# Aetiological agents of vaginitis in Nigerian women

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## Introduction

Infectious vaginitis is a common clinical problem that results in between five and 10 million gynaecological clinic visits per year worldwide.[1] Although common and easily managed, infectious vaginitis can be deceptive because symptoms are variable and often misinterpreted.[1] The most common presenting symptoms include itching, a burning sensation, abnormal odor and discharge; however, some cases are asymptomatic and remain untreated.[2]

The cause of vaginitis cannot always be determined on the basis of symptoms or physical examination. For effective management, however, accurate diagnosis depends on the use of antimicrobial agents shown to be effective against microorganisms isolated. Occasionally, treatment is prescribed on the basis of symptoms alone, but, without examination, misinterpretation of variable symptoms can lead to inaccurate diagnosis.[3]

The three main types of infectious vaginitis are bacterial vaginosis, yeast infection and trichomoniasis. Vaginal yeast infections or vulvo-vaginal candidiasis is a common cause of itching, burning and irritation of the vagina. Typical symptoms are pruritis and a thick whitish–grey discharge.[4] Yeasts are present in the vagina in small numbers and overgrowth of these organisms can occur during pregnancy, in uncontrolled diabetes mellitus, HIV and as a result of oral contraceptive antibiotic use.

Bacterial vaginosis (BV) is a gynaecological condition caused by loss of the normal vaginal flora and overgrowth of anaerobic organisms. It is the most common cause of vaginitis and accounts for about 50% of cases.[5] Some 90% of patients with symptomatic BV have vaginal discharge.[6]

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## ABSTRACT

This study focuses on the identification of aetiological agents of vaginitis in Nigerian women. Study subjects are drawn from patients presenting with lower abdominal pain, vaginal discharge and itching at the gynaecology clinic of Lagos University Teaching Hospital and at the Clinical Centre of the Nigerian Institute of Medical Research, Yaba, Lagos, between January 2001 and July 2002. A total of 250 patients gave informed consent to participate in the study. The patients also had pre- and post-test human immunodeficiency virus (HIV) counselling. Each patient completed a questionnaire in order to provide biographical data, past clinical history and socio-economic background information. A cervical swab (CS) and a high-vaginal swab (HVS) were obtained from each patient. Swab samples were examined for pH and under light microscopy by Gram's stain and as wet preparations in 10% potassium hydroxide. Subsequently, samples were cultured on appropriate media at optimal conditions and a drug sensitivity profile for all isolates was determined by standard methods. Blood samples were screened and confirmed for HIV antibodies. Bacterial, fungal and parasitic pathogens were identified or isolated in samples from 241 (96.4%) of the women. Bacterial agents (*Neisseria*, *Streptococcus* and *Staphylococcus* species) were predominant in 128 (51.2%) patients, followed by fungi in 108 (43.2%) and parasites (*Trichomonas vaginalis*) in five (2.0%). Sensitivity to ciprofloxacin was seen in 40% of *Staphylococcus* species and in 90% of *Neisseria* species. Positive HIV serology was seen in 25 (10%) of the 250 women studied, 20 (80%) of which had concurrent microbial infections. Overall, a broad spectrum of microbial agents were shown to be responsible for vaginitis in the group of patients studied.

KEY WORDS:    HIV. Trichomonas infections.
              Vaginal discharge.
              Vaginosis, bacterial.
              Yeasts

Bacteria indigenous to the female genital tract have been implicated as important opportunistic pathogens in gynaecological infections.[7] Studies have shown that these organisms are frequently isolated in mixed cultures, and *Gardnerella vaginalis* has been identified as a key organisms in BV. The predominant aerobic organisms isolated from these infections include α- and β-haemolytic streptococci, *Escherichia coli* and *Staphylococcus epidermidis*.[8]

Data on the contribution of anaerobic bacteria to gynaecological infection in Nigeria and other African countries are scanty. Risk factors that seem to increase BV

**Table 1.** Profile of identified/isolated microbial agents.

| | |
|---|---|
| Number of samples screened | 250 |
| Number positive for causative microorganisms | 241 (96%)* |
| Broad distribution of causative agents | |
| Bacterial species | 128 (53%)† |
| Fungal species | 108 (45%)† |
| Parasitic species | 5 (2%)† |

\* Percentage based on the total study population.
† Percentage based on the total number of patients positive for causative agents.

