TITLE:

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITIES OF NEISSERIA GONORRHOEAE ISOLATED FROM PATIENTS IN VARIOUS LOCATIONS OF KADUNA STATE, NIGERIA

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A Thesis Submitted to the Postgraduate School, Ahmadu Bello University, Zaria, Nigeria, in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy in Microbiology

1999

DECLARATION

This is to certify that I carried out the work reported in this thesis at the laboratories of the Department of Microbiology, Ahmadu Bello University, Zaria, under the Supervision of Dr L.E. Odama, Dr M. Galadima and Dr J.K.P. Kwaga.

This thesis is an original work carried out by myself and no part of it has been presented elsewhere for a degree or a diploma. The work of other investigators is acknowledged and referred to in this presentation.



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CERTIFICATION

This thesis titled, "PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITIES OF *NEISSERIA GONORRHOEAE* ISOLATED FROM PATIENTS IN VARIOUS LOCATIONS OF KADUNA STATE, NIGERIA" BY EDWARD DAVID JATAU, B.Sc., M.Ed, M.Sc. meets the regulations governing the award of the degree of DOCTOR OF PHILOSOPHY (MICROBIOLOGY) of AHMADU BELLO UNIVERSITY, ZARIA, NIGERIA and is approved for its contribution to knowledge and literary presentation.

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DEDICATION

This work is dedicated to my dear wife MRS FRANCA H. JATAU and our four beloved children, JOB D. JATAU, GLORIA D. JATAU, JOHN D. JATAU and JAMES D. JATAU

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ABSTRACT

A total of one thousand seven hundred and fifteen (1715) patients, consisting of nine hundred and thirty eight (938) female and seven hundred and seventy seven (777) male patients were screened for *Neisseria gonorrhoeae* infection from seven locations representing the four geographical zones into which Kaduna state was divided for the purpose of this study.

Out of the 1715 patients screened, 275 (16.03%) were found positive for *N. gonorrhoeae* infection. The prevalence rates of *Neisseria gonorrhoeae* infection per location were Zaria 22.08%, Kaduna, 21.33%, Pambeguwa, 18.35%, Kafanchan, 16.66%, Kachia, 12.66%, Giwa, 11.70% and Soba, 10.76%.

The results showed that the age group 15-20 years had the highest prevalence of infection (31.05%) followed by the age groups 36-40 years and 21-25 years with prevalence of 26.05% and 22.80% respectively. The highest prevalence in males (23.91%) occurred in the age group 36-40 years while the highest prevalence of infection in the female patients (11.18%) was found in the age group 15-20 years.

Out of the 275 gonococcal isolates, 225 (81.82%) were resistant to penicillin, 206 (74.91%) were resistant to ampicillin, 122 (44.36%) were resistant to tetracycline, 34 (12.36%) isolates were resistant to erythromycin, and 16 (5.82%) isolates were resistant to gentamicin. All the 275 *Neisseria gonorrhoeae*

isolates were sensitive to ceftriaxone, cefuroxime and ofloxacin.

A total number of 203 out of the 225 penicillin resistant isolates (90.11%) of *Neisseria gonorrhoeae* were found to exhibit multiple drug resistance. Out of, these, 106 (47.11%) were resistant to penicillin G and ampicillin, 67 (29.77%) were resistant to penicillin G, Ampicillin and Tetracycline. Eighteen (8%) isolates were resistant to penicillin G, Ampicillin, Tetracycline and Erythromycin. Five strains found possessed resistance to penicilin G, Ampicillin and Erythromycin while three strains exhibited resistance to penicillin G, Ampicillin, Tetracycline and Gentamicin. Resistance to penicilin G, Ampicillin, Tetracycline, Erythromycin and Gentamicin was each found in only one isolate. Similarly two strains simultaneously exhibited resistance to penicillin G, Ampicillin and Gentamicin while one strain showed-resistance to penicillin G, and Gentamicin.

Out of the 225 *Neisseria gonorrhoeae* isolates resistant to penicillin, 189 (84%) were positive for beta-lactamase production. The resistance in the remaining 36 penicillin resistant strains were probably chromosomally induced. The prevalence of penicillinase producing *Neisseria gonorrhoeae* (PPNG) was statistically significant ($p<0.05\%$).

This study also showed high prevalence of agents of non-gonococcal urethritits among the patients screened. *Staphylococcus aureus* was isolated from 403 (23.5%) patients. *Candida albicans from* 269 (25.7%) patients, E coli from 204 (11.9%), *Trichomonas vaginalis* from 104 (6.06%) patients and *Klebsiella* sp.

from 49 (2.8%) patients. Cases of mixed infection with agents of non-gonococal urethritis were found in a total of 298 patients. Mixed infections involving *Candida albicans* and *Staphylococcus aureus* were found in 70 (4.1 %) patients and mixed infection of *E. coli* and S. *aureus* occurred in 60 (3.5%) patients. *Trichomonas vaginalis* and *Candida albicans* mixed infection occurred in 55 (3.2%) patients.

The findings in this study showed high prevalence of *Neisseria gonorrhoeae* infection with high level of antimicrobial resistance, particularly to the drugs commonly used for the treatment of gonorrhoea. The prevalence of non-gonococcal infections was also high. The general prevalence of sexually transmitted diseases among the studied population requires urgent action by the government to contain the spread of sexually transmitted diseases that have been closely linked with the spread of HIV infection.

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CHAPTER ONE

INTRODUCTION

Gonorrhoea is a disease that has been recognized since antiquity which is still a common bacterial infection (Fekete, 1993). It is caused by *Neisseria gonorrhoeae* a gram negative coccus usually found in pairs (Krieger, 1984). The Centres for Disease Control and Prevention, Atlanta, in 1992 confirmed that in 1991 there were 586, 638 reported cases of gonorrhoea in the United States of America (CDC, 1992). According to Harrison (1984), gonorrhoea, a bacterial infection of male and female genital mucosa, has been endemic throughout the world for many centuries and that from earliest history to modern days, gonococcal urethritis has continued to be a medical problem of serious impact. Despite the advent of antibiotics specifically effective against the organism, it maintains an unenviable position as the most common reportable infectious disease in the United States of America (Krieger, 1984).

The prevalence rates of gonorrohea and its complications e.g. urethral stricture, epididymitis, pelvic inflammatory disease (P.I.D.), infertility, etc, in urban and rural areas of tropical Africa are alarmingly high (Osoba, 1981). In 1946, Willcox reported the incidence of gonorrohea among Nigerian troops in Lagos as 600 per 1000 while the World Health Organization Expert Committee on gonococcal infection in 1963 reported an infection rate of 49076 per 100,000 among adult population in Lagos. That was the highest rate in the World (W.H.O.,

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1963). According to Krieger (1984) research efforts on sexually transmitted diseases have been limited due to technical difficulties and social factors such as the stigma of working with venereal diseases (VDs) and the naive concepts that STDs cause minimal morbidity and are uniformly easy to treat. In developing countries, detection of gonococci is often cumbersome, direct microscopy of the specimen has a low sensitivity for samples from women and its culture requires adequate techniques from initial sampling step to final culture confirmation and antigenic detection tests are expensive and mostly equipment demanding (Herman et al., 1996).

The recognition of antibiotic resistance in gonoccoccus has been an especially important clinical stimulus to basic research. In the 1970s gonococci containing plasmid-borne genes for enzymes that inactivate penicillins were isolated from patients that failed standard treatment regimens. These organisms are known as Penicillinase Producing *Neisseria gonorrhoeae* (PPNG). Although PPNG have been isolated all over the world, there is evidence that they were first isolated from the Far East and parts of West Africa. PPNG originating from these areas have different penicillinase determining plasmids and differ in their nutritional requirements (Krieger, 1984). According to Perine et al., (1979), Penicillinase Producing *Neisseria gonorrhoeae* strains are known to be circulating freely among patients in West Arica with possible reservoirs existing in the urban areas of Ghana and the Ivory Coast. According to Salyers and Whitt (1994), there are two types of beta-lactam resistance that have been seen in *Neisseria*

gonorrhoeae strains, namely a chromosomal gene that encodes a penicillin binding protein that no longer binds penicillin and a plasmid gene that encodes a beta-lactamase production. Siegel et al., (1978) reported that out of 177 cases of infection with penicillinase producing *Neisseria gonorrhoeae* in the U.S.A. for which histories of sexual expossure were available, 69 (39%) were related to sexual contact in the Far East. Fifty eight of the 69 cases occurred in military personnel who had returned from that area or in their domestic sexual contacts. One civilian case was traced to sexual contact in Ghana. The United States of America witnessed a reduction in gonococcal resistance to penicillin since 1972 (Reynold et al., 1976) and in 1976 saw a substantial decline in the rate of morbidity from gonorrhoea (CDC, 1977). The isolation of penicillinase-producing *Neisseria gonorrhoeae* by Phillips (1976) and Ashford et al., (1976) threatened to offset the trends in the U.S.A. In September 1976 the CDC issued guidelines requesting state health departments to implement a system to test all positive gonococcal cultures obtained after treatment for resistance to penicillin (CDC, 1976).

An increased incidence of plasmid-mediated production of beta-lactamase was observed in the U.S. between 1976 and 1980 (Jaffe et al., 1981). Penicillinase-producing strains of *Neisseria gonorrhoeae* and intrinsically resistant strains are more common in Asia and parts of Africa where they can comprise up to 70% of isolates and in certain areas of North America where outbreaks caused by these strains have been documented (Handsfield et al., 1982).

The number of reported cases of gonorrhoea have declined substantially in several industrialized countries (Aral and Holmes, 1990) and the disease has almost been eradicated in Sweden (Swedish Institute for Infectious Diseases Control (1984). However, gonococcus is still an important sexually transmitted organism worldwide and is estimated to cause 78 million new infections annually (WHO, 1993). In Nigeria, the little information available on the prevalence of sexually transmitted diseases are mostly from the southern part of the country (Sogbetun et al., 1977, Rotimi and Somorin., 1980). Such reports from the North are scanty (Romanowski 1952, Fakunle 1979, Bello, 1982). Elegba et al (1996), in a preliminary analysis of 150 attenders of STD clinic Ahmadu Bello University Teaching Hospital (ABUTH), Zaria reported the prevalence of gonococcal infections of 14% while Ogundiran et al., (1996) in their preliminary analysis of the 115 specimens reported low prevalence of gonococcal (10%) infection among the attenders of Family Planning Clinic in Ibadan, Nigeria.

According to Hook and Hansfield (1990), gonococcal infections in women are often asymptomatic and complications include, pelvic inflammatory disease (PID), infertility and ectopic pregnancy. The worldwide distribution of *Neisseria gonorrhoeae* and the emergence of plasmid-mediated and chromosomal-mediated resistance emphasize the importance of antimicrobial susceptibility surveillance of clinical gonococcal isolates (Van Dyck et al., 1994). While the developed world was spending large sums of money on its battle against sexually transmitted diseases (STDs), the developing world was paying little or no attention to such

problems despite their widespread economic, health and social impact (Osoba 1981). Neglected for many years, programs to diagnose, treat and prevent sexually transmitted diseases are now becoming an important component of AIDS prevention in the developing world (WHO, 1993).

The objectives of this study are:

1. To isolate, characterize and identify *Neisseria gonorrhoeae* from patients presenting with urethritis or vaginitis at various clinics in Zaria, Giwa, Soba, Pambeguwa, Kaduna, Kafanchan and Kachia towns in Kaduna State, Nigeria. These locations were chosen to give even geographical distribution as well as reflecting urban and rural life pattern of the state.

2. Determine antimicrobial susceptibility of the clinical isolates by Disc Agar diffusion method and minimum inhibitory concentration (MIC) by Tube-dilution method.

3. Determine the prevalence of beta-lactamase production in penicillin resistant *Neisseria gonorrhoeae* isolates using Iodometric method.

4. Isolate and identify other aetiologic agents of non-gonococcal urethritis/vaginitis.

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CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 *Neisseria gonorrhoeae*

Neisseria gonorrhoeae is a known pathogen causing acute venereal disease in the form of urethritis or cervicitis as well as disseminated infections such as arthritis. It has also been recovered in patients with pelvic inflammatory disease (PID), mixed with other aerobic flora (McCormack et al., 1977). *Neisseria gonorrhoeae* is a gram-negative diplococcus that is oxidase-positive. It infects the mucosa of both male and female genital tract. It is highly sensitive to drying, heat and low pH and fastidious in nutritional requirements. *Neisseria gonorrhoeae* is a mucosal pathogen whose primary antioxidant defence is the production of an unusually high level of catalase, an enzyme that converts H_2O_2 to water and oxygen (Hassett et al., 1990). It is primarily a human pathogen and does not infect other animals unless administered by an abnormal route such as injection. In vivo studies of mutants have been done only with human volunteers. *Neisseria gonorrhoeae* colonizes only the columnar epithelial cells on the surface of the muccosa. In post pubescent women, genital infection is restricted to the cervix because the vaginal mucosa consists of squamous rather than columnar epithelial cells and *Neisseria gonorrhoeae* is unable to colonise squamous cells. Among the mammalian cell lines, the Chan conjunctional epithelial cell line is the most

popular. Polymorphonuclear neutrophils (PMNS) and macrophage like cell lines are used to study the fate of phagocyte ingested *Neisseria gonorrhoeae* while fallopian tissue is used as an organ culture to study effects of bacterial colonization on the fallopian tubes. *Neisseria gonorrhoeae* is naturally transformable - i.e. it takes up DNA without chemical or electroporation. But the in vitro transformation system discriminates against foreign DNA, and it is thus useless to introduce DNA cloned in *E. coli*. The conjugative transposon Tn916 and related transposons such as Tn1545 are reported to integrate into the *Neisseria gonorrhoeae* chromosomes though not completely (Salyers and Whitt 1994).

2.1.1 Pathogenicity

Gonorrhoea is characterized by the presence in the exudate of polymorphonuclear neutrophils (PMNs) in large numbers some of which are packed with gonococci. The viscous purulent discharge associated with gonorrhoeae is attributed to the lysis of many PMNs which release DNA - a viscous material. Lipooligosaccharide (Los) is thought to be responsible for most of the gonorrhoea symptoms. It triggers an intense inflammatory response. The activation of complement, sloppy feeding by phagocytic neutrophils and lysis of phagocytic cells themselves contribute to the purulent discharge. Tissue damage to the fallopian tubes is thought to be due to local production of TNF∝ (a cytokine)

induced by Lipooligosaccharide (Los) which is also responsible for serum resistance an important trait for strains that cause systemic infection (Salyers and Whitt, 1994).

2.1.2 Incidence

A review of studies conducted in Africa indicates high incidence of secually transmitted diseases (STD). Bello et al (1983), in a study conducted between 1977 and 1981 on 3089 patients attending STD clinic in Zaria, reported postpubertal gonorrhoea in 910 (28.1%) of the patients. According to World Health Organization Population Reports, studies in developing countries on women attending antenatal, family planning or gynecological clinics indicated that up to 18% of the patients had gonorrhoea. Seventeen percent had syphilis and up to 30% had trichomoniasis. It has also been reported that STDs are major public health problems in both developed and developing nations with prevalence rates being higher in the developing countries where STD treatment is less accessible. Incidence rates are also higher in developing countries e.g. the annual rate of new cases of gonorrhea in African cities is 3,000 to 10,000 per 100,000 people or as many as one in every 10 people (Aral and Holmes, 1990, CDC, 1992). An estimated 8-20% of women with untreated cervical gonorrhoea develop pelvic inflammatory Disease (PID) which could lead to infertility (Krieger, 1984).

There is general concern at the worldwide increase in sexually transmitted diseases (STDs). A review of the global prevalence of gonorrhoea, described the numbers as of epidemic proportion (Willcox, 1972). Sexually transmitted infections and their sequelae contribute significantly to morbidity and mortality. Gonorrhoea and chlamydial infections in women are responsible for acute and chronic pelvic inflammatory disease, infertility, adverse pregnancy outcomes and ophthalmia neonatorum. Perhaps, even treatable bacterial diseases, such as chancroid, that cause genital ulceration have been found to facilitate HIV-1 transmission (Cameron et al., 1989, Laga et al., 1993).

