THE POSSIBLE MICROBIAL AETHIOLOGY OF PYODERMA AMONG PRIMARY SCHOOL CHILDREN IN THE ASHANTI REGION OF GHANA

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BY

PHYLLIS VICTORIA AKOTO

SCHOOL OF MEDICAL SCIENCES

DEPARTMENT OF CLINICAL MICROBIOLOGY

UNIVERSITY OF SCIENCE AND TECHNOLOGY

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DR MRS CECILIA BENTSI
EXTERNAL EXAMINER

PROF. DR P.A.K. ADDY
SUPERVISOR

DR OHENE ADJEI
HEAD, DEPARTMENT OF CLINICAL MICROBIOLOGY

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PREFACE

Disease is known to be a departure from normal health through structural or functional disorders of the body. It often brings grave discomfort to the sufferer and this has caused man to find ways of either preventing its onset or curing it whenever it strikes.

One such disease is Pyoderma, a dermatological ailment which is also antecedent to a more debilitating renal disorder called acute glomerulonephritis. Children, particularly those in the tropical regions, are the most vulnerable or at high risk.

The essence of this thesis is to document the aetiologic agents of Pyoderma and to suggest a possible regimen of therapy for it. Again, it is to inform the public, including policy makers, of the conditions predisposing to the incidence and prevalence of pyoderma in order that necessary action may be taken to prevent its occurrence..

ABSTRACT

The possible aetiologic agents of pyoderma were studied among 310 primary school children (aged 5-14 years) drawn from two study areas, rural (Ejisu-Juaben District) and urban (Kumasi district) settings in the Ashanti Region of Ghana.

A total of 634 bacterial species was recovered. β-haemolytic Streptococci gave the highest recovery rate of 45%. Of this, 214 or 74% were identified as Streptococcus pyogenes with the remaining, 25% (or 71) representing Lancefield groups B, C, D and G. This was closely followed up by Staphylococcus aureus 32.6% (207), Staphylococcus epidermidis 10.3% (65), Pseudomonas species 10.3% (65), Proteus mirabilis 2.2% (7) and Escherichia coli 0.3% (2).

The isolation of the Pseudomonads particularly P. aeruginosa from typical pyoderma lesions was quite thought-provoking due to its innate resistance to most antimicrobial agents. Penicillin G (2mg) was effective only against the isolated species of Streptococci but ineffective against the remaining bacterial species isolated. Gentamycin (10mg), on the other hand, was the only antimicrobial agent with a 100% activity on all isolates including P. aeruginosa. The rest of the antibiotics tested on the bacterial isolates were susceptible to varying degrees.

1.0 INTRODUCTION

Pyoderma is an ubiquitous skin condition, found all over the world but is commonly associated with tropical climates which are characterized by warm, humid and sometimes dry conditions.

The clinical condition is a skin defect which may be described as a vesicular skin lesion which is often covered by a thick brownish or amber coloured crust, representing a seroexudate. Pyoderma also includes all skin infections which have resulted from secondary invasion, by Beta-haemolytic Streptococci of already existing lesions such as burns, traumatic or surgical wounds as well as scabies and similar skin lesions (Earle, 1985).

According to Belcher et al., (1977), pyoderma is a common health problem among ambulatory patients. It occurs most commonly in children of preschool age (Anthony et al., 1967; Wannamaker, 1970; Earle, 1985) and of school age (Losch et al., 1971; Allen & Taplin, 1974). This distribution pattern may be attributed to the fact that, children injure themselves more frequently with such injuries becoming contaminated with bacteria during play. Generally, children are most vulnerable to many diseases and skin infections are no exception.

Apart from children, pyoderma is also found to be common in heavy manual workers, farmers, labourers (Belcher et al., 1977; Parry, 1984) and persons associated with injury-prone activities like football, athletics (Glezen et al., 1973) and military combat (Allen et al., 1971).

For the past two decades much attention has been given to pyoderma in many temperate and tropical countries. Studies have shown that pyoderma is antecedent to acute glomerulonephritis (Anthony et al., 1967; Parker, 1969; Cruickshank et al., 1975). Several reports have also indicated that outbreaks of acute glomerulonephritis (Potter et al., 1968; Whittle et al., 1973) and in some cases chronic renal diseases (Baldwin et al., 1974) were consequent upon episodes of pyoderma.

Pyoderma is endemic in Ghana and it is rated number five after the most frequent parasitic and bacterial infections, viz., malaria, respiratory infections, diarrhoea and intestinal parasitic infections (Belcher et al., 1977). However, there is very little information about the characteristics of pyoderma and there is a paucity of reported epidemics (if any) of glomerulonephritis consequent upon β-haemolytic streptococcal infection in Ghana. Reports on microorganisms (for that matter specific β-haemolytic streptococcal strains) involved in cases of pyoderma in Ghana are also scanty.

It is in this light, therefore, that this study was conceived with the following objectives:

(a) To investigate microorganisms prevalent in lesions of pyoderma among Ghanaian children (five through fourteen years).

(b) To determine the association between the isolated organisms and pyoderma.

(c) To determine the relationship between socio-economic factors, hygienic habits and the prevalence of pyoderma among the study population.

It is envisaged that the results of this study will enlighten the entire population, especially policy makers and scientific research workers about how much at risk the infected group is to the sequelae of β-haemolytic streptococcal infection. The results will also form a basis for further research into the incidence and prevalence of glomerulonephritis in the Ghanaian population.

Again, the knowledge of the antigenic types of β-haemolytic Streptococci which are associated with pyoderma in Ghana will facilitate and promote proper application of chemotherapy in the absence of laboratory facilities. Finally, the findings of this work may serve as a basis for the promulgation of control measures for pyoderma, the consequences of which may be reckoned in terms of morbidity and mortality and naturally in terms of lost man-hours on the national economy.

2.0 LITERATURE REVIEW

2.1 SKIN AND MICROORGANISMS

The skin which forms the outer covering of man is basically made up of two distinct layers - an inner dermis and a superficial epidermis. The skin serves as a protective covering for the delicate internal organs. Being the outermost covering, it is predisposed to various kinds of dermatological assaults, viz., biological, physical/mechanical and chemical.

The skin also serves as a reservior for a variety of micro-organisms. Fungi, viruses, bacteria and to a small extent parasites may be found on the skin surface. While some of these micro-organisms are pathogenic, others are harmless commensals on the healthy skin, but may occasionally act as opportunistic pathogens in persons with defective antimicrobial defenses or lowered host - resistance.

For purposes of this study, literature citations will be limited to only some bacterial skin infections with particular reference to causal agents of pyoderma.

Micro-organisms, which are responsible for a great majority of bacterial skin infections are β-haemolytic Streptococci, Staphylococci and Corynebacterium diphtheriae (Earle, 1985). Any of these micro-organisms may appear alone or in combination with others as primary causal agents or as secondary invaders.

2.2 STREPTOCOCCUS

Streptococci belong to the family Streptococcaceae which are morphologically spherical or oval in shape with a diameter of about 1μm. With respect to oxygen utilization most Streptococci are facultative anaerobes, but some species are obligate anaerobes while yet some other species are micro-aerophilic (Cruickshank et al., 1975).

The facultative anaerobic Streptococci are divided into two groups on the basis of their ability or inability to produce difusible haemolysin on freshly prepared blood agar.

(a) Those which produce difusible haemolysin:

This group of Streptococci produce a clear zone of haemolysis on blood agar. The type of haemolysis so produced is called Beta (β) haemolysis. Beta-haemolytic Streptococci are pathogenic and are of great interest to this study. They include most species which are incriminated in primary streptococcal infections in man and in animals.

They are further divided into a number of broad groups known as Lancefield groupings A, B, C, D, F, G. etc., the individual groups corresponding with group specific carbohydrate (also called C-antigen) present in their cell wall. Streptococci belonging to Lancefield Group A, otherwise known as Streptococcus pyogenes are the most signigicant streptococcal pathogen in man (Rotta, Kereselidze and Strasser, 1985).

Aside of the above groupings, strains that belong to Strept. pyogenes (Group A) may further be categorized by specific agglutinating or precipitating sera into groups according to their surface protein antigens eg. strains M, T and R (Cruickshank et al., 1975). This categorisation is known as Griffiths Groupings (serotypes). These surface proteins (M, T and R proteins) are produced on the fimbrae (the filamentous appendages) on the surface of the Streptococci. The most important of these is the M-protein, which is directly related to the virulence of the microorganism (Slade, 1985).

(b) Those which do not produce difusible haemolysins - are categorized into two major groups:

(i) Alpha (α) haemolytic Streptococci - this group partially lyses the red blood cells in the blood agar medium creating a partial zone of haemolysis around the colonies presenting as a greenish pigmentation on the blood agar, giving the type of haemolysis known as alpha (α) haemolysis. This type of haemolysis is exemplified by Strept. viridans.

(ii) Gamma (γ) haemolytic Streptococci - This category of Streptococci produce no haemolysis on blood agar and is described as non-haemolytic or gamma (γ) haemolytic Streptococci e.g Strept. faecalis.

2.3 CLINICAL INFECTIONS OF STREPTOCOCCUS PYOGENES AND SEQUELAE

As a normal flora, Strept. pyogenes can be found commonly inhabiting the skin surface, the throat and the nose. However, they become pathogenic when the integument of the sites they occupy become breached resulting in a variety of clinical situations. A few of such diseases are briefly discussed below.

2.3.1 Upper Respiratory Tract Infections

The most common and most typical infection caused by Strept. pyogenes is acute sore throat, and depending on whether or not the tonsils are maximally or little involved, determines the type of sore throat, viz., tonsilitis or pharyngitis, respectively. Cervical lymphadenitis may also result. If the infecting Streptococcus is erythrogenic and produces toxin in copious amounts, then the sore throat would be accompanied by a generalized punctate erythema or rash, a condition known as scarlet fever.

A delayed sequel of acute sore throat is rheumatic fever which may develop into rheumatic heart disease, often causing permanent damage to the heart and leading to death (Rotta et al., 1985).

The above mentioned infections are very common in cool temperate zones with peak incidence in Winter and in Spring (Earle, 1985), and children are the most affected group (Rotta et al., 1985).

2.3.2 Skin Infections

Streptococcus pyogenes may cause two types of skin infections, viz., erysipelas and streptococcal impetigo/pyoderma.

i. Erysipelas (St. Anthony's fire):

Is a skin condition which is often preceeded by upper respiratory infections. The condition is a spreading inflammation of the dermis, seen most commonly on the face, neck and the legs (Cruickshank et al., 1975). It is caused by haemolytic Streptoccoci, usually Group A and only rarely Group C.

The full blown lesion is characterized by red, brawny thickening of the skin. On palpation, the margins present a peculiarly hard induration while all of the diseased skin has rubbery consistency due to oedema and inflamatory infiltration (Earle, 1985). Erysipelas of the face usually begins near the external nares and may spread over both cheeks in a butterfly-winged distribution. This condition is common in older age groups.

(ii) Pyoderma:

(a) Impetigo contagiosa is a form of pyoderma characterized by multiple vesiculo - pustular or ulcerative skin lesions usually on the exposed parts of the body (legs, arms and face) (Dillon, 1967). The initial lesion of streptococcal impetigo is a tiny papule followed rapidly by the formation of a vessicle filled with serous fluid and a few leucocytes. The blister formed is surrounded by a narrow rim of erythema, which is flat and has a diameter of about 2 to 3 mm. The clear vesicular fluid usually yields a pure culture of Group A Streptococci. However, the vesicle is transient and rapidly becomes

pustular with the serous fluid infecting other skin areas by direct extension, to form new vesicles. These vesicles break up and the surface becomes covered by a thick hard and brownish crust.

The type of impetigo described above differs from staphylococcal impetigo in which latter case the primary lesion is bullous rather than vesicular. (Dillon 1967; Cruickshank et al., 1975).

Another form of pyoderma is the type where existing lesions become infected by haemolytic Streptococci, (Nsanzumuhire et al., 1972). Invasion of the skin usually follows some break in the integrity of the skin caused by minor trauma such as abrasions and insect bites. Scabies, eczema, herpetic lesions and exfoliative dermatitis can also become readily infected.

2.4 EPIDEMIOLOGY OF PYODERMA

The incidence of pyoderma is highly favoured by warm humid conditions as offered by tropical climates (Rotta et al., 1985; Brahmadathan and Koshi, 1988) as opposed to the low incidence encountered in colder areas of the world (Mhalu, 1973). In a study relating pyoderma to climate, Taplin et al., (1973) reported that the prevalence rates of pyoderma and the rate of recovery of the causal agents thereof were highest in the tropics, intermediate in temperate zones and lowest in the cooler climates. It has also been found that the peak incidence of pyoderma in persons living in temperate regions is in late summer and early fall (Earle, 1985) while in the tropics peak incidence is mainly towards the end of the rainy season (Whittle et al., 1973; Earle, 1985), at which time

children are most likely to experience minor trauma and insect bites predisposing them to secondary invasion by bacteria.

A second peak has been found to occur in the tropics particularly in the late dry season (Whittle et al., 1973; Belcher et al., 1977). This latter observation may be related to increased perspiration due to low humidity, shortage of water, reduced personal hygiene and increased farming activities with corresponding injuries.

Pyoderma occurs most often in early childhood with the highest occurence being amongst the two to five year olds (Cruickshank, 1976). However, in the tropics the highest incidence is encountered amongst the five - nine year olds (Belcher et al., 1977; Acheampong et al., 1988).

Children living in poor environments are also a target group. Taplin and his colleagues (1973) noted that the standard of personal hygiene could be another factor, for, the condition was found to have a lower prevalence among people who practice good hygiene than among those in similar environment but whose hygienic practices are poor.

Taking sex into consideration, boys have been found to be affected more than girls, (Whittle et al., 1973; Brahmadathan and Koshi, 1988).

Mode of Transmission

The normal bacterial flora of the normal skin as well as the infected skin play a very important role in the spread of streptococcal pyoderma. In a report by Dudding et al. (1970), it was shown that apart from skin lesions, appreciable numbers of Streptococci were recoverable from various sites of the body. These sites included the

throat, nose, wrist, ankle and back of the trunk. The wrists and ankles gave the highest isolation rate of Lancefield Group A Streptococci. Other sources of Group A Streptococci isolation were from dirt from finger nails, clothing and beddings as well as some household pets and insects.

Mechanisms of transmission of Streptococci are not very clear but some insects such as Hippelate flavipes also called the eye gnat (Taplin et al., 1973; Bassett, 1970), the common housefly, Musca domestica and flies of the genus Siphunculina (Nsanzumuhire et al., 1972) which are known to feed on sores have been implicated as transmitters of Streptococci and other human pathogens. Cross-infection may also come about by close contact between individuals and this is not uncommon in over-crowded habitats such as occurs in refugee camps, some hospitals, schools, orphanages, borstal homes and prisons (personal observation at the Nsawam prisons between 1981 and 1984).

2.5 ACUTE GLOMERULONEPHRITIS - A SEQUEL OF PYODERMA

The association of acute-glomerulonephritis with skin infection has long been recognized (Anthony et al., 1967; Parker, 1967; Kaplan et al, 1970; Cruickshank et al, 1975). Futcher (1940) and Dillon (1967) provided evidence to support this fact. They gave excellent summaries of the epidemiology of acute glomerulonephritis in various countries including the U.S.A., with special emphasis on the site of antecedent infection. Pertinent studies cited in these reviews are shown in Tables 1 and 2.

TABLE 1: REVIEW OF SKIN INFECTION AND NEPHRITIS - BY FUTCHER (1940)

Author	Year & Location	Total Cases	Cases Following skin infection	Types of Skin Infection
Kaumheimer	1912 Germany	223	9.4%	Impetigo
Volhard	1914 Germany	204	11.0%	Erysipelas
				"Miscellanous"
Hill	1991 U.S.A	75	5.0%	Impetigo
Osman	1925 England	235	4.7%	"Wounds"
Southby	1926 Australia	103	17.0%	Impetigo
Addis	1931 U.S.A	-	20.0%	"Wounds"
Litchwitz	1934 Germany	167	17.0%	Miscellanous
Sutton	1934 U.S.A	18	28.0%	Impetigo
Futcher	1940 U.S.A.	153	17.0%	Wounds, Impetigo and

TABLE 2: REVIEW OF SKIN INFECTION AND NEPHRITIS - BY DILLON (1967) (MAINLY CASES FROM THE U.S.A).

Author	Year & Location	Total Cases	Percentage of skin infections	Infection
McCullough	1951 Alabama	124	33%	Impetigo
Callaway	1951 N.Carolina	73	51%	Impetigo
Kleinman	1954 Minnesota	63	66%	Pyoderma
Blumberg	1962 Georgia	94	68%	Impetigo, Eczema

Furthermore, there have been many and in some cases recurrent epidermics of nephritis following skin infections in Trinidad (Poon-King et al. 1967; Dillon and Wannamaker, 1971) at Red Lake, Minnesota (Anthony et al. 1967); Jamaica (Gallagher and Miller, 1967); Israel (Losch et al., 1971) and Nigeria (Whittle et al., 1973).

Acute glomerulonephritis after streptococcal pyoderma occurs mainly in young children after a latent period of three weeks or longer (Kaplan et al., 1970). This disease occurs sporadically, sometimes in epidemic forms particularly in areas where pyoderma is considered to be endemic (Potter et al., 1968; Rodriguez et al., 1977).

Clinical features of acute glomerulonephritis in warm climate countries do not differ markedly from those in other parts of the world. This nephritic syndrome is characterized by oedema (often of the face and feet), dark urine, oliguria, haematuria, proteinuria, hypertension and azotemia (Rotta et al., 1985). Nevertheless, asymptomatic cases of acute glomerulonephritis have also been reported to be much more common than cases with symptoms (Simpson et al., 1980; Seggie, 1981). In an outbreak of pyoderma and nephritis at Red Lake, Minnesota (Kaplan et al., 1970) over 50% of patients studies were asymptomatic despite evidence of acute nephritis. These researcher concluded that a significant portion of cases of epidemic nephrities may go undetected unless there is routine examination of urine for blood.

2.6 STUDIES ON PYODERMA IN THE SUB-SAHARAN REGION OF AFRICA

Although conditions that favour the onset of pyoderma in many tropical countries (like Trinidad and Tobago, Red Lake etc.) exist in most parts of Africa, (where

skin infections or rash are not uncommon), yet very few studies have been done on this subject in this part of the world.

Among the few places in Africa where research has been done on this topic with documented research findings are the following:

Ghana - Belcher et al., 1977; Acheampong et al., 1988

Nigeria - Whittle et al., 1973

Uganda - Nsanzumuhire et al., 1972

Tanzania - Mhalu, 1973

Of the researchers mentioned above, only Whittle et al., (1973) encountered some, 0.85% (ie. 17/2000) acute glomerulonephritis in their study. The explanation given for this low rate was that some of the subjects who developed mild symptoms probably did not go back to the hospital for diagnosis and the rest may have developed a asymptomatic nephritis which might have been missed in the absence of routine urine examination.

Belcher et al. (1977) and Acheampong et al. (1988) who conducted their studies confirmed the endemicity of pyoderma in Ghana but gave no report on the consequent disease, acute glomerulonephritis.

So far there are no documented reports on glomerulonephritis in Ghana, but the high prevalence of streptococcal pyoderma suggests, that pyoderma may be more common than generally assumed and that an epidemic out-break of glomerulonephritis is a possibility.

3.0 DESCRIPTION OF THE STUDY AREAS

Ejisu-Juaben and Kumasi districts were the areas chosen for the study. The choice of these areas was mainly due to the variation in social setting - Kumasi being urban and Ejisu-Juaben having rural characteristics. Other reasons for the choice were on the grounds:

i. of the proximity of the two study areas to the laboratory (Department of Clinical Microbiology, S.M.S, U.S.T.).

ii. that the Department of Community Health, S.M.S, had adopted the Ejisu-Juaben district for field work in community medicine.

3.1 EJISU-JUABEN DISTRICT

This district covers about 247.27 sq. miles or 158,250 acres of land (information from the Survey Department, Kumasi).

It shares boundaries with the Kumasi district on the west, Bosomtwe-Kwanwoma district on the south, Asante-Akim district on the east and Kwabre-district on the north-west. The district is made up of over fifty-five (55) villages which are sub-divided into four (4) circuits viz., Ejisu, Bomfa, Kwaso and Juaben circuits.

The Ejisu-Juaben district, which forms part of the Ashanti Region lies within the rainforest belt and is characterized by semi-deciduous vegetation with a number of scattered tall shady trees. The land can be described as being undulating and interspersed with a few streams and small rivers.