**Table 2.** Distribution of pathogenic agents amongst patients presenting with vaginitis in Nigeria.

| Isolates | No positive | Frequency (%) |
|---|---|---|
| *Staphylococcus* species | 55 | 23 |
| *Escherichia coli* | 14 | 6 |
| Coliform bacilli | 13 | 6 |
| *Pseudomonas aeruginosa* | 12 | 5 |
| *P. mirabilis* | 11 | 5 |
| β-haemolytic streptococci | 10 | 4 |
| *Klebsiella* species | 8 | 3 |
| *Neisseria gonorrhaea* | 5 | 2 |
| *Trichomonas vaginalis* | 5 | 2 |
| Yeasts (*Candida* species) | 108 | 44 |
| Total | 241 | 100 |

**Table 3.** Frequency of pathogens obtained from the study population (*n*=250).

| Agents isolated | No (HIV-pos) | No (HIV-neg) | Total |
|---|---|---|---|
| Bacterial | 9 | 119 | 128 |
| Yeast | 9 | 99 | 108 |
| Trichomonads | 2 | 3 | 5 |
| None | 5 | 4 | 9 |
| Total | 25 | 225 | 250 |

include multiple sex partners, intrauterine device (IUD) use, antibiotic therapy, nutritional status, hygiene and the menstrual cycle. These factors are likely to alter the type and number of bacteria found in the lower genital tract.[8] In addition to being found in 6–13% of lesbian couples,[9] BV is associated with pelvic inflammatory disease (PID) and postoperative infection following hysterectomy.[10] In pregnancy, it is considered a risk factor for preterm birth, premature rupture of membranes, post-partum endometritis and low birthweight.[11]

It has been reported that recurrent yeast vaginitis may serve as one of the initial symptoms of HIV infection.[12] *Trichomonas vaginalis* has been shown to facilitate sexual transmission of HIV infection,[13] while bacterial vaginosis may increase the survival of HIV-1 infection in the genital tract.[7] The implication of infectious vaginitis and HIV-1 infection may be confounded by multiple sexual partners, a sexual relationship with a new partner or concurrent sexually transmitted infections (STIs).

This study aims to determine a profile for the microbial agents that caused vaginitis among women in Lagos, Nigeria, and to establish which therapeutic agents should be used in its clinical management. Efforts will also be made to establish an association between vaginitis and HIV.

## Materials and methods

### Study population
The study population was selected randomly from patients who presented to the study centres, with symptoms of vaginal itching, lower abdominal pain and abnormal vaginal discharge. Informed, written consent was obtained from all patients in the study and they were precounselled for HIV testing. A total of 250 patients (age range 15–50 years) participated in the study. Pregnant women were excluded from the study. Subjects on oral antimicrobial treatment or on any form of vaginal medication during the two weeks prior to presentation were also excluded.

### Biographical data
Each patient completed a questionnaire in order to provide biographical data, past clinical history and socio-economic background information. Information on the number of sexual partners, the use of contraceptives, and the type and duration of symptoms was also sought.

### Specimen collection
High-vaginal swabs (HVS) and cervical swabs (CS) were collected from all 250 patients using sterile cotton swabs. The HVS was obtained from posterior and lateral vaginal fornices, while the CS was collected under direct view, using an unlubricated Cusco's speculum. Venous blood (10 mL) was collected from each patient.

