Microbial Pathogens Implicated in Reproductive Health Infections in a Special Treatment Clinic in Ibadan, Nigeria

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Abstract
Objective: The lack of adequate recognition of health importance of non-HIV reproductive health infections (RHIs) in Nigeria has led into this study, which was to determine clinical pathogens in non-HIV RHI in Nigeria using a tertiary health facility as case study.

Materials and Methods: A nine-year investigation was carried out between 1997 and 2005 on 4047 (n = 1626 males; n = 2421 females) patients presenting at Special Treatment Clinic (STC) of University College Hospital (UCH) Ibadan, Nigeria. Routine laboratory procedures using appropriate culture media, culture conditions, and current phenotypic taxonomic tools for classification of isolated pathogens were employed.

Results: Age (p = 0.019) and gender (p<0.0001) were related to the recovery rates of pathogens Candida species (55.6 %), Neisseria gonorrhoeae (11.1%), Gardenella vaginalis (10.3%), Escherichia coli (9.2 %), Klebsiella sp. (4.2%), streptococci (4.0%), Staphylococcus aureus (2.3%), Proteus sp., (1.8%), Haemophilus ducreyi (0.5%), Trichomonas vaginalis (0.44%) and Pseudomonas aeruginosa (0.18%). Candida and Gardenella vaginalis species were mostly recovered from female patients, while N.gonorrhoeae were mostly isolated from male patients. Age brackets for the recovery of pathogens were Neisseria gonorrhoeae (16-30 years); Gardenella vaginalis (21-25 and 31-35 years) and C.albicans (21-30 years).

Conclusion: Candida, Neisseria gonorrhoea and Gardenella vaginalis were the most recovered pathogens from patients presenting at Special Treatment Clinic of a tertiary health institution in Nigeria, and the relationship between age, gender and the aetiological agents was statistically significant.

Keywords: Age, Gender, Health policies, Reproductive health infections, Sexually transmissible infections

Introduction
Reproductive related transmitted diseases are a

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ntries. According to Røttingen et al. (3), the monitoring of STI prevalence is crucial for the evaluation of STI treatment programmes and can also provide an indirect measure of change in sexual behaviour; however, the widespread of reproductive related diseases in Africa continues to constitute a major public health problem among the developing countries (4, 5); a situation that has been compounded by non-availability of reliable data on the prevalence and pattern of these infections even for the planning control strategies. Keeping of national data and records on the detection and management of STIs and STI related infections in Nigeria is a challenge, particularly as earlier documented by Mayaud et al. (2) that management of STI like gonococcal infection is a challenge in developing countries.

As at the early 1990’s, and with a population of over 120 million people, there were only four STD clinics in the whole of Nigeria, and all located in tertiary hospitals for patients with STDs but the public health importance of non – HIV RHIs has not been adequately recognized in Nigeria (6,7). At present, however; there are no data or surveillance on non – HIV related reproductive health infections in Nigeria. In the context of the HIV/AIDS pandemic, the monitoring of sexually transmitted infections (STIs) at the population level is a public health priority and given the general scarcity of surveillance data about the epidemiology of STIs in Africa, this study will therefore be an important and timely contribution to literature. This study was needed to be undertaken since there is currently no surveillance or national data on non – HIV / AIDS reproductive health or sexually transmissible infections in the country, as such; information cannot be assessed locally or internationally. It is also more disturbing that there are a number of asymptomatic cases that are not reported or treated, since majority of the population do not seek medical assistance from the very few primary health care facilities in the country. Findings of such study can be of a proposed use in surveillance and means of control / treatment benefit to policy makers, research and medical professionals and the general populace.

Future investigations involving other such tertiary institutions in the country are under consideration, but this research study is a pilot investigation carried out to determine the microbial flora implicated in the most presented reproductive health infectious cases in Nigeria, using a Special Treatment Clinic attached to the University College Teaching Hospital, (UCH), Ibadan, a tertiary primary health care / referral centre.

