ISSN: 2536-7153 SJMLS

Sokoto Journal of Medical Laboratory Science 2023; 8(3): 8 - 17

SJMLS - 8(3) - 001

Cervical-Vaginal Dysbiosis: Clinical Query of Infections in Selected Private Medical Laboratories in Abakaliki Ebonyi State Nigeria

Nnaemeka Okorie [1,2]*, Onyekachi Ewa Ibe [2], Iyke-Ogbo Pauline [2,3] Simon Uzor [2], Victor Udoh Usanga [2], Kingsley Omogo [2], Ugomma Ude Agwu [2], Idakari Chinedu Nweke [4], Ibe Uzochukwu Chimdindu [5]

Biomedical Science Department, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia 43400 Serdang, Selangor Malaysia [1], Department of Medical Laboratory Science, Faculty of Health Sciences and Technology Ebonyi State University Abakaliki Postal code: 480214, Nigeria [2], Histopathology Department, Alex Ekwueme Federal University Abakaliki [3],

Department of Medical microbiology and parasitology, Alex Ekwueme Federal University Abakaliki [4], Department of Anatomic Pathology, Alex Ekwueme Federal University Abakaliki [5].

Author for Correspondence*: okorie.nnaemeka@ebsu.edu.ng/ gs65868@student.upm.edu.my/ +234-808-598-1168/ +60-112-425-1782. *https://dx.doi.org/10.4314/sokjmls.v8i3.2*

Abstract

Cervical-Vaginal Dysbiosis refers to an imbalance or disruption in the microbial communities inhabiting the cervix and vaginal tract, leading to an altered composition of microorganisms, often accompanied by a decline in overall vaginal health. This phenomenon contrasts with the notion of a balanced and stable vaginal microbiome, which is characterized by the predominance of Lactobacillus species, known for their protective properties against infections and maintenance of a low pH environment. This study evaluates the potential of cervical-vaginal dysbiosis among patients with clinical presentation of cervical vaginal infection in Private Medical Laboratories with limited diagnostics resources and specialization. It addresses the need for cytological investigation in addition to microbiological techniques. Clinical queries of cervical-vaginal infections of patients in twelve selected private medical laboratories in Abakaliki Ebonyi state Nigeria was studied. A total number of 38 patients who met the eligibility criteria were recruited into this study. Cervical-vaginal smear was collected from each subject, samples were fixed, and stained using Papanicolaou and Diff-Quick staining method. Out of these 38 smear samples, 17 (44.7%) had normal cytological features with no microbial features observed. Out of the remaining 21 smear samples, Trichomonas vaginalis occurred 9 times (42.9%), followed by fungi occurring 6 times (28.5%), Human papillomavirus occurring 4 times (19.1%) and Actinomyces occurring 2 times (9.5%). Notable cytological features were revealed, these included squamous intraepithelial lesions characterized by parabasal cells showing perinuclear halo, hyperchromatic nuclei, and slight nuclei polymorphism. Inflammatory lesions were also observed, characterized by the conspicuous number of superficial cells, inflammatory background, red blood cells, and infiltrating polymorphs. This study has highlighted the need to adopt a more proactive approach by evaluating the microbial profile of the cervical smear, which can be a risk factor for cervical malignancy; it has also buttressed the need to retrain and incorporate cytology services in private laboratories.

Keywords: *Microbial infection, Vaginal smear, Cytology, cervical-vaginal dysbiosis, Vagina-microbiota*.

Introduction

Women with different cervicovaginal mucosal barrier compositions experience vaginal microbiome dysbiosis and related proteome changes. A lower likelihood of having a dysbiotic vaginal microbiota was linked to higher intakes of tocopherol rich-diet, zinc, and magnesium (Borgdorff *et al*., 2016). It also opined that increased dietary intake of specific micronutrients may lower the risk of bacterial vaginosis and its aftereffects. The typical microbiota and mycobiota, which colonize the human vagina, are made up of a wide variety of microbes. The most common bacteria to be isolated from a healthy human vagina are those belonging to the genus Lactobacillus, including Lactobacillus crispatus, Lactobacillus gasseri, Lactobacillus iners, and Lactobacillus jensenii. These vaginal lactobacilli have been promoted as a means of preventing pathogen invasion by managing their population (Chee *et al*., 2020). The cervicovaginal ecosystem is altered, which leads to an overgrowth of

pathogens and intensifies complicated vaginal infections like bacterial-induced inflammation, STDs, and vulvovaginal candidiasis. The microbial community can change because of predisposing factors like pregnancy, menstruation, sexual activity, unrestrained antibiotic use, and vaginal douching (Chee *et al*., 2020). The construction of the overall defenses against potential pathogens and the maintenance of an acidic environment are both facilitated by microbiota. However, vaginal microbial dysbiosis contributes to the pathogenic process of diseases like bacterial vaginosis, preterm birth, infertility, and intrauterine adhesions. It typically manifests as a deviation from the Lactobacillus-dominated state and an increase of facultative and anaerobic organisms. Interventions aimed at changing the vaginal microbiota have been effective in managing cases of gynecological pathology (Zhao *et al.,* 2023).