Although the district is a large one, it is socially homogenous with very little class distinction among the inhabitants. A common feature found there is the unplanned settlements with most of the houses built with clay/mud and roofed with corrugated iron/aluminium roofing sheets which have become rusty with time. There are however, a few houses particularly, those along the Accra-Kumasi trunk road, built of concrete and resembling those commonly seen in urban centres.

The area has seen very little development. About 80% of the district lacks pipe-borne water supply. Water supply is however supplemented by the streams, rivulets and wells including a number of bore-holes which were recently constructed. The few villages that have pipe stands (taps), indicating a supply of pipe-borne water do not enjoy a constant flow of the commodity. Again, only a few villages, about 30%, enjoy electricity supply, which became possible through self-help projects by citizens of such villages.

One other observation made was the lack of well-constructed drainage systems in this study area. Waste water from households flow through simply dug-out gutters which are often uncemented. With regard to places of convenience, public K.V.I.P (Kumasi Ventilated Improved Pit) latrines have been constructed by the village communities through self-help to serve their various communities. Some homes have private toilets but these are mainly of the trench latrine type and are normally located about twenty to thirty metres away from houses. Only a few houses have water closet (WC) with well constructed septic tanks.

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The entire district has four health posts, the largest of which is sited at Juaben. The citizens of surrounding villages often have to travel long distances for health care services. This long distance travel is often undertaken only when the need for it is crucial; otherwise the majority of the members of the community depend on self-medication, a practice which is facilitated by the operations of chemical sellers, traditional healers, etc.

The illiteracy rate in the Ejisu-Juaben district is estimated to be quite high based on personal interaction with the people. However, about fifty-five percent (55%) of parents of the study population have had at least a primary school education. It was observed that most of them simply discontinued or dropped out of school because of economic or social constraints. One problem that seemed to plague this district socially and economically is the constant drift of the few elites in the society together with those of the productive age group (between 20-45 years) to other towns and cities for jobs, thus, depriving the Ejisu-Juaben district of her social and developmental base.

The entire district has eighty (80) public primary schools and five second cycle institutions with the Juaben and Ejisu circuits having the highest concentration of schools.

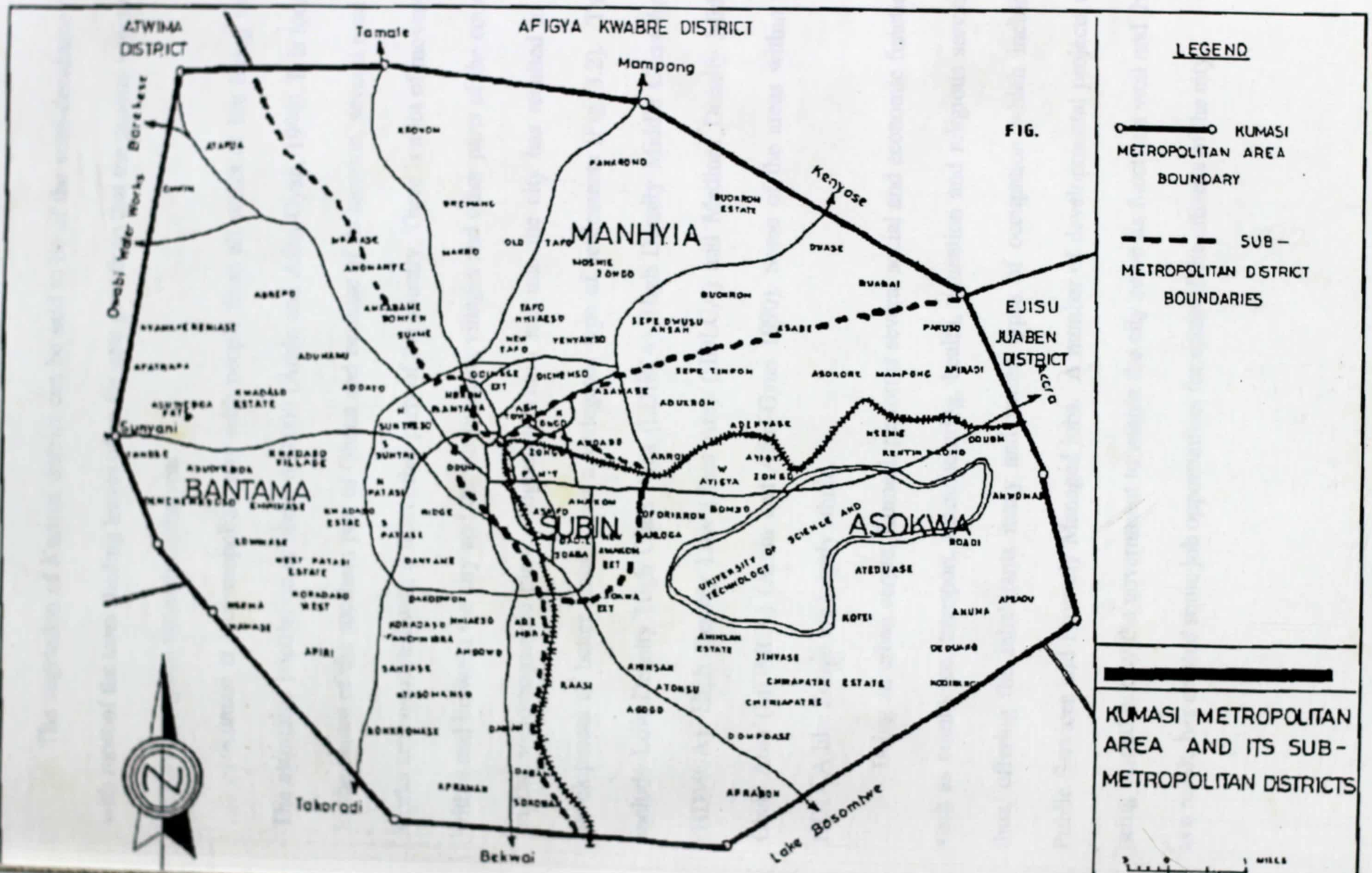
The inhabitants are predominantly farmers, growing cash crops such as cocoa, and food crops such as maize, cassava, plantain, cocoyam and some vegetables. A few are petty traders who either send food stuffs to Kumasi and other towns to sell or bring wares/goods from urban centres to the district and nearby villages to sell. Fewer still (mostly men) are artisans, drivers and mechanics.

3.2 KUMASI DISTRICT

The Kumasi area is on a low plateau of about 300 metres above sea level and covers an area of about 80.94 sq. miles or 51,800 acres of land. It shares common boundaries with Atwima (on the west), Kwabre (on the north), Ejisu-Juaben (on the east) and Bosomtwe-Kwanwoma (on the south) districts. The area constituting the Kumasi district and its boundaries are shown in Fig. 3.1.

The plateau is dissected by a number of rivulets/streams eg. Subin river, Danyame river, Kwadaso river, Wiwi river, Aboabo river etc. to mention but a few. The immediate banks of some of the streams are marshy with Colocassia sp. and other water loving plants growing there. Some of the banks are however, drained and developed.

Fig. 3.1: Kumasi Metropolitan Area and its Sub-Metropolitan Districts.

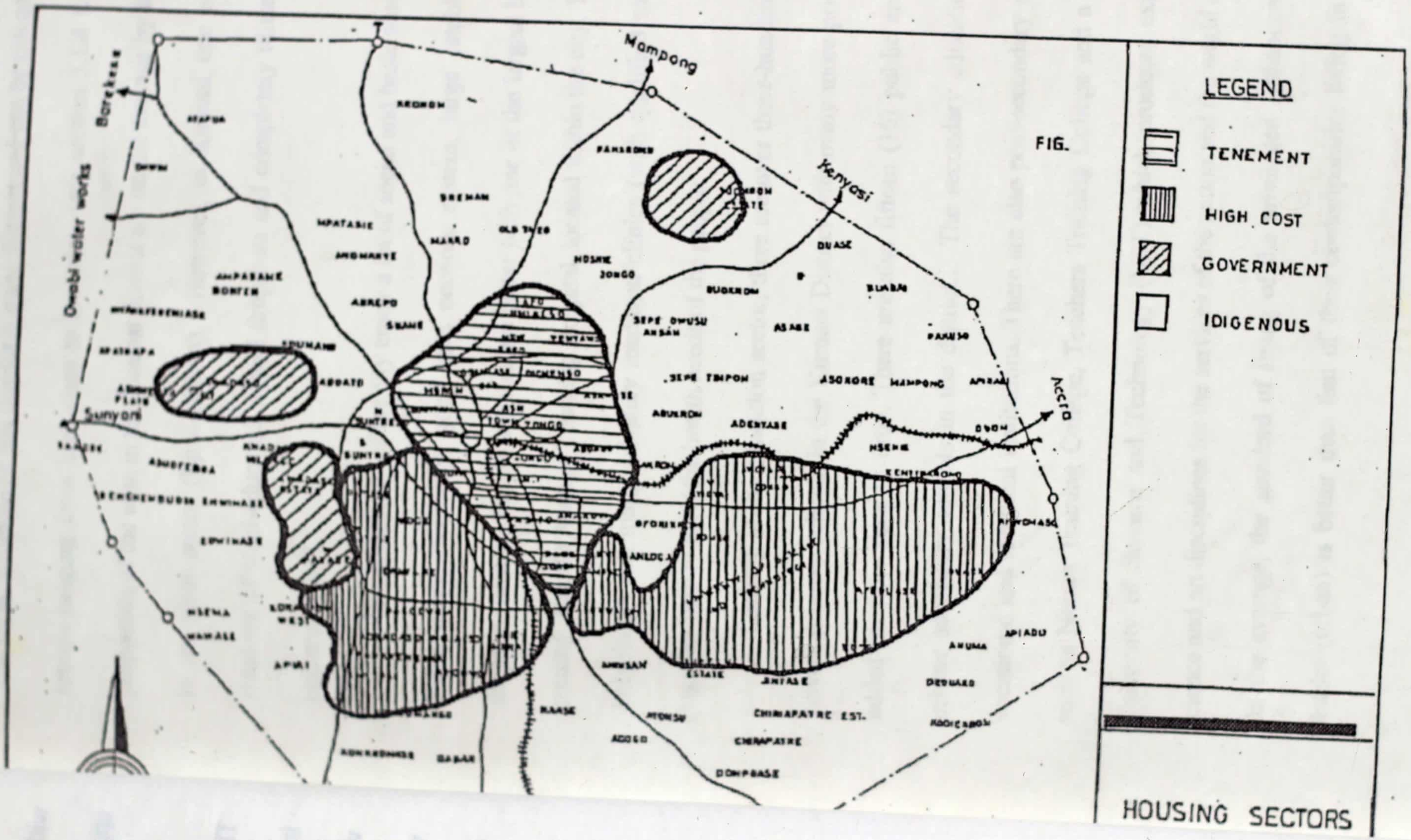


The vegetation of Kumasi district can be said to be of the semi-deciduous type with most of the trees sheding leaves often for new growth so that the general vegetation appears evergreen throughout the year.

Kumasi is a cosmopolitan city with people from all walks of life living there. The estimated population of about 600,000 (Arhin and Afari-Gyan, 1990). It is located in the centre of the southern part of Ghana and because of its location, several road net works are connected with it from other parts of the country. There is a lot of movement within and between the city and the surrounding villages and other parts of the country on the socio-economic plane. Migration within and into the city has resulted in the development of better planned but a variegated type of settlements (Fig. 3.2). These include Low Density High Class Area (LDHCA), High Density Middle Class Areas (HDMCA), High Density Low Class Area (HDLCA) and Medium Density Middle Class Area (MDMCA) (Arhin and Afari-Gyan 1990). Some of the areas within the HDLCA like Zongo are largely ethnic.

Being an urban centre, Kumasi, performs several social and economic functions such as commerce, transport, manufacturing, health, education and religious services, thus, offering the inhabitants many and varied types of occupation which includes Public Services and Privately Managed jobs. A number of developmental projects are being carried out by the government to enable the city serve its functions well and this, as a result, has created some job opportunities for some of the citizens of the city.

Fig. 3.2: Kumasi District: Housing Sectors.



The social structure of this district is quite distinct and can be categorized into four main groups ranging from high class to low class (see section 5.1.4 for the details). The proportion of the elite in the Kumasi district are on the average by far greater than those in the rural sector (Ejisu-District). However, in Kumasi one encounters countless numbers of semi-educated, school drop-outs and completely illiterate and uneducated citizens as well.

The inhabitants of the city enjoy a lot of social and basic amenities such as pipe-borne water, electricity, good road network system, large established markets and shopping centres. Health services are better with one of the largest health centres in the country, Komfo Anokye Teaching Hospital, located within the city. There are also many Polyclinics and many privately managed clinics (with qualified medical staff) which together help to meet the health needs of the people.

Looking at the education sector, there are over three-hundred (300) public and private primary schools in the Kumasi District with many more private schools being added on from time to time. There are also fifteen (15) public and twenty-one (21) private secondary schools in the district.. The secondary education sector includes vocational and technical institutions. There are also post-secondary education facilities such as Nurses Training College, Teachers Training College and a University ie. the University of Science and Technology (UST) which produces expertise in various science and art disciplines for the services of the nation and the world at large.

On the average, the standard of living of the privileged inhabitants in the Kumasi district (urban) is better than that of their contemporaries living in the Ejisu-Juaben

district (rural setting). This, therefore, puts the districts constituting the study area for this research into two distinct settings.

4.0 MATERIALS AND METHODS

The study population was selected from among Primary School children of two distinct pulations, rural and urban. Ejisu-Juaben district provided the rural setting while Kumasi trict served as the urban setting. Thus, the two study groups were drawn on the one hand m a socially and economically deprived population and on the other hand from a seemingly vantaged community. Ejisu-Juaben district consists of many small villages whilst the Kumasi strict is made up of many suburbs.

The study is basically a two-part study, encompassing:

) An epidemiological investigation whose research instrument was a non-self administered questionnaire. The study population as well as the parents thereof were interviewed in accordance with the questionnaire in Appendix 1.

) Bacteriological Investigations to determine the causal agents of pyoderma.

1 Selection Criteria

clusion criteria:

All children aged between 5 and 14 years with active vesicular or pustular lesions or ose lesions covered with brownish/amber crusts and who were willing to take part in the study ere enrolled into the study.

It is to be noted that although most of the school children examined presented with rious skin defects only a few qualified to be enlisted.

Exclusion Criteria:

(i) all those who had received any form of medication or therapy (antibiotic or local herbal preparations).

(ii) those with septic purulent or "weeping" sores, boils and all skin lesions which do not answer to the definition and description of pyoderma.

(iii) those with fresh cuts without any form of infection.

(iv) those who had the infection but were unwilling to provide specimens.

All these (with the exception of those in the `iv' exclusion category) were treated wher possible and sent away.

By simple random sampling, individuals for the study were selected. In order to facili selection of the study population, a compiled list of all primary schools in the two study ar were obtained from the District and Regional Offices of the Ghana Education Service at Ej and Kumasi, respectively.

The Ejisu-Juaben District is divided into four circuits, each having rural characterist suited for our purpose. Two of these circuits, Ejisu and Juaben were selected. From these, t circuit schools that provided individuals for the study were subsequently randomly selecte The random selection was facilitated by the use of a "Table of Random Numbers". In all a to of 20 schools were selected from the chosen circuits. A similar selection method was employ in the Kumasi district. Here, however, primary schools have been categorized into Public a Private schools. In order not to introduce any bias into the study, selection was made from bo categories of schools.

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At every selected school, skin examination of all pupils was conducted to identify children with the infection. The examination embraced all stages of the primary school ie. from class 1 through class 6.

4.2 SURVEY

Every pyoderma subject selected was interviewed using a specially designed questionnaire (Appendix 1). Questions elicited included demographic information (vital and social statistics), hygienic practices and extra-curricular activities undertaken by the study population. Also solicited and recorded were the primary causes (predisposing factors) of pyoderma, anatomical sites (where applicable) of lesions suggestive of pyoderma. Parents/guardians of study subjects were also interviewed using the questionnaire (Appendix 1) to help define their socio-economic status. Some of the questions asked sought to determine occupation, level of education and number of dependants for whom parents/guardians were responsible. Also elicited included the environmental conditions under which respondents lived including the type of houses they lived in, availability of good drainage systems and toilet facilities. The investigation also sought to determine the number of individuals sleeping in the same room with each member of the study population. The information gathered was carefully recorded and statistically analysed. The statistical methods used included Chi-square (χ^2) and Z-distribution tests.

Classification of the socio-economic groups of the study population was done according to the British system of classification which has further been modified for use in Africa (Futcher, 1940). The factors used for this classification were, levels of education, occupation, income and living conditions of parents/guardians of the children. The socio-economic status of the parents was consequently used to classify the respondents (study population).

4.3 BACTERIOLOGICAL INVESTIGATION

4.3.1 Specimen Collection

Skin defects which showed evidence of pyoderma were recorded. Surface of lesions were first cleaned with 85% (w/v) physiological saline in order to remove sand particles, debris and oily substances and to soften crusts. This was followed by the application of 70% alcohol-soaked cotton wool. With the aid of sterile forceps, crusts were removed from the surfaces of wounds. Serous discharges were carefully removed with sterile cotton swabs and immediately placed in Stuart's transport medium for onward transmission to the laboratory. Each pyogenic or pustular lesion, on the other hand, was punctured with a sterile needle and the discharge was either drawn with the syringe and needle or dabbed with sterile cotton swabs and sent to the laboratory as mentioned above.

A second cotton applicator was used to make smears of the discharges on clean, oil-free microscope slides for Gram's staining. The collected specimens (in test tube and on slide) were legibly labelled to correspond with the labelling on the questionnaire.

All collected specimens were immediately sent to the Department of Clinical Microbiology Laboratory, University of Science and Technology, (U.S.T), Kumasi, within 6 hours for processing and analysis.

4.3.2 Laboratory Processes

On reaching the laboratory, specimens for culture were seeded on pre-refrigerated and dried plates of the following media:

(a) Two (2) blood agar plates containing 7% defibrinated sheep blood.

(b) One (1) crystal violet blood agar plate containing 7% defibrinated sheep blood and 0.02% (w/v) aqueous solution of crystal violet.

(c) One (1) MacConkey agar.

One plate each of the 3 inoculated media was incubated aerobically at 37°C for 24 hours. The remaining one (1) blood agar plate was incubated anaerobically and after 24 hour incubation period, all four innoculated plates were examined and those showing no growth were re-incubated for another 24 hours. If still no growth occurred, those cultures were considered negative and were discarded. Meanwhile, slides prepared on the field were Gram-stained (Appendix 4.1) and examined under the high power (x 100) objective lens of the light microscope. The various morphological and Gram reaction types of bacteria seen were recorded. The findings were compared with the type of bacteria isolated from culture to help in the detection of exogenous organisms or contaminants.

Preliminary Tests

After incubation, culture plates were removed and carefully examined for growth and morphological characteristics. The growth characteristics observed included haemolysis, odour, colour, colony size, consistency, swarming ability and carbohydrate fermentation reactions (on MacConkey). As part of these preliminary investigations smears of various colonies from culture plates were made and Gram-stained. All results were recorded.

Confirmatory Tests

Colonies were observed for the Gram reactivity and cell morphorlogy. Arrangements of the cells were also observed. Colonies found to be Gram-positive and coccoid were subjected to the catalase test using 3% H_20_2 solution in order to differentiate Streptococci from Staphylococci, after which the different species were sub-cultured on fresh media and stored as stock culture for future analyses. Catalase-negative colonies suspected to be Streptococci were stored in 10% cooked meat medium at room temperature while catalase- positive colonies suspected to be Staphylococci were stored on nutrient agar slants and also at room temperature. Growth that showed as Gram-negative rods on Gram's staining and on microscopic examination was suspected to belong to the family Enterobacteriaceae and subsequently stored on nutrient agar slant (Cheesborough, 1985).

Gram-positive cocci in clusters that gave a positive catalase reaction were further subjected to the coagulase test (Appendix 4.3) using rabbit plasma (a product of Wellcome Reagent Limited). Both the slide and tube tests (Cheesborough, 1985) were performed with rabbit plasma instead of human plasma to avoid the effects of the possible presence of any biological or inhibitory factors in the latter which may give false positive results. This test helped to differentiate Staph. aureus from Staph. epidermidis and Staph. saprophyticus.

Catalase negative, Gram-positive, cocci in short chains from pin-point colonies which showed various types of haemolysis on blood agar were subjected to further investigations to ensure proper identification. Such bacteria (inclding those from crystal violet blood agar) were further subjected to Bacitracin (10 units) discs sensitivity test (Plate 1). The Bacitracin-sensitive cultures were preliminarily identified as belonging to Group A Streptococci. The group specific identification of these together with the Bacitracin-resistant strains as well as all other streptococcal species was performed according to the Lancefield Grouping tests using "Streptotex Kit" (Plate 2) supplied by Wellcome Reagent Limited (See also Appendix 4.9 and Plate 3).The results of the various groupings are shown in Table 3.