### Materials and Methods

Cervical swabs (CS / CX) and high vaginal swabs (HVS) of female patients 2 to 86 years old, and urethral, eye and wound swabs of male patients 2 days to 71 years old presenting at the Special Treatment Clinic (STC) of the Department of Medical Microbiology and Parasitology, University College Hospital

### Table 1: Pathogen distribution and age profiles of patients presenting at the Special Treatment Clinic (UCH) Ibadan, Nigeria (1997–2005)

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</thead>
<tbody>
<tr>
<td>Candida spp.</td>
<td>50 (7.3)</td>
<td>197 (29.1)</td>
<td>92 (13.6)</td>
<td>29 (4.2)</td>
<td>5 (0.7)</td>
<td>1 (0.1)</td>
<td>374 (55.3%)</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>8 (1.1)</td>
<td>28 (4.1)</td>
<td>18 (2.6)</td>
<td>9 (1.3)</td>
<td>6 (0.8)</td>
<td>-</td>
<td>69 (9.76%)</td>
</tr>
<tr>
<td>Staph. aureus</td>
<td>5 (0.7)</td>
<td>4 (0.6)</td>
<td>4 (0.6)</td>
<td>2 (0.2)</td>
<td>-</td>
<td>-</td>
<td>15 (2.21%)</td>
</tr>
<tr>
<td>Proteus spp.</td>
<td>4 (0.5)</td>
<td>6 (0.8)</td>
<td>4 (0.6)</td>
<td>-</td>
<td>2 (0.2)</td>
<td>-</td>
<td>16 (2.37%)</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>4 (0.5)</td>
<td>11 (1.5)</td>
<td>7 (1.0)</td>
<td>1 (0.1)</td>
<td>-</td>
<td>-</td>
<td>23 (3.40%)</td>
</tr>
<tr>
<td>N.gonorrhoeae</td>
<td>21 (3.1)</td>
<td>37 (5.5)</td>
<td>14 (2.0)</td>
<td>8 (1.1)</td>
<td>4 (0.6)</td>
<td>2 (0.2)</td>
<td>86 (13.1%)</td>
</tr>
<tr>
<td>H.ducreyi</td>
<td>-</td>
<td>2 (0.3)</td>
<td>2 (0.2)</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>04 (0.59%)</td>
</tr>
<tr>
<td>Streptococcus spp.</td>
<td>2 (0.3)</td>
<td>9 (1.3)</td>
<td>6 (0.8)</td>
<td>4 (0.6)</td>
<td>2 (0.2)</td>
<td>-</td>
<td>23 (3.40%)</td>
</tr>
<tr>
<td>G.vaginalis</td>
<td>8 (1.1)</td>
<td>28 (4.1)</td>
<td>18 (2.6)</td>
<td>9 (1.3)</td>
<td>3 (0.4)</td>
<td>-</td>
<td>66 (9.76%)</td>
</tr>
<tr>
<td>T.vaginalis</td>
<td>1 (0.1)</td>
<td>-</td>
<td>-</td>
<td>1 (0.1)</td>
<td>-</td>
<td>-</td>
<td>02 (0.29%)</td>
</tr>
<tr>
<td>Ps.aeruginosa</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>1 (0.1)</td>
<td>-</td>
<td>-</td>
<td>01 (0.14%)</td>
</tr>
<tr>
<td>C.trachomatis</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>(0.00%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>Total</th>
<th>Ad/NI</th>
<th>Grand Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤15 – &gt;66</td>
<td>679</td>
<td>437</td>
<td>1116</td>
</tr>
</tbody>
</table>

\[x^2 = 35.232; df = 20; p = 0.019\]  
Ad/NI = Ages not indicated  
Grand Total = Total patients from which pathogens were isolated
Fig. 1: Pathogen distribution profiles of patients presenting at the Special Treatment Clinic (UCH) Ibadan, Nigeria (1997 – 2005)
Exudates from the patients with eye infections were swabbed directly on appropriate sterile culture plates, while for the collection of wound swabs, the wound areas were cleaned with sterile swabs, wet with sterile saline to remove surface debris of the wounds before taking the wound swab with new sterile swabs. For the collection of cervical swabs and high vaginal swabs, sterile vaginal speculum was used to separate the vaginal walls prior to collection of high vaginal specimens with sterile swabs.