Infections incited by micro-organisms in the female genital tract are associated with cytopathic effects (Sabu *et al.,* 2017). Additionally, an inflammatory response at the affected site is invariably present and these changes may be of diagnostic importance (Kaya *et al*., 2013). According to a thorough investigation into the connection between inflammatory cervical smears and cancers, underlying infections can conceal cancerous and precancerous changes as well as hinder the detection of some invasive cervical squamous cell carcinomas (Nayar, 2015). Females have very complex biological ecosystems in their genital tract and gut with about 1013–1014 bacterial cells making up the gut microbiota. The cervicovaginal compartment is home to trillions of bacteria, whereas the upper reproductive tract-uterine cavity was previously largely thought to be sterile. The rectum, which also acts as a reservoir, has been linked to the origin of microorganisms that colonize the cervicovaginal region (Amabebe & Anumba, 2020).

Cervical cancer is a malignant tumor of the cervix that can be divided into two histological types; adenocarcinoma and squamous cell carcinoma (Torre *et al.* ,2015). It is the second most common female malignant tumor globally which seriously threatens female health (Zhang *et al*.,2020). The disease burden of cervical cancer has decreased significantly in developed countries and regions in the last decades; however, it is still serious in less developed countries and regions, and effective preventive measures in these areas still face serious challenges (Zhang *et al*.,2020). At present, various available prevention and control measures are cost-effective and scientific evidence-based to meet the needs of areas with different economic levels (Zhang *et al.,* 2020).

It is worth noting that a strategic consensus on the elimination of cervical cancer has been developed, which is targeted at accelerating the elimination of cervical cancer (Zhang *et al*.,2020). This effort is anchored on the fact that cervical cancer has been clearly linked to persistent infection of high-risk human papillomavirus (HPV) (Zhang *et al*., 2020). The clear etiology accelerated the establishment and implementation of comprehensive prevention and control system for cervical cancer. In May 2018, the World Health Organization (WHO) issued a call for the elimination of cervical cancer globally, and more than 70 countries and international academic societies acted positively immediately (Herrero, 2018).

From the mortality and incidence rates, it is seen that the developing countries are with higher burdens than the developed countries. These countries with a higher burden are not able to implement successfully organized, population-based cervical cancer screening (Tabitha *et al*., 2020), which is largely related to poverty, lack of resources and infrastructure, cum disenfranchisement of women. Even in some countries such as developed countries where screening programs are free of charge and widely available, the uptake is still very low among women. This low uptake is due to a lack of knowledge about the disease and screening practices (Ncube *et al*. 2015).

Besides screening, most factors correlated with developing cervical cancer relate to the cervical immune microenvironment (Klein *et al*., 2020). Recent research into the cervicovaginal microbiome has uncovered intricate relationships between the bacterial microbiota, HPV, HIV, and cervical cancer (Godoy-Vitorino *et al*.,2018; Huang *et al*.,2018). These relationships suggest that certain cervicovaginal microbes, or the microenvironment created by certain microbes, are cofactors of cervical cancer progression. HIV for example, is a well-studied factor in sub-Saharan Africa, which influences the cervical microbiota (Klein *et al.,* 2020). To better understand and address the

relationship between HPV, HIV, cervical microbiota, and increased cervical cancer risk, a better understanding of the diversity of infection correlated with abnormal cervical cellular features.

In 2020, an estimated 10 million cancer-related deaths were reported making it one of the leading causes of death globally. Although this number is predicted to increase worldwide, the rise is expected to occur predominantly in low and middle-income countries such as Nigeria, as they currently face the greatest challenges in tackling the cancer burden (Sung *et al*., 2022). Globally, cervical cancer is the fourth most common female cancer after breast, colorectal, and lung cancer and accounts for 600,000 new cases and 340,000 deaths annually (Cohen *et al*., 2019). Importantly, approximately 83% of all new cervical cancer cases and 88% of all deaths occur in low and middle-income countries (Arbyn *et al*., 2020). Having established that the development of cervical cancer is dependent on the persistence of HPV infection, and the fact that vaginal microbiota composition may participate in human host susceptibility to HPV infection and persistence, this study, therefore, evaluates the cellular features of samples from the cervix of patients suspected of having cervical-vaginal infections in private laboratories since virtually no private medical laboratory within the city is known to run cytological investigations; this made all the patients have been denied of these important testing. Microbial culture and sensitivity tests cannot replace the importance of cytological tests in any biomedical service laboratories. Our research seeks to advocate the inclusion of cervical evaluation in a clinical query of cervical-vaginal microbiota dysbiosis.

Material and Methods

Disposable sterile Improved Ayres' spatula was used for each patient to obtain a cervical-vaginal smear from the patients with the aid of a sterile plastic speculum that visualizes the cervical-vaginal compartment. The obtained smear was immediately transferred to grease free slide, which was labeled with the patient's confidential code identity. Duplicate smears were made and stained with Papanicolaou stain and Diff-Quick stain respectively as described by Okorie (2021). The smears prepared for Papanicolaou staining were fixed in 95% ethyl alcohol (V/V) for 15 minutes before the staining process. The smears were immersed in descending grade of ethanol from 95%, 80% to 60% (V/V) and then dipped five times each in two changes of distilled water. Then stained using Hematoxylin for 2 minutes, differentiated in 0.05% HCl alcohol solution by dipping twice. This was followed by bluing, which involves leaving it in a bath of tap water for 10 minutes, then immersed in 60%, two changes of 80% and 95% ethanol and further stained with Orange G solution (OG-6) for 2 minutes. Washed in three changes of 95% alcohol for two minutes each, and then stained with Eosin Azure (EA-36) for 2 minutes. Repeat wash in four changes of 95% alcohol for 2 minutes each. Rinsed in three changes of 100% alcohol for 2 minutes each and blotted using a sterile filter paper to dry. Subsequently, the slides were placed in three consecutive changes of Xylene for 5 minutes each, except in the last one where it was allowed till it was clear and mounted in DPX mountant as previously described by Okorie *et al.,* 2022.