2.2 *Neisseria gonorrhoeae* and Other Organism:

Saigh et al. (1978) reported that women who have contact with partners with gonorrhoea are less likely to become infected if inhibitory strains of Lactobacilli are part of their vaginal flora. He-yi et al. (1994) showed that Lactobacillus inhibits the growth of *Neisseria gonorrhoeae* due to acid production. It was also revealed that Lactobacilli that produce H_2O_2 are considerably more inhibitory than those that do not. According to Hughes and Hiller (1990), the H_2O_2 producing strains of lactobacilli (LB+) are critical to the composition of vaginal flora and should be used probiotically in the female urogenital tract as a biobarrier to vaginal infection. While receiving more attention now because of their capacity to enhance sexual HIV transmission (Wasserheit 1992, Merson 1993) STDs are also

directly responsible for a considerable amount of morbidity and mortality (Grimes, 1986, Wasserheit, 1989). According to WHO (1993), women in developing countries carry the largest share of the STD disease burden and that pregnant women and their offsprings are particularly vulnerable. Temmerman et al. (1988), cultured for sexually transmitted agents in one-third of post partum endometritis cases in Nairobi, Kenya and found that mothers were frequently transmitting the infection to their newborn. Wasserheit et al. (1992) conducted a similar study in Haiti and reported that 1 in 8 women had cervical infections that was untreated which could be transmitted to the child and could lead to upper reproductive tract infection that can cause intense chronic pelvic pains and result in severe sequelae. *Neisseria gonorrhoeae* and *Chlamydia trachomatis* are common causes of dysuria and urethral discharge in men and of cervicitis in women. Both organisms have also been isolated from the urethra in 10% or more of unselected women attending venereal disease clinics (Wallin et al., 1981). Although urethral discharge is not usually present in women infected with these organisms, they do produce dysuria and frequent urination. These complaints were reported in about 20% women with gonococcal or chlamydial genital infections attending the Seattle sexually transmitted disease clinic (Holmes et al., 1973). According to Shirley et al., (1980), chlamydiae were isolated significantly more often from women with gonorrhoea and from women who were consorts of men with non-gonococcal urethritis than from other women attending STD clinics.

According to Mabey (1991) the role of other sexually transmitted diseases

(STDs) as potential cofactors for the transmission of HIV is increasingly recognized and the individual susceptibility to HIV - infection is increased in the presence of certain STDs. He also reported that in people with HIV infection, other STDs are more resistant to treatment e.g. one-dose treatment for chancroid failed six times more often in HIV-infected patients than in patients without HIV-infection. WHO (1993), reported that six of ten (10 studies carried out in Kenya and Zaire (Democratic Republic of the Congo) found that people with genital ulcer (chancroid infection) were five times more susceptible to HIV-infection than people without ulcers and that syphilis increased the risk of HIV-infection 3-to-9 times for the heterosexual men. Gonorrhoea, chlamydia, chancroid, syphilis and trichomoniasis increase the risk of HIV transmission by nine times (Waserheit, 1992).

The link between HIV-infection and other STDs explains why HIV in heterosexual population is more prevalent in Africa than Europe and the U.S.A. where STDs are more often treated and cured (Cameron et al., 1989). Elegba et al. (1996) reported that (19.05%) of gonorrhoea patients attending STD clinic ABUTH, Zaria were HIV seropositive. Since STDs are very prevalent in many African populations, effective STD control programs will play an important role in slowing down the spread of the HIV epidemic (Mabey, 1991).

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2.3 Non-Gonococcal Urethritis (NGU)

According to Bowie (1984), non-gonococcal urethritis is diagnosed when urethritis is present and *Neisseria gonorrhoeae* can not be detected by stain or culture and that non-gonococcal urethritis arising after treatment of gonorrhoea is called postgonococcal urethritis.

The two major etiologic agents of non-gonococcal urethritis (NGU) are *Chlamydia trachomatis* and *Ureaplasma Urealyticum* which cause 30-50% and 30-40% of NGU respectively. In hetero-sexual men with acute untreated non-gonococcal urethritis, *C. trachnomatis* or *U. urelyticum* or both are often isolated from 70-80% of such patients (Harrison, 1984). According to CDC (1985), *C. trachomatis* is the most prevalent agent of mucopurulent cerivitis and of all sexually transmitted diseases (STDs) in the United States of America. It also reported that *C. trachomatis* is associated with pelvic inflammatory disease (PID), infertility and preterm births. Oriel (1983) reported that *Mycoplasma hominis* has been associated with infections in females such as P.I.D., pyelonephritis, postpartum fever, morbidity and still births in infants. He also reported the association of *Ureaplasma urealyticum* with urethral syndroms in women, reproductive failures, low birth weight of babies of infectd females and non-gonococcal urethritis. Coliform bacteria and *Pseudomonas* account for the etiology of the majority of cases of non-gonococcal urethritis in men over 35 years of age and in prepubertal boys (Ireton and Berger, 1984).

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Department of Health and Social Security, United Kingdom (1989) reported an increase in the prevalence rate of non-gonococcal urethritis particularly among the young people in Britain. It concluded that the trends in non-specific genital infections and in gonorrhoea indicated increasing heterosexual transmission of infection which was an indication that the publicity about the AIDS epidemic then had little effect on heterosexual behaviour particularly in young people. Wasserheit (1992), reported that non-gonococcal STDs like chancroid caused by *H. ducreyi*, Chlamydial infections caused by *Chlamydia trachomatis,* syphilis caused by *T. pallidium* and trichomoniasis caused by *T. vaginalis* facilitate AIDS transmission. He also reported that infertility rate is higher in women with non-gonococcal rather than gonococcal pelvic inflammatory disease and that such infertility rate increases in direct proportion to the severity of the infection.

According to Baron and Finegold (1990), homosexual practices increase common heterosexual practices of anal-genital intercourse have required that a number of gastrointestinal and systematic pathogens also be considered etiologic agents of STDS. They reported that intestinal protozoa - *Giardia lamblia, Entamoeba histolytica* and *Cryptosporidium sp* as well as some fecal pathogens such as *Salmonella sp.*, *Shigella sp.* and Coliforms are now transmitted sexually. It was also noted that oral-genital sexual practices do allow *N. meningitidis* to infect the genital tract. Other etiologic agents of non-gonococcal urethritis mentioned are, *Staphylococcus sp.* (mainly *S. saprophyticus* and *S. aureus*) *Candida sp., Proteus sp.* and group *B. Streptococci.*

2.4 Prevention andControl

Sexually transmitted diseases (STDs) have had significant adverse effects on reproductive and child health worldwide. The control of STDs such as gonorrhoea is therefore an absolute priority (Pierre et al., 1992). National STDs control programms were established in the USA during the early days of the first world war and for half a century the focus was mostly on the control of syphilis and its complications during the 1950s and 1960s. During that period, Federal assistance continued to support the tracing of sexual contacts, serological screening and patient education services (Anderson, 1965). By the late 1960s officials had become concerned with the rapidly escalating number of cases of gonorrhoea. With the dramatic increase of federal financial assistance to STD control in 1972, gonorrhoea, at the expense of syphilis, received the larger portion of the STD federal grant. By 1982, estimated expenditures for gonorrhoea control accounted for almost three-quarters of the federal STD grant (Willard et al 1984). Similar programs were established in Europe such as in Britain where annual reports from various STD clinics are sent to the Department of Health and Social Security (DHSS) e.g. reports published by DHSS showed that there was a fall in new cases of syphilis and gonorrhoea during the period of 1985-1986 (DHSS, 1989). To encourage international action in the control of STD, the World Health Organisation (WHO) identified seven components of STD intervention strategies as follows:

1. Health education and promotion
2. Detection of disease
3. Appropriate treatment
4. Partner tracing and patient counselling
5. Evaluation of clinical services
6. Professional training
7. Research

According to Hall (1980), once the diagnosis is made, treatment should be inexpensive, simple, safe, and effective. Early and adequate treatment of patients and their sexual partners is an effective means of preventing both the resistance of STD organisms to antimicrobials and the spread of STD.

Major changes in intervention strategy have accompanied the introduction of new antibiotics (such as Penicillin) and development of better detection methods (such as Thayer-Martin Media), the identification of resistant organisms (such as PPNG) and the recognition of high risk group (such as homosexuals, patients with repeated infections and women with PID). According to Willard et al. (1984) quantum leaps in STD control programs awaits the availability of rapid and inexpensive diagnostic tests for chlamydia, safe and effective treatment of genital herpes and effective vaccine against herpes and gonorrhoea. *Neisseria gonorrhoeae* can vary its surface antigens thereby preventing the development of immunity to reinfection (Davis et al 1990).

2.5 Immunity

According to Salyers and Whitt (1994), despite the massive effort that has been directed towards development of an effective vaccine against *Neisseria gonorrhoeae*, it remains one of the most difficult challenges ever faced by scientists interested in vaccine development. This is because *Neisseria gonorrhoeae* has evolved a devastating strategy for avoiding a successful host immune response by constantly changing its surface antigens so that antibody mounted by the host is rendered obsolete and that the organism makes itself appear like the host tissues thus avoiding recognition by the host immune system by acquiring fibrin of the host tissue. They further stated that the organism have a number of mechanisms for varying the amount and composition of some surface proteins such as pilj and Protein II. The expression of pil E is controlled at the level of transcription by two-component regulatory system, pil A and pil B. These are regulators (protein) of pil E transcription. Pil A is the transcriptional activator and pil B is the sensor component that phosphorylates. Pil A *Neisseria gonorrhoeae* can switch between pil^+ and pil^- by one of many mechanisms such as:

1. PilE (expressed form) is nicked at the wrong site (Ps)

2. Uneven homologous exchange between pil E and pil S (silent form) that leads to synthesis of a larger form of pil E that is not processed and assembled into Pili and

3. Slipped strand mispairing of DNA strands during synthesis leads to frameshift mutations in pil C that encode a protein involved in processing PilE (Mayer et al., 1990). According to Zhang (1992) antigenic variation of pilin is due to changes in the DNA sequences of Pil S (Silent form) and Pil E (Expressed form).

Zhang also reported that copies of the protein II gene differ in two hypervariable regions and that homologous recombination events can occur between the different copies of the gene to cause antigenic variation but phase variation of P.II is more common and because of variability, pilin and P.II are not good vaccine candidates. P.I is more promising target but for its strain-to strain variation. Penicillin-binding protein (a highly conserved surface protein) is considered a good vaccine candidate. Lack of sophisticated genetic tools coupled with the lack of a good animal model, has made working on the virulence mechanisms of *Neisseria gonorrhoeae* a challenge (Saylers and Whitt, 1994).

According to Schneider et al., (1991), the specificity of *Neisseria gonorrhoeae* for human and the subsequent lack of an animal model has led to the use of human male intraurethral challenge model to study the pathogenesis of *Neisseria gonorrhoeae*. McGee et al. (1992) in their study found that *N. gonorrhoeae* induced increased tumor necrosis factor alpha (TNF$\propto$) in explanted genital tract tissue culture supernatants within 3 hours post infection. They also found that *N. gonorrhoeae* induced local and rapid production of the cytokines

interleukin-6 (IL-6), interlaukin-8 (IL-8) and tumor necrosis factor alpha (INF∝) in response to the *Neisseria gonorrhoeae* infection. They reported that interleukin-8 (IL-8) was prominent in attracting and activating polymorphonuclear leucocytes (PMMs). There is convincing evidence from in vitro studies that piliated gonococci are much more resistant to ingestion by human polymorphonuclear leucocytes than non-piliated mutants of the same strain but the difficulty is to relate this finding to the natural infection (Roberts, 1977).

According to WHO Report (1978), humoral immunity is indicated by the presence of secretory IgA antigonococcal antibody in the urethral secretation following an infection with *Neisseria gonorrhoeae,* and that cell-mediated immune response (CMI) is characterized by lymphoblastic transformation in patients with gonorrhoea. The report concluded that the presence of a variety of potential immunological factors such as lymphocytes, serum antibodies and local secretory IgA antibody, does not protect an individual against reinfection.

2.6 Therapy of Gonorrhoea

According to Stevens (1931), rest in bed for a week, avoidance of all alcoholics, stimulating and spicy foods and condiments and abundance of water were important elements of gonorrhoea treatment. He also recommended the use of santeal oil to lessen smarting during urination and the injection of mild antiseptic

(5% argyrol) into the urethra after urination every two hours. Chronic urethritis required full dilatation of the urethra by ultro sounds, prostatic massage and irrigations with silver nitrate solution (1:10,000 to 1:4,000) while chronic salpingitis usually required excision of the fallopian tube.

Treatments for gonorrhoea before the era of antibiotics included the application of various salves and ointments to the genitalia or the instillation of various antiseptic solutions into the urethra (Cecil, 1937). Between 1936 and 1937 these remedies were discarded in favor of the then newly discovered sulfa drugs (Dees and Colsten, 1938). Cambell (1944), reported that by 1943 majority of isolates of gonocccoci were resistant to sulfadiazine. Following the discovery of penicillin, the first successfully treated cases of gonococcal urethritis with penicillin occurred in 1943 and during the subsequent 40 years the amount of penicillin needed to treat gonoccocal urethritis has steadily increased (Halverson et al., 1969).

Penicillins have been the drugs of choice for the treatment of gonorrhea until 1975 when the first cases of β-lactamase - producing *Neisseria gonorrhoeae* strains were noted in Philippines (Perine et al., 1977), followed soon thereafter by occurrence in England (Phillips, 1976) and the U.S.A. (Ashford et al., 1976). Centers for Disease Control, in the U.S.A. in 1977 reported the occurrence of Penicillinase producing strains of *Neisseria gonorrhoeae* that had acquired a plasmid coding for the production of penicillinase. These strains are reported to be spreading throughout the world thereby necessitating radical and expensive

changes in therapy of gonorrhoea (Perine et al., 1979). A single-dose of ampicillin/sulbactam (1g ampicillin/0.5g sulbactam) was reported safe and effective against both PPNG and non-PPNG strains (Hellmann et al., 1995). Earlier studies have indicated that the combination of ampicillin and sulbactam, with or without probenecid, may be effective in the treatment of both PPNG and non-PPNG strains (Kim et al., 1986, Odugbemi 1987; Baddour et al., 1992).

A few studies from the Far East have reported a high prevalence of PPNG strains that possess plasmids that induce beta-lactamase production and also chromosomally-mediated resistant strains (Clendennen et al., 1992, Putnam et al., 1992). The studies reported the prevalence of gonococcal strains with decreased susceptibility to several newer quinolone agents. According to Handsfield et al (1991), most evaluations of gonococcal strains susceptibility to cephalosphorins and quinolones have been conducted in North America where *Neisseria gonorrhoeae* strains are susceptible to the new agents. They therefore stated that the prevalence of PPNG infection is not, in itself, an indicator of prevalence of strains with chromosomally-mediated resistance in a specific geographical location. Rice and Knapp (1994), reported that in the United States of America, most PPNG strains possess plasmid beta-lactamase.

The increase in the United States of America in the number of infections caused by strains of *Neisseria gonorrhoeae* that are resistant to penicillin and tetracyline has prompted the Centers for Disease Control and Prevention (CDC) in the U.S.A. to recommend the use of broad-spectrum cephalosphorins or

fluoroquinolones in treating uncomplicated gonorrhoea (CDC, 1993). Of the fluoroquinolones a single dosage of ciprofloxacin (500mg) orally and ofloxacin (400mg per os) have been recommended as primary therapies for the treatment of uncomplicated gonorrhoea. Enoxacin 400mg, lomefloxacin 400mg and norfloxacin 800mg have been recommended as alternative therapies. However, the failure of gonococcal infections to respond to CDC recommended doses of fluoroquinollones to effectively control gonococcal infection has been reported (Corkill et al., 1991; Tapsall et al., 1992). Strains of *Neisseria gonorrhoeae* exhibiting decreased susceptibilities to ciprofloxacin and ofloxacin have been reported from Asia (Clendennen et al., 1987; Putnam et al., 1992), the United Kingdom (Gransden et al., 1990, Jephcutt and Turner, 1990), North America (Knapp et al., (1994) and Spain (Birley et al., 1994). The failure of infections caused by strains for which the MICs were 0.5, 1.0 and 16.0ug of ciprofloxacin per ml to respond to dose of at least 500mg of ciprofloxacin have been documented in Australia and the United Kingdom (Corkill et al., 1991, Tapsall et al., 1992, Birley et al., 1994).