Further grouping of the Gram-negative rods into their specific genera and species was by different biochemical test methods outlined by Cheesborough (1985), taking also into account cultural characteristics of the isolates.

Colonies with a large spreading, confluent growth with pigment or no pigment production (which are clearly seen on Nutrient Agar plates) were first tested for oxidase production as described in Appendix 4.8.

Positive reactants were tested for their ability to utilize citrate as a carbon source in Koser citrate medium (Appendices 2.6 and 4.4), the results of which are recorded in Table 4.

All isolates which swarmed on blood agar were tested for the enzyme urease production and for their carbohydrate fermentation reactions (Appendices 4.5 and 4.7). Those cultures that showed the ability to ferment carbohydrates, particularly, lactose on MacConkey agar, but gave negative citrate, oxidase and urease reactions were subsequently tested for Indole production from tryptophan-incorporated-peptone broth medium (Appendix 4.6).

All cultures made up of Gram-negative rods were innoculated onto the slope and into the butt of Kligler medium to study the pH changes (acidic and/or alkaline) and gas production following carbohydrate fermentation. The methods used for the various biochemical tests are all recorded in Appendix 4.



Plate 1: Streptococcus pyogenes on blood agar showing sensitivity to bacitracin

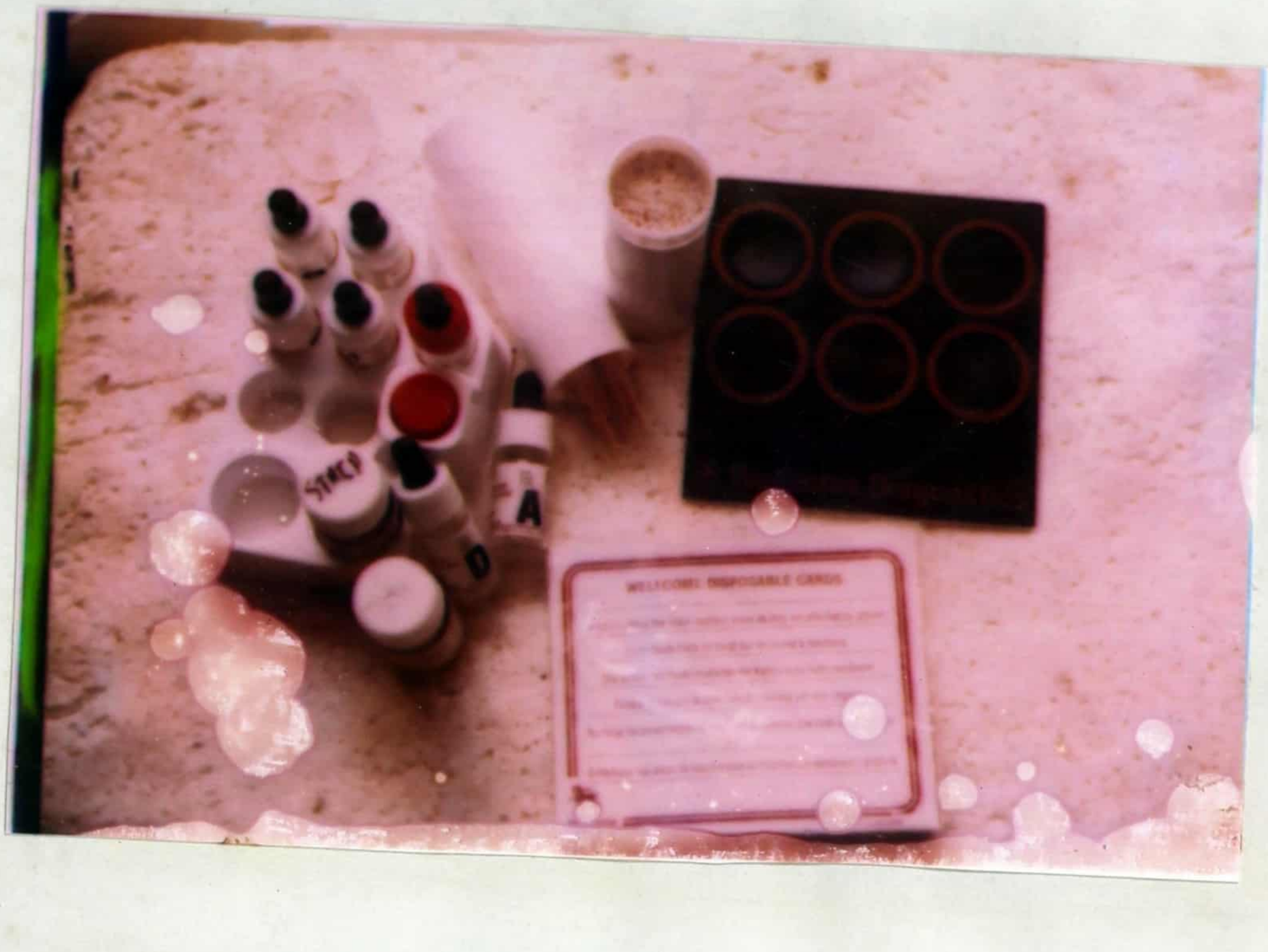


Plate 2: A 'Streptotex Kit' for Lancefield grouping of

of Streptococcus species.

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Plate 3: Agglutination Test

(A) Positive agglutination

(B) Negative agglutin

Table 3: The various Lancefield Groups of ß-Haemolytic Streptococcus Isolated

Group	No.	%
A	214	74.3
B	2	0.7
C	40	13.9
D	5	1.7
G	27	9.4
TOTAL	288	100

[redacted]

				A	B	C	D	G										
2A	β	+							+	−								Staph. aureus
3A	β	+							+	−								Staph. aureus
5A	−	+							+	−								Staph. aureus
7A	β	+							+	+								Staph. aureus
8A	−	+							+	−								Staph. aureus
2A	β	+							+	−								Staph. aureus
6A	−	+							+	−								Staph. aureus
3A	β	+							+	−								Staph. aureus
4A	−	+							+	−								Staph. aureus
3C	−	+							−	+								Staph. epidermidis
4C	−	+							−	+								Staph. epidermidis
7C	−	+							−	+								Staph. epidermidis
8C	−	+							−	+								Staph. epidermidis
1B	−	+							−	+								Staph. epidermidis
3A	−	+							−	+								Staph. epidermidis
2B	β	−	+	+	−	−	−	−										Staph. epidermidis
3B	β	+	+	−	−	−	−	−										Staph. epidermidis
B	β	−	+	+	−	−	−	−										Strept. pyogenes
7B	β	−	+	+	−	−	−	−										Strept. pyogenes
3B	β	−	+	+	−	−	−	−										Strept. pyogenes
4B	β	−	+	+	−	−	−	−										Strept. pyogenes
2B	β	−	−	−	+	−	−	−										Strept. pyogenes
4B	β	−	−	−	−	+	−	−										Streptococcus (Group A)
6B	β	−	−	−	−	+	−	−										Streptococcus (Group C)
A		−	−	−	−	−	+	−	−	+								Streptococcus (Group C)
D	β	−	−	−	−	−	+	−										Strept. faecalis
B	β	−	−	−	−	−	+	−										Streptococcus (Group D)
B	β	−	−	−	−	−	+	−										Streptococcus (Group D)
B	β	−	−	−	−	−	+	−										Streptococcus (Group D)
B	β	+	−	−	−	−	−	+										Streptococcus (Group G)
B	β	−	−	−	−	−	−	+										Streptococcus (Group G)

Isolate	Haemol-ysis	Cata-lase	Bacit-racin	[illegible]					[illegible]-lase	ose									
				A	B	C	D	G							Slope	Butt	H2S	Gas	
4D	β	+								-	+	+	-	-	R	R	-	-	(Group C) P. aeroginosa
11C	β	+								-	+	+	-	-	R	R	-	-	P. aeroginosa
12C	β	+								-	+	+	-	-	R	R	-	-	P. aeroginosa
13C	β	+							-	-	+	+	-	-	R	R	-	-	P. aeroginosa
5D	-	+							-	-	+	+	-	-	R	R	-	-	P. pseudomallei
8D	-	+							-	+	+	+	-	-	R	R	-	-	P. pseudomallei
12E	-	+							-	+	+	+	-	-	R	R	-	-	P. pseudomallei
84C	-	+							-	+	+	+	-	-	R	R	-	-	Other Pseudomonas
20C	-	-							+	+	+	-	-	+	Y	Y	-	+	Proteus mirabilis
35D	-	-							-	-	+	+	-	+	Y	Y	-	+	Proteus mirabilis
12D	-	-							-	-	+	+	-	+	Y	Y	-	+	E. coli

Key

+	Positive test
-	Negative Test
R	Red Coloration
Y	Yellow Coloration
	Test not applicable to organisms

4.3.3 ANTIMICROBIAL SENSITIVITY TESTING

All isolates were subjected to antibiotic sensitivity testing, using the agar difusion method with Dextrose Sensitivity Test (D.S.T) agar as the basal medium. For fastidious organisms such as species of Streptococci, about 7% defibrinated sheep blood was added to the D.S.T basal medium. Of the two major antibiotic test methods employed in the laboratory, the Kirby-Bauer method (Stokes & Ridgway, 1985) was used in this particular study because of its simplicity.

In performing this test care was taken to produce a semi-confluent growth of the test organism on the culture medium since any growth too thick or too thin could affect the results. To achieve this objective, a turbidity (opacity) standard (Appendix 4.10) was prepared against which a suspension of the test organism was matched. Based on the assumption that the turbidity of the prepared standard is equivalent to an overnight broth culture (Cheesborough, 1985), a suspension of the test organism was prepared by emulsifying a few colonies of the pure test strain in 1 ml of sterile peptone water. The turbidity of the suspension was compared with that of the standard one till they matched. Following this, a sterile loopful of the suspension of the test organism was placed on the test agar plate and with a dry sterile cotton swab this suspension was evenly spread over the surface of the medium and allowed to dry for some few minutes (with the lid in place). Selected antibiotic discs were then placed on the innoculated medium using a pair of sterile forceps, flaming the forceps with each manipulation. The discs were well spaced out on the medium to prevent merging of zones of inhibition which may give false positive results.

The antibiotic discs (manufactured by Oxoid Ltd., England) used, are indicated below, with selection thereof being based on the source of the isolates.

Name of Antibiotic	Concentration/ml in disc
Bacitracin	10 µg
Erythromycin	15 µg
Penicillin	2 µg
Lincomycin	2 µg
Methicillin	10 µg
Carbenicillin	100 µg
Tetracycline	10 µg
Ampicillin	10 µg
Gentamicin	10 µg
Chloramphenicol	10 µg

Susceptibility or resistance to any particular antibiotic was determined by measuring the diameter (in millimeters) of the zone of inhibition after overnight's incubation. The measurements were compared with those of a chart provided by the antibiotic disc manufacturer. The said chart is reproduced in Appendix 6.

4.3.4 Lancefield Grouping of Streptococci

Lancefield grouping of β-haemolytic Streptococci was by the latex coagglutination test technique. For this purpose a kit containing specific Lancefield grouping reagents together with a polyvalent reagent (Wellcome Reagents Limited, London) was obtained by courtesy of Robert Koch Memorial Laboratory Services, Kumasi.

Isolates belonging to the various Lancefield Groups viz., A, B, C, D, & G were identified where present. The procedure employed was as given by the manufacturer (as set out in Appendix 4.9). Positive results were indicated by a strong agglutination reaction between the antigen of the Streptococci and the latex reagent while a negative result caused the mixture of the two to remain in smooth suspension.

5.0 RESULTS

5.1 EPIDEMIOLOGY

Of a total study population of 6,017 subjects (3,195 males and 2,822 females) exaimned for pyoderma, 964 or 16.02% were found to have the diesease. But based on the exclusion criteria employed in this study, only 310 subjects qualified for further study. In this study pyoderma is defined as any lesion presenting as non-bullous amber-crusted ulcer and/or vesicles. Any such lesion which had been treated did not qualify for further study considerations.

The actual incidence rate of pyoderma encountered in this study was 16.02% based on the 6,017 patients examined. However, since only 310 of these cases fulfilled the inclusion criteria, any further studies were conducted on these 310 subjects only.

Of the 6,017 subjects, Ejisu-Juaben district (rural area) provided 3,001 subjects while Kumasi district (urban area) provided 3,016 subjects. Table 5 gives the age and sex specific distribution of the study population in the two study areas (Ejisu-Juaben and Kumasi).

5.1.1 Age and sex distribution of pyoderma

The population studied was composed of school children aged between five and fourteen years, an age range that falls within the Primary (including Kindergarteen) sector of the educational system in Ghana. From Table 6 and Fig. 5.1, it can be deduced that the 8-10 years age group recorded the highest frequency of pyoderma (40.6% or 126 of 310) and that the least occurence of pyoderma (0.6% or 2 of 310) was encountered amongst the 14 year olds.

From Table 6 it can also be seen that pyoderma is more prevalent among males than females with a male to female ratio of 3:1. This preponderance of pyoderma in males is true for both (rural/urban) study areas. Figs. 5.2 and 5.3 clearly illustrate this.

Again, from Figs. 5.2 and 5.3 it can be observed that the peak occurence of pyoderma among the male study population was among the 8-10 years age-group while the peak incidence among the female study group was in the age-group 5-7 years.

The test of significance between age and pyoderma gives a chi square(χ^2) value of 19.884 (Appendix 5.1). At 0.1% level of significance (with 3 degrees of freedom) the null hypothesis (Ho:) which states that age and pyoderma are independent is rejected. It is therefore to be concluded that age and pyoderma are dependent on each other. Likewise, the test of signifcance between sex and pyoderma (Appendix 5.2) revealed that the two (sex and pyoderma) are interdependent. From this test we can affirm that male children are more at risk for pyoderma than female children.

Table 5: Age and Sex distribution of study population (6017 subjects) irrespective of exclusion criteria for pyoderma

Age (years)	Total No. of subjects	Pyodermic Population (n=964)						Non-Pyodermic Population (n=5053)					
		Urban		Rural		Sub-Total		Urban		Rural		Sub-Total	
		M	F	M	F	No.	%	M	F	M	F	No.	%
5 - 7	1930	44	21	115	56	236	12.2	429	417	402	446	1694	87.8
8 - 10	1983	42	25	237	66	370	18.7	508	453	302	350	1613	81.3
11 - 13	1964	38	25	171	100	334	17.0	444	520	365	301	1630	83.0
14	140	5	0	11	8	24	17.1	25	20	57	14	116	82.9
Total	6017	129	71	534	230	964	16.02	1406	1410	1126	1111	5053	84.0

Table 6: Age and sex distribution of 310 cases of pyoderma fulfilling the inclusion criteria

Age (years)	Frequency of Pyoderma				Over-all total in age group	
	Males		Females			
	No.	%	No.	%	No.	%
5 -- 7	78	25.2	32	10.3	110	35.5
8 - 10	101	32.6	25	8.1	126	40.6
11 - 13	51	16.5	21	6.8	72	23.2
14	1	0.3	1	0.3	2	0.6
Total	231	74.5	79	25.5	310	100

Fig. 5.1 **AGE DISTRIBUTION OF PYODERMA IN PYODERMIC STUDY POPULATION**

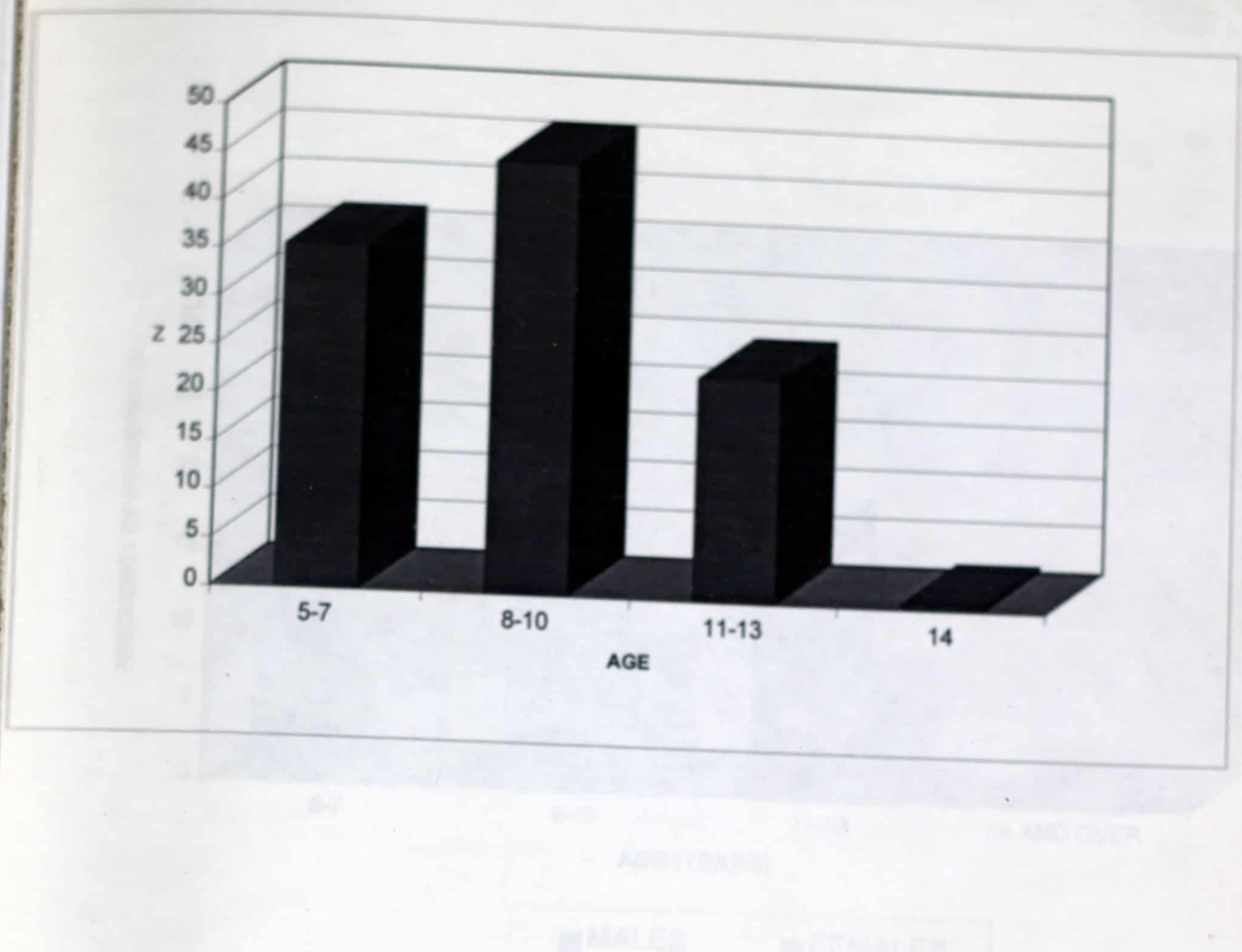


Fig. 5.2 AGE AND SEX DISTRIBUTION OF PYODERMA IN KUMASI (URBAN) DISTRICT.