The Diff-Quick staining procedure was carried out using the rapid Romanowsky stain (HD Supplies, UK). The reagents were made up of three solutions: Solution A- a fixative solution containing Thiazine dye in methanol, solution B containing Eosin Y in phosphate buffer, and Solution C containing Methylene Blue in phosphate buffer. The manufacturer's staining protocols have been followed meticulously.

The solutions were dispensed into respective Coplin jars, and the slides were immersed into the fixative solution for 30 seconds, then transferred without rinsing or drying to Solution B and stained for 30 seconds within which slow agitation of the slide in the solution was also done to enhance adequate penetration of the staining solution. Moreso, without rinsing the slides were transferred to Solution C and stained for 30 seconds, after which it was rinsed briefly in buffered water (pH 6.8) and allowed to dry.

Results

In this study, 38 participants took part, and it focused on the examination of vaginal smears through cytological and microbial analysis. Out of these 38 smear samples, 17 (44.7%) had normal cytological features with no microbial dysbiosis features observed. Out of the remaining 21 smear samples, Trichomonas vaginalis was reported in 9 samples (42.9%), followed by fungi occurring in 6 (28.5%), Human papillomavirus showed a

cytopathic effect 4 (19.1%) and Actinomyces found in 2 (9.5%) (Table 1).

Presented in photomicrographs are the microbial features as well as notable cytological features found in this study including squamous intraepithelial lesions characterized by parabasal cells showing perinuclear halo, hyperchromatic nuclei, and slight nuclei polymorphism. Inflammatory lesions were also observed, characterized by the conspicuous number of superficial cells, inflammatory background, red blood cells, and infiltrating polymorphs all reminiscence the microbiota activities in the lower reproductive region and the corresponding dysbiosis among participants in the various private laboratories.