The third-generation cephalosporins offered an excellent therapeutic choice because of their intrinsic beta-penicillinase resistance. Intramuscular administration of Ceftriaxone has been established as an ideal treatment for *Neisseria gonorrhoeae* infections (Judson, 1986) and is the recommended first-line therapy of choice (CDC, 1989).

In the absence of strains resistant to fluoroquinolones, the National

Committee for Clinical Laboratory Standards (NCCLS) in 1993 recommended interpretive criteria only for the definition of susceptible categories for the interpretation of susceptibility testing of strains of *Neisseria gonorrhoeae* for ciprofloxacin (MIC 0.06μg/ml) and mefloxacin (MIC 0.12μg/ml). However, the failures of gonococcal infections to respond to CDC recommended doses of fluoroquinolones have been reported (Tapsall et al., 1992). Birley et al., (1994) reported from Spain the isolation of high-level prevalence of resistant *Neisseria gonorrhoeae* strains to ciprofloxacin and that reports from the Far East indicate high prevalence of multiple antibiotic resistant strains of *Neisseria gonorrhoeae* (Putnam et al., 1992).

According to Clendennen et al., (1992b), periodic monitoring of the underlying susceptibility profiles of the *Neisseria gonorrhoeae* strains prevalent in areas of frequent transmission may provide clues regarding treatment options and emerging drug resistance. They reported that the commercial sex industry in Thailand sustains very high rates of transmission of sexually transmitted diseases, among which gonorrhoea is foremost in frequency and multiple antibiotics resistance is common. Traisupa et al., (1990) reported that in 1983 the high prevalence of tetracycline resistant beta-lactamase producing *Neisseria gonorrhoeae* strains led to the adoption of spectinomycin as the recommended therapy for gonorrhoeae in Thailand and that while spectinomycin resistant strains developed rapidly in both the Republic of Korea and the Philippines, spectinomycin has retained its effectiveness in Thailand and remained the drug of choice for the

treatment of gonorrhoea.

To enhance the likelihood of suscessful treatment regimens for specific diagnosis, the U.S. Public Health Service has established treatment recommendations as a standard part of its control strategy. These recommendations initially covered syphilis and gonorrhoea but have been extended to include 18 other STDs and syndromes (CDC 1982). Selective prophylactic (epidemiological) treatment plays a major role in the U.S. STDs control strategies. Antibiotics are administered when the diagnosis is considered likely based on epidemiological indication before infection is confirmed by laboratory methods. This interrupts the chain of transmission between time of testing and treatment, ensure treatment of women with negative endocervical cultures and guarantees treatment for those who might not return when notified of positive tests (Willard et al., 1984).

2.7 Antimicrobial Resistance

Adaptive changes in pathogens that cause sexually transmitted diseases have influenced the epidemiology of these diseases e.g. the evolution of resistance to antimicrobials by *Neisseria gonorrhoeae* (Holmes et al., 1984). According to Osoba, (1981), reports from tropical Africa showed that over two-thirds of the gonococcal strains in many areas had diminished sensitivity to

penicillin and a larger proportion to other antimicrobial agents mostly as a result of indescriminate use of antibiotics at subtherapeutic doses or self-medication.

Treatment of *Neisseria gonorrhoeae* infection has been complicated by the emergence of chromosomally and plasmid-mediated antimicrobial-resistant strains (Easman and Isan, `1987). According to Percival et al., (1976), plasmid-mediated resistance reported that year (1976), resulted in the emergence of penicillinase-producing *Neisseria gonorrhoeae* (PPNG). Clendennen et al., (1992) reported that antibiotic resistance among *Neisseria gonorrhoeae* isolates from Cholburi and Bangkok, Thailand, were primarily limited to penicillin and tetracycline that were no longer used to control gonorrhoea.

Self-medication among commercial sex workers (CSW) in the Philippines and Thailand have been cited as contributing to the emergence of antibiotic resistance (Harrison et al., 1984; Slack, 1989). The production of beta-lactamase is not the only mechanism by which bacteria can resist beta-lactam antibiotics. Resistance can also be due to decreased entry of beta-lactam antibiotic through the bacterial cell wall or in the case of Gram-negative bacterium, failure to reach the intracellular site of peptidoglycan (Neu, 1973). Inability of the antibiotic to bind to the penicillin-binding proteins is another mechanism of resistance and has been found in *Neisseria gonorrhoeae* and *Pseudomonas aeruginosa* (Hackenbeck et al., 1980). Inhibition of the beta-lactamase is a mechanism by which the enzyme can be inactivated. Antistaphylococcal penicillins are known inhibitors of beta-

lactamase. Specific beta-lactamase inhibitors are clavulanic acid and sulbactam and penicillanic acid sulfones. They form a stable inhibitor-beta-lactam complex that binds the enzyme and destroys it (Fisher and Knowles, 1980).

The discovery of some strains of *Neisseria gonorrhoeae* with multiple drug resistance has made the treatment of infections due to PPNG with penicillin and tetracycline pointless since the organisms will be resistant to both agents. PPNG is a major public health issue in parts of Asia and sub-Saharan Africa. Preventing the spread of penicillin-resistant strains of *Neisseria gonorrhoeae* is an important public health goal because loss of penicillin (a cheap and well tolerated antibiotic) as a treatment for gonorrhoea would make gonorrhoea an even more serious threat to public health than it already is and would make it much harder to control (Anonymous, 1993).

Widespread use of sub optimal doses of antibiotics for treating gonococcal infections has led to selection of resistant chromosomal mutants in the developing countries that have spread throughout the world (Seth and Johnston, 1980; Brown et al., 1982). The outbreaks in the U.S.A. of infection by a non-PPNG strain of *Neisseria gonorrhoeae* that was highly resistant to penicillin (Hetcher et al., 1983), raised the possibility that such organisms may be widely distributed but unrecognised even in the developed countries. Both basic and applied research are crucial to STD control.

According to Brown et al., (1982), previous research had documented high

prevalence of resistance of *Neisseria gonorrhoeae* to both penicillin and tetracyline in Thailand. Several mechanisms of resistance to quinolones have been reported (Tanaka et al., 1994). Belland et al., (1994) identified amino acid changes that are associated with quinolone resistance in the A subunit (Gyr A) and in Bar (subunit of Lipoisomerase-IV in laboratory mutants of *Neisseria gonorrhoeae*). Degushi, et et. (1995) in a preliminary study using a small number of clinical isolates of *Neisseria gonorrhoeae* found amino-acid changes in Gyr A identical to those found in the laboratory mutants. Takashi et al. (1996) examined 55 clinical strains of *Neisseria gonorrhoeae* in Japan for alterations in Gyr A and Par C and correlated such changes with antimicrobial susceptibility profiles for other agents used in the treatment of gonorrhoea. They reported that eleven strains out of the 55 had alterations in both Gyr A and Par C and were significantly more resistant to fluoroquinolones and that the MICs of cephalosporins for these strains were also significantly higher than those of other strains.

High-level tetracycline resistant *Neisseria gonorrhoeae* (TRNG) for which the MIC was 16μg/ml was first reported in the U.S.A. in 1985 (Deborah et al., 1985). West et al. (1995) reported the prevalence of this high-level tetracycline resistant *Neisseria gonorrhoeae* with MIC 16μg/ml in Northern Tanzania. Its emergence was associated with previous use of tetracycline as prophylaxis or treatment for sexually transmitted diseases (Heritage and Hawkey, 1988). Koay et al. (1996) in a study on auxotype and serogroups of tetracycline resisant *Neisseria gonorrhoeae* isolated in Malaysia between 1992 and 1994, reported that

253 out of 693 isolates of *Neisseria gonorrhoeae* strains were highly tetracycline resistant and that its emergence and spread were alarming. They attributed the high resistance to the acquisition of streptococcal tet M determinant by the gonococci at the time of colonization of the genitourinary tract resulting in high-level resistance to tetracycline. The global movement of people, particularly from countries that use tetracycline as the sole therapy for gonorrhoea, patients with repeated episodes of gonorrhoea and self-administration of tetracycline for prophylaxis and treatment of sexually transmitted diseases by sex workers and their clients, are also factors for resistance.

2.8 Prevalence of Penicillinase Producing *Neisseria gonorrhoeae*

In the 1970s gonococci containing plasmid-borne genes for enzymes that inactivate penicillins were isolated from patients that failed standard treatment regimens in England (Philips 1976) and in the United States of America (Ashford et al., 1976). These *Neisseria gonorrhoeae* strains are known as penicillinase producing *Neisseria gonorrhoeae* (PPNG). According to Krieger (1984) although PPNG have been isolated from all over the world, they originated from two separate geographical areas - that is, the Far East and parts of West Africa. Perine et al. (1979) reported that PPNG strains were known to be freely circulating in West Africa with possible reserviors in urban areas of Ghana and Ivory Coast.

A study in 1991 in Surabaya, Indonesia, documented a high prevalence rate of PPNG strains, 17.8% in low-paid sex workers and 44.4% in high-paid sex workers (Utoro et al., 1991). A similar study conducted earlier in an STD clinic in Jakarta, Indonesia, in 1982, reported an 8% prevalence of PPNG strains. Penicillin G had MIC of $\geq$128μg/ml against all the *N. gonorrhoeae* isolates, (Rockhill et al., 1982). Joesoef et al. (1994) reported a substantial increase in resistance to penicillin (89%) and tetracycline (98%) in Surabaya, which was the highest reported rate of resistance among the South-east Asian countries. They attributed the high rate of resistance to penicillin and tetracycline to widesprad self-medication of sex workers who take penicillin or tetracycline for STD prevention and treatment or for other illnesses.

A study conducted on 330 patients attending a sexually transmitted disease clinic in Kampala, Uganda reported the isolation of 24 PPNG strains (Hellmann et al., 1995). Coovadia et al. (1984) reported the prevalence of PPNG among patients attending King Edward VIII Hospital, Durban, South Africa.

In Nigeria, Bello et al. (1983) reported the isolation of seven PPNG strains from patients attending sexually transmitted disease clinic ABUTH Zaria. In 1987, it was estimated that PPNG comprised 70-80% of gonococcal isolates in Nigeria (Odugbemi, 1987). Bakare et al. (1996) reported that 59 (92.2%) of the 64 gonococcal isolates from male patients at the Special Treatment Clinic, University College Hospital Ibadan were penicillinase producing *Neisseria gonorrhoeae* and 5 (7.8%) were non-penicillinase producing *Neisseria gonorrhoeae*.

2.9 Antimicrobial Susceptibility Testings (ASTs)

The concept of in vitro testing of antimicrobial agents against bacteria dates back to the 1920s and the first paper describing the antimicrobial properties of penicillin was by Fleming (Fleming, 1929). The first description of penicillin sensitivity testing for *Neisseria gonorrhoeae* appeared in 1940 and the precursors of modern antimicrobial sensitivity testings, (ASTs) were developed along with antimicrobial agents e.g. the concept of using paper disk impregnated with antimicrobial agents was first presented in 1944 (Vineent and Vincent, 1944).

According to Stratton (1984) the quest for reproducible, standardized antimicrobial susceptibility testings (ASTs) did not follow an easy path. In 1951, Jackson and Finland showed the importance of making fundamental choices regarding inoculum size, duration of incubation and endpoints in deciding quantitative susceptibility determination. The important work of Bauer et al. (1966) brought about an agreement on the standardization for disk diffusion test. Before then, there was no agreement on whether to read the results qualitatively or quantitatively. In the 1970s there was a general agreement on condition of AST for common bacterial pathogens and that enabled the American College of Pathologists to measure the accuracy of clinical laboratories in determining the susceptibility of bacteria by comparing their results with those obtained in reference laboratories (Fekete, 1993). Maier et al. (1974) attributed the development of substantial amount of data for studying susceptibility of *Neisseria*

gonorrhoeae via a number of in vitro tests to the frequency of *Neisseria gonorrhoeae* infection. According to Putnam et al. (1992) Agar dilution and disk diffusion testing methods are most commonly done in clinical laboratories.

The College of American Pathologists report published in 1988 showed that clinical laboratories were very successful in growing and identifying *Neisseria gonorrhoeae*, but only 633 of 2,017 institutions responded to questionnaire (of nearly 8,000 laboratories surveyed) reported doing any susceptibility testing (Jones et al., 1988). Fekete, et al. (1992) reported that most laboratories do nothing more than check for the presence of beta-lactamase enzyme. They stated 14 reasons why in vitro testing of *Neisseria gonorrhoeae* has been avoided by many clinical labratories. Some of these are:

1. Testing materials are not available
2. Test materials are available and reliable but impractical
3. Susceptibility to specific antimicrobial agent is predicatable from the knowledge of the beta-lactamase phenotype.
4. Tests are too expensive
5. Tests are unnecessary because the return of symptoms always heralds, treatment failure.

6. Even in research laboratories, resources for studying *Neisseria gonorrhoeae* are better spent in other areas.

7. Tests are unnecessary because treatment are readily available.

8. Susceptibility testing is of no epidemiologic value.

A Boston study in 1963 showed that men who were given 600,000 units of penicillin had 1.4% failure rate when the MIC was less than (<) 0.125μg/ml but a 32% failure rate when the MICs was equivalent or greater than (≥) 0.125μg/ml (Kjellander and Finland, 1963). Holmes, et al. (1973) reported a good correlation between MIC and treatment failure even with higher dose of penicillin (2.4×10^6 to 4.8×10^6 μg/units).

As the case was with penicillin, there was an early appreciation of variability in MICs of tetracycline. For many strains, the MICs were sufficiently high to account for occasional treatment failures. However, in 1984 and 1985, a new high-resistance (MIC, 16μg/ml) phenotype was discovered in the United States. There were strong evidences that conjugative plasmid (also found in many strains of PPNG) was responsible for disseminating that high-level tetracycline resistance (Knapp et al., 1987).

Regional differences in susceptibility of *Neisseria gonorrhoeae* have been noticed since antimicrobial susceptibility testings were initially developed. Even before the appearance of PPNG in the U.S.A., there were substantial differences

in MICs of penicillin and tetracycline for gonococci (Jaffe et al., 1976).

An important and essential measure for the monitoring of the effectiveness of recommended drugs for the treatment of gonorrhoea is surveillence of the in vitro antimicrobial susceptibilities of clinical isolates of *Neisseria gonorrhoeae* (Gorwitz et al., 1993). According to Lind (1986), the antimicrobial susceptibility patterns of *Neisseria gonorrhoeae* may change rapidly especially in areas where ineffective treatment regimens are applied. Without a doubt, continued monitoring of *Neisseria gonorrhoeae* strains will reveal new patterns of resistance and epidemiology, and some kind of a local ongoing monitoring of ASTs will be an important way of documenting these changes.

CHAPTER THREE

3.1.0 MATERIALS AND METHODS

3.1.1 STUDY AREA COVERED

The state was zoned into four (4) geographical areas and in each zone one rural and/or one urban towns were chosen as representative of urban and rural areas for determining the prevalence of *N. gonorrhoeae* infection within the state. The geographical zoning was as follows:

Zone A: Birnin Gwari, Kaduna and Chukun. Kaduna town was chosen.

Zone B: Zaria, Giwa and Makarfi. Zaria and Giwa were the chosen towns.

Zone C: Saminaka, Soba, and Pambeguwa. Soba and Pambeguwa were chosen towns.

Zone D: Kafanchan, Zonkwa and Kachia. Kafanchan and Kachia were chosen for the study.

Zaria

Zaria lies on 11° 02[1] Latitude (North) and 7° 43[1] Longitude (East). It is an education centre having a university, college of Aviation Technology, Nigerian Railway Training School, Institute of Transport Technology and Federal College

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of Education. In addition to these Federal Institutions, it has Kaduna State Polytechnic and many post primary Institutions including Federal Government Girls College. It also has a University Teaching Hospital (ABUTH), Government General Hospital and many private hospitals and Clinics. The town is densely populated with students, civil servants and business men and women. Hence its socio-economic life-style is very similar to most urban cities like Kaduna.

SOBA

Soba lies on 10° 58¹ Latitude (North) and 8° 03¹ Longitude (EAST). It is a small town that is between Zaria and Pambeguwa. It has two post primary institutions, a government health centre, and four private clinics. The inhabitants are mostly farmers and small scale business men and women. Civil servants are mostly teachers and few medical personnel at the health centre.

