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Microorganisms implicated in vulvovaginitis among patients attending a teaching hospital in southeastern Nigeria

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Abstract

Vulvovaginitis is a common infection of the vulva and vagina with symptoms such as irritation of the genital area, itching, inflammation around the labia and perineal areas, strong-smelling vaginal discharge and discomfort while urinating. The etiological agents include bacteria, yeast, viruses, parasites, environmental factors, exposure to chemical irritants and allergens. Microorganisms implicated in vulvovaginitis among patients attending a teaching hospital in Southeastern Nigeria were analyzed using standard Microbiological procedures. High vaginal swab (HVS) specimens (100) were properly and aseptically collected from suspected cases of vulvovaginitis after ethical clearance was duly obtained from the University Teaching hospital. Pure microbial cultures were obtained after 24 hours incubation at 25 °C, on Nutrient agar, Sabouraud Dextrose agar and Blood agar plates. The isolates were identified based on their morphological, physiological, biochemical and molecular characteristics. Ninety-six percent of the HVS specimens yielded microbial growth {bacteria (54%) and fungi (46%)}; while no growth was observed in 4%. The bacteria isolates include *Staphylococcus epidermidis* (42%), *Escherichia coli* (13%), *Lactobacillus spp* (30%), and *Staphylococcus aureus* (15%); while the fungi isolates include *Candida albicans* (50%), *Candida tropicalis* (30%) and *Candida glabrata* (20%). The study revealed that bacterial and fungal organisms are the commonest etiological agents of vulvovaginitis. Adequate and proper personal hygiene is very important in prevention and management of vulvovaginitis.

Keywords: Vulvovaginitis; Etiological agents; Specimens; Hygiene

1. Introduction

Vulvovaginitis refers to the inflammation of the vulva and vagina resulting to pruritus, erythema, pain, vulva abrasions, as well as changes in the color, odor or amount of vaginal discharge (Attie *et al.*, 2016). Vulvovaginitis is a common complaint in women, accounting for 70% of all visits to gynecologists and being the most common gynecologic problem encountered by primary care providers for women (Shahinfar and Nemanpour, 2017). Inflammation of the vulva and vagina is caused by various infectious and non-infectious factors (Donders, 2007). The most common causes of infectious vaginitis are bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and trichomonal vaginitis (TV) (Workowski and Bolan, 2015).

The healthy vaginal tract of reproductive-aged women is colonized by normal microbiota dominated by Lactobacilli, which protect against pathogenic bacteria species when present in sufficient numbers. Therefore, depletion of lactobacilli distorts the balance of the vaginal microbiota and leads to an increase in anaerobic organisms, contributing to bacterial vaginitis (Ventolini, 2016). The alteration of host environment impairs its ability to control opportunistic

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pathogens contained in the resident microbes that could invade the human body and cause illness (Lloyd-price *et al.*, 2019). Although bacterial vaginitis is most commonly asymptomatic, it can be characterized by the discharge of homogeneous grayish-white smelly secretions, fishy smell after intercourse or during menstruation and an elevation of vaginal pH to above 4.5 (Paladine and Desai, 2018). The prevalence of bacterial vaginitis ranges from 8 to 51%, depending on geographical location, socioeconomic status and ethnicity (Dembélé *et al.*, 2021).

Vulvovaginal Candidiasis (VVC) is caused by the overgrowth of yeasts, mainly *Candida albicans*, which are essentially part of the vaginal flora (Shahinfar and Nemanpour, 2017). This fungus requires an oestrogenised vaginal epithelium, so it is seldom a cause of symptoms in postmenopausal women, unless they are taking hormone replacement therapy. The usual symptoms of vulvovaginal candidiasis include vaginal discharge, itching, pain, and swelling. In addition, vulvar erythema and edema with excoriations are common findings, dysuria and dyspareunia (Paladine and Desai, 2018).

Trichomonas vaginalis is a flagellate protozoan parasite that causes trichomoniasis, which is mainly characterized by severe vaginitis among symptomatic females. Its transmission is usually sexual, and frequent recurrences often occur if the male partner is not simultaneously treated. Women with *Trichomonas vaginalis* may complain of yellowish-green, foul-smelling, frothy vaginal discharge (Abdul-azizet *et al.*, 2019).

However, vulvovaginitis symptoms do not necessarily indicate an infection. Instead, they may result from other conditions that affect the vagina or vulva. For example, chemicals or other materials (such as hygiene products, bubble bath, laundry detergents, contraceptive foams and jellies, and synthetic underwear) can irritate the vagina and cause a discharge and discomfort. The inflammation that results is called non-infectious (inflammatory) vulvovaginitis. Another form of non-infectious vaginitis is atrophic vaginitis, which can occur in women after menopause because the vaginal tissue becomes dry and prone to irritation due to a decreased level of estrogen. The investigation of intimate habits such as depilation, sexual practices, types of clothing, vaginal douching, sanitary napkins and adornments may guide health professionals as to the appropriate orientation to women to avoid physiological disorders or infections. Adherence to hygienic measures and life-style changes towards medical practice is in line with medicine and public health used in disease prevention (Giraldo *et al.*, 2016).