Figure 1

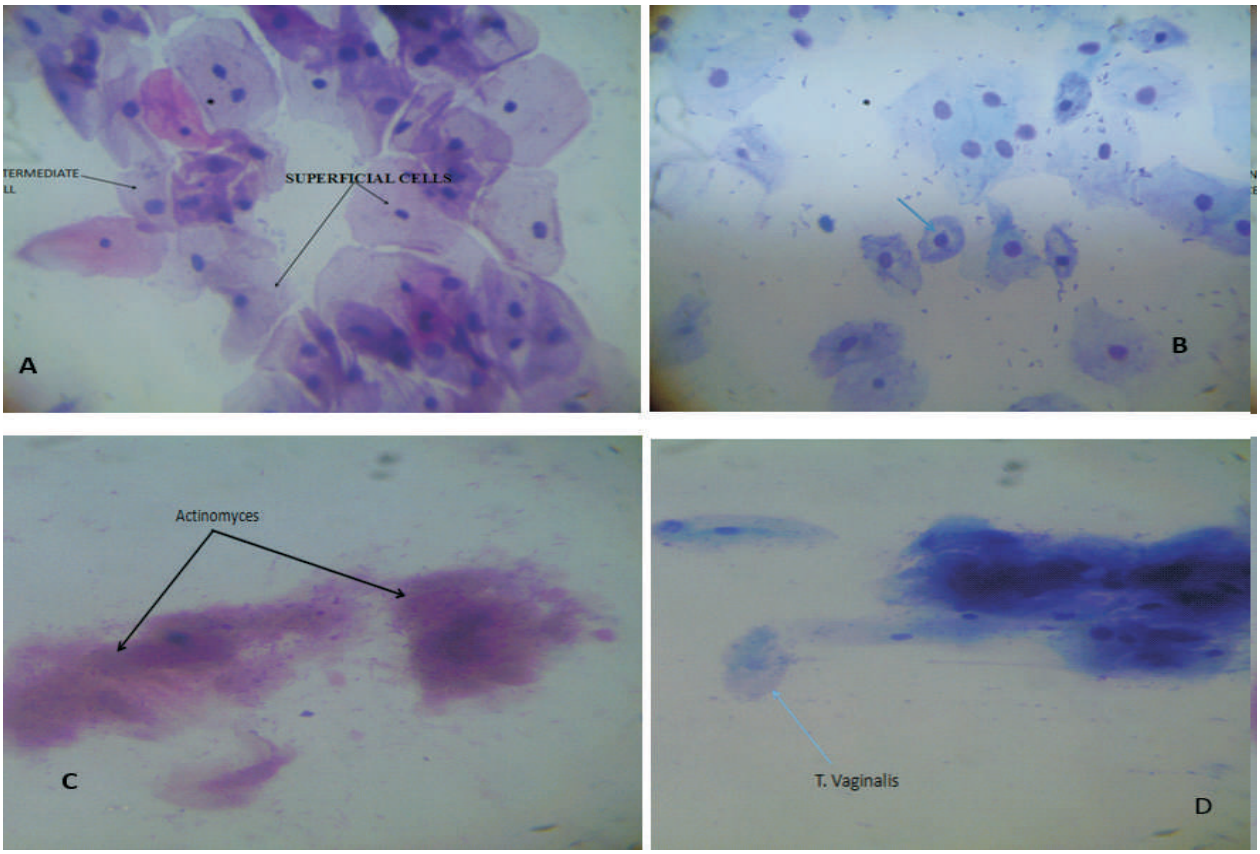

Figure 1. Plate A (Magnification X200): Cervical smear stained with PAP stain. The smear is adequate for evaluation; the cells are predominantly superficial with cytoplasm and nuclei appearing normal. No malignant feature was seen.

Plate B (Magnification X200): Cervical smear stained with Diff Quick stain. The smear is adequate for evaluation and has more intermediate and parabasal cells. The parabasal cells show perinuclei halo (arrow), hyperchromatic nuclei and slight nuclei polymorphism. These features are consistent with high squamous intraepithelial lesion caused by HPV infection.

Plate C (Magnification X200) displays a cervical smear stained with PAP stain. The stain highlights a limited number of superficial cells and points out the presence of actinomyces (indicated by the two arrows).

Plate D (Magnification X200) exhibits a cervical smear stained with Diff Quick stain. The smear reveals typical superficial cells, alongside the distinctive pear-shaped Trichomonas vaginalis.

Figure 2

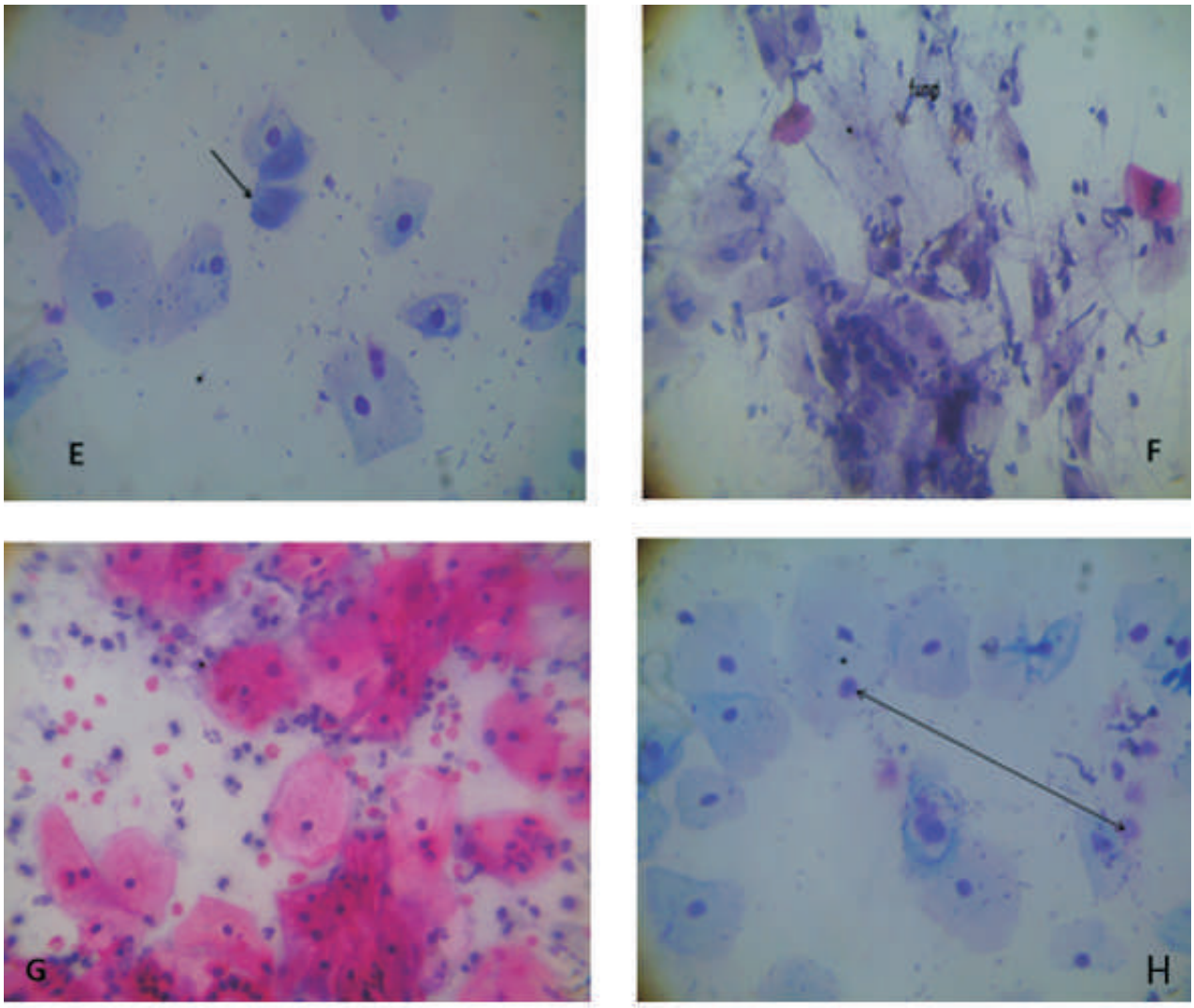

Figure 2. Plate E (Magnification X200): Cervical smear stained with Diff Quick stain. The smear is adequate for evaluation. Seen are predominantly intermediate cells with consistent perinuclei halos, as well as a pear-shaped organism at the hazy background (black arrow); this is indicative of *Trichomonas vaginalis*.

