

Prevalence of Neisseria Gonorrhoea among Under Graduate Female Students of University Of Port Harcourt Using Strand Displacement and Amplification (Sda) Technique.

Dr Wariso Kennedy T. And Dr (Mrs) Oboro Ibinabo L.

Department of Medical Microbiology and Parasitology, University of Port Harcourt.

Abstract: Background: *Neisseria gonorrhoeae* infection can occur asymptotically and due to the growth requirements in the laboratory, it can be easily missed even if it is symptomatic. Isolating the organism using conventional laboratory procedures has yielded varying degrees of sensitivity and specificity. Since there is paucity of information on the prevalence rate of the organism using nucleic acid amplification technique as the detection tool, this study became pertinent. Also, the associated risk factors to the infection on female undergraduate students were highlighted.

Methodology: Two hundred undergraduate, non-pregnant, asymptomatic female students who were above the age of 16 years but not above the age of 30 years were randomly selected and given questionnaires with self administrable vaginal swab sticks. The participants completed the questionnaires and provided the vaginal swab specimens which were analyzed using Strand Displacement and Amplification Technique.

Results: Out of the 200 sexually active participants, 10 tested positive, giving a prevalence of 5% for *Neisseria gonorrhoeae*. Some of the associated risk factors elicited were; having multiple sexual partners, irregular use of condom and past history of sexually transmitted infections

Conclusion: There is a need for a policy on routine screening for *Neisseria gonorrhoeae* since the treatment is available but the complications are danderous to reproductive health.

Key Words: Prevalence, *Neisseria gonorrhoeae*, Strand Displacement and Amplification (SDA) Technique.

I. Introduction

Gonorrhoea is a purulent infection of the mucous membranes caused by the sexual transmission of the bacterium, *Neisseria gonorrhoeae*.

This is about the second most common curable sexually transmitted infections (STI) worldwide¹. The Prevalence is only second to Chlamydia trachomatis infection. There has been a slow increase in incidence in several countries in the last 10 years especially the United Kingdom^{1,2}.

Various reasons have been deduced for this increase, one of which is the sudden propensity for unsafe sexual behavior among young people and a better diagnostic capacity unlike in the past³. It predominantly affects young people with a peak incidence in males aged 20-24 years and females aged 16-19 years. The infection is more in the developed world among homosexual and bisexual men and black ethnic minority populations.

It is a leading cause of pelvic inflammatory disease, which can result in sequelae such as tubal factor infertility, chronic pelvic pain, ectopic pregnancy and still birth⁴. Available data suggest that when it is undiagnosed or untreated, gonococcal infection can lead to a threefold or fivefold increase in risk for human immunodeficiency virus transmission or acquisition^{5,6,7}.

Reporting in the Medscape of February, 2013, the British Health Officials stated that there has been a 25% increase in resistant gonorrhoea between 2010 and 2011 (2,000 new cases). This was due to the emergence of the superbug resistant strains. In 2011, it was noted that a strain of the species found in Japan in 2008 was resistant to all of the recommended antibiotics. In United States of America (USA), the centre for disease control and prevention estimated that gonorrhoea cases totaled about 700,000 a year.

Neisseria gonorrhoeae is a Gram negative intracellular diplococcus that is not motile, not spore forming, with flattened adjacent sites. It is usually isolated by culturing on a special media such as Thayer martin, Modified New York City (MNYC) or even carbon dioxide enriched chocolate agar. This is due to its fastidious nature. It is sensitive to drying and so specimens collected should be inoculated immediately.

It is usually spread through sexual activity or perinatally. Once it attaches to the mucosal surfaces of urogenital sites (cervix, urethra and rectum), the oro- nasopharynx (throat) or the conjunctiva of the eyes; it may lead to an asymptomatic or symptomatic infection. The organism is able to evade the host defense system because of its ability to change its structure due to the antigenic heterogeneity^{7,8}.

The incubation period of *Neisseria gonorrhoeae* infection is less well defined in women but most infected persons become symptomatic within 10 days. However, average appearance of symptoms is between 2-5 days. The development of the infection in a woman may result to urethritis, cervicitis, dysuria, increased

vaginal discharge and intermenstrual bleeding. Retrograde spread may occur, leading to the sequelae stated above. In some rare cases, frank peritonitis or peri-hepatitis could result (Fitz-Hugh-Curtis syndrome).

Disseminated gonococcal infection may accompany about 1% of genital infection. About 75% of these cases occur in women who are at increased risk if mucosal infection occurs during menstruation or pregnancy.

Neonates acquiring infection intrapartum present with ophthalmia neonatorum and disseminated infection. Conjunctivitis can also occur in adults following direct inoculation of organisms and it may lead to blindness.

