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Evaluation of microorganisms associated with vaginal infections in Owo, Nigeria

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ABSTRACT

This study was designed to investigate the prevalence of pathogenic microorganisms associated with vaginitis among women of reproductive age in Owo, Nigeria and its environment. Sixty five women visiting the gynaecology unit of the Federal Medical Centre Owo were recruited for this study between December 2010 and May 2011. High vaginal swabs were obtained from the subjects following informed consent with the assistance of the consultants. The bacteria isolated were Staphylococcus aureus, S. cohnii, S. massiliensis, Micrococcus lylae, Luteococcus sanguinis, Brevibacterium epidermidis, Burkholderia cepacia, Fransciella philomiragia, Neisseria gonorrhoeae, OFBA-1, Bacillus megaterium, Citrobacterfreundii, Citrobacter spp. and Escherichia coli. Candida albicans was isolated from 17% of the subjects along with bacteria. Organisms isolated from cases of pelvic inflammatory diseases are S. aureus, S. cohnii, Bacillus megaterium, Brevibacterium epidermidis, Franciella philomiragia, Escherichia coli, Citrobacter freundii and Candida albicans. Isolates from patients with infertility include Brevibacterium epidermidis, S. aureus, Luteococcus sanguinis, Citrobacter freundii, OFBA-1 organism, Micrococcus lylae, Neisseria gonorrhoeae and Candida albicans. Burkholderia cepacia and S. aureus were isolated from cases of delay and infrequent menstruation. Organisms isolated from pregnant women were S. aureus, S. cohnii and L. sanguinis. Isolated bacteria gave varied resistant patterns to the antibiotics tested. The Gram-positive organisms were more resistant to the combination of ampicillin/cloxacillin with 21.74% susceptibility, while the Gram-negative organisms were highly susceptible to ciprofloxacin, sparfloxacin and streptomycin (91.67% each). Resistance to multiple drugs were reported among the bacterial isolates

Key words: Microorganisms, vaginitis, drug resistance.

INTRODUCTION

The human vagina normally has a mucous discharge which helps to keep the vagina moist and clean. This normal discharge is clear, has no odour and causes no irritation. However, a deviation from this norm suggests vaginitis; an inflammation or infection of the vagina. Vaginitis is a common medical complaint in women of reproductive age, especially vaginal infection of bacteria origin (bacterial vaginosis, BV) [1]. Bacterial vaginosis, one of the common causes of malodorous vaginal discharge, has a very low awareness in women probably due to the asymptomatic nature of the infection. Thus, most women self-medicate and when symptoms get complicated, they seek medical attention [1].

The vaginal microbial ecosystem is balanced by a predominance of protective lactobacilli [2], but when there is a shift in this balance, an overgrowth of vaginal anaerobes or Gram negative bacteria (such as *Atopobium vaginae, Gardnerella vaginalis, Megasphaera* spp, *Mycoplasma hominis, Ureaplasma urealyticum*), result in infection and malodorous discharge from the vagina [3]. Other microorganisms implicated in vaginosis are *Trichomonas vaginalis* (trichomoniasis), and *Candida albicans* (candidiasis). The vaginal flora varies based on different factors such as

hormonal, multiple partners, use of oral contraceptives, and use of antibiotics for infections in other body parts, use of condoms, smoking and bad habits in terms of hygiene. All these are risk factors for genital infections [4, 5].

Bacterial vaginosis has been associated with increased susceptibility to sexually transmitted infections (Herpes Simplex Virus, Human Papilloma Virus, Human Immunodeficiency Virus), and has also been linked to adverse pregnancy outcomes like premature membrane rupture, intra-amniotic infection, premature labour and delivery, abortion and low-birth-weight infants; thus, BV is of great interest to most clinicians [6, 7]. A combination of vaginal pH at >4.5, thin homogeneous vaginal discharge, clue cells in vaginal fluid when examined microscopically and amine/'fishy' odour is used in clinical diagnosis [8], while microbiological diagnosis employs the use of gram staining grading by Nugent score, which places BV at a score of 7 – 10 [8].

MATERIALS AND METHODS

Study area and population

This study was carried out at Federal Medical Centre Owo, Ondo state, Nigeria over a six month period (December 2010 – May 2011). Ethical clearance was obtained from the Ethical Review Committee of the hospital prior to carrying out this study. The investigation was carried out on women of reproductive age and high vaginal swab samples were obtained from Sixty-five patients with the use of swab sticks. Information was obtained from each patient as regards, marital status, state of pregnancy (pregnant or not pregnant) as well as infertility. Microbiological investigations were carried out on the samples obtained in the Microbiology Laboratory of Achievers University, Owo, Nigeria.