Traditionally, the diagnosis of bacterial vaginosis is based on microscopy and clinical symptoms, as reflected in Amsel criteria and the Nugent scoring system, which assigns a value to different bacterial morphotypes seen on Gram stain of vaginal secretions (Amsel *et al.*, 1983). Culture for yeast is the reference standard for diagnosing VVC. In examination of a wet mount with KOH preparation, the presence of budding yeast or hyphae on microscopy is diagnostic (Sheppard, 2020).

This study investigates the microorganisms implicated in vulvovaginitis among patients attending a Teaching Hospital in Southeastern Nigeria.

2. Materials and methods

2.1. Study area

The study was carried out at the Laboratory Unit of Department of Applied Microbiology and Brewing, Nnamdi Azikiwe University, Awka, Anambra State, South-East geopolitical zone of Nigeria, between december, 2023 – may, 2024.

2.2. Ethical considerations

Ethical Clearance was obtained from Ethical Committee of Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Anambra State. This was done to enable us access High vaginal swab specimens from patients.

2.3. Specimen collection

Hundred high vaginal swab (HVS) specimens were aseptically and properly collected from suspected cases of vulvovaginitis at Chukwuemeka Odumegwu Ojukwu University Teaching Hospital and immediately taken to the laboratory for analysis.

2.4. Isolation of Microorganisms

Wet mounts of the specimens were prepared using sterile grease-free microscope glass slide and normal saline (0.85% NaCl) to observe for the presence of pus cells, epithelia cells, yeast cells and parasite. Plates of Sabouraud dextrose agar

supplemented with chloramphenicol (50 µg/mL) and nutrient agar supplemented with nystatin (100 U/mL) were inoculated with the HVS specimens, using the spread plate method. The plates were incubated at 25 °C for 24 hours and thereafter observed for microbial growth (Pagana *et al.*, 2019).

2.5. Pure culture isolation and identification

The bacteria and fungi isolates were purified by sub culturing on fresh plates of Nutrient agar or Sabouraud dextrose agar and identified based on their morphological, physiological, biochemical and molecular characteristics. The identification tests include macroscopic appearance of the colonies, Gram' stain reactions (Oyli, 2017), Lactophenol cotton blue stain reaction, Growth on cornmeal tween-80 Agar (Pincus *et al.*, 2007), Germ tube test (Udemezie and Oyeka, 2021), Citrate utilization, Catalase, Coagulase, Indole and Oxidase tests (Dawodu and Akanbi, 2021), Motility (Jain *et al.*, 2020), Voges-Proskauer (VP) and sugar fermentation tests (Cheesbrough, 2010); and Nucleic acid sequence analysis (Campbell *et al.*, 2013).

3. Results

Table 1 Morphological and biochemical characteristics of the bacteria isolates

isolate	Colony	Shape	Gram Reaction	Catalase	Citrate	Motility	Indole	Coagulase	Hemolysis	Suspected Organism
1	Yellowish Cocci, Convex & opaque	Cocci	+	+	+	-	-	+	+	<i>S. aureus</i>
2	Grayish Rod and Moist	Rod	-	-	-	+	-	+	+	<i>Escherichia coli</i>
3	Whitish Cocci Raised Convex & opaque	Cocci	+	+	-	-	-	-	-	<i>S. epidermidis</i>
4	Rough, rod Opaque, & creamy white	Rod	+	+	-	-	-	-	-	<i>Lactobacillus spp</i>

Table 2 Microscopic Morphologies and Germ tube test

Characteristic features	Isolates		
	<i>C. albicans</i>	<i>C. tropicalis</i>	<i>C. glabrata</i>
Germ tube	+	-	-
Chlamydo spores	+	+	-
Pseudohyphae	+	+	-
Blastospores	+	+	+
Arthrospores	-	-	-

+ = positive
- = Negative

Table 3 Sugar Fermentation of Bacteria Isolates

Sugars	<i>S. epidermidis</i>	<i>E. coli</i>	<i>S. aureus</i>	<i>L. spp</i>
Fructose	+	+	+	-
Sucrose	+	+	+	-
Lactose	+	+	+	-
Glucose	+	+	+	+
Maltose	+	+	+	+
D-xylose	-	+	-	-
Galatose	-	+	-	-
Cellebiose	-	-	+	-
Dextrose	-	-	-	-
Trehalose	+	+	+	+
Raffinose	-	-	-	-
Mannose	+	-	+	-