Plate F (Magnification X200): Cervical smear stained with PAP stain. The smear is adequate for evaluation. The cells are predominantly intermediate cells, and few normal superficial cells with pyknotic nuclei. Most significantly revealed in the smear are the fungi with pseudo hyphae.

Plate G (Magnification X200): Cervical smear stained with PAP stain. The smear is adequate for evaluation. It shows a conspicuous number of superficial cells, inflammatory background with red blood cells and marked polymorphs. This is consistent with inflammatory lesion.

Plate H (Magnification X200): Cervical smear stained with Diff Quick stain. The smear shows relatively poor nuclei details of the superficial cells and unclear perinuclei details.

Figure 3

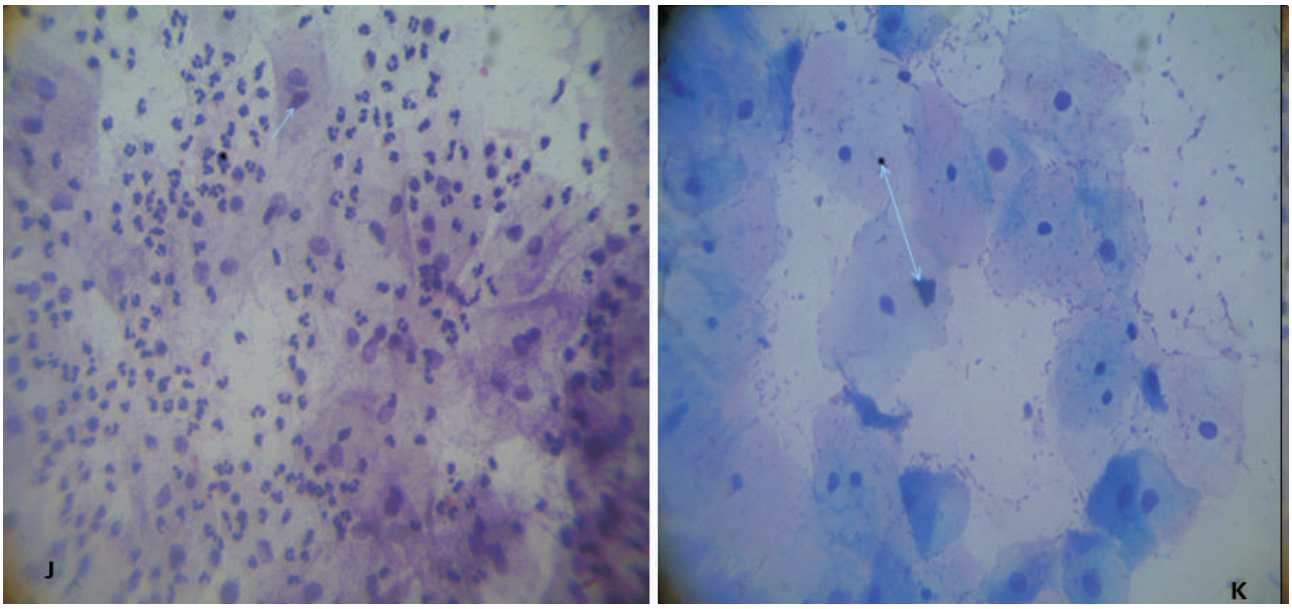

Figure 3. Plate J (Magnification X200) displays a cervical smear stained with PAP stain. Noteworthy features include a blue cytoplasmic bleb (indicated by the arrow) and the presence of infiltrating polymorphs in the background.

Plate K (Magnification X200) exhibits a cervical smear stained with PAP stain. It is worth noting that an unidentified cytoplasmic inclusion is indicated by the arrow.

Table 1. The frequency of Infective microbiota causing dysbiosis

Organism	Frequency n = 38	Percentage
Trichomonas vaginalis	9	23.7
Human Papilloma Virus	4	10.5
Pseudo-hyphae	6	15.8
Actinomyces	2	5.3

Discussion

Previous studies reported that microbiota plays an increasingly significant role in the occurrence and development of cancer (Bhatt *et al*., 2017). Meanwhile, numerous reports have shown that microbial groups promote cancer by inducing inflammatory reactions in recent years. The development of endometrial cancer, for instance, could be accelerated by PID (Yang *et al*., 2015). Cervicovaginal microbiological disorders have become a key factor in inflammation, HPV infection, and cervical cancer. Human papillomavirus is known to be a pivotal factor in the development of cervical cancer. HPV causes various diseases, including cervical cancer and precancerous lesions (Li *et al* ., 2020). There are low-risk and high-risk HPV, depending on their carcinogenic potential (Curty *et al* ., 2019). Low-risk HPV is associated with the development of anogenital warts, while high-risk HPV types are associated with cervical intraepithelial neoplasia (CIN) and cervical cancer (Curty *et al* ., 2019). In this study, parabasal cells were seen with perinuclear halo, hyperchromatic nuclei, and slight nuclei polymorphism. These features are consistent

with high-grade squamous intraepithelial lesions (HSIL) caused by HPV infection. Though not all HSIL will progress to cancer, HSIL is a precancerous lesion and therefore is usually treated aggressively (Khieu and Butler, 2022).