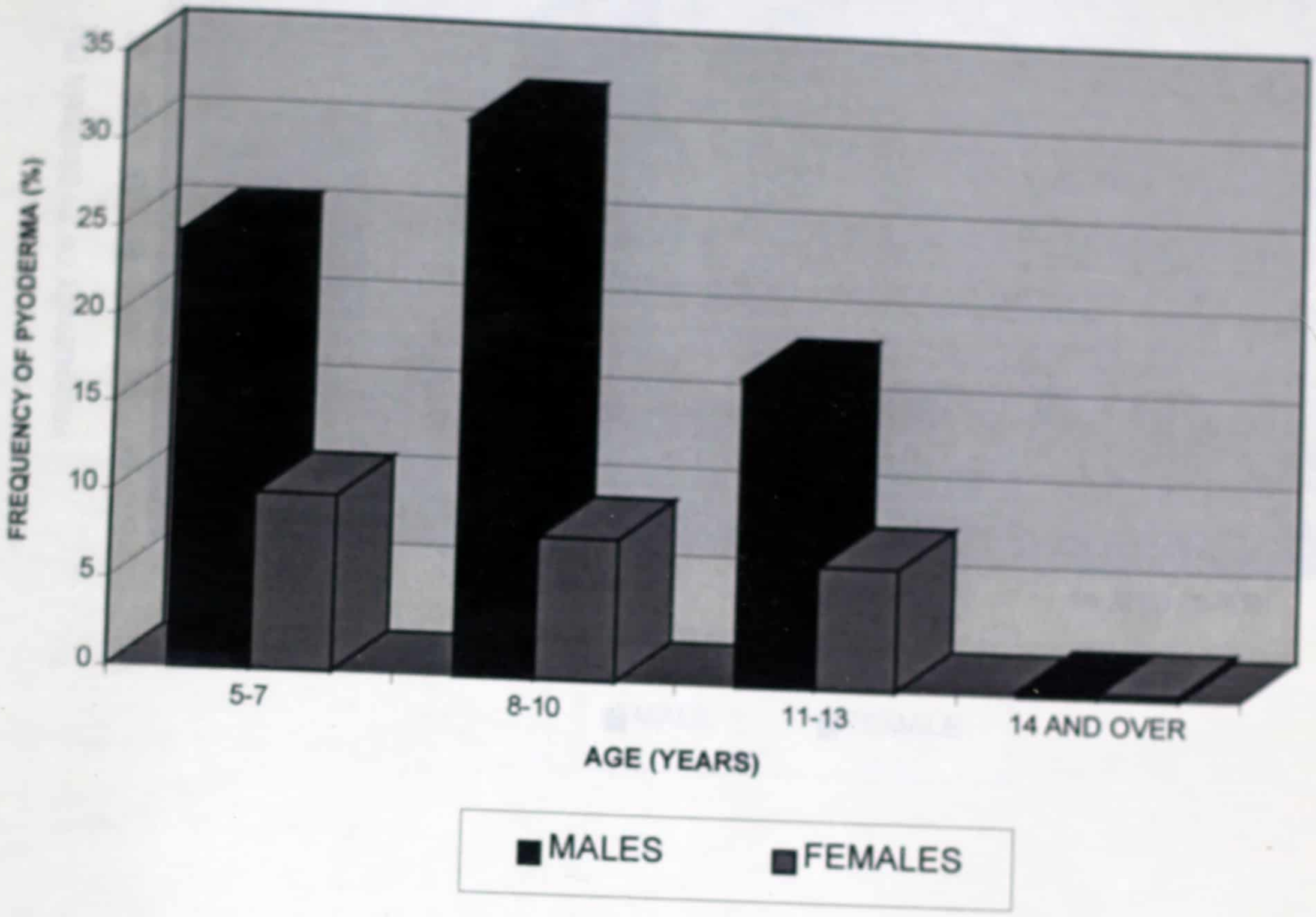
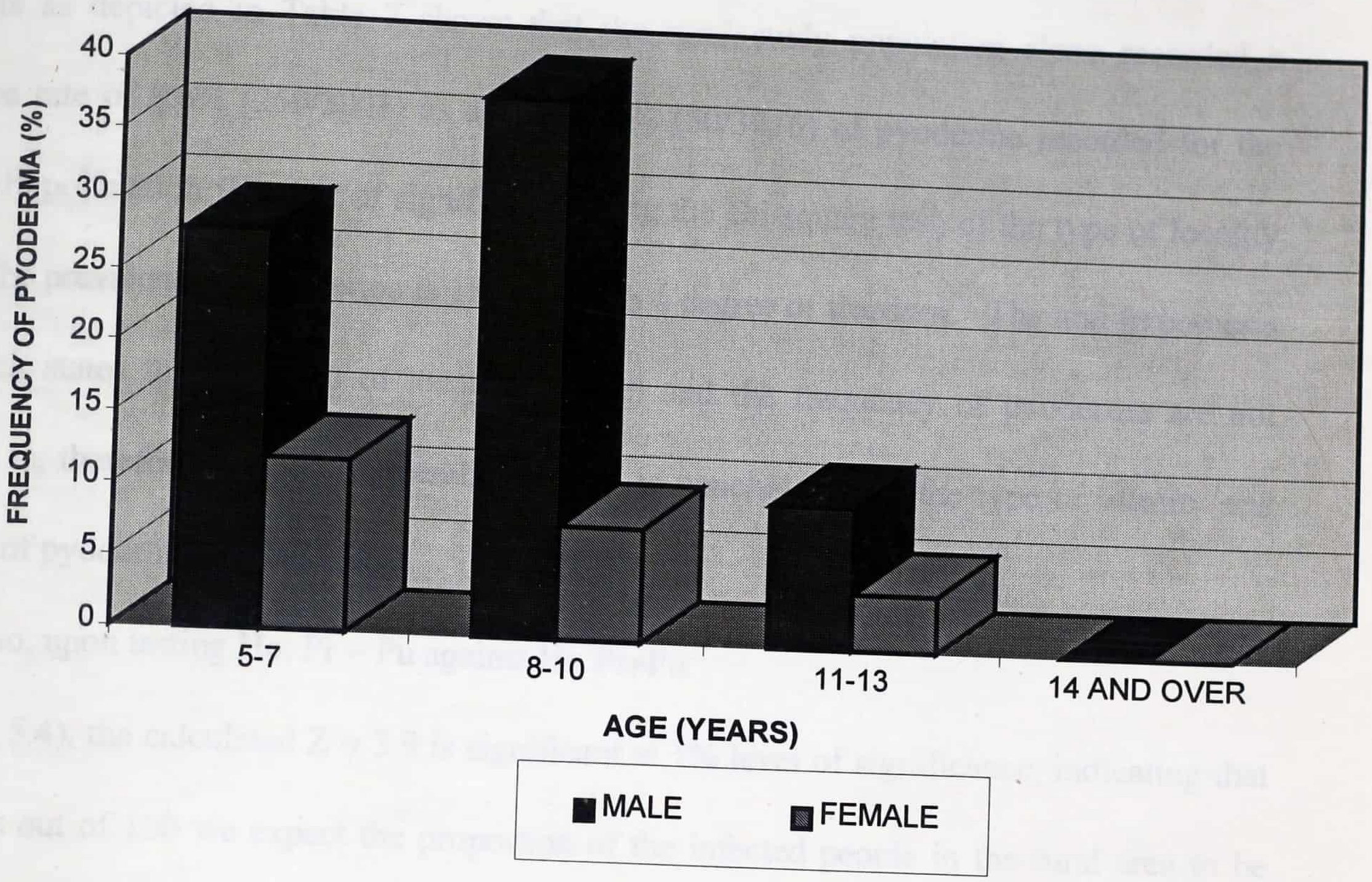


Fig. 5.3 **AGE AND SEX DISTRIBUTION OF PYODERMA IN EJISU-JUABEN (RURAL) DISTRICT.**



5.1.2 Type of locality and pyoderma

With approximately equal numbers of subjects drawn from each of the two study areas, the results as depicted in Table 7 shows that the rural study population alone recorded a prevalence rate of 8.6% (260/3001) as against 1.7% (50/3016) of pyoderma recorded for the urban study population. The test of significance using the chi-square test, of the type of locality vis-a-vis the prevalence of pyoderma is 151.091 with 1 degree of freedom. The null hypothesis (Ho) which states that the type of locality (abode) and the frequency of pyoderma are not dependent is, therefore rejected (Appendix 5.3). It is concluded that, the type of locality and frequency of pyoderma are dependent.

Also, upon testing Ho: Pr = Pu against Hi: Pr≠Pu

(Appendix 5.4), the calculated Z = 3.9 is significant at 1% level of significance, indicating that in 99 cases out of 100 we expect the proportion of the infected people in the rural area to be higher than those in the urban area.

Table 7: Association between study locality (Rural/Urban) and Pyoderma among the 310 selected patients

Locality	Pyodermic Population No.	%	Non-Pyodermic Population No.	%	Total
Urban	50	1.7	2966	98.3	3016
Rural	260	8.6	2741	91.3	3001
Total	310		5707		6017

Ho: Locality and pyoderma are independent.

$\chi^2 = 151.091$

Degrees of freedom = 1

At 0.1% level of significance, the Ho is rejected with the conclusion that type of locality and pyoderma are not independent

5.1.3 Personal Hygiene and Pyoderma

The data obtained by consideration of the hygenic practices of the 310 patients with pyoderma were grouped into 3 categories as follows; above average, average and below average (see Chapter 4 for definitions).

The results as recorded for each of the three (3) defined categories are shown in Table 8, from which it can be deduced that of the 310 selected patients, as many as 189 or 60.96% patients were those whose personal hygiene was categorized as being below average. This group of patients carried six times more the risk of contracting pyoderma than those with high (above average) hygienic practices. The probability of those classified as average contracting pyoderma was two (2) times more than those with high standards of hygiene.

5.1.4 Socio-economic standing and pyoderma

Four (4) social classes were derived from the data collected. The classes are as follows:

Social class 1: Jobs requiring higher education to the tertiary level eg. lectures, doctors, lawyers, company directors, senior civil servants and which jobs are associated with the highest income.

Social class 2: Skilled occupations requiring post-secondary education eg. school teachers, technicians, nurses, the police, prison services and the armed forces.

Social class 3: Occupations requiring upper primary or lower secondary education e.g auxillary nurses, drivers, mechanics, machine operators, post office clerks, messengers.

Social class 4: Unskilled labour with no education or formal training e.g gardeners, labourers, watchmen, street cleaners, peasant farmers. This group earns the lowest income.

Table 9 shows the number of pyoderma patients who fell under the various social classes with regard to urban or rural origin. From this table it can be recognised that as many as 208 patients or 80% (208/260) of the rural patients belonged to the lowest social class. Fifteen (15) patients out of fifty (50) or 30% of urban patients were classified under this class (social class 4) in the urban sector alone. Patients from the two localities (urban and rural) put together and who fitted into the social class 4 numbered two hundred and twenty three (223), giving an overall percentage of 71.94% (223/310). Social class 1 (highest class) recorded the lowest number of patients, that is, three (3) patients or 0.97%. All three (3) patients came from the urban area with none from the rural area.

Table 8: Classification of 310 selected patients in relation to the level of rural and urban hygienic practices.

Schools	Hygienic Practices			
	Below Average	Average	Above Average	Total
RURAL				
Juaben L/A Primary	25	6	3	34
Akyawkrom Methodist Primary	8	4	0	12
Asotwe L/A Primary	15	2	0	17
Apromase Methodist Primary	12	3	0	15
Ejisu Experimental Primary	15	13	10	38
Manhyia L/A Primary	9	0	0	9
Kwamo Anglican Primary	21	11	2	34
Besease Roman Catholic Primary	30	6	0	36
Fumesa Anglican Primary	24	5	3	32
Onwe L/A Primary	12	5	0	17
Edwenase L/A Primary	6	0	0	6
Abenase L/A Primary	3	0	0	3
Abankro L/A Primary	7	0	0	7
Subtotal	187	55	18	260
URBAN				
U.S.T. Primary School	0	16	4	20
Central International School	2	11	5	18
Nyhiaeso International School	0	7	3	10
Ridge School	0	1	1	2
Subtotal	2	35	13	50
Grand Total	189	90	31	310

Table 9: Association of pyoderma with socio-economic standing

Locality	Social Classes				Total
	Class 1	Class 2	Class 3	Class 4	
Urban	3	11	21	15	50
Rural	-	9	43	208	260
Total	3	20	64	223	310

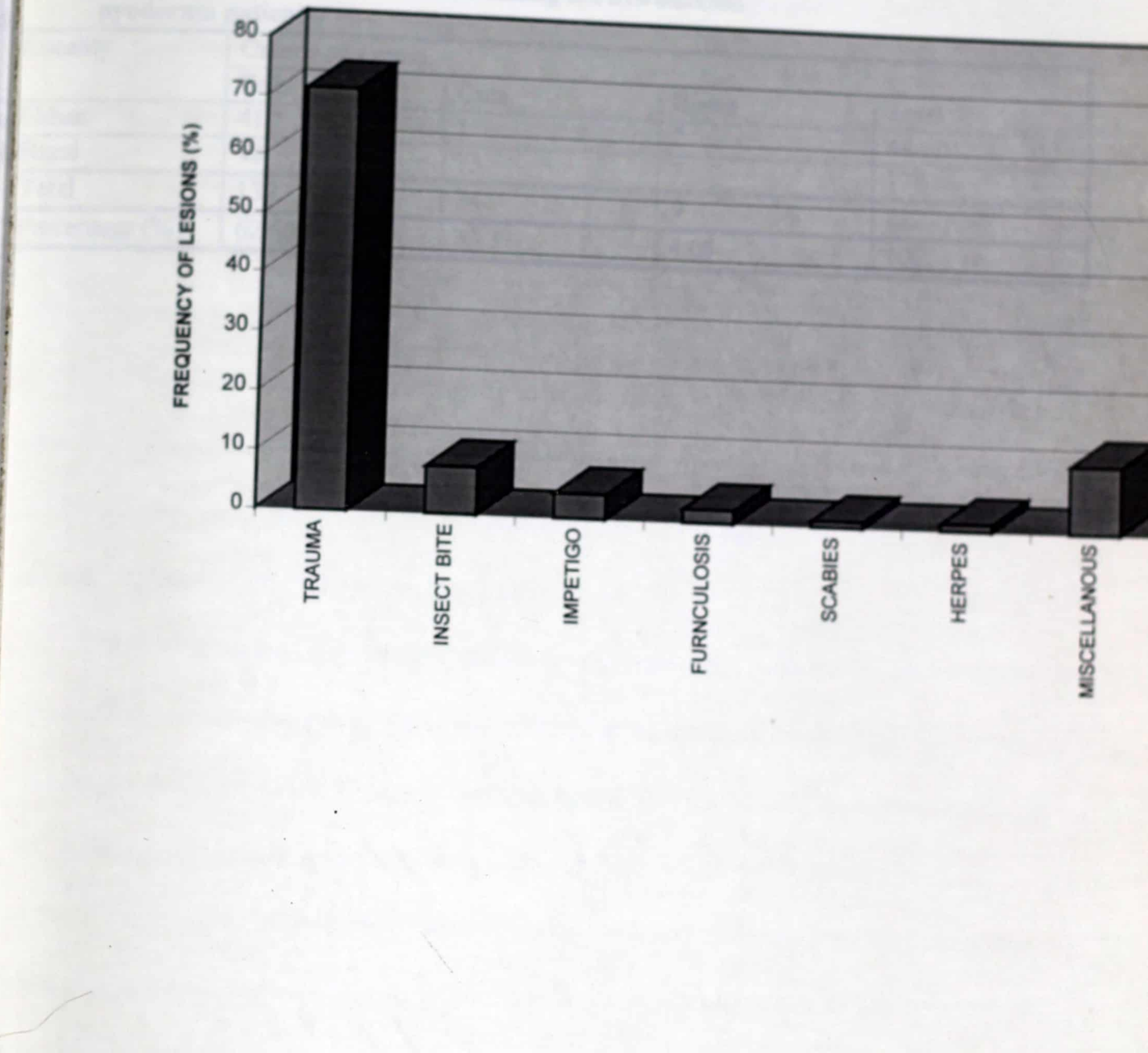
5.2 SOME FEATURES OF PYODERMA IN THE STUDY

The distribution pattern of pyoderma in relation to predisposing factors revealed that pyoderma resulting from traumatised tissue ranked highest, standing at 71.6% (223/310) with the rural population suffering the most. The detailed distribution in terms of factors predisposing to pyoderma is shown in Table 10 and Fig. 5.4. From these illustrations (Table 10 and Fig. 5.4) it is obvious that trauma was the most dominant (71.6%) factor predisposing to pyoderma, for which reason, Table 11 is set out to show the breakdown of the aetiology of the trauma. From this table, it is clear that the most predominant cause of traumas in the study was falls' (62.6%), followed by `cuts' (33.33%). The incidence of pyoderma due to burns was only 4.05%.

Table 10: Distribution pattern of Pyoderma in relation to pedisposing factors.

Predisposing factors/lesion	Number Surveyed					
	Urban		Rural		Total	
	M	F	M	F	No.	%
Trauma	35	9	145	33	222	71.6
Insect bite	-	-	11	14	25	8.1
Impetigo	1	-	9	3	13	4.2
Furunculosis	-	-	4	3	7	2.3
Scabies	-	-	2	1	3	1.0
Herpes	-	-	2	2	4	1.3
Miscellanous	2	3	20	11	36	11.6
Total	**38**	**12**	**193**	**67**	**310**	**100**

Fig. 5.4 **THE DISTRIBUTION OF PYODERMA IN RELATION TO FACTORS PREDISPOSING THERETO**



able 11: Causes of Traumatic lesions among the 310 selected pyoderma patients.

ocality	Causes of Truma			
	Falls	Cuts	Burns	Total
rban	41	1	2	44
ural	98	73	7	178
otal	139	74	9	222
ercentage (%)	62.60	33.3	4.05	100

Also connected with the acquisition of traumas which predispose to pyoderma were the pes of extracurricular activities engaged in by the selected 310 respondents. The activities and e number of patients who frequently undertake them are all recorded in Table 12. From this ble it can be seen that, 99.4% (308/310) of the total number of patients with pyoderma, gaged in games as part of their extracurricular activies. Games here refers to outdoor games ke playing of football, running, jumping etc. A further breakdown shows that 82% (41 of 50) the urban study group engaged in games only, while the remaining 18% (9 of 50) engage in mestic work (household chores) in addition to playing games. In the rural area the set of tivities that ranked highest among the study poplulation of 260 were games and farming tivities (30.4% or 79/260) followed by games, farming and domestic activities together 5.8% or 67/260), then games and domestic work only (23.5% or 61/260). Nineteen point six rcent (19.6% or 51/260) of patients undertook only games while two (2) out of the 260 (0.7%) tients did carpentary and at other times made cement blocks as part of their extracurricular tivities. This latter group did not play any games at all.

Table 10 again has other features that are worth mentioning. Aside of traumas, the rates recurrence of other skin lesions which also predispose to pyoderma are shown in this table hich shows that 4.2% (13/310) patients had impetigo, 2.3% (7/310) had furunculosis and 1.3% /310) had herpes. Scabies was the lowest (1.0% or 3/310) recorded skin lesion.

Sex differential of pyoderma (Table 10) in the study indicated that boys are more prone acquiring traumatic lesions, impetigo, furunculosis and scabies than their female counterparts om the same localities.

Table 12: Extracurricular Activities engaged in by the 310 selected Pyoderma patients.

Activity	Urban (n=50)		Rural (n=260)	
	No.	%	No.	%
Games only	41	82	51	19.6
Games and farming	-		79	30.4
Games and domestic work	9	18	61	23.5
Games, farming and domestic work	-		67	25.8
Others	-		2	0.7
Total	50	100	260	100

5.2.1 Distribution of Pyoderma by Anatomical sites

The lower extremeties show the greatest predisposition to pyoderma than any other anatomical sites. Of the 310 selected patients with pyoderma, 243 (78.4%) patients had pyoderma lesions on their legs only (Table 13). No lesions were found on the neck of study patients. The pattern of distribution of lesions on the bodies of the selected patients (310) shows in descending order of frequency that, the lower limbs, followed by the upper limbs, combinations of upper and lower limbs, and the scalp and face with the trunk taking the rear.

Table 13: Frequency distribution of Pyoderma in relation to antomical sites

Site on Skins	Number (n=310)	Percentage (%)
Lower Limbs	243	78.4
Upper Limbs	32	10.3
Upper and Lower Limbs	19	6.1
Scalp and Face	12	3.9
Trunk	4	1.3
Total	310	100

5.3 BACTERIOLOGICAL FINDINGS

A total of 310 specimens of pus were collected and cultured, out of which a total of 634 bacterial isolates were obtained. The breakdown of this figure in terms of the numbers of specific bacterial groups are as follows: Streptococci, 288 (45.4%) (Plate 1 and 4), Staphylococci, 272 (42.9%) (Plates 5, 6 and 7), Pseudomonas, 65 (10.3%) (Plate 8), Proteus, 7 (1.1%) (Plate 9) and Escherichia coli, 2 (0.3%) (Plate 10). A detailed isolated bacterial distribution is shown in Table 14.

Further analysis showed that from 60 of the 310 specimens of pus cultured, pure cultures of 38 ß-haemelytic Streptococci (Plates 1 and 4) and 22 Staphylococcus aureus (Plate 5) were isolated. The rest of the isolates which came from 250 lesions were mixed cultures. Table 15 gives the numbers and porportions of pure cultures and the various combinations of mixed cultures.

It can be observed that 288 (45.4%) of the 634 isolates made, belonged to the bacterial group Streptococcus, 74.3% (214) of which belonged to Lancefield Group A or Streptococcus pyogenes. It was further found that of the remaining 25.7% (74), 13.9% and 9.4% belonged, respectively, to Lancefield Groups C and G. Details of the Lancefield groupings of the isolates made in this study are shown in Table 3.

The results of the physico-chemical characterization of the various isolates are tabulated in Tables 4 and 16. Table 4 gives detailed results of biochemical identification of the isolates to species level and Table 16 shows the macroscopic and microscopic features of the isolates.

