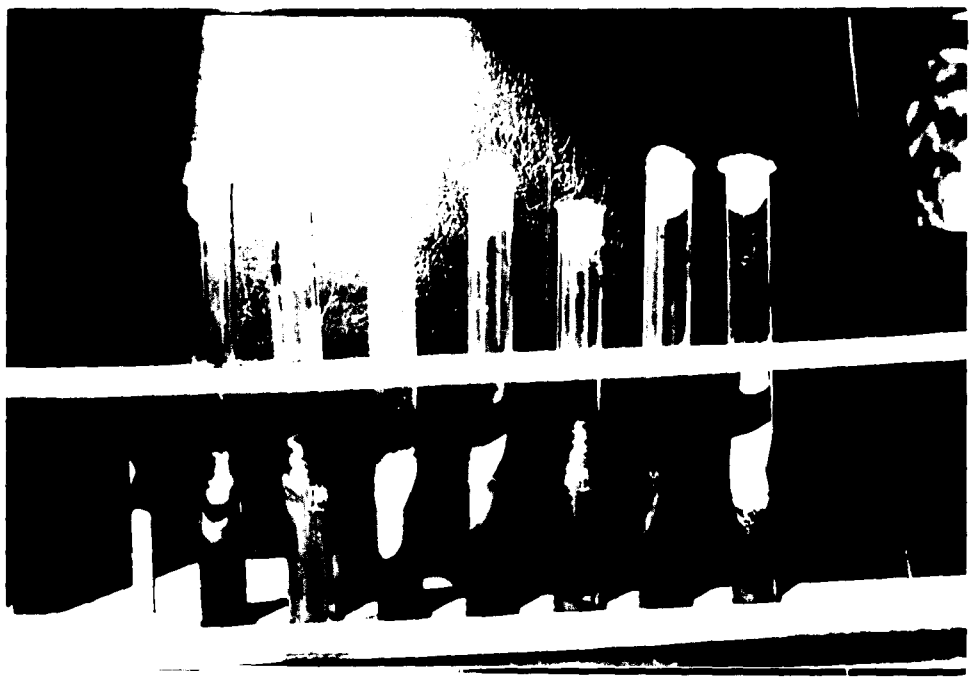

TABLE V: incidence of the most common Pathogens Isolated from the four Health Centres in Minna.

Pathogen	No.of Isolates	No.of Isolates Male	Occurring in Female	% of Total (N=160)
Bacillus anthracis	6	5	1	3.8
Coryaebacterium diphtheriae	32	18	14	20.0
Klebsiella pneumoniae	18	12	6	11.3
Staphylococcus aureus	78	47	31	48.8
Pseudomonas aeruginosa	12	9	3	7.5
Streptococcus pyogenes	14	8	6	8.8

Similar trend of the incidence of the most common pathogens isolated from the individual health centres is given on tables VI (from the General Hospital Minna), VII (from the Taimako Clinics), VIII (from Bisi Clinics) and IX (from the Clinics in the Federal University of Technology Minna).

In all the cases, S aureus predominated followed by C diphtheriae. Others that followed are K pneumoniae, Str pyogenes, P aeruginosa and B anthracis in decending order.

Plate 3.0 *Pseudomonas aeruginosa* growth on blood agar. Typical pigment formation showing.