Infection occurring during pregnancy may be associated with abortion, preterm labour, early rupture of the membrane and perinatal mortality⁹.

The diagnosis of *Neisseria gonorrhoeae* in older infants and young children is often associated with allegations of sexual abuse.

The genital infections are the most common unlike the pharyngeal, rectal and eye infections. Pharyngeal and rectal infections are mostly asymptomatic but they constitute reservoirs for disease transmission and are also under diagnosed in general practice^{10,11}.

Detection of *Neisseria gonorrhoeae* by culture is highly specific and it is the gold standard but it is not highly sensitive because of the fastidious nature of the organism. For the pharyngeal and rectal samples, the sensitivity of culture detection is also low because there are fewer organisms at these sites and the growth requirement competition from other bacteria even selective media occur¹¹.

The finding of Gram negative diplococci within leucocytes from a direct Gram staining of urethral discharge is diagnostic.

In disseminated infections, specimens such as joint effusions, blood and cerebro spinal fluid should be collected and send for culture and Gram staining where appropriate. Negative cultures do not rule out disseminated infection.

Antibiotic treatment of gonorrhoea is with a third generation Cephalosporin, which is the drug of choice¹². For uncomplicated infections, an intramuscular injection with 250mg of Ceftriaxone single dose or 400mg orally of Cefixime is adequate. However, if there is co-infection with other STIs, which is common, the patients and their other partners should be screened and treated.

Due to the growing increase in antibiotic resistance in USA, they have adopted the routine use of combination therapy of two anti gonococcal drugs with different mechanisms of action. The combination of Ceftriaxone 250mg and Azithromycin 1gm or Doxycycline 100mg twice daily for 7 days. Japan has stopped using Cefixime because of drug resistance. When appropriate treatment is given, the cure rate can be as high as 98.9%.

There is a dearth of studies on the prevalence of gonorrhoea in the third world countries and its natural history¹³ but Gopal Rao et al found a prevalence of 3.8%¹⁴. In the University of Port Harcourt Teaching Hospital, there is no data on the prevalence and even in the whole of the Niger Delta region of Nigeria.

Strand Displacement and Amplification (SDA) technique is a type of Nucleic Acid Amplification Technique (NAAT) with a high specificity and sensitivity in the diagnosis of *Neisseria gonorrhoeae*¹⁵. The technique allows for the use of self administered vaginal swab as the clinical specimen^{16,17}.

The urine based screening test using NAAT has a comparable sensitivity and specificity to cervical and urethral specimens^{18,19}.

This study is aimed at determining the prevalence of asymptomatic *Neisseria gonorrhoeae* infection among under graduate female students of the University of Port Harcourt. It will also identify risk factors that are associated with genital gonococcal infection on the chosen population.

II. Materials And Methods

The study was done between January and June 2009 at the University of Port Harcourt Teaching Hospital, Port Harcourt, Rivers state, Nigeria. The ethical approval was obtained from the relevant boards at both the University and the Teaching Hospital.

The inclusion criteria were that the subjects should be healthy female under graduates of the University who are aged 16- 30 years. They should not have any obvious vaginal discharge, not on any antibiotic and should respond appropriately to the questionnaire. They must give their consent to participate. The sample size determined using the Kish formula was 200 with prevalence rate of 3.8% at 95% confidence interval, and degree of accuracy as 0.05.

Informed and written consent was obtained from every participant. Those who consented were required to fill in questionnaires which detailed the predisposing/risk factors associated with *Neisseria gonorrhoeae*. They were also given vaginal swabs for self administered vaginal swabbing. The process of specimen collection was explained to each participant by trained clinical students. All the self admissible vaginal swabs (Genital BBL culture swab EZ) were quickly transported to the laboratory and analyzed by SDA²⁰.

III. Results

Out of the 200 students screened, only 10 of them were positive for Neisseria gonorrhoeae infection. These constitute about 5% of the total students screened. It was noted from the data obtained that students aged between 18 and 20 years have a significantly increased chance of contracting Neisseria gonorrhoeae infection when compared to the other age ranges.(Table 1), with a P value of <0.05, $\chi^2=22.081$ for linear trend and odds ratio of 0.6.

1. Prevalence Of Students With Neisseria Gonorrhoeae Infection According To Age Range

TABLE 1.

AGE RANGE	NEISSERIA GONORRHOEAE		NOT TESTED
	(positive) YES	(negative) NO	
[16-18]	4 (28.6%)	10 (71.4%)	NIL
[19-21]	2 (4.8%)	40(95.2%)	NIL
[22-24]	2(3.2%)	60(96.8%)	NI;
[25-27]	0(0%)	60(100%)	NI;
[28-30]	2(9.1%)	20(90.9%)	NIL
TOTAL	10(5%)	190(95%)	

2. ASSOCIATED RISK FACTORS FOR THE INFECTION.

A. Prevalence Of Students With Neisseria Gonorrhoeae Infection In Relation To Number Of Sexual Partners

Table 2.