Urethral swabs were collected after cleaning the penile tip and area with sterile swab soaked in sterile saline. The collected specimens were securely kept in appropriate specimen containers to avoid contamination prior and during processing. T. vaginalis was grown in a conventional broth medium followed by observation of saline preparation for motile trichomonads under the microscope (wet mount examination). Specimens such as high vaginal swabs and urethral swabs were processed immediately after collection, while those that were not processed immediately after collection were kept in the refrigerator. All specimens were processed within 24–48 hrs after collection.

The swabs were cultured on appropriate media (blood agar, chocolate agar, MacConkey agar, eosin methylene blue agar and Sabouraud dextrose agar) under conditions optimal for their growth, while the microbial pathogens obtained from the clinical specimens were stored on brain–heart infusion agar slants as bench and stock cultures. The isolates were examined microscopically and identification and grouping of the isolates were based on standard phenotypic, microbiological taxonomic tools (8-10) of the laboratory of the Department of Medical Microbiology, University College Hospital Ibadan, Nigeria.

### Results

Out of the total four thousand and forty seven patients that presented at the Special Treatment Clinic (STC) of the Department of Medical Microbiology and Parasitology, University College Hospital (UCH) Ibadan, Nigeria, between May 13 1997 and November 29 2005, 1626 (40.7%) were males, while 2421 (59.3%) were females. The ages of the patients were between below 15 years and above 66 years but majority of the patients were in the age brackets of 21–25 years (22.7%); 26-30 years (23.9%); 31–35 years (16.0%) and 36–40 years (11.9%), while 12.7% were below 20 years and 0.78 % above 60 years age groups respectively. About 25.8 % of the patients however did not specify their precise ages (Table 1).
Results obtained in this study indicated that age was related to the pathogen recovery rates among the patients \((p = 0.019)\). The age bracket at which most of the pathogens were recovered was between 16 and 40 years, however, the highest recovery rates were between 21–25 \((25.7\%)\) and 26–30 \((22.6\%)\) (Fig. 1).

*Neisseria gonorrhoeae* was mostly recovered from 16–30 year old patients; *Gardennella vaginalis* from 21–25 and 31–35 year old patients while *C.albicans* from 21–30 year old patients. The oldest male patient from whom pathogen was isolated was a 71–80 year old man while oldest female patient from whom pathogen was isolated was an 86 – year old woman (Table 1).

Recovery of pathogen was also gender – related \((p < 0.001)\) as shown in Table 1 and Figure 1, which shows the percent of total recovery rates. More of the isolated pathogens were prominent in females than males except *Staph. Aureus*, *Streptococcus spp.* and *Neisseria gonorrhoeae*. *Candida* species were the pathogens mostly recovered from female patients, while *N.gonorrhoae* were mostly isolated from the
male patients. About eleven genera of pathogens were isolated from the various clinical specimens of the patients. The most recovered pathogens from the patients were *Candida* species (55.6%), *Neisseria gonorrhoeae* (11.1%), *Gardnerella vaginalis* (10.3%) and *Escherichia coli* (9.2%). Other isolated pathogens were Klebsiella spp. (4.2%) β–haemolytic streptococci (2.3%), *Staphylococcus aureus* (2.3%), *Proteus sp.* (1.8%), non–haemolytic streptococci (1.3%), *Haemophilus ducreyi* (0.5%), *Trichomonas vaginalis* (0.4%), α–haemolytic streptococci (0.4%) and *Pseudomonas aeruginosa* (0.2%) (Fig. 2). Clue cells were also observed in a total of 1934 (47.7%) of the patients irrespective of recovery of other pathogens. The clue cells obtained from the clinical specimens were in the total range of 42.5% from cervical swabs; 52.2% from high vaginal swabs and 5.3% from urethral swabs (Table 2).