GIWA

Giwa lies on 11° 16¹ Latitude (North) and 7°26¹ Longitude (East). It has two post primary institutions (Government Girls Secondary School and Government Day Secondary School), a Government Health Centre and three private clinics. Its population is mostly farmers, traders and civil servants.

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PAMBEGUWA

Pambeguwa lies on 10° 40¹ Latitude (North) and 8° 28¹ Longitude (East). It has a Government Day Secondary School, Government Health Centre and five private clinics. Most of the inhabitants are farmers, business men and women with a few civil servants.

KADUNA

Kaduna, an industrial town and the capital of the state, lies on 10° 50¹ Latitude (North) and 7° 25¹ Longitude (East). It has many industries, Government ministries (both state and Federal), a polytechnic, Federal Training Centre, many post primary institutions including a Federal Government College and Military formations including Nigerian Defence Academy (NDA). It also has University Teaching Hospital (ABUTH Kaduna), two state government hospials and many private hospitals and clinics. Being an urban town, it has in addition to dense population of business men and women, large population of civil servants and students. Its socio-economic life-style is like that of any urban city in Nigeria.

KACHIA

Kachia lies on 7° 59¹ Latitude (North) and 9° 51¹ Longitude (East). Kachia has a general hospital, a few private clinics and two secondary schools. The inhabitants are predominantly farmers, civil servants and traders.

KAFANCHAN

Kafanchan lies on 9° 36¹ Latitude (North) and 8° 20¹ Longitude (East). It has Kaduna State College of Education, four secondary schools, and Railway Station. It also has a government general hospital, a Nursing School and a few dprivate hospitals and clinics. The population is mainly farmers, civil servants and business men and women.

These chosen areas are shown in the map of Kaduna State below (Fig. 1).

3.1.2. Materials

One thousand seven hundred and fifteen (1,715) samples consisting 938 Endocervical Swabs (ECS) and 777 urethral swabs (US) were collected from the study towns in the four zones. The number of samples collected per location is shown in Table 1.

3.1.3 Reference Organisms

Neisseria gonorrhoeae reference strains A, B, C, D, and E were obtained from Statens Seruminstitut, Centre for Prevention and Control of Infection Diseases and Congenital Disorders, World Health Organization (WHO)

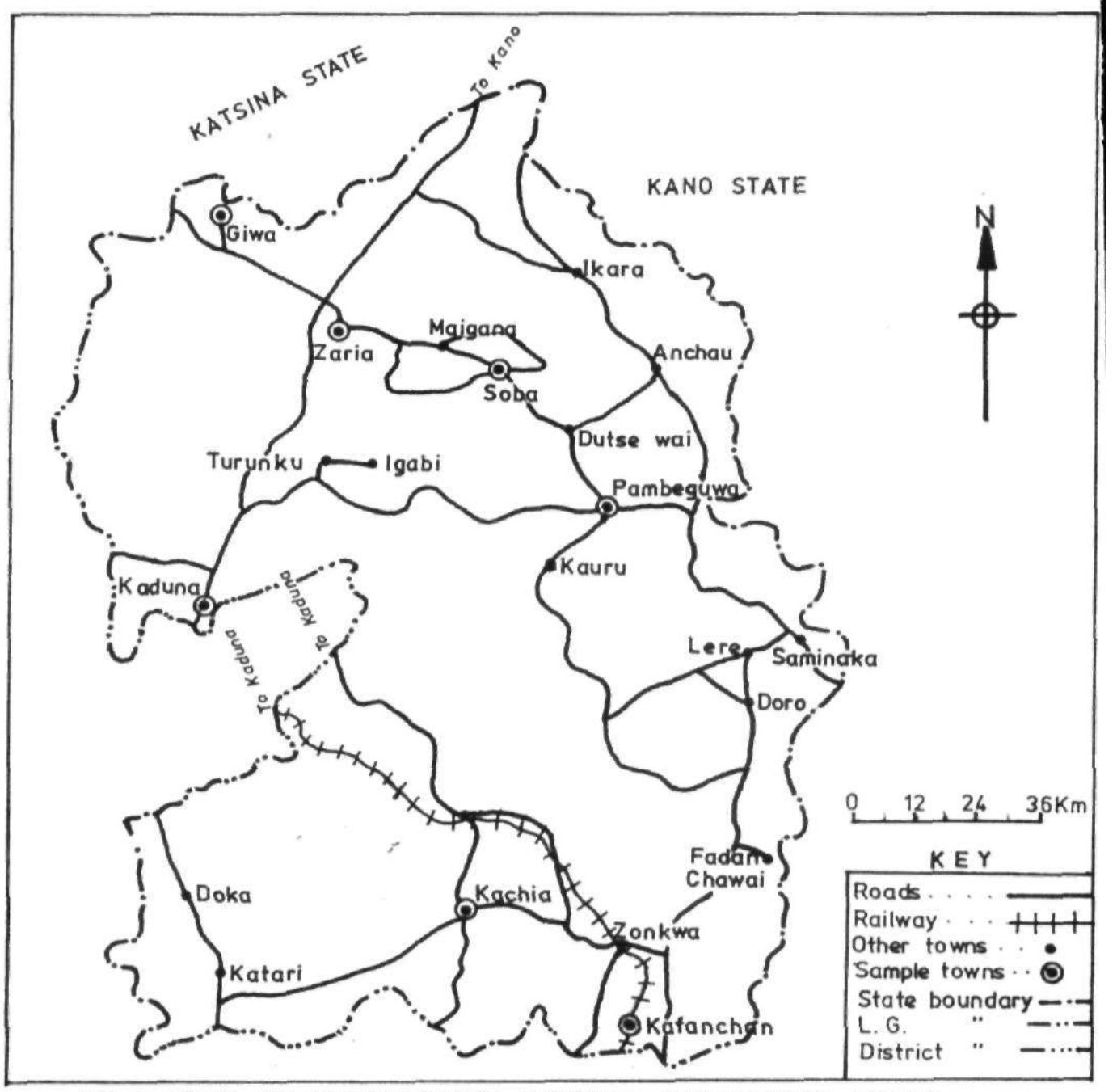


FIG. 1 PART OF KADUNA STATE SHOWING THE SAMPLED TOWNS

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Collaborating Centre for Reference and Research in Gonococci, Copenhagen, Denmark. After resuspension of the lyophilized cultures, they were maintained under moist state by periodic transfer (subculturing) onto fresh chocolate agar plate at the interval of every four days of storage at 4°C.

3.1.4 Media

Thayer-Martin Medium (Oxoid G.C. Agar Base, and Oxoid Haemoglobin powder and Oxoid G.C. Selective Supplement), Blood Agar Base (Oxoid), Chocolate Agar using Oxoid Agar, Oxoid urea Agar were purchased from Oxoid, Unipath Ltd, Basingstoke, Hampshire, England, MacConkey Agar, was purchased from Biolife Miland, Italy. Staphytococcus Medium 110, Sabouraud Dextrose Agar, Corn meal Agar and Stuart's transport medium were purchased from Difco Laboratories, Detroit, Michigan, U.S.A. All these media were prepared according to the manufacturer's instructions. The detailed preparation procedures are given in appendix 1.

3.1.5 Biochemicals and Reagents

Carbohydrates - Glucose, Maltose, Sucrose and Lactose were purchased from BDH, U.K. Starch soluble, Methyl red, Iodine solution and Urea powder were

purchased from Oxoid, Unipath Ltd. U.K. Kovac's reagents, Oxidase reagent, Voges-Proskauer reagent and Gram-straining reagents were prepared as directed in the manual of Clinical Microbiology by American Society for Microbiology, 5th Edition 1991, Human Plasma was obtained locally by centrifuging aseptically expired anti-coagulated blood collected from Blood Bank, at ABUTH Zaria. The plasma was dispensed with small plastic bottle and stored in freezer until needed for use.

Antibiotics

The following antibiotic discs and their therapeutic forms (powder or vials) were purchased from Oxoid, Unipath Ltd. U.K.:

Penicillin G 2.4μg, Ampicillin 10μg, Tetracycline 10μg, Gentamicin 5μg, Erythromycin 5μg, Ceftriazone 0.1μg, Cefuroxime 10μg and Ofloxacin 10μg.

3.2.0 METHODS

3.2.1 COLLECTION OF SAMPLES

Table 1: *Sampling sites and number of samples collected per season.*

LOCATION	NUMBER OF SAMPLES COLLECTED		
	RAINY SEASON	DRY SEASON	TOTAL
ZARIA	167	150	317
PAMBEGUWA	166	150	316
SOBA	166	150	316
GIWA	166	150	316
KADUNA	150	NC	150
KAFANCHAN	150	"	150
KACHIA	150	"	150
TOTAL	1115	600	1715

NC = No collection

3.2.2 Preparation of Media

All media used in this study were prepared as directed by the manufacturers. Biochemical solutions and reagents were prepared as stated in the Manual of Clinical Microbiology, 5th edition by American Society for Microbiology 1991. The detailed preparation techniques are given in appendix 1.

3.2.3 Phosphate Buffer pH 5.8

Potassium dihydrogen phosphate (6.25g) and dipotassium hydrogen phosphate (0.69g) were dissolved in one litre of distilled water and stored at 4°C for use. The buffer solution was fulter-sterilized before storage.

3.2.4 Penicillin G. Solution

A solution of 10,000 units per ml (0.6 microgram = 1.0 International unit of penicillin) was made in phosphate buffer and dispensed in small aliquots into small screwcapped plastic tubes and frozen until used.

3.2.5 Carbohydrates

A twenty percent (20%) stock solution of glucose, sucrose, lactose, and maltose were made in sterile distilled water respectively. Each carbohydrate was filter sterilized and dispensed in 2ml volume into appropriately labelled tubes and stored at -20°C until needed for use.

3.2.6 Buffered Salt Solution (Bss)

Dipotassium hydrogen phosphate 0.04g, potassium dihydrogen phosphate 0.01g, potassium chloride 0.80g were dissolved in 100ml of distilled water and 0.2ml of 1% aqueous solution of phenol red was added, mixed and stored at 4°C until used.

3.2.7 *Collection of Endocervical Swabs (Ecs) and Urethral Swabs (US)*

Using sterile cotton wool swabs, two endocervical swabs were collected from each female patient who presented at the clinic with vaginitis with the assistance of the medical personnel. The swabs were processed by making wet mount preparations for microscopic examination for *Trichomonas vaginalis* and Gram-stained and inoculated directly onto Thayer-Martin medium where facilities were available or inoculated into transport medium for transportation to the

laboratory.

A thin sterile cotton wool swab was used in collecting urethral swab. The urethral swabs were also processed by preparing smears which were gram-stained as well as inoculating Thayer-Martin medium (MTM) directly where facility was available or inoculating the transport medium for onward transfer to the laboratory.

All MTM plates inoculated with the ECS and US samples were incubated at 36°C under 5% CO_2 condition for 24-48 hours after which period they were examined for the growth of pathogenic *Neisseria*. All swabs inoculated into transport medium had to reach the laboratory within 48 hours for subculturing onto MTM medium.

Subculturing onto Thayer-martin medium was made immediately each swab sample arrived in the laboratory. The inoculated plates were also incubated under 5% CO_2condition at 36°C for 24-48 hours and then examined for the presence of *Neisseria gonorrhoeae*. The urethral smear made from each patient was Gram-stained and examined for the presence of *Neisseria sp* and other pathogens.

The swabs obtained from both male and female patients were also inoculated onto appropriate media for the isolation of other non-gonococcal agents of urethritis and vaginitis.

3.3.1 Processing the Ecs and Us for the Isolation of *Neisseria gonorrhoeae* and Other Micro-organisms

Each ECS and US gram-stained smear was examined microscopically for the presence of aetiologic agents of non-gonococcal urethritis/vaginitis. The presence of gram-negative rods in each case was confirmed by culture (colonial morphology) on MacConkey agar plate after 24 hours incubation. Each isolate was further identified and speciated using a few standard biochemical tests such as Indole, Methyl red, Voges-Proskauer and Citrate utilisation in addition to pinkish red colonies on MacConkey. The non-Lactose fermenters (NLF) were identified by colourless colonies on MacConkey agar, and greenish pigmentation in the case of *Pseudomonas sp* and urease production by *Proteus sp*. The detailed description of method used in identifying these pathogens is given in appendix 2.

3.3.2 Staphylococcus aureus

Each gram-stained ECS and US smear was microscopically examined for the presence of gram-positive cocci in clusters resembling *Staphylococci*. Positive cases were confirmed by production of good growth on *Staphycococcus* medium 110 after 18-24 hours incubation at 36°C. The culture was gram-stained and examined for typical staphylococcal morphology under the microscope. Isolates were identified, using human plasma for coagulase test. A drop of plasma

(human) was placed on a clean glass-slide and a small portion of the suspected bacterial colony was emulsified in the plasma using sterile wire-loop. Coagulation of the plasma indicated coagulase production.

3.3.3 Candida Albicans

Each gram-stained smear of ECS and US was examined for the presence of yeast cells. The wet mount preparation was also examined for the budding yeast cells. The positive cases were confirmed by luxuriant growth on Sabouraud Dextrose Agar (SDA) after 24 hours incubation at 36°C. The isolates were identified morphologically under the microscope, and germ-tube formation using sterile bovine serum.

3.3.4 Identification of Trichomonas Vaginalis

A wet mount preparation of each ECS was examined for the presence of ovoid, motile-flaggellated protozoan that moves with characteristic wobbling and rotating motion (Heine and Mcgregor, 1993). Positive microscopic cases were indicative of the presence of *Trichomonas vaginalis.*

3.3.5 Identification

Preliminary identification of all *Neisseria gonorrhoeae* isolates were done by colonial morphology and Gram staining characteristics. Typical colonial characteristics of *Neisseria gonorrhoeae* on modified Thayer-Martin (MTM) medium are evidenced by opaque, convex, gray-white, glistering colonies after 24 to 48 hours incubation at 36°C under increased CO_2 tension (5%). The typical colonies on MTM medium were gram-stained and examined microscopically for the presence of typical gram-negative diplococci.

3.3.6.0 Biochemical Confirmatory Test of *Neisseria Gonorrhoeae*

3.3.6.1 Oxidase Test

A drop of oxidase reagent (tetramethyl-phenylene diamine (TMPD) 6.0g dissolved in 100ml of dimethyl sulfoxide (DMSO) in brown bottle was placed on a piece of filter paper and a portion of the suspected colony was smeared across the spot. A positive reaction resulted in the paper turning purple within ten (10) seconds. Absence of change in colour indicated negative reaction (Balows and Hauster, 1991).

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3.3.6.2 Carbohydrate Utilization by *Neisseria gonorrhoeae*

The gonococcal isolates were characterized by their ability to utilise maltose, lactose, sucrose and glucose (Brown, 1974).

A heavy suspension of each pure *Neisseria gonorrhoeae* isolate was made from a 24 hour-culture on chocolate agar by scraping the entire surface growth into 0.3ml of Buffer Salt Solution (BSS) contained in a Khan tube. One drop (0.1ml) of each carbohydrate tested was added to appropriately labelled tube containing 0.1ml of BSS and a drop of heavy *Neisseria gonorrhoeae* suspension was added to each tube and mixed well. All inoculated tubes were incubated in a 35°C water bath for four hours.

Interpretation:

The development of yellow colour after exactly 4 hours indicated positive carbohydrate utilization by the isolate. Absence of colour change indicated negative utilization of carbohydrate by the organism. Carbohydrate utilizing and non-carbohydrate utilizing *Neisseria gonorrhoeae* strains obtained from WHO at Copenhagen, Denmark, were also used in the test as standards.

3.3.7.0 Antimicrobial Susceptibility Testing

3.3.7.1 Disc Diffusion Method

The method used was that recommended by WHO Expert Committee on Biological Stadardization (WHO/Bs/81-1337 Rev.1). It has been found useful for testing of gonococci, especially for screening for beta-lactamase production and for high level resistance (Biddle et al., 1978, Riou et al., 1981).

The antimicrobial discs containing penicillin G Ampicillin, Tetracycline, Erythromycin, Gentamicin, Ceftriaxone, Cefuroxime and Ofloxacin were supplied by Oxoid, Unipath, Basingstoke, Hampshire, England.