There was the presence of inflammatory lesions as shown by the result of our study. Inflammation is a kind of defense mechanism to various stimuli. Tissue damage and various contributing factors can trigger inflammation. When the host body shows inflammatory signs, it results in elevated cellular metabolism, vessel wall dilatation, the release of soluble mediators, and increased blood flow (Ferrer-Miliani *et al*., 2007). Persistent inflammation contributes to chronic inflammation which could be explained by lymphocytic infiltration (Zhou *et al.,* 2021). Antibodies or cytokines are secreted by T and B lymphocytes, which are involved in tissue damage and inflammatory cell recruitment (Zhou *et al.,* 2021). It was observed in this study that there were infiltrating polymorphs also indicative of inflammation. Persistent inflammation can result in chronic inflammation, which is one of the inducing factors of the tumor (Zhou *et al.,* 2021). In the presence of chronic inflammation, the individual's susceptibility increases, making the cells prone to cancer (Zhou *et al*., 2021). Trichomonas vaginalis is the most common, prevalent, curable non-viral sexually transmitted infection (STI) worldwide (Bbabazhistu and Adegboro, 2022). Several studies suggest that some sexually transmitted infections such as trichomonas vaginalis might play important roles in cervical carcinogenesis (Ghosh *et al*., 2017). It is believed that the inflammatory process and modulation of host metabolism caused by TV predisposes epithelium to carcinogenesis by HPV (Mercer and Johnson, 2018). In this study, TV was identified in the cervical smear as a pear-shaped feature. Zhang *et al*.(2020) stated that TV infects the squamous epithelium of the vagina, and that cervical epithelium disruption is due to the inflammation process caused by TV (Babazhitsu and Adegboro, 2022). The presence of TV in this study may probably be due to vaginal contamination. This, however, does not undermine the role of TV in cervical cancer.

Another notable organism found in this study is actinomyces. Actinomyces spp is an anaerobic Gram-positive bacterium that causes pelvic actinomycosis, a rare, chronic, suppurative, and granulomatous disease (Saramago *et al*., 2019). Actinomyces exist in normal oral and gastrointestinal flora and female genital tract colonization is also not uncommon (Seramago *et al.,* 2019). In a study by Kim *et al.* (2004) the incidence of Actinomyces-like organisms in Pap smear was 0.26% and 81.1% of the positive cases were users of intrauterine device (IUD). This present study did not, however, collect any data on the lifestyle of the study participants concerning IUD usage. According to the Gynecology, Long-Acting Reversible Contraception Work Group (2017), the finding of Actinomyces on a PAP smear is considered incidental, and the asymptomatic patient does not require antimicrobial treatment or removal of the IUD. However, pap smear lacks specificity in identifying Actinomyces, and only half the diagnosis made through pap smears are culture positive (Saramago *et al*., 2019).

Also revealed in this study are fungi with pseudohyphae. Among the various fungal species, Candida is the most common pathogen seen in cervical-vaginal smears. Kini *et al.* (2017) in their study noted that while candidiasis is the most frequently encountered fungal infection in cervicovaginal smears, contaminants are quite common. As observed in some smear samples containing fungal elements, there were no significant features of inflammation. The cells were predominantly intermediate, and few normal superficial cells with pyknotic nuclei. Kini *et al.* (2017) noted that to distinguish contamination from true infection, the latter is usually supported by positive clinical findings of inflammation in the smears

Conclusion and recommendations

Cytological analysis of the cervical smear using PAP and Diff Quick techniques can provide useful information about microbial infections in the cervix. Cervical cancer, a preventable and treatable cancer, remains the cancer with the highest incidence in women and the leading cause of cancer death in women. There is therefore an urgent need to roll out better cervical

screening programs. To accomplish this, a better understanding of microbial factors contributing to cervical cancer is necessary to improve the identification and treatment of at-risk individuals before the onset of the disease. Current cervical screening approaches and daily practice focus on studying cytological features of cervical smears to identify morphological abnormalities. This study has highlighted the need to adopt a more proactive approach by evaluating the microbial profile of the cervical smear, which can be a risk factor for cervical malignancy.

This study has identified the presence of microorganisms that potentiate dysbiosis in cervical-vaginal smear and further microbial examination of the cervix should go along with the traditional and routine PAP smear, seeing that microorganisms can also predispose persons to the development of cervical cancer. Secondly, subsequent studies should consider patient lifestyle and other data to correlate the presence of microorganisms with any lifestyle risk factor. Also, the sample size should be increased in subsequent studies (this was part of the limitation of this present study) and may be carried out among cervical cancer patients to ascertain the correlation of these microorganisms with cervical cancer.

Ethical Approval

A letter of introduction was submitted to the directors of the different laboratories requesting permission to obtain the patients' cervical-vaginal smear samples after ethical committee approval of the faculty. All processes were carried out in line with research ethical best practices in maintaining patients' confidentiality.