+ = positive; - = Negative

Table 4 Sugar Fermentation of Fungi Isolates

Sugars	<i>C. albicans</i> strain M52C	<i>C. albicans</i> strain M188B	<i>C. glabrata</i>	<i>C. tropicalis</i>
Fructose	+	+	+	+
Sucrose	+	+	-	+
Lactose	-	-	-	-
Glucose	+	+	+	+
Maltose	+	+	+	+
D-xylose	+	+	-	+
Galatose	+	+	-	+
Cellebiose	-	-	-	+
Dextrose	+	+	+	+
Trehalose	+	+	+	+
Raffinose	-	-	-	-
Mannose	-	-	-	-

+ = positive ; - = Negative

Table 5 Distribution of fungi Isolates from High Vaginal Swab Specimens

Isolates	No. of isolates	Percentage (%)
<i>Candida albicans</i>	22	50
<i>Candida tropicalis</i>	13	30
<i>Candida glabrata</i>	9	20
Total	44	100

Table 6 Distribution of Bacteria Isolates from High Vaginal Swab Specimens

Isolates	No. of isolates	Percentage (%)
<i>Staphylococcus epidermidis</i>	22	42
<i>Escherichia coli</i>	7	13
<i>Lactobacillus spp</i>	16	30
<i>Staphylococcus aureus</i>	8	15
Total	52	100

4. Discussion

The study investigated microorganisms implicated in vulvovaginitis among patients attending Chukwuemeka Odumegwu Ojukwu University Teaching Hospital, Awka, Southeastern Nigeria. A total of 100 HVS specimens were properly and aseptically collected from suspected cases of vulvovaginitis. After 24 hours incubation, 96% of the specimens yielded microbial growth; 54% bacteria and 46% fungi.

The bacteria isolates include *Staphylococcus epidermidis* (42%), *Escherichia coli* (13%), *Lactobacillus spp* (30%), and *Staphylococcus aureus* (15%) as shown in table 6. Similar results were also obtained in studies by Okiki *et al.* (2015) and Thais *et al.* (2020). Thais *et al.* recorded higher rates of bacterial vaginosis with prevalence rate of 83%. *Staphylococcus epidermidis* is usually a symbiont that is harmless in its natural environment. However, it is an opportunistic pathogen that can cause virulence once it invades the human body via medical and prosthetic devices (Kleinschmidt *et al.*, 2015). Thus, *Staphylococcus epidermidis* found in reproductive women in this study could be suggestive of the use of intra-uterine device. *Staphylococcus aureus* is a pathogenic bacterium not commonly found in vagina but however has been implicated in vaginitis. Close proximity of the anus to the vagina brings about infection of the vagina by intestinal flora such as *Escherichia coli*, which is quite common.

Lactobacillus species accounted for 30% of the total bacterial isolates. Witkin and Linhares, (2017) noted that *Lactobacillus species* play protective and probiotic roles in the treatment and prevention of vaginal infections by producing antagonizing compounds and is regarded as safe for humans. It is worthy of note that *Lactobacillus species* are the main source of L- lactic acid and D-lactic acid that keep the pH value of the vagina area lower than 4.5. Furthermore, whenever *Lactobacillus species* is displaced by an overgrowth of pathogens like *Escherichia coli*, Group B Streptococci, *S. aureus* and *Enterococcus faecalis*, clinical signs and symptoms such as itching, burning sensation, dyspareunia, yellowish discharge and increased pH are triggered (Okiki *et al.*, 2015).

The study revealed that vulvovaginal candidiasis is the second common cause of vulvovaginitis. The overall prevalence of *Candida spp* was 46%: *Candida albicans* (50%), *Candida tropicalis* (30%) and *Candida glabrata* (20%) as shown in table 5. The finding is in accordance with Patrick *et al.*, (2022) who observed a higher prevalence of *Candida albicans* (67%) and opined that this could be attributed to the enhanced ability of this fungus to adhere to the vaginal mucosa, a fundamental step in the establishment of vulvovaginal candidiasis. Udemezie and Oyeka (2021) recorded a similar occurrence frequency among *Candida species*. The findings above are in concordance with the fact that *Candida* is an opportunistic pathogenic fungus that normally inhabits various anatomical sites of the human body including the gastrointestinal and genitourinary tracts.

5. Conclusion and recommendation

It is important to know the etiological agents of vulvovaginitis and observe appropriate treatment and preventive procedures to duly manage its health outcomes. High prevalence of vaginal infections points to the need for greater monitoring, evaluation and guidance regarding the prevention and management of these infections.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

Statement of ethical approval

The present research work does not contain any studies performed on animals/humans subjects by any of the authors'.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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