Collection and processing of samples

Exposing the posterior fornix with a sterile vaginal speculum, a sterile swab stick was inserted to pick a high vaginal swab. The swab stick was immediately replaced in its casing and labeled appropriately. Each specimen was refrigerated at 4^{o}C as soon as it was collected.

Inoculation, isolation, characterization and identification

A microscopy examination was first carried out on the High Vaginal Swab (HVS) specimen by adding normal saline into the swab sticks and placing a drop on a glass slide for viewing under the microscope in order to check for pus cells, epithelia cells, yeast cells etc. Another few drops of the sample was placed in the nutrient broth and incubated at 37^{o}C for 24h. A sterile wire loop was used to inoculate from the nutrient broth on nutrient, blood and chocolate blood agar plates. The plates were incubated at 37^{o}C for 24h and thereafter observed for obvious microbial growth (colonies) on the surface of the culture plate. Subsequent sub-culturing in selected media was carried out to further purify the isolates. Cultures were Gram-stained and morphologies of the organisms observed under the microscope.
Biochemical tests were carried out on the bacterial isolates as described by Barrow and Feltham [10]. Identification of microorganisms, based on cultural, microscopic and biochemical characteristics was determined using an online bacteria identification system, the Gideon Informatics [11], with reference to Barrow and Feltham [10], and Garrity et al [12].

Antibiotic susceptibility test

Susceptibility test was determined using antibiotic disc after due sub-culturing. Briefly, the isolates, adjusted to McFarland standard, were inoculated in Muller Hilton agar plates, streaked evenly using cotton swabs and allowed to set at room temperature. Antibiotic discs were placed on the set agar plates, allowed to equilibrate at room temperature for 15 minutes and finally incubated at 37^{o}C for 24h. Thereafter, the plates were observed for obvious zone of clearing, and recorded as either susceptible or resistant, as described by Clinical and Laboratory Standards Institutes [13, 14]. The antibiotics tested were Amoxycillin, AMX 20µg , Augumentin (Amoxycillin/clevulanic acid), AUG 20/10µg, AmpicillinCloxacillin, AMC 10/10µg , Ceftriaxone, CRO 30µg, Gentamycin, GEN 10µg, Ciprofloxacin, CIP 5µg, Perfloxacin, PEF, Ofloxacin, OFL 5µg, Erythromycin, ERY 15µg, Trimethoprim/ Sulphamethazole, COT 1.25/23.75µg, , Chloramphenicol, CHL 30µg, Cefuroxime, CXM 30 µg, Streptomycin, STR 10µg, Sparfloxacin,SPA 5µg.

RESULTS AND DISCUSSION

A total of sixty five vaginal swabs were obtained from 26 singles and 39 married women. Microorganisms were isolated from the vagina of all the women studied. The bacteria isolated were *Staphylococcus aureus*, *S. cohnii*, *S. massiliensis*, *Micrococcus lylae*, *Luteococcus sanguinis*, *Brevibacterium epidermidis*, *Burkholderia cepacia*, *Fransciella philomiragia*, *Neisseria gonorrhoeae*, *OFBA-1*, *Bacillus megaterium*, *Citrobacter freundii*, *Citrobacter spp.* and *Escherichia coli*. *Candida albicans* was isolated from 17% of the subjects along with bacteria. The prevalence of these microbial isolates was as presented in Figure 1.

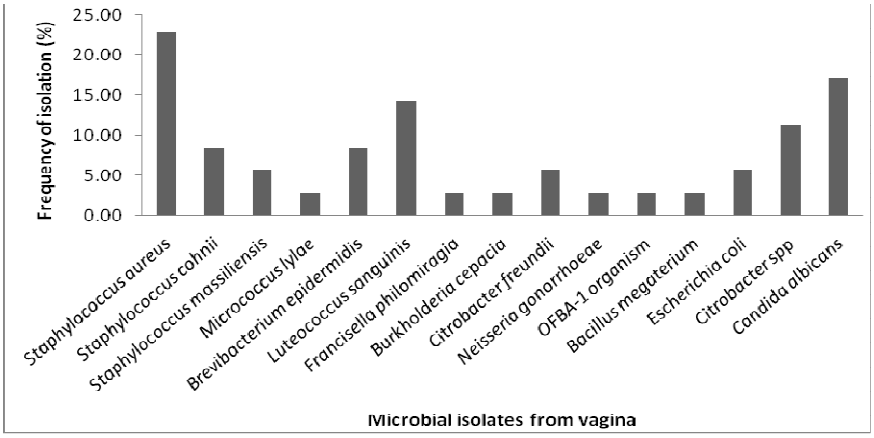

Figure 1: Frequency of isolation of microorganisms from vagina

TABLE1: Distribution of microbial isolates from high vagina swab along marital status