The differentiation of Staph. epidermidis (Plate 7) from Staph. saprophyticus was based on the ability of the former to grow under strict anaerobic conditions whilst the latter does not. Looking at the Pseudomonas species, apart from P. aeruginosa (Plate 8) and P. pseudomallei which were identified to species level, the rest described in Table 14 as "other Pseudomonas" species could not be identified that far due to lack of adequate laboratory supplies and time constraints.

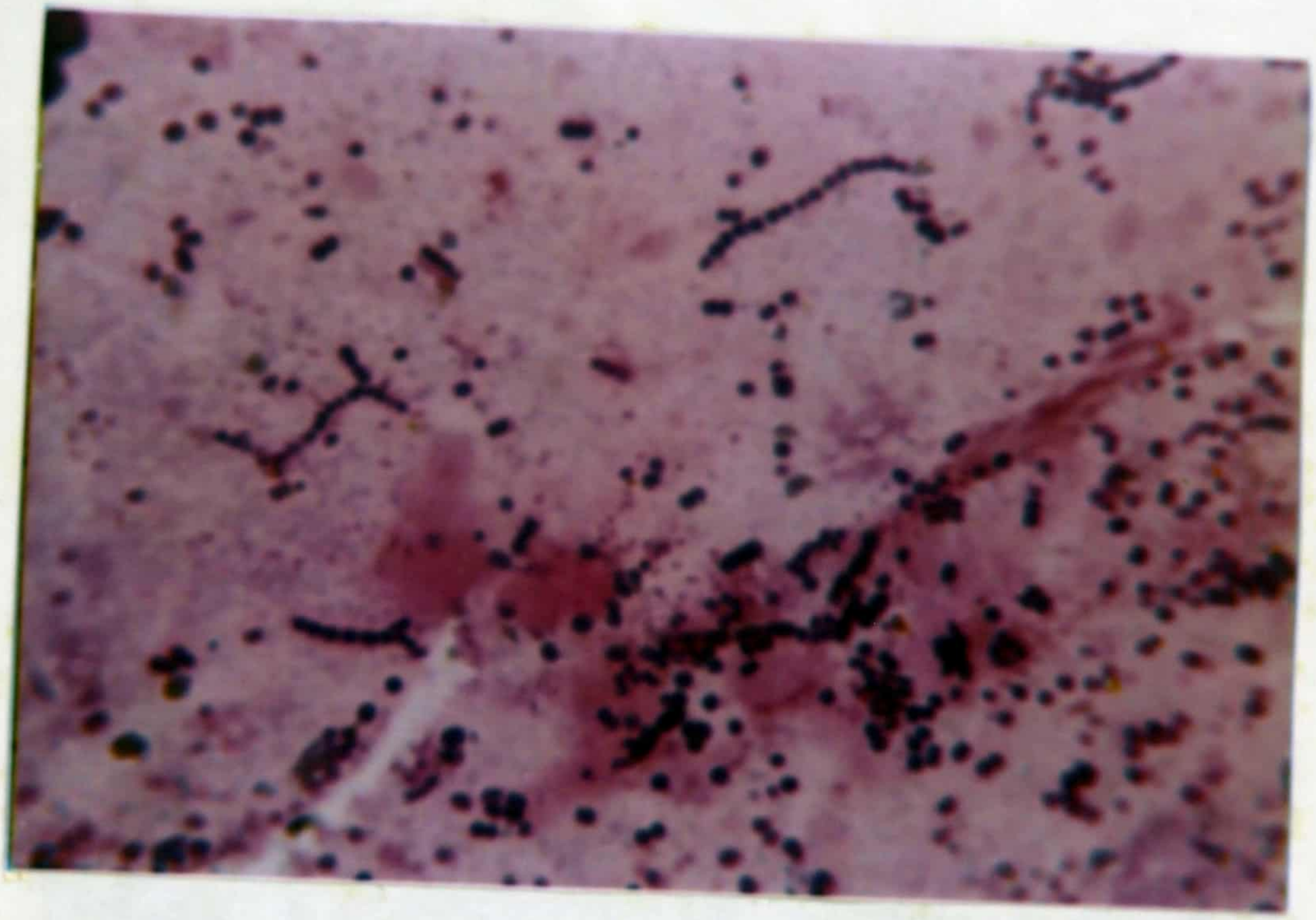


Plate 4: A micrograph showing short chains of Streptococcus Species with pus cells.

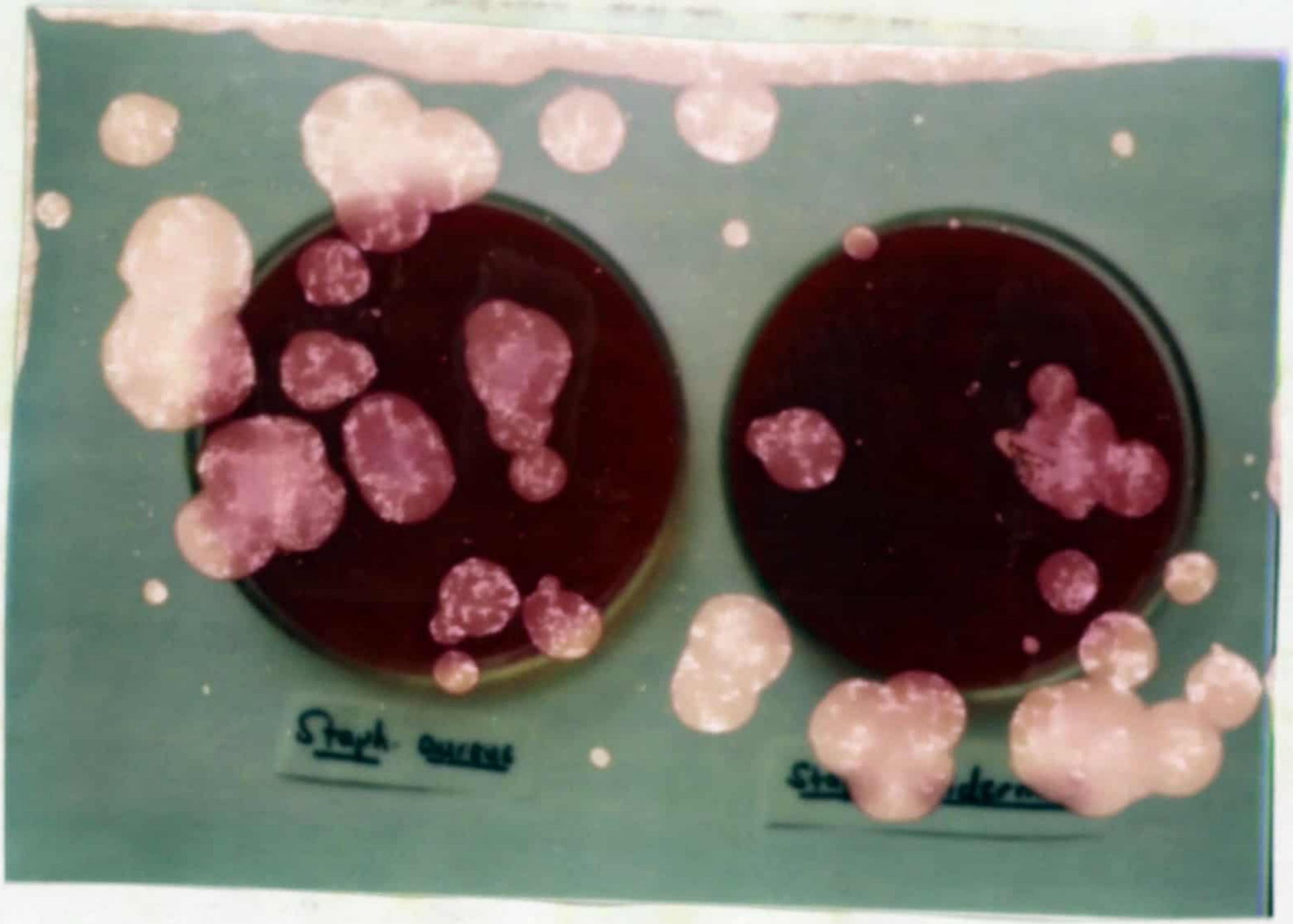


Plate 5: Discreet colonies of Staphylococcus aureus and Staphylococcus epidermidis on blood agar medium.

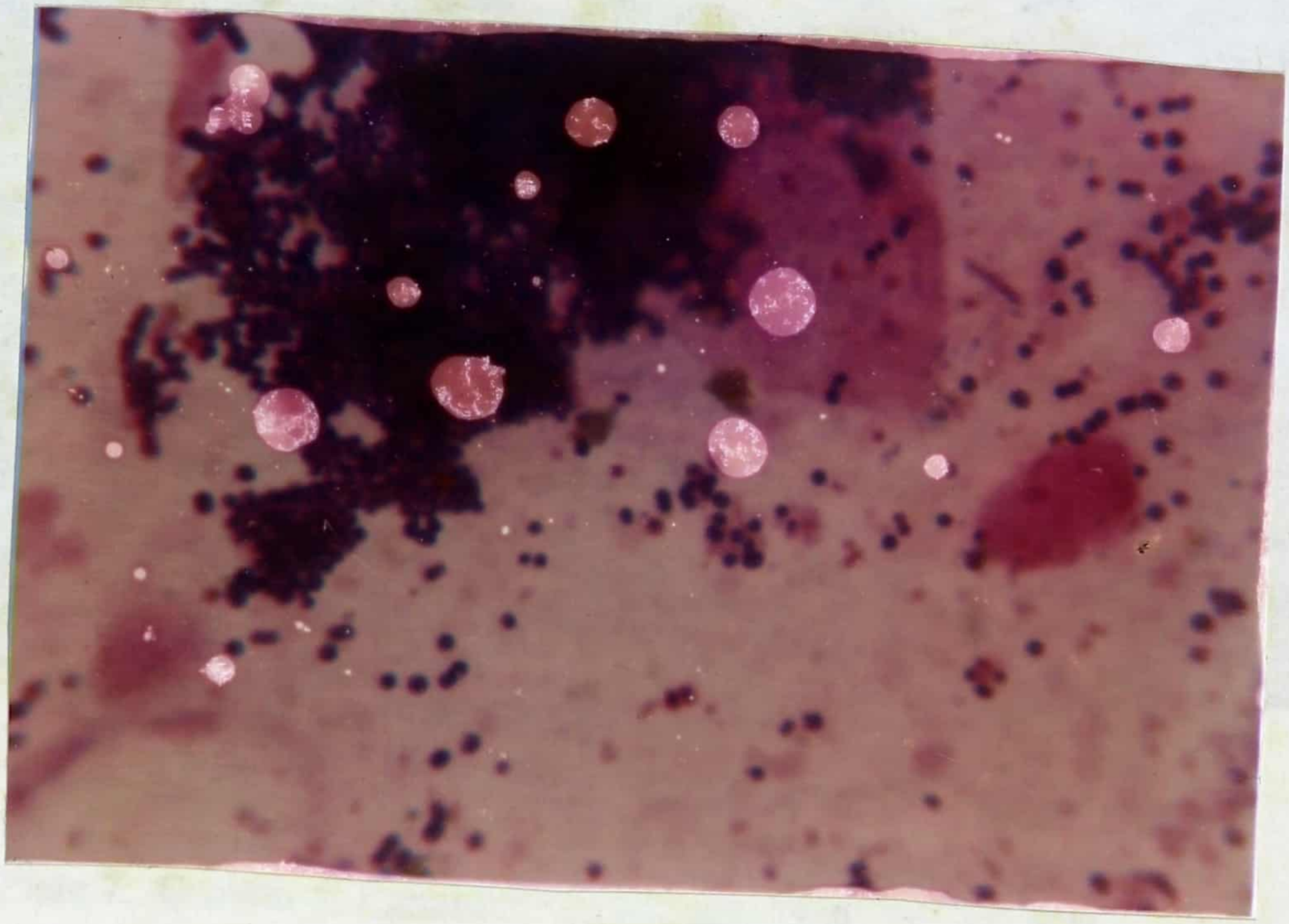


Plate 6: A micrography of Stapylococcus species in clusters with pus cells.

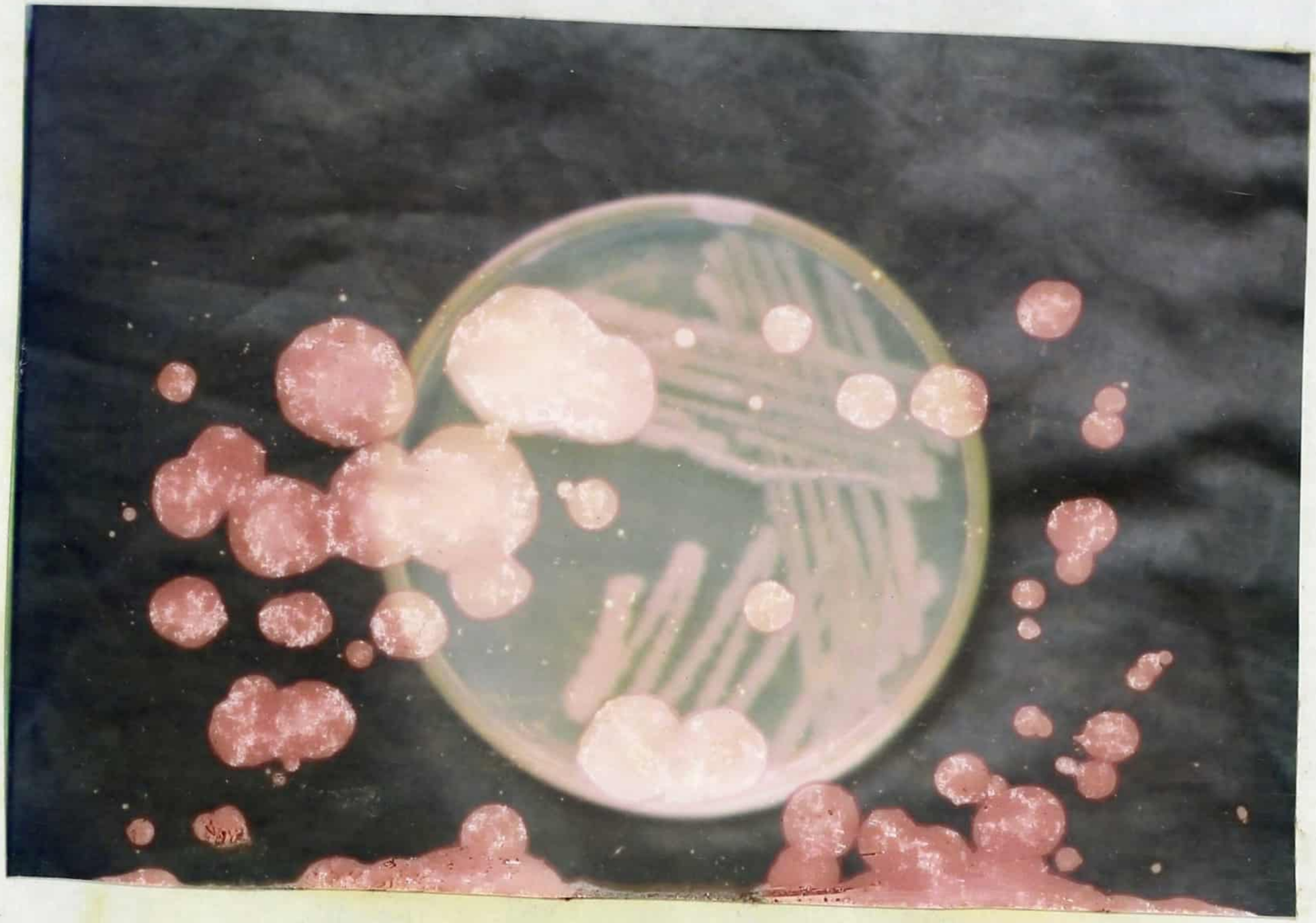


Plate 7: Growth of Pseudomonas aeruginosa on nutrient agar showing the greenish-blue coloration of pyocyanin (pigment)

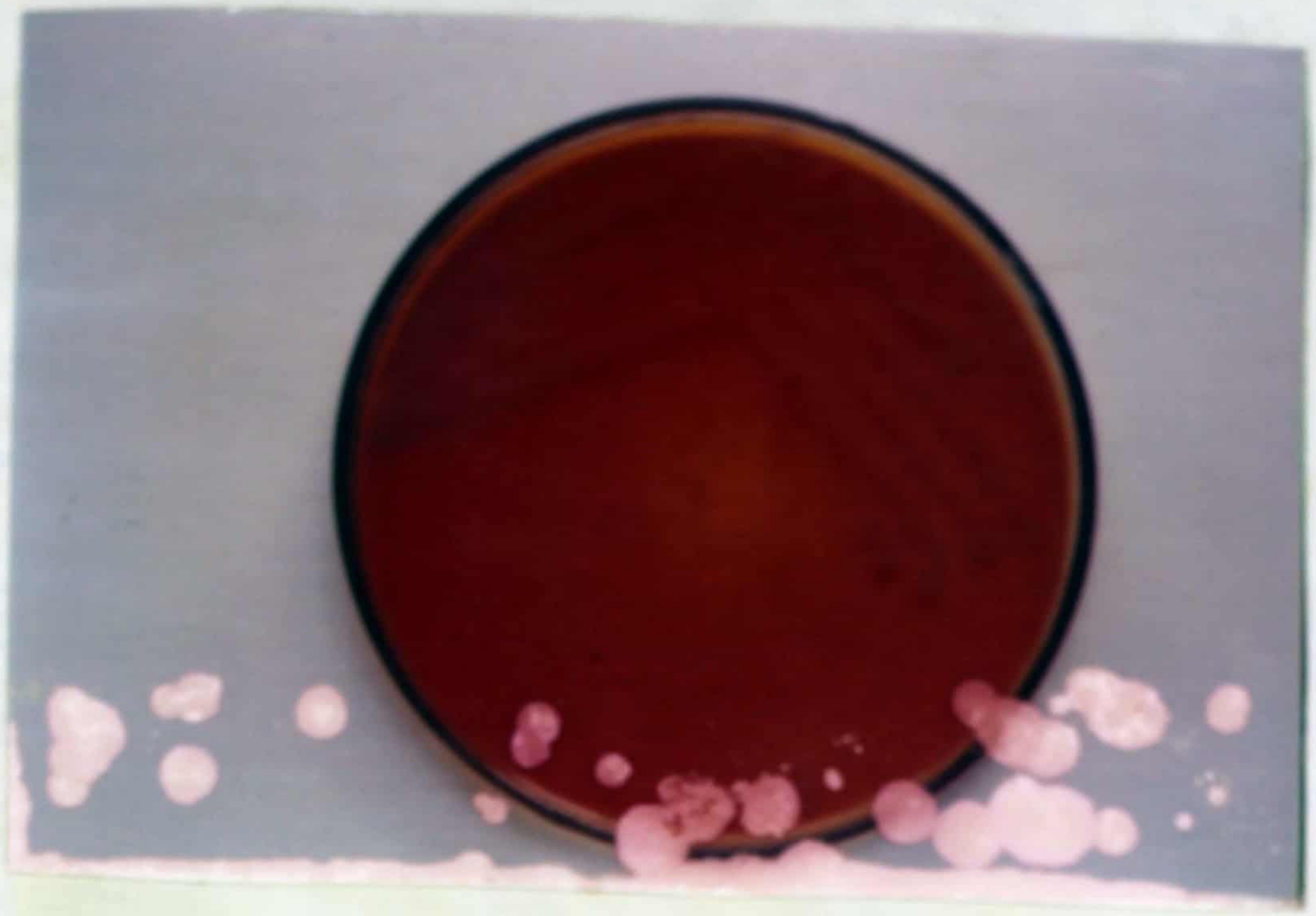


Plate 8: Colonies of Proteus mirabilis on MacConkey Agar Medium.