**Discussion**

Sexually transmitted diseases (STDs) are a major public health problem (10), and according to Johnson et al. (5), surveillance of the prevalence of sexually transmitted infections (STIs) is increasingly recognised as a key priority in public health. The surveillance report of Victorian Infectious Diseases Bulletin (12) also affirmed that prompt notification of infectious diseases is an integral component of prompt public health action; and that there is high risk of STIs in the absence of monitoring. In Nigeria, however, there are no nationally representative microbiological, epidemiological, nor any sentinel surveillance studies on non–HIV related STIs, thus, any such study conducted may only be carried out as private investigative research or in few teaching hospitals in urban areas such as the University College Hospital, Ibadan, where studies conducted are usually among users of such public health facilities. The finding of this present study confirms the earlier report of Christian et al. (13), which claimed that epidemiology of sexually transmitted infections (STI) in rural, developing world populations is poorly understood (1, 14) and same is the case of Nigeria.

In this study, a wider age bracket (21–30) for acquiring most STIs in Nigeria was indicating, as compared with that of WHO report, in which adolescents and young adults (15–24) were at the greatest risk of acquiring an STI, with 3 million becoming infected each year (1). Chancroid, caused by *Haemophilus ducreyi* is a significant cause of genital ulcers in Africa and Asia (15, 16), accounting for over 50% of genital ulcers disease (GUD) in males, and a substantially lower proportion in females. In this present study, only 0.56% recovery rate was recorded for *Haemophilus ducreyi* in patients presenting at the clinic in 1997 and 1998, which also confirms the earlier report of Johnson et al. (5) that there appears to have been a significant decline in the proportion of ulcers attributable to chancroid in recent years.

Johnson et al. (5) also reported that gonorrhoea accounts for the vast majority of urethritis cases in men, and that the men attending STI clinics also have extremely high prevalence rates. The prevalence of the disease in women was found to be significantly higher (10–31%) in high – risk groups such as sex workers and women attending STI clinics (17). In this study however, *N.gonorrhoeae* was detected in 7.72% of the male patients and in 3.41% of the female patients. Although gonorrhoea was the second most encountered infection among all the patients, having recorded a total of 11.1% recovery rates, the prevalence rates were relatively low in comparison with the earlier report of Douglas (18), which stated that the probability of acquiring gonorrhoea is about 25% in men and about 40% in women. Meanwhile, in the earlier finding of Christian et al. (13), it was reported that *N.gonorrhoeae* rates were low among women in rural population of Nepal, having detected *N.gonorrhoeae* in only 2.3% of the rural Nepal women. A migratory study carried out by Ogbuale et al. (7) in Nigeria for example, also reported that gonorrhoea was one of the most transmitted sexual diseases among their study groups. In a special study carried out by Bakare et al. (17), out of 155 patients that were found to have gonococcal infection, 118 (76.1%) were males while 37 (23.9 %) were females. Sixty-four (54.2%) of the male patients and 19 (51.4%) of the female patients positive for gonorrhoea were aged between 20 and 29 years, while 21.2% of the males and 16.2% of the females were in the age bracket of 40 years and above. Douglas (19) also reported that gonorrhoea occurs worldwide and generally affects persons aged 15-29 years. Similarly, in this study, *N.gonorrhoeae* was mostly isolated from patients aged between 16 and 30 years. However, it is necessary to note that the results obtained in this study are not significant to represent a national data, especially since most of those attending the clinic are those exposed to the opportunity of presenting at such facility.