Single	Married
Staphylococcus aureus *Bacillus megaterium* *Staphylococcus massiliensis* *Brevibacterium epidermidis* *Francisella philomiragia* *Burkholderia cepacia* *Citrobacter freundii* *Candida albicans*	*Brevibacterium epidermidis* *Micrococcus lylae* *Staphylococcus aureus* *Citrobacter freundii* *Staphylococcus cohnii* *OFBA-1* *Neisseria gonorrhoeae* *Escherichia coli* *Luteococcus sanguinis* *Candida albicans*

TABLE 2: Distribution of microbial isolates from high vagina swab along diagnosis

PID/vaginal discharge	Infertility	Delay/infrequent menstruation	Pregnancy
Bacillus megaterium *S. aureus* *Brevibacterium epidermidis* *Francisella philomiragia* *S. cohnii* *Escherichia coli* *Candida albicans*	*Brevibacterium epidermidis* *S. aureus* *L. sanguines* *Citrobacter freundii* *OFBA-1* *Neiseria gonorhoeae* *Candida albicans*	*S. aureus* *Burkholderia cepacia* *Candida albicans*	*S. aureus* *L. sanguinis* *S. cohnii* *Micrococcus lylae* *Candida albicans*

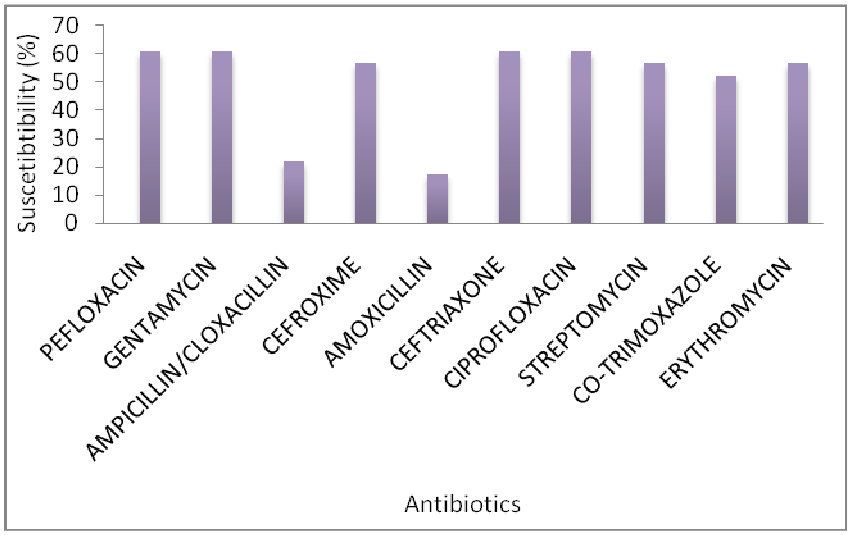

Figure 2: Susceptibility of Gram positive bacterial isolates from vagina to antibiotics

The distribution of the isolated microorganisms along marital status and clinical diagnosis are presented in Tables 1 and 2 respectively. Organisms isolated from cases of PID are *S. aureus, S. cohnii, Bacillus megaterium, Brevibacterium epidermidis, Franciella philomiragia, Escherichia coli, Citrobacter freundii* and *Candida albicans.*

Isolates from infertility patients include *B. epidermidis, S. aureus, Luteococcus sanguinis, Citrobacter freundii, OFBA-1 organism, Micrococcus lylae, Neisseria gonorrhoeae* and *Candida albicans. Burkholderia cepacia* and *S. aureus* were isolated from cases of delay and infrequent menstruation. Organisms isolated from pregnant women were *S. aureus, S. Cohnii, M. lylae* and *L. sanguinis* (Table 3).