An inoculum of 10^9 c.f.u. ml (equivalent to McFarland Standard No.1) was prepared from a 24 hours old pure culture of *Neisseria gonorrhoeae* in sterile Saline. A plate of oxoid Diagnostic sensitivity Test Agar at room temperature with a dry surface was flooded with 2ml of the standardized suspension and the excess fluid was pipetted out. The inoculated plates were allowed to dry for 15 minutes before the antimicrobial discs were placed and slightly pressed down using sterile forcep. The inverted plates were incubated in a moist atmosphere containing 5% CO_2 at 36°C for 18-24 hours. The zones of inhibition were measured and interpreted according to WHO's standard i.e. a zone of inhibition measuring eighteen (18mm) and above was reported as susceptible and any measurement below 18mm was reported as resistant which was recommended by the NCCLS. protocol M_2-T_4 and quoted by Baron and Finegold, (1990).

Another interpretation of antimicrobial susceptibility testing of *Neisseria gonorrhoeae* based on clinical efficcacy of the drug used for the treatment of gonorrhoea was given by CDC in 1987 as follows:

1. The lowest antimicrobial concentration that predicts a 95% success rate with standard therapy is the break point for the highly susceptible range.

2. The concentrations that predict 85-95% cure make up of the moderately susceptible range.

3. The concentrations that predict 85% cure are in the resistant range (CDC, 1987).

3.3.8 Rapid Iodometric Test for Beta-lactamase Production in *Neisseria gonorrhoeae*

A heavy suspension of a 24 hour pure culture of each *Neisseria gonorrhoeae* isolate was made in penicillin-buffer solution contained in a tube to obtain 10^9 cells per ml. The mixture was stirred and allowed to stand at room temperature for 30 minutes. 0.05ml of 1% soluble starch solution was added and mixed. Iodine reagent (0.02ml) was then added and mixed. A blue colour developed immediately due to the reaction of the iodine and starch.

Rapid decoloration indicated the production of beta-lactamase. Absence

of decoloration for over 10minutes indicated non-production of the enzyme beta-lactamase. Both PPNG and Non-PPNG strains obtained from WHO in Copenhagen Denmark were used in the test.

3.3.9.0 Determination of Minimum Inhibitory Concentrations (Mics) of Antibiotics on the *Neisseria Gonorrhoeae* Isolates

3.3.9.1 Preparation of Antibiotic Stock Solutions

A stock solution of each of the following antibiotics was prepared as follows:

Penicillin G 64μg/ml was prepared in phosphate buffer, pH 6.5, dispensed in 2ml volumes into plastic screwcapped tubes and stored at -20°C until needed for use. The same procedure and storage were used for the preparation of ampicillin, Erythromycin and the Cephalosporins (Ceftriazone, Cefuroxime and Ofloxacin). Tetracycline hydrochloride 64μg/ml was prepared in sterile distilled water, dispensed in 2ml volumes into plastic screw-capped tubes and stored at -20°C until needed for use. The same techniques was employed in preparing 64μg.ml of Gentamicin. At the time of use, once thawed, the antibiotic solution was not re-frozen as recommended by WHO Expert Committee on antibiotic susceptibility testing of *Neisseria gonorrhoeae* (Lind et al., 1994).

3.3.9.2 Tube Dilution Method

The tube dilution method according to Kenny and Brachman, (1994) was used.

A row of ten (10 tubes each containing 1ml of Muller-Hinton broth was set up for each antibiotic tested against each *Neisseria gonorrhoeae* isolate. To tube 1 of the appropriately labelled set of tubes, 1ml of thawed antibiotic solution (64 μg/ml) was added and mixed and 1ml transferred to tube 2. The content of tube 2 was mixed and 1ml transferred to tube 3. The process was carried out to tube 9 after which 1ml was discarded. To each tube, 0.1ml of *Neisseria gonorrhoea* suspension equivalent to 10^9cells/ml was added. The inoculated tubes were incubated at 36°C under 5% CO_2condition for 24 hours. The highest dilution that showed no vissible growth was taken as the MIC of the drug for the *Neisseria gonorrhoeae* isolate. The procedure was repeated in determining the minimum inhibitory concentration of all antimicrobial agents used against all the *Neisseria gonorrhoeae* isolates. In all the tests, tube 10 served as the negative control (without antibiotic added to the Muller-Hinton broth). In addition, both susceptible and resistant strains obtained from the World Health Organization were set up during each test. The range of antimicrobial concentrations in micrograms/ml were: 0.125, 0.25, 0.5, 1.0, 2.0, 4.0, 8.0, 16.0, 32.0mcg.

CHAPTER FOUR

4.1.0 RESULTS

4.1.1 ISOLATES FROM CLINICAL SAMPLES

Out of the 1715 patients screened, 275 (16.03%) were found positive for *Neisseria gonorrhoeae* infection. The prevalence per location and the percentage of such prevalence at each location are shown in Table 2. The prevalence rate per location as shown in the same table 2 showed Zaria having the highest rate of 22.08% of patients screened, followed by Kaduna with 21.33%, Pambeguwa 18.35%, Kafanchan 16.66%, Kachia 12.66%, Giwa 11.70% and Soba 10.76% respectively. The number of postive gonococcal cases per location/sex and their sample numbers are given in appendix 3.

Table 2: Prevalence of *Neisseria gonorrhoeae* infection in various Locations in Kaduna State.

Location	No. Of sample	No. positive for *Neisseria gonorrhoeae*	Percentage Prevalence
ZARIA	317	70	22.08
PAMBEGUWA	316	58	18.35
GIWA	316	37	11.70
SOBA	316	34	10.76
KADUNA	150	32	21.33
KAFANCHAN	150	25	16.66
KACHIA	150	19	12.66
Overall	1715	275	16.03

4.2.0 Statistical Analysis of the Data

4.2.1 Statistical Significance of Prevalence

Statistical analysis of the results shown in Table 3 using chi-square (X^2) shows that X^2 computed (X^2_c) was greater than the X^2 table value at 5% alpha level of significance where X^2 table value was 3.84. The computed X^2_c value obtained was 25.23 against the table value of 3.84. The statistical level of significance of prevalence at each location is also shown in the same Table 3.

The formula used in statistical analysis was Chi-square =

$$X^2 = \left\{ \frac{O-E}{E} \right\}^2 \quad \left\{ \frac{\text{Observed-Expected}}{\text{Expected}} \right\}^2$$

The X^2c value overall is greater than X^2 table value at alpha level of significance of 5% (=0.05). Hence it is concluded that the over all prevalence of *Neisseria gonorrhoeae* infections among the screened population is statistically significant. The statistical significance of prevalence was dependent upon the location (Appendix 4).

Table 3: Chi-square analysis of statistical significance of prevalence of *Neisseria gonorrhoeae* Infection in Kaduna State.

	Positive Cases		Negative Cases				
Location	Observed Value	Expected Value	Observed Value	Expected Value	Total	X^2c Value	5% Signi., Level
Zaria	70	50.83	247	266.17	317	8.17	3.84
Giwa	37	50.47	279	265.33	316	3.99	3.84
Soba	34	50.67	282	265.33	316	6.15	3.84
Pambeguwa	58	50.67	258	265.33	316	1.10	3.84
Kachia	19	24.05	131	125.95	150	1.03	3.84
Kafanchan	25	24.05	125	125.95	150	0.01	3.84
Kaduna	32	24.05	118	125.93	150	2.75	3.84
TOTAL	275		140		1715	25.23	3.84

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4.2.2 PREVALENCE OF GONOCOCCAL INFECTIONS PER SEX PER LOCATION

The prevalence of *Neisseria gonorrhoeae* per sex per location is shown in Table 4. The prevalence rate was higher among the male patients than among the female patients. Statistical analysis was carried out with the view to determine the effect of location on the sex prevalence of gonoccoccal infections among the studied population. Chi-square (X^2) analysis showed that sex prevalence of *Neisseria gonorrhoeae* infection is independent of location, that is higher gonorrhoea infection among male patients than among female patients was common in all locations.

The $X^2c = 0.485$ against the alpha level of significance at X^2 tab. value = 3.84 at 5%. That is $X^2c < X^2$ tab. Hence the sex prevalence is independent of location (Appendix 5).

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Table 4: Prevalence of *Neisseria Gonorrhoeae* Infection per Sex per Location in Kaduna State

LOCATION	Samples Collected	TOTAL NO. POSITIVE	NO. OF MALE POSITIVE	NO. OF FEMALE POSITIVE	TOTAL PERCENTAGE PREVALENCE
ZARIA	317	70	48	22	22.08
GIWA	616	37	26	11	11.70
SOBA	616	34	24	10	10.75
PAMBEGUWA	616	58	40	18	18.35
KADUNA	150	32	22	10	21.33
KACHIA	150	19	12	7	12.66
KAFANCHAN	150	25	18	7	16.66
TOTAL	715	275	190	85	16.03

Out of the 938 female patients screened, 85 (9.07%) were found positive for gonococcal infection. 190 out of 777 (24.46%) male patients were found positive for *Neisseria gonorrhoeae* infections. Thus gave a prevalence ratio of male-to-female patients at about 2:1.

4.2.3 Gonococcal Infection among Different Age-groups

The prevalence of *Neisseria gonorrhoeae* infection per age-groups and sex is shown in fig.2. The results show that age group 15-20 years old has the highest prevalence of gonorrhoea indicating that the age-group is the most sexually active group. The prevalence went on the decline from 21-25 age-group through 31-35 age-groups. The second highest prevalence rate of gonorrhoea was among the

36-40 year-old age-group with 12 (26.08%) of 46 patients screened. The prevalence was zero for the 41-45 and 46-50 age-groups. This perhaps indicates that human sexual activities decline with age coupled with awareness of medical dangers of indiscriminate sex. The statistical significance of the prevalence on the basis of age-groups is shown in appendix 6. The Chi-square computed value was greater than the table value ($X^2c = 83.7$ against the X^2table = 12.83 at 5% ($\propto$ = 0.05). It is thus concluded that the prevalence is significant and depends on age-group.

4.3.0 Antimicrobial Susceptibility Testings (Asts)

4.3.1 Disc Diffusion Method (Bauer-kirby Method)

The resistance patterns of *Neisseria gonorrhoeae* to various drugs tested is as presented in Table 5. Out of the two hundred and seventy five (275) *Neisseria gonorrhoeae* isolates two hundred and twenty five (225) were resistant to penicillin, two hundred and six (206) were resistant to ampicillin, one hundred and twenty two (122) were resistant to tetracycline, thirty four (34) isolates were resistant to erythromycin and sixteen (16) isolates were resistant to gentamicin. All the 275 *Neisseria gonorrhoeae* isolates were sensitive to Ceftriaxone, Cefuroxime and Ofloxacin. Generally, there was high level of multi-drug resistance among the resistant strains particularly to Penicillin, ampicillin and tetracycline. The multi-drug resistance pattern is shown in Table 5. The susceptibility pattern of the *Neisseria gonorrhoeae* isolates from each location to each antimicrobial agent is shown in Table 6.

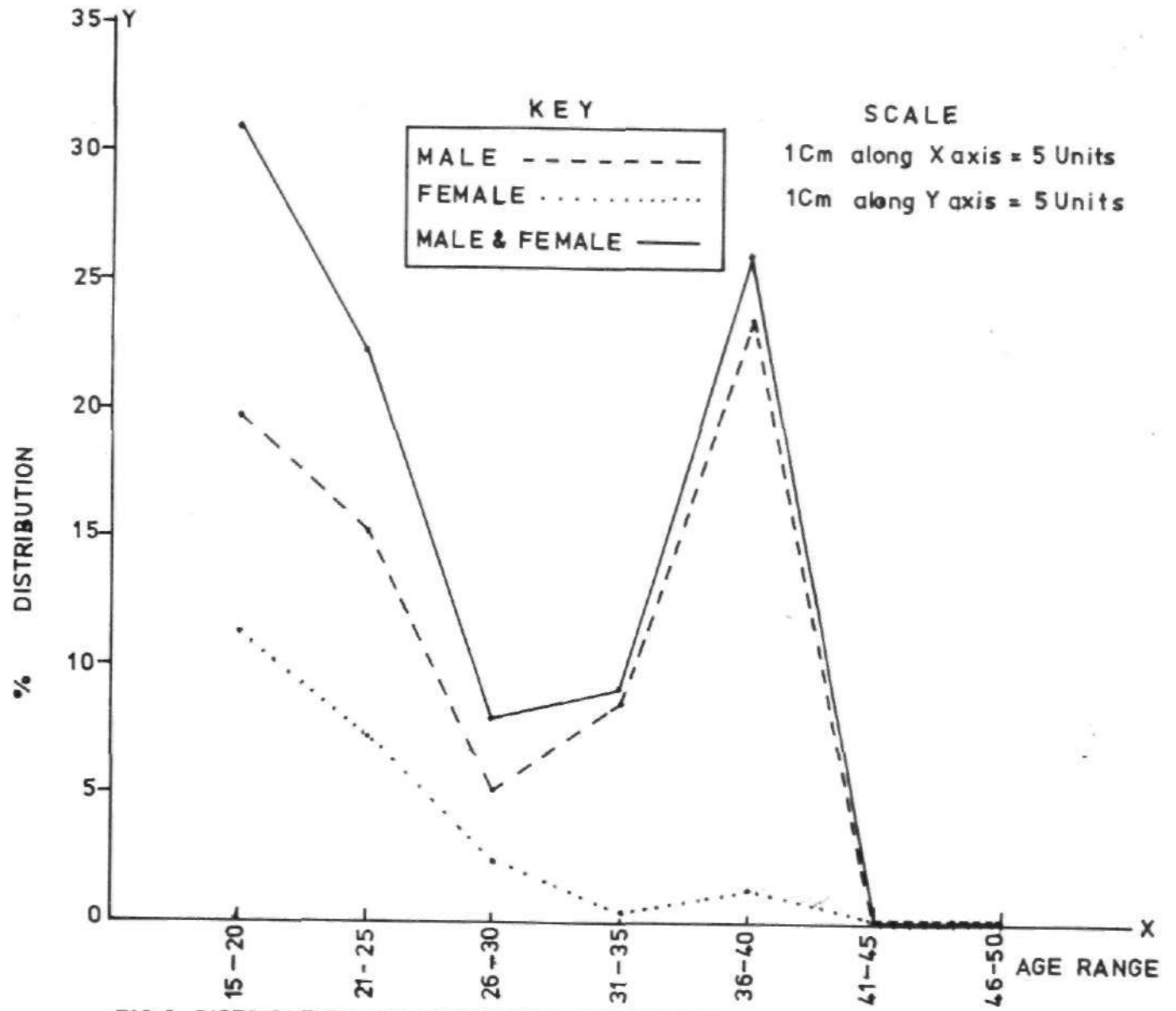


FIG.2 DISTRIBUTION OF NEISSERIA GONORRHOEAE INFECTION AMONG AGE GROUPS BASED ON SEX.

Table 5: Number of isolates of *Neisseria gonorrhoeae* from each location showing multi-drug resistance.

Location	PG& Amp.	PG.& Amp & TC	PG., Amp & E	PG., Amp TC & GM	PG., Amp, TC & E	PG., Amp Tc, E & GM	PG, Amp & GM	PG., & GM
Zaria	25	16	2	-	10	-	2	1-
Giwa	18	7	-	-	-	-	-	-
Pambeguwa	23	18	1	-	3	-	-	-
Soba	16	6	-	-	-	-	-	-
Kafanchan	7	8	-	1	2	1	-	-
Kachia	6	5	1	1	-	-	-	-
Kaduna	11	7	1	1	3	-	-	-

KEY: PG - Penicillin G
Amp - Ampicillin
TC - Tetracycline
E - Erythromycin
GM - Gentamicin

Table 6: Antimicrobial Susceptibility of *N. gonorrhoeae* Per Location

Location	Total Number of Isolates	Penicillin		Ampicillin		Tetracycline		Erythromicin		Gentamicin		Cefuroxime		Ceftriazone		Ofloxacin	
		R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S
ZARIA	70	63	7	60	10	29	41	14	56	3	67	1	69	-	70	-	70
GIWA	37	26	11	26	11	9	28	-	37	-	37	-	37	-	37	-	37
PAMBEGUWA	58	47	11	46	12	22	36	5	53	-	58	-	58	-	58	-	58
SOBA	34	25	9	24	10	6	28	-	34	-	34	-	34	-	34	-	34
KACHIA	19	16	3	13	6	6	13	1	18	1	18	-	19	-	19	-	19
KADUNA	32	28	4	26	6	12	20	40	28	2	30	-	32	-	32	-	32
KAFANCHAN	25	21	4	19	6	12	13	3	22	2	23	-	25	-	25	-	25

KEY: S = Susceptible

R = Resistant

4.3.2 Beta-lactamase Production

Out of the two hundred and twenty five (225) penicillin resistant isolates tested for the production of beta-lactamase, one hundred and eighty nine (189) were beta-lactamase positive, that is they were penicillinase producing *Neisseria gonorrhoeae* (PPNG) and thirty six (36) isolates were beta-lactamase negative that is non-penicillinase producing *Neisseria gonorrhoeae* (Non-PPNG). The prevalence of PPNG and Non-PPNG is shown in Table 7. Analysis shows that the prevalence of PPNG in this study is quite significant, with $X^2c = 12.25$. Hence the PPNG prevalence is statistically significant. This calculation is shown in appendix 7.