Earlier studies, including those of Fortenberry et
Pathogens of reproductive infections

al. (20), Orr et al. (21), DiClemente et al. (22) and Kissinger et al. (23) had noted that gonorrhoea and chlamydia are common among young females. Johnson et al. (5) similarly reported that chlamydia was found to be more prevalent than gonorrhoea in low risk groups, but its prevalence was lower than that of gonorrhoea in individuals with STI symptoms. This earlier report is further supported by the results of the present study in which the prevalence rate of Chlamydia (0.18%) was lower than that of gonorrhoea in individuals with STI symptoms (22). Although infections of the genital tract caused by Gram-negative bacteria are typically not classified as sexually transmitted infections, their recovery from the clinical specimens analysed in this study may be explained by strong overlap between pathogens of the urinary tract infections and those of STIs. Presence of species within the genera, Proteus, Providencia and Morganella in body fluids and in some deep or superficial lesions would lead one to suspect their potential aetiologic nature (24). Similarly, Escherichia coli has been found to be the causative agent in 80.0% of urinary tract infections (UTIs), though other enteric bacteria such as Pseudomonas aeruginosa from faeces can also cause such infections. E. coli has been reported to cause nearly half of nosocomial UTIs, while Proteus mirabilis and E. coli also cause prostatitis in men but the clinical implications of such pathogens, as STI related in Nigeria cannot be ascertained yet.

Trichomonas vaginalis, a parasitic protozoan, which is the aetiologic agent of trichomoniasis, is a sexually transmitted disease (STD) of worldwide importance, and one of the most common non-viral STD associated with many perinatal complications in male and female genitourinary tract infections (17, 25, 26). In the study of Mayaud et al. (2) on the prevalence of STDs among 964 women attending antenatal clinics in a rural area of the United Republic of Tanzania, a total of 378 (39%) of the women were infected with at least one STD pathogen, 97 (10%) had syphilis, and 81 (8%) had N. gonorrhoeae (NG) and/or Chlamydia trachomatis (CT) infection. Recent data have shown that the annual incidence of trichomoniasis is quite high worldwide and more disturbing however, is the number of asymptomatic cases that are not treated (26). Although trichomoniasis is regarded primarily as a disease of women, few studies on trichomoniasis have been conducted in men and it would appear that this disease accounts for less than 5%–20% of male urethritis cases (5, 26). In women, however, the disease is highly prevalent, with prevalence rates typically in excess of 20% (5). T. vaginalis has been frequently found among the sexual partners of patients with proven infection, while the pathogen has been demonstrated in 30–40% of male sexual partners of infected women (27) and in approximately 20% of all cases of non-gonococcal urethritis (28).

In the study of Bakare et al. (17), trichomoniasis was present in 23.9% of the female patients, and the majority of female patients positive for trichomoniasis were between the ages of 20-29 (38.8%) and 30-39 (37.9%), while 8.7% were between 40–49 years and 2.9% were above 50 years of age. In this present study, there was a low prevalence (0.44%) of T. vaginalis, although, inadequate cultural methods may introduce bias in the recovery rates of the pathogen. Low prevalence may also be explained by the illustration made by Petrin et al. (26) that the pathogenesis of T. vaginalis is indeed complex and its interaction with the members of the resident vaginal flora and certain stress responses affect the organism’s survival in its changing environment. This present study confirms some of the earlier findings since T. vaginalis was isolated only in female patients. The World Health Organisation (WHO) has developed a risk assessment approach to identify cervical infections among women complaining of vaginal discharge and the prevalence of any vaginal or cervical infection was 68%, while the prevalence rates of various pathogens were: C. albicans (39%), T. vaginalis (16%), bacterial vaginosis (24%), N. gonorrhoeae 2.3%, C. trachomatis (5.9%), N. gonorrhoeae and/or C. trachomatis (7.4%).