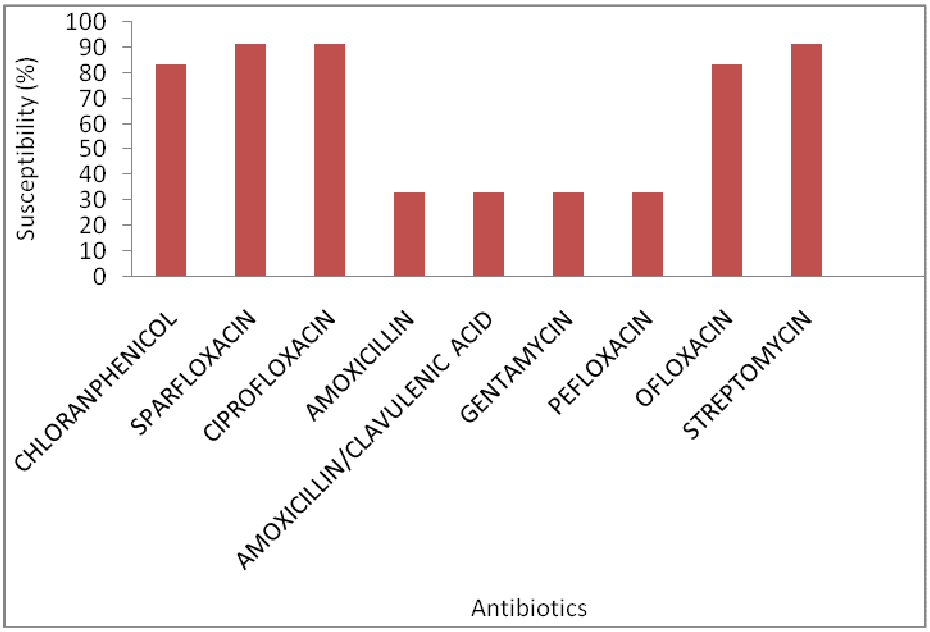

Figure 3: Susceptibility of Gram negative bacterial isolates from vagina to antibiotics

Table 3: Multi-drug resistant patterns exhibited by bacterial isolates from high vaginal swab

DRUG COMBINATION	FREQUENCY
Gram positive	
PEF/GEN/AMC/AUG/CXM/CRO/CIP/STR/COT/ERY	7
AMC/AUG/CXM	4
AMC/AUG	3
NO RESITANCE	6
Total	20
Gramm negative	
COT/CHL/SPA/CIP/AMX/AUG/GEN/PEF/OFL/STR	2
COT/CHL/AMX/AUG/PEF/OFL	3
COT/AMX/AUG/GEN/PEF	3
AMX/AUG/GEN/PEF	11
AMX	3
No resistance	8
Total	30

NR = No Resistance to any of the antibiotics used

Isolated bacteria showed varied resistant patterns to the antibiotics tested. The Gram-positive organisms were more resistant to ampicillin/cloxacillin with 21.74% susceptibility. The Gram-negative organisms were highly susceptible to ciprofloxacin, sparfloxacin and streptomycin (91.67% each). High prevalence of multi-drug bacteria was reported. Seven (35%) of the 20 Gram-positive isolates tested were resistant to all the antibiotics used, while 19 (63%) of the 30 Gram-negative bacteria were resistant to 3-10 antibiotics (Table 4).

In this study of microorganisms associated with vaginal infections, both bacterial and fungal isolates have been implicated. *Staphylococcus aureus* belongs to those pathogenic bacteria not commonly present in the vagina but however, have been implicated in vaginitis. Infection of the vagina by intestinal flora is quite common due to the close proximity of the anus to the vagina [15]. Furthermore, it was also reported that whenever *Lactobacillus* species is displaced by an overgrowth of pathogens like *Escherichia coli*, Group B *Streptococcus, S. aureus* and *Enterococcus faecalis* (all organisms classed as aerobic vaginitis, AV pathogens), clinical signs such as itching/burning sensation, dyspareunia, yellowish discharge and increased pH are triggered. In a study of 631 patients attending routine prenatal care, 7.9% have moderate to severe AV signs and symptoms [15]. In another study of 3000 women, 4.3% were found to have severe AV, in which 49.5% of them were postmenopausal [16]. Aerobic vaginitis was also recorded at 12.6% in another study of 215 women [17]. This study records the presence

of plausible AV pathogens in all the women attending the clinics, although the pathogenicity of these organisms were not determined as many of them could constitute vaginal microflora, but being drug resistant is a serious concern. While vulvovaginal candidiasis was recorded at 17% of the subjects, of noteworthy is the occurrence of *S. aureus* in all the categories of women.

The therapy for AV should include an antibiotic with an intrinsic activity against the majority of bacteria of faecal origin. Optimal treatment includes antibiotic with little/no effect on normal flora, especially *Lactobacillus* species, while eradicating the enteric and other species of contamination. In a study that measured the MIC of prulifloxacin, ciprofloxacin, ofloxacin, erythromycin, doxycycline, clindamycin, ampicillin, kanamycin and vancomycin against some vaginal *Lactobacillus* species, kanamycin, ciprofloxacin and ofloxacin were reported to show the best MIC result and in a concentration range potent enough against AV pathogens [18]. The fluoroquinolones (ciprofloxacin, ofloxacin and pefloxacin) have been reported by other researchers to have good clinical successes, with little effect on the normal flora of the vagina, allowing for rapid recovery [19, 20]. This study also recorded a high percentage potency of fluoroquinolones against AV pathogens at 60.87% and 91.67% for gram positive and gram negative respectively.

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