The HVS and CS samples were cultured and then examined by light microscopy. Drug sensitivity patterns of isolated organisms were also determined. Plasma samples were tested for the presence of HIV antibodies.

At sample collection, the characteristics of the discharge (eg quantity, colour and odour) were recorded. The pH of each sample was determined with pH 1–14 litmus paper. Production of a fishy odour on addition to the sample of two drops of 10% potassium hydroxide was recorded as a positive amine test.

### Microscopy
Wet preparations from the HVS and CS samples were prepared in physiological saline and examined by light microscopy (x10 to x40 objectives). These was used to determine the presence of pus cells, clue cells, normal epithelial cells, yeasts cells and trichomonads. Smears from the swab samples were prepared on clean, grease-free glass slides then fixed and stained by Gram's technique and examined (x100 oil-immersion objective) for the presence of bacterial and yeast cells.

**Fig. 1.** Age distribution and frequency of the three broad classes of agents that caused vaginitis.
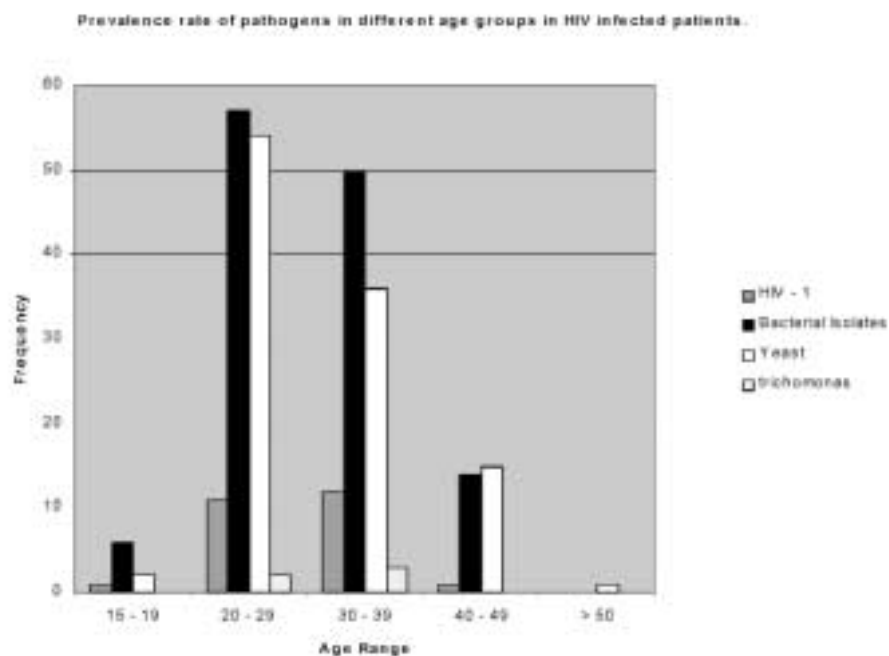
## Culture

Immediately upon collection, HVS and CS samples were inoculated on sterile chocolate, MacConkey and blood agar plates. Chocolate plates were incubated in an atmosphere of 5–10% carbon dioxide, while the MacConkey and blood agar plates were incubated conventionally. All plates were incubated at 37°C for 24–72 h. Resultant growths were identified by culture characteristics, microscopy and biochemical tests. Gram's stain was used to establish the morphology and staining of the culture organisms. Haemolytic properties of blood and chocolate agar plates were also used to aid culture identification.

## Susceptibility testing

Microbial sensitivity tests were performed using Stokes' disc diffusion technique. Briefly, light suspensions of pure culture were inoculated evenly on the entire surface of sterile sensitivity agar plates. Gram-negative and -positive antibiotic discs (ABTECK Biological, Liverpool, UK) were placed on the inoculated agar plates and these were incubated at 37°C for 24 h. The plates were examined for zones of inhibition. S. aureus (ATCC 25923) was used as the positive control.