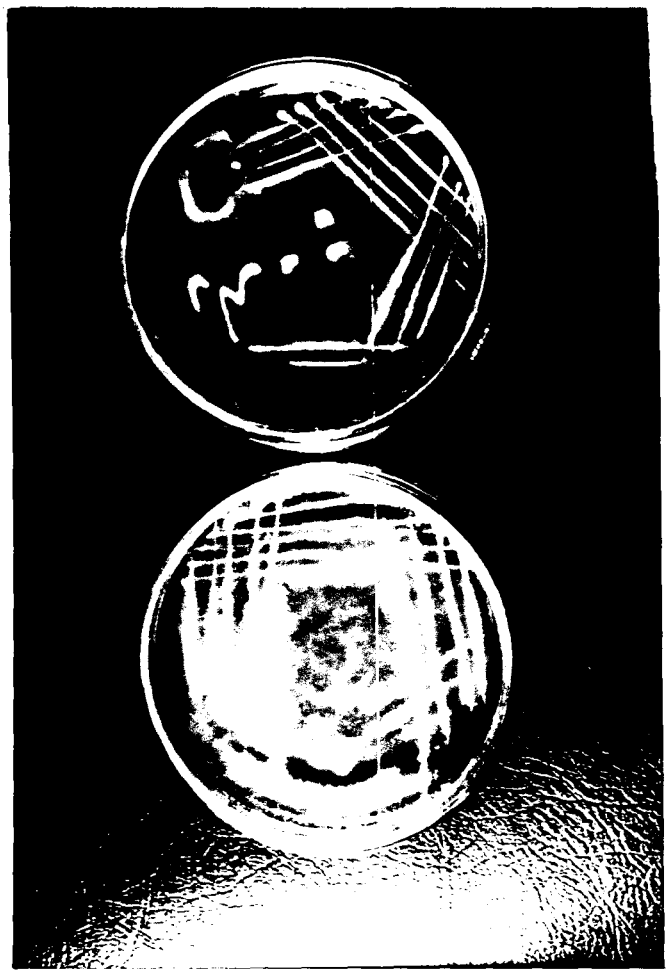

TABLE VI: Incidence of the Pathogens Isolated in General Hospital.

Pathogen	No.of Isolate	Patients Male	Female	Total (N=100)
B anthracis	3	3	0	3
C diphtheriae	22	12	10	22
K Pneumoniae	13	9	4	13
S aureus	47	29	18	47
P aeuginosa	11	8	3	11
Str. pyogenes	4	8	3	4

TABLE VII: Incidence of the Pathogens Isolated from Taimako Clinic.

Pathogen	No.of Isolates	Patients Male	Female	% Total (N=30)
B anthracis	1	0	1	3.3
K pneumoniae	5	3	2	16.7
S aureus	18	14	4	60.0
Str.pyogenes	6	5	1	20.0

Plate 4.0 Bacillus and Streptococcus species on blood and chocolate media. Typical mixed cultures showing.

Plate 4.1 Mixed cultures of Klebsiella and Bacillus species on the left, and mixed cultures of Pseudomonas and Streptococcus species on the right.

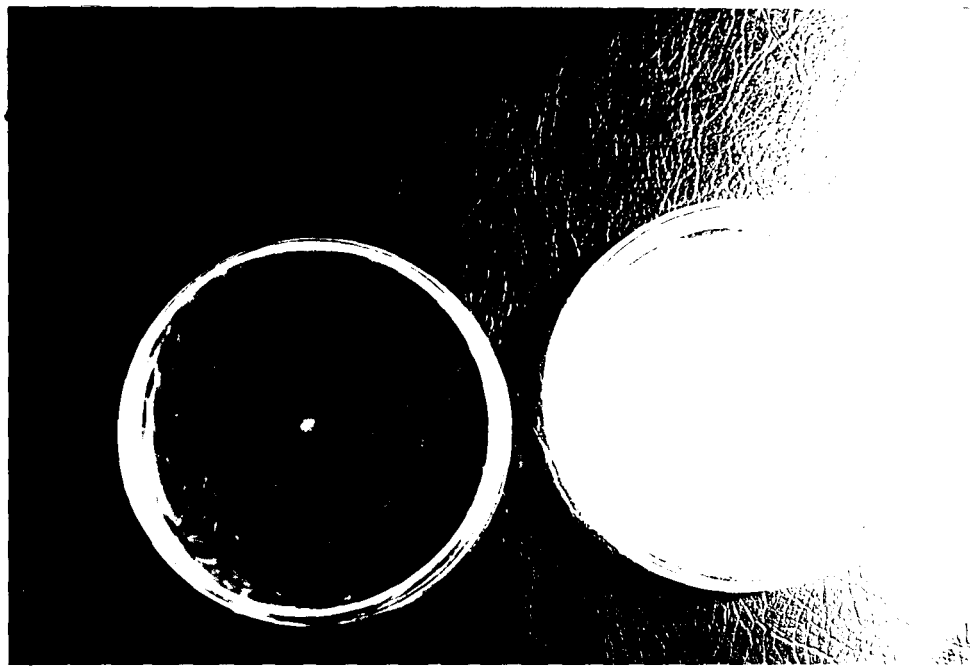

TABLE VIII: Incidence of the Isolated Pathogens in Bisi Clinics

Pathogen	No.of Isolates	Patients Male	Female	% Total (N=20)
B anthracis	2	2	0	10
C diphtheriae	6	2	4	30
S aureus	8	2	4	40
P aeruginosa	1	1	0	5
St.pyogenes	3	1	2	15

TABLE IX: Incidence of the Isolated Pathogens from the Campus Clinics.