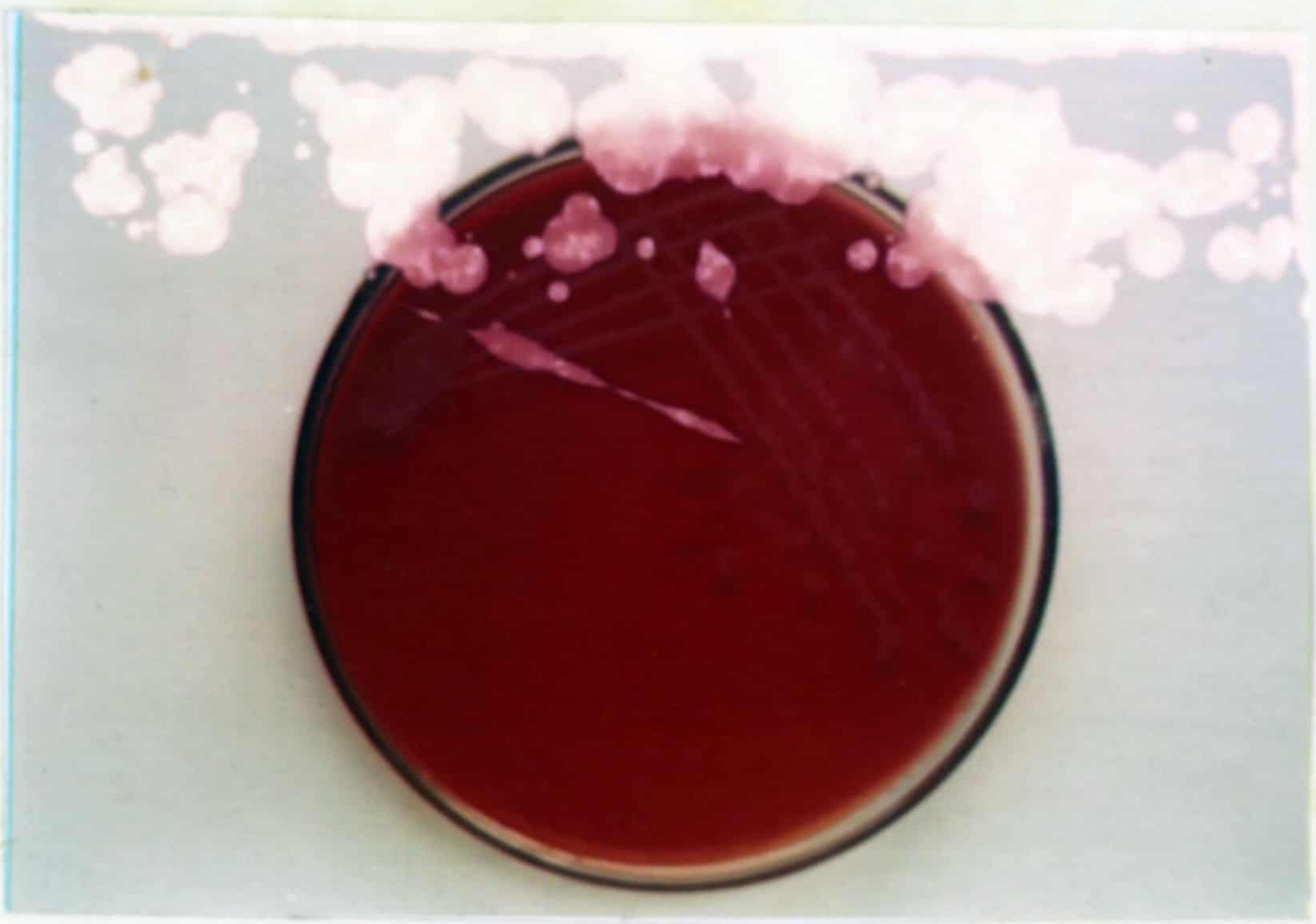


Plate 9: Colonies of Escherichia coli MacConkey Agar Medium

Table 14: Species distribution of 634 bacterial isolates

Bacteria	No. Isolated	%
β-Haemolytic Streptococcus	285	45.0
γ-Haemolytic Streptococcus (S. faecalis)	3	0.5
Staphylococcus aureus	207	32.6
Staphylococcus epidermidis	65	10.3
Pseudomonas aeruginosa	36	5.7
Pseudomonas pseudomallei	11	1.7
Other Pseudomonas Species	18	2.8
Proteus mirabilis	7	2.2
Escherichia coli	2	0.3
Total	634	100

Table 15: Numbers and proportions of pure as well as mixed cultures encountered in the study.

Bacterial Isolates	No.of lessions	%
Pure Cultures		
Streptococcus	38	12.3
Staphylococcus aureus	22	7.1
Mixed Cultures		
Streptococci and Staphylococci	183	59.0
Strept., Staph. and Pseudomonas	58	18.7
Strept., Staph., and Escherichia coli	2	0.6
Strept., Staph., Pseudomonas and Proteus	7	2.3
Total	310	100

Table 16: Macroscopic and Microscopic Characteristics of isolates

Species	Macroscopic Appearance on Medium			Microscopic Appearance
	Blood Agar	Crystal Violet	MacConkey	
Streptococcus pyogenes Group A See Plate 1 and 4	Pin-point colonies, βeta-haemolytic (more marked when incubated under anaerobic conditions)	Growth with pin-point colonoies	No growth	Gram-positive cocci, in chains of various lengths
Streptococcus agalactiae Group B	Colonies slightly larger than those ofgroup A with smaller zone of haemolysis	No growth	No growth	Same as above
Stept. Group c	Similar to group A colonies	No growth	No growth	Same as above
Enterococci Strept. Group D	Pin-point colonies, βeta-haemolytic	No growth	No growth	Same as above
Streptococcus faecalis Group D	Glossy white pin-point colonies non-haemolytic	No growth	Magenta-Pink pin-point colonies	Same as above
Streptococcus Group G	Similar to group A colonies	No growth	No growth	Same as above
Staphylococcus aureus See plates 5 and 6	Smooth, Golden-yellow, βeta-haemolytic(some strains are non-haemolytic) colonies of between 1-3 mm in diameter	Some strains grow	More golden in colour and slightly smaller in size than that on blood agar. Some straings are lactose fermenting.	Gram-positive occci, grape-like clusters
Staphylococcus epidermdis See Plate 5	Whitish(non-haemolytic) colonnies of about 2mm in diameter	No growth	Mostly weakly lactose fermenting	Gram-positive cocci, grape-like clusters
Proteus mirabilis See Plate 8	Swarming growth.	Swarming growth	Non-lactose fer-menting, about 1m in diameter.	Gram negative rods
Escherichia coli See Plate 9	Whitish colonies of about 3mm in diameter, slightly mucoid in appearance.	No growth	Pink (lactose fermenting) colonies of about 2mm in diameter	Gram negative rods
*Pseudomonas aeruginosa See Plate 7	Large,flat,haemoly-tic colonies produces greenish (pyocyanin) pigment	No growth	Large, flat, Non-lactose ferm-enting colonies	Gram-negative pleomorpic rods
*Pseudomonas pseudomallei	Relatively small non-haemolytic ringed and striated colonies with no pigment, (produces brown pigment on Nutrient agar).	No growth	Rough, large flat colonies	Gram-negative rods with bipolar straining
Othe Pseudomonas	Large flat, non-haemolytic colonies	No growth	Confluent colonies mucoid in nature	Gram-negative rods

* Gives off a characteristic fishy smell

5.4 BACTERIAL SUSCEPTIBILITY TO ANTIMICROBIAL AGENTS

Table 17 gives the patterns of antimicrobial sensitivity of all isolates made in the study to nine (9) selected antibiotics. With the exception of ß-haemolytic Streptococci which showed susceptibility to all nine antibiotics, the rest of the isolates were susceptible or resistant to a variety of antibiotics. Penicillin (2µg) did not have any antimicrobial effect on any of the isolates except against ß-haemolytic Streptococci. Gentamycin (10µg) gives a different picture, with all isolates completely succumbing to its action. The Pseudomonas species showed resistance to about 50% of the drugs tested. However, P. pseudomallei and "other Pseudomonas" species responded favourably to Carbenicillin, Chloramphenicol and Tetracycline (P. pseudomallei only).

Bacteria Species	No of Strains tested	Penicillin 2µg	Lincomycin 2µg	Ampicillin 10µg	Methicillin 10µg	Erythromycin 15µg	Carbenicillin 100µg	Gentamycin 10µg	Tetracyclin 10µg	Chloram-phenicol 10µg
β-haemolytic streptococci	285	99.3	100	76	100	100	98.2	100	73.8	100
Strept. faecalis	3	0	N.T.	100	100	100	0	100	0	100
Staph. aureus	207	0	98	28.6	0	100	64.5	100	90	83
Stapt. epidermdis	65	0	0	22.3	0	9	N.T.	100	0	0
P. aeruginosa	36	0	0	9.4	0	0	3.8	100	0	51
P. pseudomallei	11	0	0	36.4	N.T.	37.5	80	100	90	100
Other Pseudomonas	18	0	0	N.T.	0	0	78	100	30	100
Proteus mirabilis	7	0	0	100	0	0	100	100	71.4	100
E. coli	2	0	0	100	0	0	100	100	0	100

N.T. - Not tested

6.0 DISCUSSION

It is evident from this study that pyoderma, a common health hazard, exists among our child population. The incidence rate of pyoderma calculated was 16.02% but which figure was reduced to 5.2% when the exclusion criteria employed in this study disqualified as many as 654 patients with the condition.

These 654 subjects with skin ulcers suggestive of pyoderma were rejected on the grounds that they had some form of treatment including antibiotic application (directly from the capsules) or application of local herbal preparations to the lesions. Such prior treatment of the lesions was found to interfere with the bacterial isolation attempts.

The type of pyoderma accepted for the study were therefore of the two major types, namely the non-bullous, amber-crusted ulcers and the vesicular types which have had no prior treatment, whatsoever. Despite the reduction in the pyoderma study population from 964 or 16.02% to 310 or 5.2% for the purposes of this study, yet the occurrence of as many as 964 in a population of 6017 is an indication that pyoderma is still common among our child population. An earlier report by Belcher, et al., (1977) on pyoderma in Danfa, Southern Ghana, has shown that pyoderma is endemic in Ghana. Our results confirm this view.

From the results of this study, it became clear that male children are prone to suffer from pyoderma more than their female counterparts, with a male/female ratio of 3:1. The preponderance of pyoderma in boys may be attributed, to a large extent, to the rather vigorous playful activities, eg. running, tree climbing and climbing of other tall structures, playing of football etc boys indulge in. These activities almost invariably

earn them injuries which become infected with microorganisms, if such wounds are not kept clean. In contrast to boys, girls generally, prefer less vigorous and less injury-prone games (personal observation made from interview).

In this study peak incidence of pyoderma was found among the eight to ten year old age group, an age group which constituted 40.6% of the 310 selected pyoderma study population. The results as presented in Table 6 and Fig. 5.1 compares favourably with the results of other workers (Belcher et al, 1977; Rotta et al, 1985; Acheampong et al, 1988). The fourteen years age-group (0.65%) was the least likely group to have pyoderma. From these results (those of this study and of those from workers cited above), it appears that the rate of pyoderma decreases with increase in age, the disease generally, being common in younger children than in older ones. Taplin et al, (1973) also found that except for those adults who engage in injury-prone occupations like manual labourers and jungle combating soldiers, pyoderma is least encountered in adults.

Comparing the results obtained for the two defined settlements, the economically disadvantaged rural study population recorded a higher rate (8.7% or 260/3001), of pyoderma, than that recorded for the economically privileged urban study population (1.7% or 50/3016). The reason for the disparity in the pyoderma rates between populations of the two areas studied is again attributable to a higher tendency of acquiring injuries in the rural area than in the urban area. The main occupation in the rural study area is farming, which is manually carried out with simple and crude implements. This job employs the services of all able-bodied persons in the family

including children. Hence for most school children, farming forms part of their extracurricular activities. Children in the rural study group were no exception and as many as 146 (56% of 260) of the rural pyoderma study group have it as an extracurricular activity. The nature of the farming, therefore, exposes those who engage in it, especially children who are normally careless to a lot of risks, including injuries. The results shown in Table 11 lend credence to this fact, for of the total number of rural patients with pyoderma resulting from trauma 73 were due to cuts sustained through farm work.

In the urban area, however, none of the pyoderma patients was engaged in farm work. Besides, farming (tilling of the land) did not form part of the main occupations of the urban study population and, therefore, children of this area were not exposed to injuries from farm work.

A report by Cruickshank (1976) says that, when socially considered, pyoderma is common among the low social class, particularly among the under-privileged who live under poor unhygienic conditions, often exacerbated by over crowding. The results of this study is in conformity with this report. As high as 71.9% of the 310 selected pyoderma patients belonged to social class 4 (the lowest social class). Interviews with parents of the study population revealed a common feature among this class of study subjects, all families studied slept on the average, seven persons to a small room (approximately 2' x 4') which was also poorly ventilated. As mentioned earlier, overcrowding encourages the spread of pyoderma, particularly, those of the vesicular type, among individuals and siblings. It was observed that seven impetigo patients and

three scabietic pyoderma patients who lived in overcrowded homes had severe infection. Again, the three scabietic patients had siblings who also suffered from the infection.

One important factor that might have contributed immensely to the high rate of incidence of the infection is the standard of personal hygiene; this proved, among others, to be the principal determinant of the high infection rate of incidence among the people, regardless of their social status. Thus, people with high social standing can be affected if they fail to observe good personal hygiene.

From the results of this study (Table 4) pyoderma was six times more common among people with poor hygienic practices than among those with good hygienic practices. It appears, therefore, that pyoderma has a predilection for people with poor personal hygiene.

Dudding et al, (1970) recorded high rates of group A Streptococci, from normal skin (uninfected skin), clothing and beddings. They asserted that the individual's hygienic practices and sanitation of his immediate surroundings, if poor, become good reservoirs for the causal organisms of pyoderma and that from these sources the causal agents could infect the inner tissues of the skin through a break in the integument of the skin.

Clinically, the pyoderma diagnosed in this study were of traumatic type, mostly due to mechanical injuries (71.6%), insect bites (8.1%), impetigo (4.2%), furunculosis (2.3%) herpetic lesions (1.3%) and scabietic lesions (1.0%). The remaining pyoderma lesions (11.6%) designated as `miscellaneous' were those lesions whose origin could not be ascertained and whose history could also not be obtained from the interview of both

subjects and their parents. From the results, it is obvious that trauma due to mechanical injuries and insect bites, more frequently, results in pyoderma. Some early investigators have reported the same findings (Wannamaker, 1970; and Taphin et al, 1973). These minor traumas invariably predispose to bacterial infection culminating in pyoderma.

Pyoderma occurs mostly on the lower extremities. In all, 84.5% (262) of the 310 selected patients had active lesions on their legs. Of the above number seventy-eight percent (78.4%) of the 310 patients had lesions on the legs only, while 6.1% patients had the lesions on both legs and arms. The remaining 15.5% (48/310) patients had the lesions distributed on other anatomical sites as depicted in Table 13. The propensity of pyoderma to affect the legs is attributed mainly to the multiple injuries frequently sustained on the legs which subsequently result in pyoderma, and not that the pathogens involved have a special predilection for the legs.

The main pathogens encountered in lesions of pyoderma in this study was ß-haemolytic Streptococci. Ninety-three percent (93%) of the 310 lesions studied, were due to the ß-haemolytic Streptococci. In all, 288 streptococcal species were isolated and of this figure, 214 belonged to Lancefield Group A or Streptococcus pyogenes (See Table 3). The second highest causal pathogen recovery rate of 66.8% was for Staphylococcus aureus, (207 of 310 lesions studied). Twenty-two (22) of the 207 lesions yielded pure cultures of Staph. aureus while the remaining (185) lesions produced mixed cultures including Streptococci.

The ability of Staph. aureus alone to initiate pyoderma has been doubted by some workers (Nsanzumuhire et al., 1972) but its association with streptococcal

pyoderma have long been established (Barrow, 1955; Parker et al, 1955; Dillon, 1968 and Allen et al., 1971).

In spite of their co-existence, Streptococci, particularly of Lancefield Group A (Strept. pyogenes) appear to play a more significant role in initiating the infection than does Staph. aureus. Evidence supporting this assertion is provided by Ferrieri et al.(1972) who reported that a high frequency of Streptococcus pyogenes often occurs on the normal skin before the onset of pyoderma. Prior to this report, Dudding et al. (1970) had analyzed serial cultures obtained from the same individuals, at weekly intervals, and had found that strains of Group A Streptococci isolated from skin lesions of these individuals had appeared on normal skin two weeks prior to the appearance of the pyoderma lesions. In another study, Dajani et al. (1972) recovered pure cultures of Group A Streptococci from vesicular and/or pustular lesions and also from the early stages of the disease rather than from the later stages.

From the results of this study it is also evident that, exudate from 38 lesions (made up of impetigo, furunculosis and some skin ulcers) when cultured, gave pure cultures of Streptococcus pyogenes (Table 15) with the remaining of Streptococci strains isolated being from mixed cultures.

According to Dajani and colleagues (1972), a correlation exists between the duration of lesions and the type of bacteria found in them. They pointed out that whereas early-staged-lesions yielded mostly Streptococci, late or older lesions gave more Staphylococci and bacteria other than Streptococci. In this study, since the ages of the various lesions were not studied, it cannot be proved that the 22 lesions (all non-bullous

skin ulcers) which yielded pure cultures of Staphylococcus aureus had been initiated by this bacterial species.

Clinically, lesions caused by Staph. aureus are mostly characterized by abscess formation (Youmans, 1985) for example, staphylococcal impetigo presents with persistent bullous lesions (Earle, 1985). In this study, however, no consideration was given to bullous lesions. It is, therefore, possible that these lesions which yielded pure cultures of Staph. aureus were older lesions. Based on the above information (literature and results of this study) it can be concluded that ß-haemolytic Streptococci, particularly Strept. pyogenes, is the primary causative agent of pyoderma with Staphylococcus aureus serving as secondary aetiologic agent. The frequency and aetiological significance of their co-existence in pyoderma cannot also be over-emphasized. In 250 of the 310 lesions studied ß-haemolytic Streptococci and Staphylococci existed together.

Although, Strept. pyogenes, (Group A Streptococcus) has been found to be the possible initiator of the infection, it should not be the only concern of researchers as it appeared to have been in the past. The possibility of Lancefield Groups C and G Streptococci in initiating the infection should not be overlooked. In this study the rates of recovery of these pathogens from pyoderma were quite significant; there were 40 Group C strains as against 28 of Group G, (Table 3). Moreover, no Group A Streptococcus was isolated from the lesions in which Groups C and G occurred. Some earlier researchers have also achieved higher isolation rates of Group C and Group G in Ghana (Belcher et al., 1977) and Group C in Nigeria (Ogumbi, 1971). These reports,

coupled with the results of this study do suggest the capability of Group C and G Streptococci to cause pyoderma.

Another common staphylococcal species frequently isolated in this study was Staphylococcus epidermidis. It was obtained from 65 active lesions and in combination with other bacterial species. The aetiologic role of Staph. epidermidis in pyoderma is not fully understood. Since it forms part of the normal resident flora of the skin, it is thus believed to have found its place in the lesions as a mere contaminant. Dajani et al, (1972) who isolated this species in late lesions of pyoderma have speculated that Staph. epidermidis probably produces some antibiotic - like substance(s) which is/are bactericidal to Streptococci. Hence its infrequent association with Streptococci.

Apart from Streptococci and Staphylococci other bacteria isolated from pyoderma lesions in the study were Pseudomonas (pathogenic and non-pathogenic species), Proteus mirabilis and Escherichia coli. These were not isolated in pure cultures but rather in combinations with Streptococci and Staphylococci (See Table 15).

Although Proteus mirabilis and E. coli are known to cause serious infections in humans, their aetiologic significance in pyoderma has not been reported. Being part of the resident flora of soil and polluted waters, they had probably entered the lesions as contaminants rather than as pathogens.

Pseudomonas species were recovered from 65 of 310 or 21% of the lesions studied. Of this number 36 lesions produced Pseudomonas aeruginosa, 11 others gave Pseudomonas pseudomallei and the rest, 18 lesions, produced other species of Pseudomonas whose identification could not be completed due to exhaustion of

laboratory supplies. All said, the presence of P. aeruginosa and P. pseudomallei in typical amber crusted pyoderma was particularly disturbing because of their grave pathogenetic changes caused to wounds. Their adaptability and innate resistance to most antibiotics and disinfectants (Govan, 1992) can pose a serious danger to the patient, in that, the infection may be protracted and its cure delayed, being transformed into a more debilitating skin ulcer.

An interesting observation made which was also deluding was that, lesions which carried P. aeruginosa in them did not show the clinical characteristics often associated with this organism in skin ulcers. The clinical indication, which is, a greenish discoloration of particularly burn dressings due to pyocyamin production was not visible. Instead the lesions in question were similar in every way to non - P. aeruginosa induced lesions. Pyoderma lesions of this kind can be elusive when it comes to the administration of proper therapy in the absence of laboratory diagnosis since the presence of P. aeruginosa may not be suspected.