Conflict of interest

The authors declare no conflict of interest.

Funding

No grant was given for this research by funding organizations in the public, private, or not-for-profit sectors.

Acknowledgement: We hereby acknowledge the understanding and compliance of our study participants; most sincerely we appreciate the efforts and technical supports of private medical laboratory directors in Ebonyi state and indeed has promised to avail it facilities for such research that stand to improves patients' diagnosis.

***Abbreviations:** PID; stands for Pelvic Inflammatory Disease, STDs; Sexually Transmitted Diseases, HPV; Human papillomavirus, HIV; Human Immunodeficiency Virus, STI; Sexually transmitted infection, HSIL; high-grade squamous intraepithelial lesions, TV; Trichomonas vaginalis, IUD; intrauterine device.

References

Amabebe E, & Anumba (2020). Female Gut and Genital Tract Microbiota-Induced Crosstalk and Differential Effects of Short-Chain Fatty Acids on Immune Sequelae. *Frontiers in Immunology*; *11*. DOI:10.3389/fimmu.2020.02184 .

Arbyn M, Weiderpass E, Bruni L, de Sanjosé S, Saraiya M, Ferlay J, Bray F. (2020). Estimates of incidence and mortality of cervical cancer in 2018: a worldwide analysis. *Lancet Global Health*;**8(2)**: e191-e203. doi: 10.1016/S2214-109X (19)30482-6. Epub 2019 Dec 4. Erratum in: Lancet Glob Health. 2022 Jan;10(1): e41. PMID: 31812369; PMCID: PMC7025157.

Babazhitsu M and Adegboro B. (2022). Does trichomoniasis play any role in the pathogenesis of cervicalcarcinoma? *Sokoto Journal of Medical Laboratory Science; **7(1): 56 - 60.***

Bhatt AP, Redinbo MR, and Bultman SJ (2017). The role of the microbiome in cancer developmentand therapy.*CA Cancer Journal for Clinicians,* 2017;*67*: 326–344.

Borgdorff H, Gautam R, Armstrong, SD, Xia D, Ndayisaba GF, van Teijlingen NH, Geijtenbeek TBH, Wastling JM, & van de Wijgert JHHM. (2016). Cervicovaginal microbiome dysbiosis is associated with proteome changes related to alterations of the cervicovaginal mucosal barrier. *Mucosal Immunology*; ***9*(3)**: DOI: 10.1038/mi.2015.86.

Chee WJY, Chew SY, & Than, LTL. (2020). Vaginal microbiota and the potential of Lactobacillus derivatives in maintaining vaginal health. *Microbial Cell Factories; **19*(1)**: 203. DOI; 10.1186/s12934-020-01464-4.

Cohen PA, Jhingran A. and Oaknin A (2019). Cervical cancer. *Lancet; **393***: 169–182.

Curty G, de Carvalho PS and Soares MA. (2020). The role of the cervicovaginal microbiome on genesis and as a biomarker of premalignant cervical intraepithelial neoplasia and invasive cervical cancer. *International Journal of Molecular Sciences; 21*: 222.

Ferrero-Miliani L, Nielsen OH, Andersen PS, Girardin SE. (2007). Chronic inflammation: importance of NOD2 and NALP3 in interleukin-1beta generation. *Clinical Experimental Immunology;* **147(2)**:227-35. doi: 10.1111/j.1365-2249.2006.03261.

Ghosh I, Muwonge R, Mittal S, Banerjee D, Kundu P, Mandal R, Biswas J. and Basu P. (2017). Association between high-risk human papillomavirus infection and co-infection with *Candida* spp. and *Trichomonas vaginalis* in women with cervical premalignant and malignant lesions. *Journal of Clinical Virology;* ***87***: 43-48.

Godoy-Vitorino F, Romaguera J, Zhao C, Vargas-Robles D, Ortiz-Morales G, and Vazquez Sanchez F. (2018). Cervicovaginal fungi and bacteria associated with cervical intraepithelial neoplasia and high-risk human papillomavirus infections in a hispanic population. *Frontiers in Microbiology;* ***9***: 2533.

Gupta, R.; Dutta, T. K.; Kundu, S. S.; Chatterjee, A.; Gautam, M.; Sarkar, S. (2016). Nutritional evaluation of tree leaves of Ayodhya Hills of Purulia District, West Bengal. *Indian Journal of Animal Nutrition*; **33 (4)**: 404-410

Herrero R. (2018). Elimination of cervical cancer in Latin America. *Salud Publica Mexicana;* ***60***: 621–623.

Huang X, Li C, Li F, Zhao J, Wan X, and Wang K. (2018). Cervicovaginal microbiota composition correlates with the acquisition of high-risk human papillomavirus types. *International Journal of Cancer;* ***143***: 621–634.

Kaya D, Demirezen Ş, Hasçelik G, Gülmez KD. and Beksaç MS. (2013). Comparison of PCR, Culturing and Pap smear microscopy for accurate diagnosis of genital *Actinomyces*. *Journal of Medical Microbiology*, 2013; ***62*****(5)**: 727-733.

Khieu M and Butler SL. (2022). High grade squamois intraepithelial lesion. In: tatPearls [Internet]. Treasure Island (FL); StatPearls Publishing.