Pathogen	No.of Isolates	Patient Male	Female	% Total (N=10)
S aureus	5	2	3	50
St.pyogenes	1	1	0	10

CHAPTER IV

DISCUSSIONS

The incidence of the pathogens, isolated from different clinics showed that Staphylococcus aureus has the highest occurance rate of 48.8%. This is in line with the findings of Lynne and Bernard (1991) in their investigations of 45 centres from eight African countries including Nigeria in which 2,888 bacterial isolates were obtained from patients with community acquired infections. They found that S aureus predominated with a rate of 29% (837) of the 2,888. This implies that infections due to S aureus is on the increase in most of the communities in Africa. This may be possible as a result of the unique position S aureus occupies among the microbial pathogens. It lies in close association with man and have achieved extensive colonization of man, particularly of the nasopharynx and skin (Hauge, 1980). In children, colonization with S aureus is a predictable event occurring in almost 90% of infants during the neometal period and mainly in the nostrils and on the umblical stump (Cerati, 1990). Another reason may be attributed to the resistance of S aureus to most of the drugs used in treatment which only exerts temporary activity on the organism. This also corresponds with the works of Lynne and Berrnard (1991) in which the isolates obtained from Nigeria showed 90%-98% resistance to penicillin and 89% to Ampicillin. This may simply mean that drugs used in Nigeria have either been adulterated or there is indiscriminate use of antibiotics which impart resistance to the organism and hence high rate of occurrence.

The incidence rate of Streptococcus pyogenes showed 8.8% out of the cases suspected to be due to the S pyogenes. This indicates that infections of the respiratory tract due to S pyogenes has not gone beyond the management level. The very low number might even be as a result of contamination with this organism since several species of them have their habitat in the upper respiratory tract of man. So also the severity of Streptococal infections has become steadilymarkedly reduced in many countries in the past century. The strongest evidence in support of this statement comes from the mortality rates for scarlet fever which has long been a notifiable disease have fallen approximately from 1000 per million of population in the decade 1860-1869 to virtually zero at the present time (Mackie and Cartney, 1978). This corresponds with the report of Dudding (1986) who reported that the management of Streptococcal upper respiratory tract infections are relatively simple because only a throat culture is required to rule out the possibility of whether the patient has a streptococcal or non-streptococcal infections. Generally two antibiotics have been recommended by the American Academy of pediatrics, i.e. penicillin and erythromycins.

The incidence rat of Pseudomonas aeruginosa showed 7.5% out of the 160 patients screened. This organisms is not known to be a normal flora of man; but can infect almost any external site or organ in the body (Eugene et al, 1983). This ability may be responsible for its getting into the respiratory tract. P aeruginosa prolonged carriage is often associated with the emergence of the mucoid form and further pulmonary

deteriorations. Acquisition of P aeruginosa in patients in hospital is very rapid and transmission may occur directly of indirectly via the hands of medical staff or contaminated apparatus. Hence this may mean that the infection is primarily, but is present as a secondary invader (Flenly, 1981). Pneumonia due to P aeruginosa presents a radiological pattern like staphylococcal pneumonia, often being a hospital acquired infection in patients receiving multiple antibiotic therapy or during mechanical ventilation through a tracheostomy tube where the organism may be acquired from contaminated humidifiers. Patients with impaired polymorphonuclear leucocyte responses are particularly at risk.

The findings of Klebsiella pneumoniae in this work showed an incidence rate of 11.3% out of the 160 patients. Unlike P aeruginosa, K pneumoniae is found in the respiratory tract and feces of 5 - 10% of healthy individuals and is frequently present as a secondary invader in lungs of patients with chronic pulmonary disease. However, the invasive properties of this organism depends on the antiphagocytic effects of its capsule. The destructive action of the unphagocytosed organism on the pulmonary tissues (abscess formation and necrosis) interferes with antimicrobial therapy. This often results in chronic lung abscess requiring surgical operations (Weatherall et al, 1983). Other Klebsiella species have been implicated in chronic inflamatory diseases of the upper respiratory tract. K ozaenae has been associated with ozena a progressive fatid atropy of the nasal mucosa. So also a species called as K rhinoscleromatis was found in rhinoscleroma, a destructive granuloma of the nose and

pharynx (Weatherall *et al*, 1983).

Bacillus anthracis has the occurrence rate of 3.8% out of the 160 patients screened. Anthrax is a zoonosis i.e. a disease of animals transmissible secondarily to man. The annual incidence of human anthrax throughout the world was not known accurately due to a large number of cases not being notified, but probably is between 20,000 and 100,000 cases mostly in rural areas. The world health organization reported that anthrax remains a human and animal health problem in Africa, and causes a significant economic looses, and that the true incidence and effect of the disease in many African countries is uncertain. But in many developed nations where strong action is taken to prevent the disease, anthrax has almost been eradicated (WHO Memorandum, 1994). Biting insects may contribute to the transmission of anthrax among wild and domestic animals and this implies that when many has been bitten by such insects, the possibility of the disease transmission is certain (WHO Memorandum, 1994). Since this is not a normal flora of the man's respiratory tract, symptoms due to this organism which simulate other known normal flora may confuse diagnosis in our hospitals.