According to Cheesborough (1985) about 4% of P. aeruginosa strains do not produce the characteristic bluish-green pyocyamin pigment at all. It therefore, would have sufficed us to attribute the lack of visible pyocyanin production by the P. aeruginosa strains isolated from the thirty-six (36) pyoderma lesions to belong to the 4% non-pigment producing P. aeruginosa. However, this attribution cannot be done in this study on the grounds that all thirty-six (36) P. aeruginosa isolated, ironically, readily produced the pigment on nutrient agar plate when aerobically cultured. To this end, it can be speculated that there could have been some extracellular agents produced by the

other bacterial species present, particularly, Streptococci and Staphylococci, which might have suppressed or masked the production of pyocyanin by P. aeruginosa in the lesions.

Another unsuspecting pathogenic species of Pseudomonas isolated was P. pseudomallei. This pathogen is known to cause melioidosis, a condition characterised by pus-filled nodules and abscesses in the lungs, spleen, liver, joints and skin or subcutaneous tissues (Cheesborough, 1985) in man. The infection is via open wounds. With this pathogen in wounds, patients stand the risk of developing melioidosis which may gravely affect their health.

The above given reasons make the isolation of Pseudomonads in the study very fascinating and at the same time quite disturbing. What is more disturbing is their general resistance to most of the common antibiotics. Earlier workers on pyoderma did not give any indication as to their (Pseudomonas species) possible existence in pyodermic lesions. Studies on pyoderma have for the past few decades bordered on Streptococci and Staphylococci. One, therefore, wonders if these pathogens (the Pseudomonas) were ever isolated in addition to what were often reported; if earlier workers encountered this species in their research but left them out then it is possible that these workers only treated these organisms as mere contaminants. In this study the rate of isolation of pathogenic Pseudomonas species was quite significant and therefore, their significance could not have been easily glossed over, considering their pathogenic capabilities. However, further research would be needed to eliminate this uncertainty

function. As such, it should be administered with great caution amidst frequent tests for changes in the haematopoetic system (Corcoran, 1985).

A further investigation to expose the specific types of Lancefield group A Streptococcus, (ie. nephritogenic or non-nephritogenic) involved in the study could not be conducted because of lack of adequate laboratory supplies. As a result, the extent to which our child population is exposed or prone to the sequel of pyoderma, namely, acute-glomerulonephritis, could not be ascertained.

7.0 SUMMARY

This research sought to expose the possible aetiologic agents of pyoderma among children in the Ashanti Region of Ghana; the findings of which, it was envisaged would help medical practitioners in their choice of appropriate therapy for pyoderma, particularly, in the absence of proper laboratory diagnosis, and to serve as a basis for future research on the risk of a possible outbreak of acute glumerulonephritis, an infection consequent upon pyoderma, in our child population.

Of a total population of 6,017 drawn from an urban (Kumasi) and a rural (Ejisu-Juaben) setting, 964 (16.02%) patients had the infection but only 310 (5.2%) patients out of this number qualified to be included in the study.

Pyoderma was more common among the rural children (ie. 260/310) than among the urban children (ie. 50/310) and among boys than among girls with a ratio of 3:1. The peak incidence of the disease was among the 8-10 year olds. About 72% of the 310 patients belonging to the low social class and who also lived under poor living conditions were mostly affected. The habit of keeping poor personal hygiene was identified as the principal determinant of pyoderma. In this study, pyoderma was six times more common among those who observed poor personal hygienic practices than among those whose hygienic practices were good.

The type of lesions which predisposed to pyoderma in this study were mostly traumatic lesions due to various mechanical injuries, (71.6%). The remaining 28.4% of

the pyoderma lesions resulted from Impetigo, Herpes, Furunculosis, Scabies, Trauma due to insect bites and others. In all, about 634 bacterial species made up of Streptococci (288), Staphylococci (272), Pseudomonas (65), Proteus (7) and E. coli (2) were recovered from the lesions studied.

The Streptococci were mainly of the β-haemolytic type with about 74% belonging to Lancefield Group A. This Group was found to be the primary causative agent of pyoderma with Staphylococcus aureus serving as secondary aetiologic agent and often co-existing with the former. Of the 65 Pseudomonas species isolated, 36 were P. aeruginosa and 18 were P. pseudomallei; this latter opportunistic bacterium is capable of causing serious secondary infections. In addition, they are generally resistant to the common antibiotics (the penicillins) and this characteristic make their presence in pyoderma quite disturbing. What could not be ascertained was their aetiologic significance to pyoderma.

The reactivity of various antibiotics viz, Penicillin, Erythromycin, Lyncomycin, Ampicillin, Methicillin, Tetracycline, Carbenicillin, Gentamycin and Chloramphenicol was observed. Of these, Penicillin (2mg) showed a hundred percent antimicrobial action against β-haemolytic Streptococci only, exhibiting no antimicrobial action on the other bacterial isolates. Gentamycin (10mg) on the other hand, showed a 100% action against all isolates in the study.

8.0 RECOMMENDATIONS

This research has indeed added to our knowledge of the possible aetiologic agents to expect in pyoderma in the Ashanti Region of Ghana. It has also provoked our thoughts on the aetiologic significance of some bacterial pathogens in pyoderma and the possible secondary infections they could cause. With this knowledge, it is now appropriate to discuss the possible treatment and control of the disease.

8.1. Suggested Therapy (Arndt, 1989; Fitzpatrick et al., 1987)

Impetigo (pyoderma) caused by both Streptococci and Staphylococci may be treated with either topical or systemic antibiotics; such treatment is necessary although the disease is often self-limited and that there is no proof that treating pyodermas will prevent subsequent glomerulonephritis.

Systemic treatment is at times justified for the following reasons:

1. that pyoderma sometimes does not resolve for a very long time and may become widespread before it does, and
2. that antibiotics can shorten the healing time and decrease the number of recurrences.

Treatment failures with oral antibiotics have been reported in 24% of patients treated with Penicillin V, 4% of patients treated with Erythromycin estolate, and no patients treated with Cephalexin, although the number of resistant strains is increasing.

The following are some suggested therapy:

1. Penicillin is the drug of choice in the treatment of streptococcal pyoderma (Fitzpatrick et al., 1987; result this project's antibiotic testing – pg 75). Penicillin can be administered either as a single injection of long-activity benzathine penicillin (300,000 to 600,000 units for children; 1,200,000 units for adults) or orally (25,000 to 100,000 units/kg per day in divided doses every 6 hours for 10 days.

2. Alternatively, where widespread or recalcitrant pyoderma (impetigo) exits Treatment with erythromycin (30 to 50 mg/kg per day by mouth in divided doses every 6 hours for children; 250 to 500 mg by mouth every 6 hours for 10 days can be administered).

3. Mupirocin (bactroban) has been described as a safe and effective treatment for pyoderma. It may be superior to oral erythromycin in the eradication of Staph aureus, including antibiotic-resistant strains. Mupirocin is applied tid for 7 to 10 days and its more costly than a 10-day course of erythromycin. Extensive pyoderma should be treated with oral antibiotics.

a. Topical antibiotics may be useful in preventing streptococcal pyoderma applied tid for minor traumas, especially in children at increased risk for such infection.

8.2 Preventive Measures

Good personal hygiene is a great preventive tool against recurrence of pyoderma in the patient and against the passing on of the infection to siblings in close contact. A few suggestions are outlined below:

1. Regular bathing habits (at least once a day) by both patient and family members is very useful. A bacteriocidal iodine (eg. Betadine skin cleanser) or bacteriostatic soap or solution containing hexachlorophene or chlorhexidine (Hibiclens) are recommended for use at all bath times.
2. Keep the patient's fingernails cut short and hands washed frequently. Other members of the family should also wash hands carefully after touching any infected area.
3. Disinfection of household surfaces with sodium hypochlorite solution (*chlorox* or *parozone,* mixed as directed by the manufacturer), may be needed to prevent spread of the infection.
4. Patient should not share towels, bedclothes and bedding with others.

8.3 Future Research

For effective control of the disease which is endemic in our locality, future research work should focus on the following:

1. The aetiologic significance of the Pseudomonads in pyoderma.
2. The effects of extracellular products of certain pathogens on others, particularly, on P. aeruginosa in Pyoderma.
3. The serotypes of Streptococcus pyogenes involved in pyoderma in Ghana.

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APPENDIX 1

QUESTIONNAIRE

A study of pyoderma among primary school children in the Ejisu-Juaben and Kumasi Districts.

Study No.....

School Name .....

Location .....

PERSONAL DATA

(a) Name:.....

(b) Age:..... (c) Sex: Male / / Female / /

(d) Class:..... (e) Place of residence.....

(f) Whom do you stay with?

1. Parents / / 2. Other relative / / 3. Non-relatives / /

(g) Description of residence

1. Mud/Wooden building //
2. Concrete house (compound) //
3. Concrete house (apartment) / /

PERSONAL HABITS

(a) Source of domestic water

1. Pipe borne / / 2. Well / / 3. Stream/river / /

4. Other (specify).....

(b) How often do you bathe in a day

1. Once / / 2. Twice / / 3. Three times or more / /

4. Not daily in a week / /

(c) How often do you use soap in bathing times

1. Always / / 2. Often / / 3. Occasionally / /

(d) What type of soap do you use?.....

(e) Extracurricular activities: 1 Games only / /
2. Games and farming / / 3. Games and domestic work / /
4. Games, farming and domestic work / /
5. Other (specify).....

CLINICAL DATA

(a) Predisposing factors of pyoderma

1. Trauma - A fall / / B. Cut / / C. Burns / /
2. Insect bite / /
3. Scabies / /
4. Other (specify).....

(b) Site (anatomical) and number of pyoderma lesions.

1. Scalp/face ..... 2. Upper limbs.....

3. Trunk..... 4. Lower limbs.....

(c) Site (on skin) of specimen collection

(d) Colour of pus.....

(e) Date of specimen collection.....

FAMILY DATA

Father/Mother

(a) Age..... (b) Occupation (Primary).....

(Secondary, if any).....

(c) If you are a farmer, what main crop do you grow?.....

..... minor crop (if any)_____

(d) Level of education:

1. None / / 2. Primary/middle / / 3. Secondary / /
4. Post Secondary/Tertiary / /

(e) How many children/dependants do you have?

Male..... Female.....

Observation/Comments by Interviewer:.....

.....

LABORATORY INFORMATION

Study No. .....

1. Date of processing of specimen.....

2. Findings from gram's staining of smear from:

   a. Lesion:.....

   b. .....

   c. Culture:.....

3. Description (Microscopically) of organisms isolated in the following culture media:

   (a) Blood agar

      i. incubated aerobically.....

      ii. incubated anaerobically.....

   (b) MacConkey agar.....

   (c) Cooked meat broth.....

3. Colony morphology on:

   (a) Blood agar: i. aerobic:.....

ii. anaerobic_____

(b) MacConkey agar:_____

5. Some biochemical tests performed:

Test :	Positive	Negative
Catalase :		
Coagulase:		
Oxidase :		

APPENDIX 2.0

CULTURE MEDIA PREPARATION

2.1 BLOOD AGAR

Blood agar, an enriched medium is made from nutrient agar and 7% sheep blood. It is used to culture a wide variety of microorganisms including fastidious ones like Streptococcus species. It can also serve as a differential medium particularly for the various Streptococcal species based on haemolysis.

Needed ingredients (for 500ml)

Nutrient agar	- 14g
Distilled water	- 500ml
Sterile defibrinated sheep blood	- 35ml
pH	- 7.2 - 7.4

Method of Preparation

14g of dehydrate nutrient agar (Oxoid medium CM3) was weighed and transferred into a 1 litre capacity conical flask. To this, 500ml of distilled water was added, shaken vigorously to ensure thorough mixing and then boiled to bring it into solution . It was later autoclaved at 121°C for 15 mins and allowed to cool to 50°C. At this temperature 35ml of defibrinated sheep blood was added aseptically, mixed thoroughly by gently shaking and dispensed into $9cm^3$ sterile petri dishes. They were

allowed to set and the plates were labelled and dated. Packaging was done by inverting the plates and kept in clean polythene bag and stored at 4°C. The shelf-life was 21 days.

2.2 Crystal Violet Blood Agar Medium

This is a selective medium for Group A ß-haemolytic Streptococci or Streptococcus pyogenes. Crystal violet at a concentration of 1 in 500,000 inhibits the growth of most microorganisms.

Needed Ingredients (500mls)

Dehydrated Nutrient Agar

Medium (Oxoid, CM3) - 14g

Distilled water - 500ml

Sterile defibrinated sheep blood - 35ml

0.02% w/v aqueous solution of crystal violet - 5ml

Method of Preparation

Medium was prepared as described for blood agar above. After the addition of the defibrinated blood, 5ml of 0.02% crystal violet solution was aseptically added. Storage was at refrigerator temperature.

2.3 MacConkey Agar Medium

MacConkey agar is a differential medium used to distinguish between lactose fermenting and non-lactose fermenting bacteria. Lactose fermentors have the ability to ferment the carbohydrate, lactose, to produce acid and carbon dioxide. The acid released react with an indicator incorporated in the medium to change the colour of the medium.

In MacConkey, neutral red is the indicator used which at an acid, organisms grown on it pick up the colour making their colonies appear pink or red. An example of a lactose fermentor is Escherichia coli.

Ingredients Needed (500ml)

Peptone - 10.0g

Lactose - 5.0g

Bile salt - 2.5g

Sodium chloride - 2.5g

Neutral red - 0.04g

Agar - 6.0g

pH - 7.2-7.6

Method of Preparation

Twenty-six grams (26g) oxoid MacConkey dehydrated medium was transferred to a 1 litre capacity conical flask containing 500ml of distilled water. The flask was plugged with cotton wool and the contents brought to boil for complete dissolution of the agar. This was later sterilized by autoclaving at 121°C for 15 mins. The medium was cooled to 50°C and dispensed into sterile $9cm^3$ petri dishes. The poured medium was allowed to set; the plates were labelled, dated and stored in clean polythene bags at 4°C. The shelf life was 28 days. The pH was between 7.2 and 7.4.

2.4 Cooked meat medium

This is an enriched broth medium used for the rapid growth and maintenance of microorganisms, particularly, anaerobes. It also helps in the differentiation of saccharolytic organisms (which produce acid and gas with reddening of meat) from proteolytic organisms (which also decompose meat with blackening of the meat).

Ingredients used (Oxoid dehydrated medium - CM81).

Heart muscle	-	454g
Peptone	-	10g
Lab-Lemco powder	-	10g
Sodium chloride	-	50g
Dextrose	-	2g
pH	-	7.0

Method of Preparation

1g of Oxoid cooked meat dehydrated medium (CM-81) was dispensed into 20ml capacity screw-capped Bijou bottles. Ten (10)ml of distilled water was added and allowed to soak for about 5 mins. and then, sterilised by autoclaving at 121°C for 15 minus with caps loosely screwed on. Medium was allowed to cool and caps were then tightly screwed on. Medium was stored in a cool dark place. Cooked meat medium was also used to maintain Streptococcus species isolated in the study. It was subcultured after every 3 months.

2.5 Kligler Iron Agar (KIA)

This medium is a differential slope medium which helps in the identificatioin of the Enterobacteriaceae. KIA reactions are based on the fermentation of some carbohydrates (lactose and glucose) with the production of gases e.g Hydrogen sulphide. The fermentation of the carbohydrates give acid or alkaline reaction which often show as yellow or pink colour change of the medium. Cracks and air bubbles in the stem of the tube containing the medium indicate gas production from glucose fermentation. H_2S production is indicated by blackening along the stab line or thoughout the medium.

Needed Ingredients contained in 1 litre of medium:

Lab-Lemco powder	-	3.0g
Yeast extract	-	3.0g
Peptone	-	20.0g
Sodium chloride	-	5.0g
Dextrose (glucose)	-	1.0g
Ferric citrate	-	0.3g
Sodium thiosulphate	-	0.3g
Agar	-	12.0g
Phenol red	-	0.05g
Distilled water	-	1000ml
pH	-	7.2-7.4

Method of Preparation

Forty-nine grams (49g) of powder was suspended in 1 litre of distilled water. The suspension was brought to boil swirling intermittently for complete dissolution of the powder. The liquid medium was then dispensed into test tubes, plugged with cotton wool and autoclaved at 121°C for 15 minutes. After sterilizing, the medium was allowed to set in slopes with about 3ml butts.

2.6 Koser Citrate Medium (Fluid)

This fluid medium is used for the differentiation of organisms that have the sole ability to utilize citrate as the only source of carbon and ammonium as the only source of nitrogen.

Ingredients Needed:

Oxoid dehydrated medium	-	C65
Sodium ammonium phosphate	-	1.5g
Potassium dihydrogen phosphate	-	1.0g
Magnesium sulphate	-	0.2g
Sodium citrate	-	2.5g
Bromothymol blue	-	0.016
Distilled water	-	1 litre
pH	-	6.8

Method of Preparation

5.2g of the dehydrated medium was dissolved in 1 litre of distilled water. The solution was distributed into clean screw-capped Bijou bottles and sterilized by autoclaving for 15 minutes at 121°C. When cooled the medium in the Bijou bottles were stored in the refrigerator till needed.

2.7 Peptone Water

Peptone water is basically used to culture a wide range of microorganisms, and particularly for indole test when enriched with tryptophan.

Ingredients in 250mls of medium (Oxoid dehydrated medium - CM9)

Peptone (oxoid L37)	-	2.5
Sodium chloride	-	1.25
Distilled water	-	250mls
pH	-	7.2

Method of Preparation

3.75g of dehydrated medium was dissolved in 250mls of distilled water and 5mls of it was distributed into 20ml-test tubes. The filled test tubes were plugged with cotton wool and sterilized by autoclaving at 121°C for 15 minutes.

2.8 Stuart Transport Medium (STM)

Stuart transport medium is a modified non-nutrient medium specially prepared for the preservation of micro-organisms (especially those that are easily lost through dryness) in transit between the place of collection and the laboratory. Most microorganisms can be kept in this medium up to about 48 hours at 4°C.

Ingredients needed: Oxoid Dehydrated Medium CM111

Sodium glycerophosphate	-	10.0g
Sodium thioglycollate	-	0.5g
Cysteine - HCL	-	0.5g
Calcium chloride	-	0.1g
Methylene blue	-	0.001
Agar No. 1 (oxoid L11)	-	5.0g

Method of preparation

8g of the dehydrated STM was suspended in 500mls of distilled water. The suspension was brought to boil to dissolve completely. It was then dispensed in 5ml into test tubes, tightly plugged with cotton wool and sterilized by autoclaving at 121°C for 15 minutes.

2.9 Christensen's Urea Agar

This is a medium used in the differentiation of urease producing organisms such as some strains of Proteus.

Formular:

Oxoid Dehydrated Urea Agar base CM

Sterile urea solution (40% w/v) 5ml (ample)

Distilled water 95ml

Method of Preparation (for 100ml)

2.4g of the dehydrated urea agar base was suspended in 95mls of distilled water. The suspension was brought to boil to dissolve completely. Then it was sterilized by autoclaving at 121°C for 20 mins. When cooled to about 50°C, 5ml of sterile urea solution (40% w/v) was aseptically added to the agar base, carefully mixed by shaking the bottle. It was later dispensed in 10ml into 20ml capacity test tubes and allowed to set in a slant position.

3.6 Oxidase Reagent

Volume prepared at a time - 10ml

Tetramethyl-p-phenylenediamine dihydrochloride - 0.1g

Distilled water - 10ml

Method of Preparation

0.1g Tetramethyl-p-phenylenediamine dihydrochloride crystal was dissolved in 10mls distilled water in a screw cap bottle. By shaking the bottle and its contents the reagent dissolved faster. The reagent now a solution was ready for use.

N.B: A freshly prepared oxidase reagent was always used.

3.7 Turbidity (opacity) Standard

This is a Barium chloride suspension which acts as a standard for the turbidity of the suspension of the test organism when matched. The turbidity of the standard suspension is equivalent to an overnight's broth culture. If the turbidity of the test organism's suspension matches with the standard suspension, the inoculum should produce a semi-confluent growth on the agar plate.

APPENDIX 3.0

REAGENTS: Preparation and Storage

3.1 Gentian Violet Stain (0.5% w/v)

Gentian Violet (powder) - 5g

Distilled water - 1 litre

Preparation

5gm of Gentian violet powder was weighed on a fresh filter paper (preweighed) and dissolved in a litre of distilled water. The prepared solution was then stored in a brown 1 litre capacity bottle.

3.2 Neutral Red Solution (0.1% w/v)

Neutral Red (powder) - 0.5g

Distilled water - 500mls

0.5g of neutral red powder was weighed using a pre-weighed filter paper and transferred into a 1 litre capacity bottle. To this 200mls of distilled water was first added and carefully mixed by stirring to ensure complete dissolution. The remaining 300mls of distilled water was added giving 500mls of stain. The solution was filtered using filter

Method of Preparation

A 1% v/v solution of sulphuric acid was prepared by adding 1ml of concentrated sulphuric acid to 99ml of distilled water. Then, 2.35g of dehydrated barium chloride ($BaCl_2.2H_2O$) was dissolved in 200ml of distilled water to obtain about 1.18% w/v solution of barium chloride.