Kim YJ, Youm J, Kim JH and Jee BC. (2004). Actinomyces-like organisms in cervical smears: The association with intrauterine device and pelvic inflammatory diseases. *Obstetrics and Gynecology Science;* ***57*****(05)**: 393–396

Kini H, Kini JR, Suman E and Rai S. (2017). Fungal spores and fruiting bodies in cervicovaginal smears: contaminant or infection? *Diagnostic Cytopathology;* ***45*****(3)**: 191-194.

Klein C, Kahesa C, Mwaiselage J, West J T, Wood C and Angeletti P C. (2020). How the cervical microbiotacontributes to cervical cancer risk in Sub-Saharan Africa. *Frontiers in Cell Infection Microbiology*; ***10***: 23.

Li Y, Yu T, Yan H, Li D, Yu T, Yuan T. (2020). Vaginal microbiota and HPV infection: novel mechanistic Insights and therapeutic strategies. *Infection and Drug Resistance;* ***13***: 1213–1220.

Mercer F. and Johnson PJ. (2018). *Trichomonas vaginalis*: pathogenesis, symbiont interactions, and host cell immune responses. *Trends in Parasitology;* ***34*****(8)**: 683-693.

Nayar R. (2015). The Bethesda system for reporting cervical cytology. In Nayar R, Wilbur DC (eds). Definitions, criteria, and explanatory notes. Third edition. Switzerland: Springer: 45-49.

Ncube B, Bey A, Knight J, Bessler P, Jolly PE. (2015). Factors associated with the uptake of cervical cancer screening among women in portland, Jamaica. *North American Journal of Medical Science*; **7(3)**:104-13. doi: 10.4103/1947-2714.153922.

Okorie N. (2021). Basic Histopathology techniques: instructional manual for staff and students (1st edition). Shibboleth Prints, Makurdi, Nigeria, 2021. ISBN: 978-978-978-617-4: 203-205

Okorie N, Obeagu EI, Odigbo CN, Ibe OE, Usanga VU, Jacob IC and Obi IA. (2021). Cytological Evaluation of Urinary Samples among Vesicovaginal Fistula Patients in National Obstetrics Fistula Centre, Southeastern Nigeria. *Asian Journal of Medicine and Health*; 20(10): 136-146. DOI: 10.9734/AJMAH/2022/v20i1030514

Sabu S, Nayak DM, Nair S. and Shetty R. (2017). Role of Papanicolaou smear in the diagnosis of pathologic flora in asymptomatic patients in rural health care set-up. *Journal of Clinical and Diagnostic Research;* ***11*****(10)**: EC10-EC13

Saramago SM, Cominho JC, Proença SSM, Conde PJCC and Nunes FMP. (2019). Pelvic actinomycosis mimicking pelvic malignancy. *Revista Brasileira de Ginecologia e Obstetricia;* ***41***: 463–466.

Sung H, Ferlay J, Siegel JL Laversanne M, Soerjomataram I, Jemal A and Bray F. (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA: A Cancer Journal for Clinicians:* DOI: 10.3322/caac.21660.

Tabitha Amere Leslie, Odunayo Rebecca Adigun, Tayo Emmanuel Olajide, Margaret Ihunanya Okwuikpo, Tata Leslie Danlami and Kaneng Mary Dalyop.(2020). Perception And Acceptance of Cervical Cancer Screening Among Women of Childbearing Age Attending Postnatal Clinic in Ifako-Ijaiye General Hospital, Lagos State. *IAR Journal of Medical Sciences;* **2(1)**: 64-69.

Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J and Jemal A. (2012) Global cancer statistics, 2012. *CA Cancer Journal for Clinicians;* ***65***: 87–108.

Yang TK, Chung CJ, Chung SD, Muo CH, Chang CH and Huang CY. (2015). Risk of endometrial cancer In women with pelvic inflammatory disease: a nationwide population-based retrospective cohort Study, *Medicine;* ***94***: e1278)

Zhang Z, Li Y, Wang S, Hao L, Zhu V, Li H, Song X, Duan Y, Sang Y and Wu P. (2020). The molecular characterization and immunity identification of trichomonas vaginalis adhesion protein 33 (AP33). *Frontiers in Microbiology;* ***11***: 1433.

Zhao X, Liu Z, & Chen T. (2023). Potential Role of Vaginal Microbiota in Ovarian Cancer Carcinogenesis, Progression and Treatment. *Pharmaceutics;* ***15*****(3)**: DOI: 10.3390/pharmaceutics15030948.

Zhou ZW, Long HZ, Cheng Y, Luo HY, Wen DD and Gao LC. (2021). From microbiome to inflammation: The key drivers of cervical cancer. *Frontiers in Microbiology*; ***12***: 767931.

Citation: Nnaemeka Okorie, Onyekachi Ewa Ibe, Iyke-Ogbo Pauline, Simon Uzor, Victor Udoh Usanga, Kingsley Omogo, Ugomma Ude Agwu, Idakari Chinedu Nweke, Ibe Uzochukwu Chimdindu. Cervical-Vaginal Dysbiosis: Clinical Query of Infections in Selected Private Medical Laboratories in Abakaliki Ebonyi State Nigeria. ***Sokoto Journal of Medical Laboratory Science*****; 8(3): 8 - 17**. https://dx.doi.org/10.4314/sokjmls.v8i3.2