Vaginitis is known to be caused by G. vaginalis, although various anaerobic bacteria may work in combination with it. Several organisms however, account for a share of vaginitis cases. Infected males do not exhibit clinical symptoms but nevertheless are capable of transmitting the bacteria. The results obtained in this study however, confirms the earlier reports that G. vaginalis is a feminine infection, since the recovery rates were more among female (7.91%) than male (2.60%) patients. Similarly, Oni et al. (29) earlier reported that the prevalence of G. vaginalis was found to be 7.4% in male patients, but could be cultured from approximately one third of normal women, and up to 90% of the male sexual partners of infected women who harbour G. vaginalis in the urethra. In the present study, candidiasis and vaginitis (G. vaginalis) were the most presenting STI
among females. Bakare et al. (17) earlier reported that some of the STDs in their order of frequency were candidiasis (61.3%), bacterial vaginosis (19.3%) and gonorrhoea (15.5%). Other STI – implicated pathogens such as *Treponema pallidium* were not isolated from the clinical specimens in the course of this study due to the non – availability of advanced diagnostic techniques such as appropriate selective media or isolation kits for such pathogens. This therefore, also introduces a bias in the study. The geographical distribution of this study is generally not proportional to that of the entire Nigerian population and this also introduces a geographical bias. There is a need therefore, for studies that are more nationally representative. There is also a need for more cross sectional studies conducted periodically in the same population, using more advanced diagnostic techniques, which can be used to monitor trends in STI prevalence and for STI treatment initiatives more reliably as previously suggested by Wilkinson et al. (30).

Studies from other African countries, which suggested that there have been significant declines in the prevalence of curable STIs in recent years, include those of O’Farrell et al. (31) and Nagot et al. (32). STI prevalence rates in South Africa for example, have been claimed to be high, even when compared with other African countries. Currently, the South African Department of Health conducts annual surveys and also collects data on numbers of STI cases treated at public STI clinics (33). As large as Nigerian population is, there is no Nigerian Department of Health that conducts annual surveys, nor collects data on numbers of STI cases presented/treated at public or private STI clinics. The only awareness programme presently targeted in Nigeria is the HIV / AIDS awareness programme, while data on the prevalence of other STIs, STI symptoms, improved microbiological, surveillance and drug resistance monitoring for different STI syndromes are grossly lacking. In Nigeria, monitoring and evaluation, as well as comparison of historical and current clinical data of such infections would also aid in their control. This present study concludes that among the patients presenting for non – HIV, reproductive health infections, STI pathogens were sex and age – related and the most recovered pathogens were *Candida* species, *N.gonorrhoea* and *G.vaginalis*. *Candida* and *G.vaginalis* were more prominent in females while *N.gonorrhoea* were more prominent in males. Other pathogens recovered in lower proportions were *G.vagi-nalis, E.coli Klebsiella, Proteus* and *Streptococcus* species.

The clinical diagnosis indicated that most of the female patients presented for infertility and pelvic inflammatory diseases. In as much as this study did not implicate any of the recovered STI pathogens in infertility or pelvic inflammatory diseases, it is still important to raise caution on the related clinical significance of these associated pathogens. As an example, infectious complications in pregnancy and delivery are still very serious problem in obstetrical, gynecological and neonatological practice (34), with the main part caused by the anaerobic organisms associated with bacterial vaginosis as well from sexually transmitted pathogens. In the study of Chervenkova et al. (34), data were presented about the frequency of spreading of bacterial vaginosis and vaginitis in pregnant women aged between 15 and 35 at different stages of pregnancy. Their results showed high frequency of spreading of bacterial vaginosis and cervico – vaginitis in pregnant women and that 14% of them harbored more than one such pathogen.

Besides bacteria, fungi (*Candida*) and protozoans (*Trichomonas*) are very common STI pathogens and produce characteristic findings. When present during pregnancy, these infections have been linked to low birth weight and obstetric disorders (35). There are also documented reports indicating possible association between cervicovaginal infections (CVI) and preterm delivery (36–39). It has be found as well that urologic complaints in both men and women may be related to sexually transmissible infectious agents that frequently cause vaginitis in women and that vaginal infections in women often have their counterparts in men (40). There is a need therefore, for studies that are more nationally representative in other to monitor and evaluate the prevalence of STIs in Nigeria. Such studies can justify or debunk the implication of such STDs in infertility and other obstetrical, gynecological and neonatological problems. This study is based on laboratory data only, which serves as a limitation. There is therefore, the need for more detailed sociodemographic and clinical data. It is also recommended that microbiological surveillance system for STIs be incorporated in the health care delivery at every stage of governance in Nigeria.

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References

Pathogens of reproductive infections