The incidence rate for *Corynebacterium diphtheriae* was 20%. This is next in occurrence to the *S aureus*. This organism is a commonsal in the oropharynx and on the skin of man. This implies that its presence in sputum samples from one man is not enough to confirm the respiratory tract disease suffered by the patient was due to *C diphtheriae*. But in such cases where large number of patients reporting to the hospital are found to have this organism, then confirmation can be made (Report, 1980).

From the above explanations, one can readily agree that due to so many symptoms that simulate one another between the isolated organisms, and also the presence of some in both the healthy and the diseased states, there is the possibilities of the following:

(a) Misdiagnosis - In this case, the true pathogen may be mistaken or confused for another entirely different organism especially when new organisms are encountered that are not known to be the normal flora. Or it may be dismissed on the ground of a contaminant.

(b) Improper diagnosis - This may result due to too much pressures from the doctors and patients on the laboratory staff seeking results for a particular test carried out on them. This leads to overworking of the laboratory staff. This is more so when one looks at the present state of our laboratories in the hospitals throughout Nigeria and some African countries, except of course very few, where diagnosis were based on presumptive and not confirmatory analysis due to inadequate facilities as a result of the economic situations.

(c) Resistance - Some of the organisms are not thoroughly screened for resistance. This can be dangerous because certain organisms like P aeruginosa and staphylococcus species are now known to be multi-resistant to drugs (Richmond et al, 1964 and Richmond, 1969). Thus this may lead to repeated diagnosis and treatments which may not yield a better result at the end.

With all these problems, there is the need to adopt the following in our hospitals or laboratories:

i. Proper sample collection: Doctors should assist where swabs are needed for throat culture by obtaining the specimen themselves. Patients should be educated on why such specimen are sought from them. This is because in some quarters, patients may not present the true specimen but may instead fetch from their children or wives for fear of occulticism. The laboratories should endeavour to sterlize containers for sputum collection before giving it to the patient.

ii. Sample analysis: This should include analysis to investigate bacteria, viruses, protozoa and fungi that may be responsible for any of the prevailing symptoms. Other cultural media for organisms like Mycobacterium tuberculosis, Mycoplasma and some protozoa should be forced as part of the normal routine in our hospitals.

iii. Biochemical characterization: This is very important because without it most of the species cannot be pinpointed. This should also extend up to sensitivity tests and serology.

iv. Time: At least there should be a minimum of three days before reports are released from the laboratories back to the doctors. This will allow for culture, biochemicals, sensitivity and if possible serology.

CONTROL MEASURES

The control of human anthrax depends on the control of the disease in animals, and suspected cases of an anthrax should be treated and dead carcasses be buried deeply to prevent the spread to new pastures. Among the measures adopted by health agencies

to protect susceptible individuals are the large scale immunization programmes; the quarantine of carriers and the daily use of appropriate antibiotics may help prevent transmission of the disease until their systems are free (WHO Memorandum, 1994).

Control measures for streptococcus sore throat include pasteurization of milk, isolation of infected persons and proper disinfection or disposal of objects contaminated by the discharges of infected persons. Such patients should not be allowed to come into contact with products for consumption during either the acute or convalescent stage of the disease.

There are three guidelines to control infections due to P aeruginosa as follows:

a- Patients under high risk category should not be admitted to a ward were other infections existed but should be isolated.

b- The period of isolation of such patients should last until the infection has been eradicated.

c- A general requirements demands that all cases of hospital acquired infections can be eradicated by sterilizing instruments, dressing and so on.

Similar measures for S pyogenes can be applied to C diphtherise. But Quarantine measures are not necessary for Staphylococcus and Klebsiella species.

CONCLUSION

In general six organisms have been isolated from 160 patients that reported to four different health centres in Minna. Four of these organisms Staphylococcus aureus, Klebsiella pneumoniae, Staeptococcus pyogenes and Corynebacterium

diphtheriae, are known to either directly or indirectly be in close association with man, and they form the normal flora in man's respiratory tract. The bacteria discovered that are not the normal flora in this research are the Bacillus anthraces and Pseudomonas aeruginosa. Considering the high rate of occurrence, S aureus predominated (48.8%) followed by C diphtheriae (20%). Others are K pneumoniae (11.3%), Str pyogenes (8.8%), P aeruginosa (7.5%) and B anthracis (3.8%). Since other organisms that are not known to be pathogens of the respiratory tract are discovered, this may actually confirm misdianosis in our hospitals and the persistance nature of some respiratory tract infections in patients.

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