Table 7: PENICILLINASE AND NON-PENICILLINASE PRODUCING *Neisseria gonorrhoeae* ISOLATES FROM VARIOUS LOCATIONS IN KADUNA STATE

LOCATION	TOTAL REISTANT ISOLATES	NO. OF PPNG	NO. OF NON-PPNG
ZARIA	63	**47**	16
GIWA	26	**24**	**2**
SOBA	24	**24**	0
PANBEGUWA	47	**37**	10
KADUNA	28	**24**	4
KAFANCHAN	21	**18**	3
KACHIA	16	**15**	1
TOTAL	225	189	36

4.3.3 The Mic of Antimicrobial Agents on Clinical Isolates (Tube Dilution Method)

The MICs of penicillin and ampicillin against most of the *Neisseria gonorrhoeae* isolates were higher than 0.125 μg/ml. The MIC values of 0.125mcg/ml of tetracycline, erythromycin and gentamycin were effective against

19,100 and 119 isolates respectively. Ceftriaxone, Cefuroxime and Ofloxacin had MIC value of 0.125 μg/ml for all the isolates. For five isolates, penicillin had MIC of 32.0 mμg/ml and tetracycline had MIC of 32.0μg/ml for three isolates. Generally, the MICs of most of the drugs commonly used for the treatment of gonorrhoea were high. The minimum inhibitory concentrations of the antimicrobial agents used in this study against the *Neisseria gonorrhoeae* isolates are shown in Table 8.

Table 8: MINIMUM INHIBITORY CONCENTRATIONS (MICs) IN μg/ml OF COMMONLY USED ANTIBIOTICS ON CLINICAL ISOLATES OF *Neisseria gonorrhoeae*

	Concentration in μg/ml								
Antibiotics	0.125	0.25	0.5	1.0	2.0	4.0	8.0	16.0	320
Penicillin G	**0**	12	31	4	1	2	120	107	5
Ampicillin	**0**	26	26	6	4	12	162	33	0
Tetracycline	**19**	75	69	6	6	5	15	77	3
Erythromycin	100	86	38	7	10	17	17	0	0
Gentamicin	119	84	40	18	6	4	3	1	0
Ceftriaxone	275	0	0	0	0	0	0	0	0
Cefuroxime	275	0	0	0	0	0	0	0	0
Ofloxacin	275	0	0	0	0	0	0	0	0

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4.4.0 PREVALENCE OF AGENTS OF NON-GONOCOCCAL URETHRITIS AND VAGINITIS

From some of the patients screened for *Neisseria gonorrhoeae* the following aetiologic agents of non-gonococcal urethritis/vaginitis were isolated and identified: *Escherichia coli* was isolated from 204 (11.9%) made up of one hundred and nineteen females and eighty five males. *Candida albicans* was isolated from 269 (15.7%) patients consisting of two hundred and sixty females and nine males, while *Staphylococcus aureus* was isolated from four hundred and three 403 (23.5%) consisting of three hundred and forty females and sixty three males. *Trichomonas vaginalis* was isolated from 104 (6.06%) female patients only, and *Proteus sp* from 5 (.3%) patients made up of one female and four male, *Klebsiella sp* was isolated from 49 (2.85%) patients consisting of fourty five females and four males and *Pseudomonas sp* isolated from 2 (0.12%) female patients. The prevalence of each aetiological agent is presented in Table 9.

Table 9: The number of ECS and US that were positive for various agents of non-gonococcal urethritis

LOCATIONS	SPECIMEN TYPE	*E. Coli*	*C. albicans*	*T. vaginalis*	*S. aureus*	*Proteus sp*	*Klebsiella sp*	*Pseudomonas sp.*
ZARIA	ECS	16	40	14	16	-	1	1
	US	15	1	-	45	-	-	-
GIWA	ECS	21	56	18	7	-	11	-
	US	12	2	-	15	-	1	-
PAMBEGUWA	ECS	16	54	21	3	-	8	-
	US	13	2	-	70	2	3	-
SOBA	ECS	23	41	20	14	-	11	-
	US	19	5	-	73	-	-	-
KAFANCHAN	ECS	13	23	8	14	-	5	-
	US	9	-	-	19	1	-	-
KACHIA	ECS	18	23	9	3	1	5	-
	US	11	1	-	29	1	-	-
KADUNA	ECS	12	21	14	6	-	4	1
	US	6	-	-	29	-	-	-
TOTAL		204	269	104	403	5	49	2
		11.9%	15.7%	6.06%	23.5%	0.3%	2.85%	0.12%

4.4.1 INCIDENCE OF MIXED STD. INFECTIONS AMONGST THE STUDIED POPULATION

The organisms that were encountered in the mixed infections were *Trichomonas vaginalis*, *S. aureus*, *C. albicans*, *E. coli* and *Klebsiella sp.*

The most common mixed infections encountered were *C. albicans* and *S. aureus* (4.1%); *E. coli* and *S. aureus* (3.5%), and *Trichomonas vaginalis* and *C.*

albicans (3.2%). Mixed infections of *Trichomonas vaginalis; E. coli* and *S. aureus* were the least encountered (0.12%). Out of 289 cases of mixed infections, 86 cases (29.75%) involved males and 203 cases (70.24%) involved females (Table 10).

Table 10: No. Of cases of mixed infections with agents of gonococcal and non-gonococcal urethritis

LOCATIONS	SPECIMEN TYPE	*T. vaginalis* + *S. aureus*	*T. vaginalis* + *C. albicans*	*T. vaginalis* + *E. coli*	*T. vaginalis* + *M. gonorrhoeae*	*T. vaginalis* + *E. coli* + *S. aureus*	*N. gonorrhoea* + *S. aureus*	*C. albican* + *S. aureus*	*C. albican* + *E. coli*	*C. albican* + gonorrhoeae	*E. coli* + *S. aureus*	*N. gonorrhoeae* + *E. coli*	*Kleb. Sp.* + *S. aureus*	*N. gonorrhoea* + *C. albican* + *Kleb. Sp.*
ZARIA	ECS	9	10	-	-	2	5	17	9	3	4	2	1	-
	US	-	-	-	-	-	7	2	-	-	5	4	3	-
GIWA	ECS	-	9	-	-	-	2	8	2	-	10	-	2	-
	US	-	-	-	-	-	-	7	1	-	5	-	-	-
PAMBEGUWA	ECS	-	11	1	1	-	1	5	7	3	7	2	-	2
	US	-	-	-	-	-	5	2	-	-	4	1	-	-
SOBA	ECS	-	10	1	-	-	-	5	3	-	5	-	1	-
	US	-	-	-	-	-	-	11	1	-	8	-	-	-
KAFANCHAN	ECS	4	4	-	-	-	3	-	1	1	2	-	-	-
	US	-	-	-	-	-	1	-	1	-	-	2	-	-
KACHIA	ECS	1	5	-	-	-	1	3	2	-	2	1	-	-
	US	1	-	-	-	-	-	3	1	-	1	1	-	-
KADUNA	ECS	-	6	-	-	-	4	2	1	-	7	-	-	-
	US	-	-	-	-	-	3	5	-	-	-	1	-	-
TOTAL		15	55	2	1	2	32	70	30	7	60	14	8	2
		0.8%	3.2%	0.12%	0.06%	0.12%	1.7%	4.1%	1.7%	0.4%	3.5%	0.81%	0.4%	0.12%

CHAPTER 5

DISCUSSION

The findings in this study have shown a high prevalence rate (16.03%) of gonococcal infection among the population studied. The prevalence rate of gonococcal infection was significantly higher among male than female patients (P>0.05). In this study, patients who presented at the various clinics with urethritis or vaginitis were screened for *Neisseria gonorrhoeae*. That could account for the higher number of female patients than male patients seen at the screening level.

The prevalence of *Neisseria gonorrhoeae* infection was found to be higher in the population screened in Zaria than in those from other locations, although the prevalence rate (22.08%) was slightly lower than the 28.1% reported by Bello et al., (1983). The findings, however confirmed the high prevalence rate of gonorrhoea among the inhabitants of Zaria. Sogbetun et al., (1977) reported 19.5% prevalence rate of postpubertal gonorrhoea in Ibadan while Rotimi and Somorin (1980) reported 19% prevalence rate of gonorrhoea cases among 276 patients referred to an STD clinic in Lagos. The 21.33% prevalence rate for Kaduna was higher than those reported from both Ibadan and Lagos from the studies cited above. Pambeguwa had 18.35% rate of prevalence of gonorrhoea. Pambeguwa is a junction town on the Jos-Kaduna highway where long distance vehicle drivers stop-over in the course of their journey. It is generally believed that these long distance vehicle drivers spend their nights with prostitutes. Hence, the

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high prevalence rate of *Neisseria gonorrhoeae* infection in such communities is often attributed to their activities. The 11.7% infection rate of gonococci for Giwa is slightly higher than that found in Soba (10.76%). Being a local government headquarter and a commercial town close to both Zaria and Funtua, the socio-economic life-style of Giwa is likely to be influenced by what obtains in Zaria and Funtua towns. It is a known fact that greater freedom of movement and socialization in urban centres contribute to the spread of sexually transmitted diseases (STDs).

The prevalence rate of gonococcal infection for Kafanchan was 16.66% Kafanchan is the third largest town in Kaduna State. The prevalence rate of 12.66% of gonococcal infection recorded for Kachia is among the moderately high cases of gonorrhoea among the communities studied in Kaduna State. Kachia has been a local government headquarter since 1976. It has thus been a centre of social activities over a long period of time. Among the communities investigated for gonococcal infection in Kaduna State, Soba has the lowest (10.76%) prevalence rate.

Statistical analysis of the prevalence of *Neisseria gonorrhoeae* infection on the basis of sex showed a significant difference in prevalence rate between male and female patients. Such significant difference in prevalence of *Neisseria gonorrhoeae* infection between the sexes was shown in all the locations studied.

During the course of collection of samples, some of the patients confessed that they preferred private hospitals to government hospitals because of the

prompt attention they received and because they felt that private hospitals were better at maintaining secrecy than the government hospitals. It is known that government hospitals have better trained personnel and cheaper medical services and yet people with sexually transmitted diseases (STDs) prefer private clinics. This behaviour is in agreement with World Health organization (1993) report which found that many people with STDs seek health care from private providers where they may pay up to one-third of their monthly earnings for drugs (WHO, 1993).

The prevalence of *Neisseria gonorrhoeae* infection among different age-groups was found to be highest among 15-20 years old age-group in which 50 (31.05%) out of 161 patients screened were found positive. This was followed by the 21-25 years old age-group where 124 (22.67%) out of 547 patients screened were found positive for gonococcal infection. Out of 586 patients in the 26-30 year-old age-group only 47 (8.02%) were found positive for gonorrhoea. The details of this prevalence on the basis of age-groups is shown in fig. 2. The highest prevalence found among the 15-20 year-old age-group is in agreement with reports from the United States of America where it was found that people between the ages of 15 to 19 years have the highest prevalence of gonorrhoea (CDC, 1992).

The antimicrobial susceptibility patterns of the isolates showed that most were resistant to those antibiotics that are commonly used in the treatment of gonorrhoea. There were fewer isolates that were resistant to erythromycin and gentamicin than to penicillin, ampicillin and tetracycline. All the *Neisseria*

gonorrhoeae isolates were susceptible to ceftriazone, cefuroxime and ofloxacin. The resistance exhibited by these isolates to penicillin, ampicillin and tetracycline could be attributed to indiscriminate use of antibiotics and self-medication that results in sub-therapeutic dosage, thereby stimulating the development of resistant mutants (Odugbemi, 1987). Many of the isolates exhibited multiple-drug resistance patterns. One hundred and six (106) *Neisseria gonorrhoeae* isolates were found to be resistant to both penicillin G and ampicillin, 67 isolates were simultaneously resistant to penicillin G, ampicillin and tetracycline. This is in conformity with the high prevalence rate (84%) of penicillinase producing *Neisseria gonorrhoeae* found in this study (Table 7). Similar results were reported by Love and Finland (1955) who isolated *Neisseria gonorrhoeae* strains that were resistant to penicillin, ampicillin, tetracycline and erythromycin. The high prevalence of multi-drug resistant strains of *Neisseria gonorrhoeae* has also been reported from South East Asia (Joesoef et al., 1994). According to Joesoef and colleagues, the reasons for the frequent emergence in South East Asia of *Neisseria gonorrhoeae* strains that are highly resistant to antimicrobial agents are not well known. Antimicrobial resistance mechanism are of two major types, (I) the chromosomal resistance which results from serial changes in the structure of penicillin-binding proteins and/or outer membranae permeability and (2) the production of beta-lactamase by plasmid bearing strains of *Neisseria gonorrhoeae* that was first reported in 1976 (Fekete, 1993).

Penicillin had MIC of 32.0μg/ml against five (5) *Neisseria gonorrhoeae* while

tetracycline had MIC of 32.0μg/ml against three (3) *Neisseria gonorrhoeae* isolates. The high MICs of penicillin, ampicillin and tetracycline confirmed the high level of resistance by the isolates to these antibiotics. All the 225 penicillin resistant isolates were sensitive to Ceftriazone, Cefuroxime and Ofloxacin at MIC values of 0.125 μg/ml (Table 8). High MIC levels of antibiotics against multiple-drug resistant *Neisseria gonorrhoeae* strains have been similarly reported from South East Asia (Joesoef et al., 1994). Penicillinase production was determined by Iodometric method and was found positive in 189 (84%) of the 225 penicillin ressistant strains. The remaining 36 (16%) of the 225 penicillin resistant isolates were beta-lactamase negative (table 7). The prevalence of penicillinase producing *Neisseria gonorrhoeae* (PPNG) found in this study is in agreement with the earlier report that PPNG constitutes 70-80% of *Neisseria gonorrhoeae* isolates in Nigeria (Odugbemi, 1987). It has been firmly established that certain drug-resistance genes reside upon sequences of DNA which have the ability of translocating themselves from plasmid to plasmid (Saunders, 1975). The production of penicillinase by *Neisseria gonorrhoeae* is plasmid-induced (Ashford, et al., 1976). The initial reports on the epidemiology of PPNG strains showed that there was a spread of these strains from Asia and Africa to other parts of the world (Fekete, 1993). Their penicillin-resistance could be chromosomal, that is due to genetic mutation of the organisms or altered outer membrane permeability (Roberts, 1977). The high prevalence rate of PPNG among patients screened, poses a serious public health problem because the enzyme (beta-lactamase) has been found to be identitical to the one in *Haemophilus influenzae* which is reported to

have acquired the resistant-plasmid from other enteric gram-negative bacilli (Fekete, 1993). The susceptibility of both PPNG and NPPNG to fluoroquinolones is in agreement with report from United Arab Emirate that PPNG isolates from there are susceptible to these newer drugs (Al-Hattawi, and Ison, 1996). However, there have been reports of gonococcal infections not responding to treatment with fluoroquinolones and cephalosporins in North America (Knapp et al., 1994), United Kingdom (Joephcutt and Turner 1990) and in Asia (Putnam et al., 1992).