No. of sexual partners	NEISSERIA GONORRHOEAE		Not tested
	(positive) YES, infected	(negative) Not infected	
NONE	0(0%)	40(100%)	NILL
ONE	2(3.2%)	60((96.8%)	NILL
TWO	2(3.2%)	60(96.8%)	NILL
THREE	2(6.3%)	30(93.7%)	NILL
FOUR	4(100%)	0(0%)	NILL
TOTAL	10(5%)	190(95%)	

While no student in the study who had no sexual partner was infected at the time of study, those with four sexual partners were all infected.

$P < 0.05$, using Firscher's test of exactness, $\chi^2 = 28.10$. $RR = 19.00$ ($7.32 < RR < 49.32$).

B. Prevalence Of Students With Neisseria Gonorrhoeae Infection In Students With A Previous History Of Sexually Transmitted Infection

Table 3.

PREVIOUS STI	NEISSERIA GONORRHOEAE		Not tested
	YES(infected)	NO(not infected)	
YES	8(11.4%)	62(88.6%)	nil
NO	2(1.5%)	128(98.5%)	nil

Of the 70 students that had a previous history of STI, 8 were infected while only 2 students were infected out of 130 students who had no previous history of STI. $\chi^2 = 4.58$, $P < 0.05$

On the question of regular condom use, 112 students use condom regularly while 39 students infrequently use condom. None of those that use condom regularly was infected while 10 of the 39 students who do not use condom regularly were infected. This was very significant with a P value of < 0.05 , $\chi^2 = 21.99$

IV. Discussion

The Prevalence rate of Neisseria gonorrhoeae infection amongst undergraduate female students in the University of Port Harcourt between January and June 2009 was 5%. This result is comparable to those of Franceschi S et al²¹ and Gopal et al¹⁴ who had 2.6% and 3.8% respectively but it was significantly different from that of Alli JAO et al who had 25%²². It sample size in this study was small compared to what obtained in all the studies sited above but this did not significantly affect the study negatively. It can be said that the result of 5% prevalence is high due to the high risk of transmission from one individual to another and the attendant complications if undetected and treated.

The age ranges for infection from the study is lower than those for Chlamydia trachomatis²⁰. While the study found an age range of 16-18 years having a higher infectivity rate, the age range for Chlamydia trachomatis infection was highest between 28-30 years.

The associated risk factors for increased *Neisseria gonorrhoeae* infection were comparable to other sexually transmitted infective organisms. It was observed that most of the infected students has had previous episodes of STI, had multiple sexual partners and did not use condom regularly as a preventive device.

Most researches done on *Neisseria gonorrhoeae* using NAAT have been limited to a few centers in Nigeria and the out of these researches have not been adequately disseminated. There is therefore paucity of knowledge on the outcome of most of these studies. It will be useful and helpful if these research findings are made public regularly so that policy makers can apply them during policy formulations. Screening for asymptomatic *Neisseria gonorrhoeae* infection among the populace should be a welcome development by the government of Nigeria considering the health implications of an asymptomatic infection of the organism to an infected individual.

V. Conclusion

Due to the complications of the infection if left undiagnosed or not treated and since NAAT provides a highly sensitive and specific diagnostic accuracy, there should be a generalized screening policy by the government of Nigeria for *Neisseria gonorrhoeae* using NAAT on the target population. This will help to reduce the reproductive health challenges posed by asymptomatic infection of *Neisseria gonorrhoeae*.

Acknowledgement:

Female undergraduate students of University of Port Harcourt, Head of Department and staff of Medical Microbiology and Parasitology of the University of Port Harcourt Teaching Hospital.

References:

- [1]. Velicko I and Unemo M: Increase in reported gonorrhoea cases in Sweden, 2. *Euro Surveill* 2009; 14: 1-5.
- [2]. ESSTI. sexually transmitted infections in Europe. Health Protection Agency, 2008; 3: p.27-54.
- [3]. Janier M, Lassau F, Casin I and Morel P. Pharyngeal gonorrhoea: The forgotten reservoir, *Sex Transm Infect* 2003; 79:345.
- [4]. Hook EWI and Handsfield HH. Gonococcal infections in adults. In: Holmes KK, Sparling PF and Mardh PA, et al, eds. *Sexually Transmitted Diseases* 3rd ed. San Francisco, CA: Macgraw-Hill. 1999 : 451-466.
- [5]. Fleming DT and Wasserheit JN. From epidemiological synergy to public health practice: the contribution of sexually transmitted disease to sexual transmission of HIV infection. *Sex Transm Infect* 1999; 75: 3-17.
- [6]. Chem A, Boulton IC, Pongoski J, Conchrane A and Gray-Owen SD. Induction of HIV-1 long terminal repeat mediated transcription by *Neisseria gonorrhoeae*. *AIDS* 2003; 17: 625-628.
- [7]. Schieh ISD. National Public Health Service for Wales. Renewing the focus, HIV and other sexually transmitted infections in the United Kingdom in 2002. London: Health Protection Agency, 20002.
- [8]. World Health Organization Task force on Prevention and Management of Tubal Infertility: Serological relationship to Post Chlamydia and Gonococcal infections. *Sex Transm Dis.* 1995; 22: 71-77.
- [9]. Killick V, Kell P, Plumb H, Hurd N and Turner P. Screening for *Neisseria gonorrhoeae* using the BD Probe Tec Nucleic acid amplification test in a low prevalence genitourinary medicine clinic. *Int. J.STD AIDS.* June 2002; vol 23 no. 6 e1-e3.
- [10]. Clinic- based testing for rectal and pharyngeal *Neisseria gonorrhoeae* and Chlamydia trachomatis infections by community-based organizations-five cities, United States, 2007. *MMWR Morb. Mortal Wkly Rep* 2009; 58: 716-719.
- [11]. Schachter J, Chernesky MA, Willis DE, Fine PM, Martin DH, Fuller D, et al. Vaginal swabs are the specimens of choice when screening for Chlamydia and *Neisseria gonorrhoeae*: results from a multicenter evaluation of the APTIMA assays for both infections. *Sex Transm Dis.* 2005; 32: 725-728.
- [12]. Jatau ED, Galadima M, Odama LE and Kwaga JKJ. Prevalence and anti microbial susceptibility of *Neisseria gonorrhoeae* isolated from patients in various locations of Kaduna state, Nigeria. *Nig J Surg Res* 2003; 5: 50-56.
- [13]. Barlow D. Commentary. *Sex Transm Infect* 2008; 84: 121.
- [14]. Gopal RG, Bacon I, Evans J, et al. Prevalence of *Neisseria gonorrhoeae* infection in young subjects attending community clinics in South London. *Sex Transm Infect* 2008; 84: 117-121.
- [15]. Akduman D, Ehret JM, Messina K, Ragsdale S and Judson FN. Evaluation of a Strand Displacement and Amplification assay (BD Probe Tec-SDA) for detection of *Neisseria gonorrhoeae* in urine specimens. *J Clin Microbiol* 2002; 40: 281-283.
- [16]. Ryan C, Kudesia G, McIntyre S, Davies S, Zadik P and Kinghorn GR. BD Probe Tec ET assay for the diagnosis of gonorrhoeae in a high-risk population: a protocol for replacing traditional microscopy and culture techniques. *Sex Transm Infect* 2007; 83: 175-179.
- [17]. Black CM. Current methods of laboratory diagnosis of Chlamydia trachomatis and *Neisseria gonorrhoeae* infection. *Clin Microbiol. Rev* 1997; 10:160-184.
- [18]. Schepetiuk S, Kok T and Martin L, et al. Detection of Chlamydia trachomatis and *Neisseria gonorrhoeae* in urine samples by nucleic acid tests. Comparison with culture and enzyme immunoassay of genital swab specimens. *J. Clin Microbiol* 1997; 35: 3355-3357.
- [19]. Cook RL, Hutchison SL, Ostergard L, Braithwaite RS and Ness RB. Systemic review, non invasive testing for Chlamydia trachomatis and *Neisseria gonorrhoeae*. *Ann Intern Med* 2005; 142: 914-925.
- [20]. Wariso KT, Odigie JO and Eyearu SG. Prevalence of Chlamydia trachomatis infection among female undergraduates of the University of Port Harcourt using Strand Displacement and Amplification (SDA) Technique. *TNHJ*, vol. 12. No 2, April-June; 2012.
- [21]. Franceschi S, et al. Cervical infection with Chlamydia trachomatis and *Neisseria gonorrhoeae* in women from ten areas in four continents. A cross sectional study. *Sex Transm Dis.* 2007 Aug, 34(8): 563-9.
- [22]. Alli JAO, Okonko LO, Odu NN and Kolade AF. Detection and prevalence of genital pathogens among attendees of STI clinic of a tertiary care Hospital in Ibadan, South Western Nigeria. *World Journal of Medical Sciences* 2011; 6(3): 152-161.