The turbidity standard was prepared by adding 0.5ml of the barium chloride solution to 99.5ml of the sulphuric acid. The mixture was carefully mixed, the resulting solution showed as a turbid liquid. About 5ml of the turbidity standard solution prepared was then transferred into a Bijou bottle with a screw cap, carefully sealed and kept for future matchings with emulsions of test organisms.

APPENDIX 4

BIOCHEMICAL TESTS

4.1 Gram's Staining Technique

This technique serves as a preliminary identification test for most bacteria. It employs the reactivity of the bacterial cell wall to two different stains - Gentian/Crystal violet stain and Neutral Red stain / Safranin.

Bacteria that stain purple with the Gentian violet stain and are not de-colorized by acetone and/or alcohol are described as Gram positive organisms. Those bacteria that stain red with neutral red stain with this technique are called Gram negative organisms.

Reagents Needed:

Gentian violet/crystal violet stain

Lugol's iodine

Acetone-alcohol decolorizer

Neutral red (0.1% w/v)

<u>Procedure</u>

1. Make a smear of the test organism on a clean, oil free microscope slide
2. Air dry the `wet smear'
3. Fix the dried smear by passing the slide three times through a bunsen flame so that the back of the slide feels warms to the touch of the back of the hand.

4. Cover the fixed smear with a few drops of the Gentian/Crystal violet stain for 60 seconds.
5. Rapidly wash off the stain with slow running water
6. Tip off all traces of water and cover the smear with the Lugol's iodine solution for 30-60 seconds.

   (N.B: The iodine solution acts as a mordant for the gentian violet/crystal violet stain).
7. Wash off iodine with slow running water.
8. Decolorize rapidly (few seconds) with acetone alcohol. Wash immediately with clean water.

   Caution: Decolorizer is highly inflammable. Use well away from open flame.
9. Cover the decolorized smear with neutral red stain for 2 minutes.
10. Wash off stain with water
11. Carefully blot dry with a filter paper
12. Examine the stained smear with the x 100 objective lens and with a drop of immersion oil

<u>Findings:</u>

Dark purple/violet stained organisms	-	Gram positive
Pink stained organisms	-	Gram negative

4.2 Catalase Test

This test is used to differentiate bacteria that produce the enzyme catalase from non-catalase producing bacteria.

Principle

Catalase acts as a catalyst to liberate oxygen from hydrogen peroxide solution with the occurence of vigorous bubbling.

Reagent: 3% Hydrogen peroxide solution (H_20_2.

Procedure

1. Pour about 2ml of 3% H_20_2 solution into a test tube.
2. With the aide of a sterile wooden stick or glass rod, remove a good growth of the test organism and immerse in the H_20_2 solution.

Results

Active bubbling indicates a positive reaction which means the test organisms produces catalase. No release of bubbles shows a negative reaction. Staphylococci is catalese positive while Streptococci is catalese negative.

4.3 Coagulase Test

Coagulase activity is essentially limited to Staphylococci. By it Staphylococcus aureus is differentiated from the other pathogenic Staphylococci species.

Coagulase, an enzyme, is produced by S. aureus only and forms clots with plasma by converting fibrinogen in the plasma to fibrin.

There are two types of coagulase tests:

- the slide test which detects bound coagulase.

- the test tube method which detects free coagulase.

Reagents:

1. Plasma (human or rabbit)
2. Physiological saline
3. Test organism

Procedure 1 - slide method.

A few drops of physiological saline were placed on a clean glass slide. In this, a heavy growth of the Staphylococci was emulsified to form a thick suspension. Then a drop of fresh plasma was added and mixed gently.

Results

A positive test showed clamping within 10 seconds which implied that the test organism was Staph. aureus. A negative test showed no change in the consistency of the suspension.

Procedure II - Test tube method

0.5 ml of 0.1% plasma (0.2ml plasma in 1.8ml physiological saline) was put into a test tube. Then about 5 drops of pre-cultured broth (18-24 hours) of the test organism was added to the diluted plasma. The mixture was carefully mixed and incubated at 37°C. After the first hour the test tube was examined for clotting, then at every 30 minutes intervals up to 4 hours. The fibrin clot when formed could be seen clearly by gently tilting the test tube.

Results

Clot of fibrin in the test tube indicates a positive coagulase test and the test organism is Staph. aureus.

NB: Cultures that gave a negative slide test were again tested using the test tube method for confirmation.

4.4 Citrate Test

This test is used for the identification of enterobacteria on the basis of citrate utilization. Ability to utilize citrate as carbon source and ammonia as source of nitrogen is shown by turbidity due to growth and colour change of the indicator from light green

to pale blue. The colour change is caused by an alkaline reaction resulting from the citrate utilization.

Procedure

A growth of the test organism was transferred into Koser citrate medium (Appendix 2.6) in screw capped bottle using sterile straight wire (pre-flamed). The innoculated medium was incubated at 37°C. At every 24 hours intervals for up to 4 days, the culture medium was examined for any colour change and growth or turbidity.

Results

A positive citrate test showed turbidity and colour change from light green to blue, while a negative test showed no change in the citrate medium.

Pseudomonas species are citrate positive.

4.5 Urease Test

This is one of the many biochemical tests used for the differentiation and identification of enterobacteria. It involves the activity of the enzyme urease on urea contained in the medium. Urease hydrolyses urea to release carbon dioxide and ammonia. The free ammonia changes the pH of the medium to alkaline which is seen by a change in the colour of the indicator from straw colour to pink.

Procedure

Urea agar medium (Appendix 2.9) in test tubes was `stabbed' with the test organism using a sterile straight wire. The stab was made by pushing the straight wire with the test organism through the butt of the agar medium. 2 or 3 streaks were also

made on the slope as the wire was removed. The innoculated medium was incubated at 37°C for 24 hours.

Results

A change of the colour of the medium to reddish-pink indicated a positive urease test. A negative test showed no colour change of medium. Proteus species are urease positive.

4.6 Indole Test

This test is ideal for the identification of species of enterobacteria that have the ability to break down tryptophan, an amino acid, to release indole - a gaseous substance. The production of the indole is detected by its reaction with 4-dimethylaminobenzaldehyde contained in Kovac's reagent to give a red coloured compound.

Procedure

About 4 mls of peptone water containing tryptophan was innoculated with the test organism using a sterile straight wire. The test tube was tightly plugged with cotton wool to prevent the escape of any gases produced and to prevent contamination by extraneous organisms. The innoculated medium was incubated for 24 hours after which time 2 drops of Kovac's reagent were added and the mixture was observed for any reaction.

Results

Within some few minutes the Kovac' reagent which is insoluble changed from a yellowish-brown colour to a red layer on top of the culture; this is a positive test. Escherichia coli is indole positive.

4.7 Kligler Iron Agar Test (KIA)

KIA is a differential medium used for the identification of the various enterobacteria species.

Fermentation of sugars and the production of gases form the basis of the test. KIA medium contains sugars like glucose (dextrose) and lactose, ferric citrate and an indicator phenol red. Different species give different butt and/or slope colour changes. Gases produced are seen as cracks or bubbles in the medium mainly in the butt and as black compound when the gas produced is hydrogen sulphide.

Procedure

KIA medium (Appendix 2.5) was innoculated with the test organism by stabbing the butt and streaking the slope using a sterile straight wire. This was incubated at 37°C for 24 hours.

Results

1. Both butt and slope assumed a yellow coloration and the agar cracked creating a space below the medium. This resulted mainly from glucose fermentation with gas production. E. coli is responsible for this reaction.

2. A yellow butt (acid production) with a red slope (a reversion of acid production under aerobic conditions) with H_2S production indicated the presence of Proteus species.

3. A red butt and a red slope with no gas production indicated an oxidation of carbohydrates by Pseudomonas species.

4.8 OXIDASE TEST

This test is used to identify oxidase (enzyme) producing organisms such as Pseudomonas, Neisseria etc.

Principle

The phenylene diamine in the oxidase reagent (tetramethyl-p-phenylenediamine dihydrochloride) is easily oxidized by the enzyme oxidase to form a purple coloured compound.

Method

A piece of filter paper was soaked with of freshly prepared oxidase reagent (about 2-3 days old). Then with a sterile stick or plastic loop a colony of the test organism was picked and smeared on the soaked portion of the filter paper.

Within a few seconds the smear changed to a deep purple colour indicating positive oxidase reaction. A negative reaction showed no change in the colour of the smear.

4.9 LANCEFIELD GROUPING TEST (STREPTOTEX)

This test is specifically used for grouping ß-haemolytic Streptococci. It is a rapid latex test which uses an enzyme digestion technique in the extraction of the antigen.

Principle

Most species of Streptococcus possess group-specific antigens which are usually carbohydrate structural components of the cell wall. The antigen is extracted and identified using polystyrene latex particles which have been coated with group-specific antibodies (Balows et al., 1991 and Collins et al., 1995). The latex particles (contained in the group-specific antisera labelled A, B, C, D, F and G) agglutinate strongly in the presence of homologous antigen or remain in smooth suspension in the absence of homologous antigen.

Reagents provided in the commercial latex kit are as follows:

1. Freeze-dried extracted enzyme
2. Polyvalent positive control
3. Six bottles, labelled A, B, C, D, F and G, each containing about 1.2ml of the group specific antiserum.

Method (According to the manufacturer's instrutions)

To reconstitute the freeze-dried enzyme, 11mls of sterile distilled water was added to a bottle containing the enzyme. When fully dissolved about 0.4ml of the enzyme solution was pippetted into each of a series of test tubes and using a sterile loop,

a few colonies (about 5 colonies) each of the test organisms were added to each test tube and then vigorously shaken for a few seconds to form a suspension. The suspensions were incubated at 37°C in a water bath for a period of between 10 minutes to 1 hour. After the first 5 minutes of incubation the tubes were shaken again and then left to go through the rest of the incubation period; however, a minimum of 10 mins. incubation is enough for the enzyme to be extracted..

Before any test was conducted on the extracts, the potencies of the various latex suspensions provided were ascertained by mixing each in turn with a drop of the polyvalent antiserum. The presence of agglutination indicated potency of latex suspensions. Being satisfied with the potency of the various antisera/latex suspensions A, B, C, D, F, and G, extracts from the different Streptococcus strains were then tested. A drop of the latex suspension was first put on a clean reaction card (provided by the manufacturer) then a few drops of the extract from the test organism was added. This was carefully mixed for a maximum of one minute, during which time agglutination of the mixture was formed.

Results

The labelled latex suspension which produced an agglutination with the test organism was the group specific name of the streptococcal species whose enzyme extract was tested.

APPENDIX 5.0

CALCULATIONS IN DETAIL

5.1 Test of Significance between age and prevalence of pyoderma among study population using the chi square (χ^2).

Ho: Age and pyoderma are independent

Hi: Age and pyoderma are not independent

χ^2 Table

Age	Pyodermic Population		Non-Pyoderma Pop.		Total
	O	E	O	E	O
5-7	110	90.93	1655	1674.07	1765
8-10	126	110.67	2022	2037.33	2148
11-13	72	101.19	1892	1862.81	1964
14+	2	7.21	138	132.79	140
Total	310	-	5707	-	6017

O - Observed cases

E - Expected cases

$$\text{Chi-square},\chi^2 = \sum \frac{(0i,j - Ei,j)^2}{Ei,j}$$

$$\frac{(0i,j - Ei,j)^2}{Ei,j} = \frac{(110 - 90.93)^2}{90.93} = 3.99$$

$$\cong 4.0$$

$$\chi^2 = 4.0 + 0.217 + 2.125 + 0.115 + 8.419 +$$

$$0.457 + 3.756 + 0.20$$

From the χ^2 tables 0.1% level of significance with 3 d.f is 16.27.

Conclusion

From the above calculation $\chi^2 = 19.294$ which is below χ^2 value at 0.1% significant level with 3 d.f. Therefore we reject the null hypothesis (Ho) at 0.1% significant level and conclude that age and pyoderma are not independent.

5.2 Test of significance between sex and pyoderma using χ^2 analysis test.

Ho: Sex and pyoderma are independent

Hi: Sex and pyoderma are not independent

χ^2 Analysis Table

Sex	Pyodermic Population O	E	Non-Pyoderma Pop. O	E	Total O
Male	231	164.609	2964	3030.391	3195
Female	79	145.391	2743	2676.609	2822
Total	310	-	5707	-	6017

$$\chi^2 = \frac{\sum (0ij - Eij)^2}{Eij}$$

$$= 60$$

Degree of freedom = 1

χ^2 value (from tables) at 0.1% significant level with 1 d.f. = 10.83.

Conclusion

At 0.1% level of significance with 1 d.f, χ^2 value from the calculation is below χ^2 value from the tables. Therefore we reject the null hypothesis (Ho) at 0.1% significant level and conclude that sex and pyoderma are not independent.

5.3 Test of significance between type of locality and incidence of pyoderma.

Ho: Type of locality and the incidence of pyoderma are independent.

Hi: Type of locality and the incidence of pyoderma are not independent.

χ^2 - analysis table

Type of Locality	Pyodermic Population O	E	Non-Pyoderma Pop. O	E	Total O
Urban	50	155.386	2966	2860.614	3016
Rural	260	154.614	2741	2846.386	3001
Total	310	-	5707	-	6017

O - Observed cases
E - Expected cases

$$\chi^2 = \sum \frac{(0i,j - Ei,j)^2}{Ei,j} = 151.091$$

Degree of freedom = 1

Conclusion

At 0.1% level of significance with 1 d.f, χ^2 value is 10.83 but that from the calculation is 151.091 which is far below 10.83 in the table. We therefore reject the null hypothesis (Ho) that the type of locality and pyoderma are independent and conclude that they are not independent.

5.4 Testing the significance of the difference in the frequency of pyoderma between the urban and rural study areas, using the Z distribution test.

Ho: $Pr = Pu$

Hi: $Pr \neq Pu$

where,

Pr is the proportion of infected rural children in the study

Pu is the proportion of infected urban children in the study.

$$Z = \frac{Pr - Pu}{\sqrt{P(1 - P)(\frac{1}{nr} + \frac{1}{nu})}}$$

where $P = \frac{Xr + Xu}{nr + nu}$

Definition

P = sample of infected children/pooled proportion

Xr = number of infected children in the rural population

Xu = number of infected children in the urban population

nr = total number of rural study population

nu = total number of urban study population.

Calculating for P

$$= \frac{260 + 50}{3001 + 3016}$$

$$= 0.052$$

Also,

$$Pr = \frac{Xr}{nr} = \frac{260}{3001}$$

$$Pu = \frac{Xu}{nu} = 0.0166$$

Therefore,

$$Z = \frac{0.08664 - 0.01658}{\sqrt{0.05152\ [1-0.05152](0.00033)+(0.00033)}}$$

$$= \frac{0.07006}{\sqrt{0.0003225}}$$

$$= 3,9009$$

From the statistical table Z value, 3.9009 gives a percentage of 0.005.

APPENDIX 6
INTERPRETATION OF ZONES OF INHIBITION

Antibiotic	Disc content	Diameter (millimeters) of zones of inhibition		
		Resistant	Intermediate	Susceptible
Amikacin	30 mcg	14 or less	15-16	17 or more
Ampicillin[1] when testing gram-negative micro-organisms and enterococci	10 mcg	11 or less	12-13	14 or more
Ampicillin[1] when testing staphylococci and penicillin G-susceptible micro-organisms	10 mcg	20 or less	21-28	29 or more
Ampicillin[1] when testing *Haemophilus* species	10 mcg	19 or less		20 or more
Bacitracin	10 units	8 or less	9-12	13 or more
Carbenicillin when testing *Proteus* species and *Escherichia coli*	100 mcg	17 or less	18-22	23 or more
Carbenicillin when testing *Pseudomonas aeruginosa*	100 mcg	13 or less	14-16	17 or more
Cefamandole	30 mcg[6]	14 or less	15-17	18 or more
Cefotaxime	30 mcg[6]	14 or less	15-22[12]	23 or more
Cefoxitin	30 mcg[6]	14 or less	15-17	18 or more
Cephalothin when reporting susceptibility to cephalothin, cephaloridine and cephalexin	30 mcg	14 or less	15-17	18 or more[2]
Cephalothin when reporting susceptibility to cephaloglycin	30 mcg	14 or less		15 or more
Chloramphenicol	30 mcg	12 or less	13-17	18 or more
Clindamycin[3] when reporting susceptibility to clindamycin	2 mcg	14 or less	15-16	17 or more
Clindamycin when reporting susceptibility to lincomycin	2 mcg	16 or less	17-20	21 or more
Colistin	10 mcg	8 or less	9-10	11 or more
Erythromycin	15 mcg	13 or less	14-17	18 or more
Gentamicin	10 mcg	12 or less	13-14	15 or more
Kanamycin	30 mcg	13 or less	14-17	18 or more
Methicillin[5]	5 mcg	9 or less	10-13	14 or more
Nalidixic Acid	30 mcg[7]	13 or less	14-18	19 or more
Neomycin	30 mcg	12 or less	13-16	17 or more
Nitrofurantoin	300 mcg[7]	14 or less	15-16	17 or more
Oxacillin	1 mcg[5]	10 or less	11-12	13 or more
Penicillin G when testing staphylococci[8]	10 units	20 or less	21-28	29 or more
Penicillin G when testing other micro-organisms[8]	10 units	11 or less	12-21[9]	22 or more
Polymyxin B	300 units	8 or less	9-11	12 or more[4]
Streptomycin	10 mcg	11 or less	12-14	15 or more
Sulphamethoxazole/Trimethoprim	23.75/1.25 mcg[7,11]	10 or less	11-15	16 or more
Sulphonamides	250 or 300 mcg[11]	12 or less	13-16	17 or more
Tetracycline[10]	30 mcg	14 or less	15-18	19 or more
Ticarcillin when testing *P. aeruginosa*	75 mcg	11 or less	12-1·	15 or more
Tobramycin	10 mcg	12 or less	13-14	15 or more
Trimethoprim	5 mcg[7,11]	10 or less	11-15	16 or more
Vancomycin	30 mcg	9 or less	10-11	12 or more

1. The ampicillin disc is used for testing susceptibility to both ampicillin and hetacillin.
2. Staphylococci exhibiting resistance to the penicillinase-resistant penicillin class discs should be reported as resistant to cephalosporin class antibiotics. The 30 mcg cephalothin disc cannot be relied upon to detect resistance of methicillin-resistant staphylococci to cephalosporin class antibiotics.
3. The clindamycin disc is used for testing susceptibility to both clindamycin and lincomycin.
4. Colistin and polymyxin B diffuse poorly in agar and the accuracy of the diffusion method is thus less than with other antibiotics. Resistance is always significant but when treatment of systemic infections due to susceptible strains is considered, it is wise to confirm the results of a diffusion test with a dilution method.
5. The methicillin disc is used for testing susceptibility to all penicillinase-resistant penicillins, that is, methicillin, cloxacillin, dicloxacillin, oxacillin and nafcillin.
6. Cefamandole, cefoxitin and cefotaxime are recently released cephalosporins having a wider spectrum of activity against gram-negative bacilli than do other previously approved cephalosporins. Therefore, the cephalothin disc cannot be used as the class disc for these three drugs.
7. Susceptibility data for nalidixic acid, nitrofurantoin, sulphonamides and trimethoprim apply only to organisms isolated from urinary-tract infections.
8. The penicillin G disc is used for testing susceptibility to all penicillinase-susceptible penicillins except ampicillin and carbenicillin; that is, penicillin G, phenoxymethyl penicillin and phenethicillin.
9. This category includes some organisms such as enterococci and gram negative bacilli that may cause systemic infections treatable with high doses of penicillin G. Such organisms should only be reported susceptible to penicillin G and not to phenoxymethyl penicillin or phenethicillin.
10. The tetracycline disc is used for testing susceptibility to all tetracyclines; that is, chlortetracycline, demeclocycline, doxycycline, methacycline, oxytetracycline, rolitetracycline, minocycline and tetracycline.
11. The 250 or 300 µg sulfisoxazole discs can be used for any of the commercially available sulphonamides. Blood-containing media, except media containing lysed horse blood, are not satisfactory for testing sulphonamides. The Mueller-Hinton agar should be as thymidine-free as possible for sulphonamide and/or trimethoprim testing. (See Sec. 3.1.1.).
12. The category "Intermediate" should be reported. Infections with bacteria of "intermediate" susceptibility may be considered moderately susceptibile and may respond to antimicrobial agents with a wide safe dosage range.