This study also revealed high prevalence of mixed gonococcal and non-gonococcal infections among the population studied. The most common of such mixed infections were *Neisseria gonorrhoeae* and *S. aureus* accounting for 1.7% and *Neisseria gonorrhoeae* and *E. coli* (0.8%) out of the total 1,715 sampled.. The ability of some organisms such as *S. aureus* to produce beta-lactamase does provide protection for other beta-lactam antibiotic susceptible organisms in the mixed infection (Brook and Apichaun, 1989). The prevalence of mixed non-gonococcal infection were found to be high in cases of candidiasis and *S. aureus* (4.1%, *E. coli* and *S. aureus* (3.5%) and candidiasis and trichomoniasis (3.2%) (Table 10). It was found that the most common single etiologic agents of non-gonococcal urethritis or vaginitis were *Staphylococcus aureus* (23.5%), followed by *C. albicans* (15.7%), *E. coli* (11.9%), *Trichomonas vaginalis* 6.06%) and *Klebsiella sp* (2.8%). The prevalence of Staphylococcal infection was found to be higher than that of *gonococci* in this study. The prevalence of candidiasis was found to be almost as high as that of gonorrhoea among the studied population.

In general, gonorrhoea and other STDs are very prevalent in Kaduna State. The highest prevalence of 31.05% was found among the 15-20 year-old age-group (table 5). This should be a matter of serious concern as STDs are known to have serious consequences on health and reproduction and could lead to death. The high prevalence rate of antimicrobial resistant *Neisseria gonorrhoeae* isolates encountered in these areas may result in high cost of treatment for patients who due to treatment failure with commonly used drugs would spend more money on the newer drugs to get cured. There is also the problem of these newer drugs not being available in the rural areas of the state.

Recommendation

1. In view of the high prevalence rate (16.03%) of gonococcal infection amongst the population studied, government should use its information media to educate the public on the dangers of gonorrhoea and other sexually transmitted diseases.

2. From this study females within the age of 15-20 years old had highest infection rate (31.05%) of *Neisseria gonorrhoeae*. *Neisseria gonorrhoeae* infection is known to have serious consequences including ophthalmia neonatorum, abortion and infertility. It is therefore recommended that all expectant mothers should be screened for STDs at their various antenatal clinic in an effort to avert sequelae of STDs.

3. Sexually transmitted diseases clinics should be established in various parts of the state for effective diagnosis and treatment of these diseases for which most general hospitals are ill-equipped to handle.

4. In view of the high level multi-drug resistance shown by *Neisseria gonorrhoeae* isolates in this study, government should enforce the regulations (laws) governing the handling and sale of drugs (antibiotics) to avoid possible indiscriminate use of drugs or self-medication that could lead to subtherapeutic dosage thereby enhancing the development of resistant mutants.

5. Government should sponsor research on the epidemiology and drug resistance of *Neisseria gonorrhoeae* and other sexually transmitted diseases on regular basis. This will provide adequate information on these diseases for effective control.

6. Gonorrhoea, is one of the sexually transmitted diseases that are linked with human immunodeficiency virus - AIDS. There is need for continuous surevillance and antimicrobial susceptibility patterns of gonococci as a routine activity by every laboratory service.

7. Priority should be given to research towards the development of a low-cost transport medium for gonococcal specimens that will ensure viability for at least a week in hot climates and a long shelf-life without refrigeration.

Presently the transport media used allows for 48 hours holding time which requires speedy transportation to the laboratory.

8. Highly sensitive diagnostic equipment should be provided for effective diagnosis and treatment of sexually transmitted diseases at every clinic. Such provision coupled with well trained personnel will surely curb the spread of both STDs and the HIV AIDS that are becoming epidemic in the society.

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APPENDIX 1: PREPARATION OF MEDIA AND BIOCHEMICALS

MODIFIED THAYER-MARTIN MEDIUM

Eigtheen (18g) grams of oxoid G.C. Agar Base was dissolved in 240ml of distilled water, heated to boiling to dissolve the agar, and autoclaved at 121°C for 15 minutes. A 2% solution of soluble haemoglobin powder obtained from oxoid U.K. was prepared by dissolving 5 grams of the powder in 250ml of warm distilled water and added to the molten G.C. Agar. The mixture was autoclaved at 121°C for 15 minutes. A vial of oxoid G.C. selective supplement was properly dissolved in 15ml of warm distilled water at 45oC and aseptically added to 485ml Thayer-Martin medium at 50°C. The selective medium was aseptically poured into sterile petri dishes, allowed to gel, labelled and stored at 4°C for use when needed.

STUAT'S TRANSPORT MEDIUM

Fourteen (14g) grams of dehydrated Difco stuart's transport medium (Difco, Detroit, Mechigan, U.S.A.) was dissolved in a litre of cold distilled water and 1% oxoid bacteriological charcoal was added and heated to dissolve completely. The preparation was dispensed into bijou bottles and autoclaved at 121°C for 15 minutes. The addition of bacteriological charcoal was to neutralize the effect of fatty acids of the cotton wool on the *Neisseria gonorrhoeae* while in the transport medium.

CHOCOLATE AGAR

Forty (40g) grams of oxoid dehydrated blood Agar Base was dissolved in a litre of distilled water and autoclaved at 121°C for 15 minutes. Seven (7%) percent of sterial sheep blood was added and heated to make chocolate. The

medium was dispensed into sterile plates, allowed to solidify and stored at 4°C for use.

STAPHYLOCOCCUS MEDIUM 110 (DIFCO)

One hundred and forty nine grams (149g) was dissolved in a litre of distilled water, heated to dissolve completely and autoclaved at 121°C for 10 minutes. The slective medium was aseptically dispensed into sterile petri dishes, allowed to solidify and stored for use at 4°C.

SABOURAUD DEXTROSE AGAR (DIFCO)

Sixty five (65g) grams of Difco dehydrated sabouraud Dextrose Agar was dissolved in 1 litre cold freshly distilled water, heated to dissolve, autoclaved at 121°C for 15 minutes, poured into sterial petri dishes allowed to solidify and kept at 4oC until used.

SIMMONS CITRATE AGAR (OXOID)

Twenty three (23g) grams of the dehydrated medium was suspended in a litre of distilled water, boiled to dissolved fully and autoclaved at 121°C for 15 minutes. Slants were made in bijou bottles and stored at 4°C for use.

MacConkey AGAR (BIOLIFE)

Fifty (50g) grams of the dehydrated medium was dissolved in 1 litre of distilled water and autoclaved at 121°C for 15 minutes. The sterile medium was dispensed into sterile petri dishes, labelled and stored at 4°C for use when needed.

LODINE REAGENT

Iodine (2.03g) and potassium iodine (53.2g) were dissolved in 100ml of distilled water and stored in brown bottles for use.

STARCH INDICATOR

One (1.0g) gram of soluble starch was dissolved in 100ml of distilled water. It was always prepared freshly for use.

PHOSPHATE BUFFER pH 5.8

Potassium dihydrogen phosphate (6.25g) and dipotassium hydrogen phosphate (0.696g) were dissolved in one litre of distilled water and stored for use.

PENICILLIN G. SOLUTION

A solution of 10,000 units/ml of penicillin G was made in phosphate buffer and dispensed into small screw capped tubes and stored at 4°C for use.

OXIDASE REAGENT

Six grams (6g) of Tetramethyl phenylene diamine (TMPD) was dissolved in 100ml of dimethyl sulfoxide (IMSC) in a brown bottle and stored away from direct light at room temperature.

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APPENDIX 2: TESTS FOR THE IDENTIFICATION OF AGENTS OF NON-GONOCOCCAL URETHRITIS

1. Indole test

To 4ml of 48h tryptophane broth culture of each isolated 0.5ml of Kovacs' reagent was added and gently shaken. A red colour in the alcohol layer indicated a positive reaction.

Methyl-red test

To 2ml of 48h old MR-VP broth culture of each isolate, 0.1ml of methyl red reagent was added, mixed and read immediately. A bright red colour indicated positive while yellow colour meant negative.

Voges-proskauer test

To 4ml of 48h old MR-VP broth culture of each isolate 1ml of 40% potassium hdroxide was added. Followed by addition of 3ml of 5% alpha-naphthol solution in absolute ethanol. The mixture was shaken and allowed to stand for 5 minutes. Positive reaction was indicated by the development of pink colour within 2-5 minutes.

Citrate utilization test

Each isolate was inoculated unto Simmons' citrate agar slant and incubated at 37°C for 48 hours. Blue colour indicated positive and the original green colour meant negative.

Hydrogen sulphide production

Each of the non-lactose fermenting isolates was inoculated unto Triple Sugar Iron (TSI) agar slant using straight sterile inoculating wire and incubated at 37°C for 24 hours. The development of blackening indicated production of H_2S.

Urease production test

Pure culture of each isolate was inoculated unto urea agar slant entire slope and incubated at 37°C. The incubated slants were examined four-hourly for 4 days before discarded as negative. The development of pink colour indicated positive while no colour change meant negative.

APPENDIX 3: LIST OF POSITIVE CASES OF *N. gonorrhoeae* infections and other agents of urethritis

GIWA: RAINY SEASON

SAMPLE NO.	SPECIMEN	SEX	AGE	ISOLATE(S)
4	US	M	20	*N. gonorrhoeae*
15	ECS	F	22	*N. gonorrhoeae*
24	US	M	21	*N. gonorrhoeae*
31	US	M	23	*N. gonorrhoeae*
38	US	M	21	*N. gonorrhoeae*
46	ECS	F	26	*N. gonorrhoeae*
53	US	M	27	*N. gonorrhoeae*
67	US	M	23	*N. gonorrhoeae*
81	US	M	21	*N. gonorrhoeae*
88	US	M	19	*N. gonorrhoeae*
94	ECS	F	23	*N. gonorrhoeae*
99	US	M	21	*N. gonorrhoeae*
113	US	M	21	*N. gonorrhoeae*
112	US	M	28	*N. gonorrhoeae*
130	US	M	28	*N. gonorrhoeae*
136	US	M	27	*N. gonorrhoeae*
143	ECS	F	24	*N. gonorrhoeae*
151	ECS	F	24	*N. gonorrhoeae*
158	US	M	27	*N. gonorrhoeae*
164	ECS	F	20	*N. gonorrhoeae*
165	US	M	22	*N. gonorrhoeae*

ECS 6

US 15

21

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GIWA: DRY SEASON

SAMPLE NO.	SPECIMEN	SEX	AGE	ISOLATE(S)
8.16224e+35	US	M	20	*N. gonorrhoeae*
	US	M	20	*N. gonorrhoeae*
	ECS	F	20	*N. gonorrhoeae*
	US	M	21	*N. gonorrhoeae*
	US	M	21	*N. gonorrhoeae*
	ECS	F	23	*N. gonorrhoeae*
	US	M	20	*N. gonorrhoeae*
	US	M	24	*N. gonorrhoeae*
	ECS	F	22	*N. gonorrhoeae*
	US	M	23	*N. gonorrhoeae*
	ECS	F	21	*N. gonorrhoeae*
	US	M	21	*N. gonorrhoeae*
	US	M	25	*N. gonorrhoeae*
	ECS	F	22	*N. gonorrhoeae*
	US	M	19	*N. gonorrhoeae*
	US	M	28	*N. gonorrhoeae*

ECS = 5

US = 11

16

Total = 21 + 16 = 37 for GIWA

SOBA: POSITIVE CASES, N.G.: DRY SEASON

SAMPLE NO.	SPECIMEN	SEX	AGE	ISOLATE(S)
6.18254e+32	US	M	25	*N. gonorrhoeae*
	ECS	F	20	*N. gonorrhoeae*
	US	M	22	*N. gonorrhoeae*
	US	M	20	*N. gonorrhoeae*
	US	M	25	*N. gonorrhoeae*
	ECS	F	23	*N. gonorrhoeae*
	US	M	26	*N. gonorrhoeae*
	ECS	F	23	*N. gonorrhoeae*
	US	M	19	*N. gonorrhoeae*
	US	M	26	*N. gonorrhoeae*
	US	M	24	*N. gonorrhoeae*
	US	M	20	*N. gonorrhoeae*
	US	M	21	*N. gonorrhoeae*
	US	F	21	*N. gonorrhoeae*
	US	M	25	*N. gonorrhoeae*

F = 3
M 12
15

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SOBA: RAINING SEASON

SAMPLE NO.	SPECIMEN	SEX	AGE	ISOLATE(S)
1.02025e+44	US	M	19	*N. gonorrhoeae*
	ECS	F	22	*N. gonorrhoeae*
	US	M	24	*N. gonorrhoeae*
	US	M	29	*N. gonorrhoeae*
	ECS	F	19	*N. gonorrhoeae*
	US	M	20	*N. gonorrhoeae*
	US	M	20	*N. gonorrhoeae*
	ECS	F	77	*N. gonorrhoeae*
	US	M	20	*N. gonorrhoeae*
	US	M	21	*N. gonorrhoeae*
	US	M	24	*N. gonorrhoeae*
	US	M	25	*N. gonorrhoeae*
	ECS	F	18	*N. gonorrhoeae*
	US	M	21	*N. gonorrhoeae*
	US	M	20	*N. gonorrhoeae*
	US	M	25	*N. gonorrhoeae* *Staph. aureus*
	ECS	F	18	*N. gonorrhoeae*
	US	M	20	*N. gonorrhoeae*
163	ECS	F	24	*N. gonorrhoeae*

M = 13

F = 6

19

Total 15 + 19 = 34

PAMBEGWA: (DRY SEASON)

SAMPLE NO.	SPECIMEN	SEX	AGE	ISOLATE(S)
2	ECS	**F**	24	*N. gonorrhoeae C. albicans Klebsiella sp.*
4	US	**M**	32	*N. gonorrhoeae*
10	ECS	**F**	27	*N. gonorrhoeae, C. albicans*
15	US	**M**	31	*N. gonorrhoeae*
19	US	**M**	40	*N. gonorrhoeae, S. aurous*
24	ECS	**F**	22	*N. gonorrhoeae*
29	US	**M**	31	*N. gonorrhoeae*
40	ECS	**F**	16	*N. gonorrhoeae*
42	ECS	F	21	*N. gonorrhoeae*
45	ECS	**F**	23	*N. gonorrhoeae, E. coli*
47	US	M	25	*N. gonorrhoeae, S. aureus*
52	US	**M**	26	*N. gonorrhoeae,S. aureus*
59	US	**M**	20	*N. gonorrhoeae*
64	US	**M**	40	*N. gonorrhoeae, S. aureus*
67	US	**M**	30	*N. gonorrhoeae*
71	ECS	F	22	*N. gonorrhoeae, C. albicans*
73	US	**M**	28	*N. gonorrhoeae, S. aureus*
76	ECS	**M**	20	*N. gonorrhoeae*
79	ECS	**F**	19	*N. gonorrhoeae*
83	US	**M**	30	*N. gonorrhoeae, E. coli*
85	ECS	**F**	20	*N. gonorrhoeae, T. vaginalis*
91	US	M	40	*N. gonorrhoeae, S. aureus*
92	ECS	F	22	*N. gonorrhoeae, C. albicans, Klebsiella sp.*
100	ECS	F	A	*N. gonorrhoeae, N. gonorrhoeae*
103	US	M	25	*N. gonorrhoeae*
112	US	M	31	*N. gonorrhoeae*
119	US	**M**	27	*N. gonorrhoeae*
129	US	**M**	21	*N. gonorrhoeae*
142	ECS	**F**	19	*N. gonorrhoeae*
144	US	**M**	28	*N. gonorrhoeae*
147	US	**M**	35	*N. gonorrhoeae S. aureus*

SAMPLE NO.	SPECIMEN	SEX	AGE	ISOLATE(S)
153	US	M	35	*N. gonorrhoeae*
158	ECS	F	19	*N. gonorrhoeae*
161	US	M	35	*N. gonorrhoeae, S. aureus*
165	US	M	24	*N. gonorrhoeae*
4	US	M	19	*N. gonorrhoeae*
6	US	M	A	*N. gonorrhoeae*
16	US	M	24	*N. gonorrhoeae*
23	US	M	A	*N. gonorrhoeae*
30	US	M	A	*N. gonorrhoeae*
37	ECS	F	20	*N. gonorrhoeae*
41	US	M	A	*N. gonorrhoeae*
47	US	M	20	*N. gonorrhoeae*
51	US	M	A	*N. gonorrhoeae, S. aureus*
57	US	M	35	*N. gonorrhoeae, S. aureus*
67	US	M	28	*N. gonorrhoeae*
68	ECS	F	23	*N. gonorrhoeae Staph. aureus*
74	US	M	19	*N. gonorrhoeae*
78	US	M	A	*N. gonorrhoeae, S. aureus*
88	US	M	A	*N. gonorrhoeae*
94	US	M	23	*N. gonorrhoeae*
103	US	M	22	*N. gonorrhoeae*
111	ECS	F	21	*N. gonorrhoeae*
122	US	M	28	*N. gonorrhoeae*
129	US	M	24	*N. gonorrhoeae, S. aureus*
140	ECS	F	26	*N. gonorrhoeae*
146	US	M	A	*N. gonorrhoeae, E. coli*
149	ECS	F	A	*N. gonorrhoeae*