## Serology

Plasma samples were screened for the presence of HIV antibodies using the Immunocomb rapid test kit (Yaune, 70650, Israel). Positive samples were confirmed by enzyme-linked immunosorbent assay (Immunoconfirm kit) for HIV-1 and HIV-2. Serology for Chlamydia trachomatis and syphilis were not performed; however, samples were stored at -80°C for future testing, if this proved necessary.

## Data analysis

The data obtained were subjected to statistical analysis by $\chi^2$ analysis to test the significance between infectious vaginitis and HIV.

## Results

Although the age range of the study population was 15–50 years, more of the patients fell within the 20–29 age range. All the patients complained of abnormal vaginal discharge and 120 (48%) had PID. Of the latter group, 20% complained of infertility (primary infertility 12%, secondary infertility 16%). Eighty (32%) complained of itching and 10 (4%) suffered a septic abortion. Vaginal pH >5.0 was recorded in 150 (60%) of the patients and moderate to profuse discharge was recorded in 170 (68%). A positive amine test was found in 100 patients (40%), while the presence of clue cells was recorded in 70 (28%).

Microscopy and culture results are shown in Table 1. The distribution of specific microbial agents and their frequency are reflected in Table 2. Figure 1 shows age distribution and the frequency of isolation of the three broad classes of causative agents. Bacterial and fungal agents were responsible collectively for infection in 96% and 72%, respectively, in HIV-negative and HIV-positive patients (Table 3).

## Discussion

The most common infectious causes of infectious vaginitis are BV, trichomoniasis and candidiasis. Of the bacterial causes of vaginitis in the present study, *Staphylococcus* species accounted for the highest number of cases (23%) and agrees with a previous study by Egwari *et al.*,[13] who showed that *S. epidermidis* and *E. coli* gave the highest isolation rate (35%).

The prevalence of *N. gonorrhoeae* in this study was quite low (2%) and this could be attributed to the fact that the organism is quite fastidious and thus difficult to culture, although *N. gonorrhoeae* infections among Nigerian women are on the decline. However, in a previous study,[13] the

prevalence of *N. gonorrhoeae* was 10% in patients not on antibiotic treatment and was absent from those on antibiotics.

*G. vaginalis*, one of the most important aetiological agents of BV, was not isolated in this study; however, Rotimi *et al*.[14] identified it by Gram's stain in all of their patients with BV. *G. vaginalis* identification by Gram's stain is regarded as the gold standard,[15] being a more sensitive technique than culture or the examination of a wet preparation, and McCormack *et al*.[16] isolated it from 30% of woman without BV. The absence of *G. vaginalis* among the women in the present study could be as a result of its decreasing incidence among Nigerian women.

Depletion of lactobacilli in patients with BV in this study supports others who suggest that a reduction in the number of lactobacilli occurs when the vagina becomes infected with other organisms.[6,17]

In the present study, bacteria and fungi were more prevalent among patients in the 20–39 age range. The factors related to this increase could include sexual activity, number of sexual partners, menstrual cycle, socio-economic factors, concomitant infections, personal hygiene, use of contraceptives and antibiotics, and the greater likelihood of abnormal uterine bleeding.[18]

The prevalence of *T. vaginalis* was low (2%) and consistent with the findings of Kent,[9] who noted that the incidence of trichomoniasis is declining and currently is responsible for less than 25% of vaginal infections.[19] However, the presence of *T. vaginalis* has been associated with an increased risk of HIV-1 infection.[18]

Yeast infections showed the second highest prevalence (44%) in this study and the questionnaire revealed that approximately 5% of patients with such infections were diabetic.

In the study group, 25 (10%) were HIV-1-seropositive, 80% of which had infectious vaginitis.

Overall, a broad spectrum of microbial agents was shown to be responsible for vaginitis in the group of patients studied. Molecular epidemiological studies on the organisms isolated are in progress; however, future study should focus on anaerobic bacterial vaginosis. □

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