ECS = 6
US = 22
27 Total 27 + 31 = 58

POSITIVE *N. gonorrhoeae* Cases: Zaria Raining Season

SAMPLE NO	SPECIMEN	SEX	AGE	ISOLATES
54	ECS	F	AD	*N. gonorrhoeae, Staph aureus*
2	ECS	F	26	*N. gonorrhoeae*
7	US	M	24	*N. gonorrhoeae*
8	ECS	F	29	*N. gonorrhoeae*
9	US	M	34	*N. gonorrhoeae*
10	US	M	A	*N. gonorrhoeae,*
13	US	M	38	*N. gonorrhoeae, S. aureus*
14	US	M	36	*N. gonorrhoeae,*
15	US	M	20	*N. gonorrhoeae*
18	ECS	F	24	*N. gonorrhoeae*
33	US	M	24	*N. gonorrhoeae*
46	US	M	36	*N. gonorrhoeae, Staph aureus*
51	US	M	25	*N. gonorrhoeae, E. coli*
52	US	M	A	*N. gonorrhoeae*
48	ECS	F	A	*N. gonorrhoeae, C. albicans*
58	US	M	28	*N. gonorrhoeae*
65	US	M	24	*N. gonorrhoeae, E. coli*
69	ECS	F	A	*N. gonorrhoeae*
78	ECS	F	A	*N. gonorrhoeae, E. coli*
84	US	M	22	*N. gonorrhoeae*
89	ECS	F	AD	*N. gonorrhoeae*
90	US	M	19	*N. gonorrhoeae*
92	US	M	30	*N. gonorrhoeae*
96	US	M	35	*N. gonorrhoeae, S. aureus*
99	ECS	F	24	*N. gonorrhoeae, Staph. aureus*
107	US	M	22	*N. gonorrhoeae, E. coli*
110	US	M	24	*N. gonorrhoeae*
113	ECS	F	21	*N. gonorrhoeae*
117	US	M	27	*N. gonorrhoeae*
128	US	M	28	*N. gonorrhoeae*
139	US	M	21	*N. gonorrhoeae*
146	ECS	F	19	*N. gonorrhoeae S. aureus*
153	US	M	24	*N. gonorrhoeae*
156	US	M	22	*N. gonorrhoeae S. aureus*
156	ECS	F	26	*N. gonorrhoeae*
160	US	M	21	*N. gonorrhoeae*

ECS = 12
US = 24
36

ZARIA: DRY SEASON

SAMPLE NO	SPECIMEN	SEX	AGE	ISOLATES
3	US	M	A	*N. gonorrhoeae,Staph aureus*
5	US	M	A	*N. gonorrhoeae*
6	US	M	A	*N. gonorrhoeae*
9	US	M	30	*N. gonorrhoeae*
12	US	M	24	*N. gonorrhoeae,*
13	US	M	28	*N. gonorrhoeae,*
16	ECS	F	A	*N. gonorrhoeae, Staph aureus*
19	ECS	F	22	*N. gonorrhoeae*
20	ECS	F	20	*N. gonorrhoeae*
23	US	M	28	*N. gonorrhoeae*
24	ECS	F	A	*N. gonorrhoeae*
26	US	M	A	*N. gonorrhoeae*
28	US	M	26	*N. gonorrhoeae*
32	US	M	A	*N. gonorrhoeae, Staph aureus*
36	US	M	36	*N. gonorrhoeae*
40	ECS	F	40	*N. gonorrhoeae*
41	US	M	22	*N. gonorrhoeae*
45	US	M	18	*N. gonorrhoeae*
47	US	M	A	*N. gonorrhoeae*
50	ECS	F	25	*N. gonorrhoeae*
52	US	M	40	*N. gonorrhoeae*
61	US	M	21	*N. gonorrhoeae*
66	US	M	A	*N. gonorrhoeae*
70	ECS	F	A	*N. gonorrhoeae, S. aureus*
72	US	M	25	*N. gonorrhoeae, E. coli*
75	US	M	40	*N. gonorrhoeae*
76	US	M	30	*N. gonorrhoeae*
78	US	M	35	*N. gonorrhoeae, S. aureus*
81	ECS	F	30	*N. gonorrhoeae, C. albicans*
83	US	M	40	*N. gonorrhoeae, E. coli*
87	US	M	A	*N. gonorrhoeae*
91	US	M	26	*N. gonorrhoeae*
93	US	M	25	*N. gonorrhoeae*
127				

ECS = 9
US = 25
34

Total 36 + 34 = 70

KAFANCHAN:

SAMPLE NO.	SPECIMEN	SEX	AGE	ISOLATES
2	ECS	**F**	**28**	*N. gonorrhoeae, Staph. aureus*
5	**US**	**M**	**31**	*N. gonorrhoeae*
9	ECS	**F**	**24**	*N. gonorrhoeae, S. aureus*
11	US	**M**	**22**	*N. gonorrhoeae, Staph. aureus*
14	**US**	**M**	**26**	*N. gonorrhoeae,*
22	**US**	**M**	**28**	*N. gonorrhoeae,*
28	**US**	**M**	**24**	*N. gonorrhoeae,*
30	ECS	**F**	**23**	*N. gonorrhoeae*
36	**US**	**M**	**25**	*N. gonorrhoeae*
42	**US**	**M**	**24**	*N. gonorrhoeae*
40	**ECS**	**F**	**21**	*N. gonorrhoeae, C. albicans*
53	US	**M**	**28**	*N. gonorrhoeae*
62	ECS	**F**	**24**	*N. gonorrhoeae*
70	US	**M**	**28**	*N. gonorrhoeae*
75	US	**M**	**25**	*N. gonorrhoeae*
79	US	**M**	**30**	*N. gonorrhoeae*
88	US	**M**	**26**	*N. gonorrhoeae, E. coli*
98	US	**M**	**25**	*N. gonorrhoeae, S. aureus*
106	US	**M**	**28**	*N. gonorrhoeae*
116	US	**M**	**AD**	*N. gonorrhoeae*
123	US	**M**	**24**	*N. gonorrhoeae*
128	US	**M**	**24**	*N. gonorrhoeae, Coliform Sp.*
132	US	**M**	**30**	*N. gonorrhoeae*
142	US	**M**	**24**	*N. gonorrhoeae*
147	ECS	**F**	**24**	*N. gonorrhoeae*

ECS = 6

us = 19

25

KADUNA:

SAMPLE NO.	SPECIMEN	SEX	AGE	ISOLATES
4	US	M	18	*N. gonorrhoeae*
7	US	M	24	*N. gonorrhoeae*
11	ECS	F	21	*N. gonorrhoeae*
12	US	M	22	*N. gonorrhoeae*
17	ECS	F	19	*N. gonorrhoeae*
22	US	M	24	*N. gonorrhoeae*
25	US	M	22	*N. gonorrhoeae*
30	ECS	F	21	*N. gonorrhoeae, s. aureus*
31	US	M	23	*N. gonorrhoeae*
422	ECS	F	22	*N. gonorrhoeae*
46	US	M	26	*N. gonorrhoeae*
50	US	M	19	*N. gonorrhoeae*
54	ECS	F	23	*N. gonorrhoeae*
59	US	M	21	*N. gonorrhoeae*
63	US	M	23	*N. gonorrhoeae, S. aureus*
67	ECS	F	20	*N. gonorrhoeae*
74	US	M	24	*N. gonorrhoeae*
78	US	M	19	*N. gonorrhoeae, S. aureus*
83	ECS	F	23	*N. gonorrhoeae*
87	US	M	21	*N. gonorrhoeae*
96	US	M	24	*N. gonorrhoeae*
103	US	M	24	*N. gonorrhoeae*
106	ECS	F	18	*N. gonorrhoeae, S. aureus*
114	US	M	30	*N. gonorrhoeae, E. coli*
119	ECS	F	22	*N. gonorrhoeae*
121	US	M	24	*N. gonorrhoeae*
124	US	M	20	*N. gonorrhoeae*
133	US	M	34	*N. gonorrhoeae*
141	US	M	21	*N. gonorrhoeae*
145	ECS	F	17	*N. gonorrhoeae*
35	US	M	25	*N. gonorrhoeae*
39	US	M	24	*N. gonorrhoeae*

Total = 32 (ECS 10 + US 22).

KACHIA:

SAMPLE NO.	SPECIMEN	SEX	AGE	ISOLATES
22	**US**	M	35	*N. gonorrhoeae*
7	ECS	F	23	*N. gonorrhoeae*
11	**US**	M	20	*N. gonorrhoeae*
17	**US**	M	21	*N. gonorrhoeae, E. coli*
30	**US**	M	23	*N. gonorrhoeae,*
36	**US**	M	A	*N. gonorrhoeae*
48	**US**	M	A	*N. gonorrhoeae, Staph aureus*
53	ECS	F	26	*N. gonorrhoeae*
60	US	M	31	*N. gonorrhoeae*
139	ECS	F	19	*N. gonorrhoeae*
69	ECS	F	22	*N. gonorrhoeae*
77	US	M	25	*N. gonorrhoeae*
86	US	M	21	*N. gonorrhoeae*
93	US	M	24	*N. gonorrhoeae*
107	ECS	F	25	*N. gonorrhoeae*
110	US	M	25	*N. gonorrhoeae*
120	US	M	23	*N. gonorrhoeae*
130	US	**M**	25	*N. gonorrhoeae*
143	ECS	F	24	*N. gonorrhoeae*

Total = ECS 7 + US 12 = 19

APPENDIX 4

CHI-SQUARE ANALYSIS OF STATISTICAL SIGNIFICANCE OF PREVALENCE

LOCATION	POSITIVE OBSERVED	CASS EXP.	NEG. OBS. VALUE	CASES EXP. VALUE	TOTAL NUMBER SAMPLED
ZARIA	70	50.83	247	266.17	317
GIWA	37	50.47	279	265.33	316
SOBA	34	50.67	282	265.33	316
PAMBEGUWA	58	50.67	258	265.33	316
KACHIA	19	24.05	131	125.95	150
KAFANCHAN	25	24.05	125	125.95	150
KADUNA	32	24.05	118	125.93	150
TOTAL	275		1440		1715

Formula

$$\frac{(\text{Observed Value-Expected Value})^2}{\text{Expected}}$$

$$X^2 = \sum \frac{(O - E)^2}{ej}$$

= 7.23 + 3.69 + 5.48 + 1.06 + 1.06 + 0.04 + 1.63 + 1.38 + 0.70 + 1.05 + 0.20 + 0.20 + 0.007 + 0.50 = 25.227

X^2_c = 25.227.

X^2 Computed X^2 table value for any α-level of 5% (level of significance) hence the prevalence of *Neisseria gonorrhoeae* is significant and depends on the location.

APPENDIX 5

STATISTICAL ANALYSIS OF PREVALENCE OF *N. GONORRHOEAE* INFECTION PER SEX/LOCATION

LOCATION	OBSERVED	EXPECTED	OBSERVED	EXPECTED	TOTAL
ZARIA	22	21.63	48	48.36	70
GIWA	11	11.44	26	25.56	37
SOBA	10	10.51	24	23.49	24
PAMBEGUWA	18	17.93	40	40.07	58
KADUNA	10	9.89	22	22.11	32
KACHIA	7	5.87	12	13.13	19
KAFANCHAN	7	7.73	18	17.27	25
TOTAL					

X^2 = 0.006 + 0.017 + 0.025 + 0.003 + 0.0012 + 0.217 + 0.069 + 0.0027 + 0.0076 + 0.011 + 0.001 + 0.005 + 0.097 + 0.031 = 0.4854

X^2 = 0.4854

This is not significant hence sex prevalence is independent of location

APPENDIX 6

STATISTICAL SIGNIFICANCE OF PREVALENCE OF *NEISSERIA GONORRHOEAE* DISTRIBUTED AMONG AGE-GROUPS

AGE-GROUP	POSTIVE OBSERVED	CASES EXP.	NEGATIVE OBSERVED	CASES EXP.	TOTAL
15-20	50	25.86	111	135.14	161
21-25	124	87.87	423	459.13	547
26-30	47	94.13	539	491.13	586
31-36	20	34.21	193	178.79	213
37-40	12	7.39	34	38.61	46
Adult	22	25.54	137	133.46	159
TOTAL					

X^2 = 22.53 + 14.86 + 23.60 + 5.90 + 2.88 + 6.49 + 4.31 + 2.80 + 4.52 + 1.13 + 0.55 + 0.09 = 83.7.

X^2 = 83.7.

X^2_c X^2 table value for any level hence it is significant and the prevalence therefore depends on age-group.

APPENDIX 7

ANALYSIS OF PREVALENCE OF PPNG AND NON-PPNG

	PPNG		NON -PPNG		
LOCATION	OBS.	EXP.	OBS.	EXP.	TOTAL
ZARIA	47	52.92	16	10.08	63
GIWA	24	21.84	2	4.16	26
SOBA	24	20.16	0	3.84	24
PAMBEGUWA	37	39.48	10	7.52	47
KADUNA	24	23.52	4	4.48	28
KAFANCHAN	18	17.64	3	3.36	21
KACHIA	15	13.44	1	2.56	16
TOTAL					

X^2 = 0.66 + 0.21 + 0.73+ 0.16 + 0.009 + 0.007 + 0.18 + 3.48 + 1.12 + 3.84 + 0.82 + 0.05 + 0.039 + 0.95 = 12.2558.

X^2 = 12.2558.

X^2_c = $>X^2$ table value at α = 0.10, it is thus significant hence PPNG prevalence is statistically significant.

APPENDIX 8

CONTIGENCY TABLE

LOCATION 1 - ZARIA

SEASON	PREVALENCE	NON-PREVALENCE	TOTAL
R.S.	36	131	167
D.S.	34	161	150
TOTAL	70	247	317

$$X^2_c = \frac{317\,(436)\,(116) - (131)\,(34)^2}{70\,(247)\,(167)\,(150)}$$

$$= \frac{317\,(4176 - 4454)^2}{4.331145 \times 10^8}$$

$$= \frac{317 \times 77284}{4.331145 \times 10^8}$$

$$= \frac{24499028}{4.331148 \times 10^6}$$

$$= 0.0566$$

At $\propto = 0.05$, x^2 computed < x^2 table value hence it is concluded that the prevalence in Zaria is independent of the season.

APPENDIX 9

LOCATION 2 - GIWA

SEASON	PREVALENCE	NON-PREVALENCE	TOTAL
R.S.	20	146	166
D.S.	17	133	150
TOTAL	37	279	316

$$x^2 = \frac{316\ 20(133) - 17(146)^2}{37\ (279)\ (166)\ (150} = \frac{316\ (2660 - 2482^2}{2.570427 \times 10^8}$$

$$= \frac{316 \times 31684}{2.570427 \times 10^8}$$

$$= 0.0389512$$

Prevalence is independent of season in Giwa at $\propto = 0.05$.

APPENDIX 10

LOCATION 3 - SOBA

SEASON	PREVALENCE	NON-PREVALENCE	TOTAL
R.S.	18	148	166
D.S.	16	134	150
TOTAL	34	286	316

$$x^2 = \frac{316\,(18(134) - 16(148^2} {34 \times 282 \times 148 \times 134} = \frac{316 \times 1736}{1.9 \times 10^8}$$

$$= \frac{611776}{1.9 \times 10^8} = 0.003217$$

Prevalence is independent of season in Soba.

APPENDIX 11

LOCATION 4 - PAMBEGUWA

SEASON	PREVALENCE	NON-PREVALENCE	TOTAL
R.S.	27	139	166
D.S.	31	119	150
TOTAL	34	258	316

$$x^2 = \frac{316\,(27)\,(119) - 31(139)^2}{58 \times 258 \times 166 \times 150}$$

$$\frac{3.7958426 \times 10^8}{3.726036 \times 10^8} = 1.0187$$

Prevalence is independent of season in PAMBEGUWA.