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Resistant bacteria in vaginal microbiota in reproductive-age women and Its Clinical

Implications - A Pilot Study

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Abstract

Purpose

The vaginal microbiota plays a crucial role in maintaining women's health and reproductive well-being. This pilot study aims to examine the composition and antibiotic susceptibility of the vaginal microbiota in reproductive-age women from the South-South region of Nigeria. This research seeks to identify the most common microbial species and their resistance patterns to fill important gaps in our knowledge of vaginal health in this specific population.

Methodology

High vaginal swab samples from 55 patients were analysed using standard microbiology techniques to identify bacteria and their susceptibility to antibiotics.

Findings

The results from this study showed that Lactobacilli was the most dominant in 29.1% of the vaginal samples, followed by *Staphylococcus aureus* (20%), non-pathogenic staphylococci (23.6%), *Gardnerella vaginalis* (14.5%), and *Escherichia coli* (12.7%) were also present. The study revealed significant antibiotic resistance patterns, with *Staphylococcus aureus* showing 100% resistance to Pefloxacin, Zinnacef, Amoxicillin, Rocephin, and Streptomycin. *Gardnerella vaginalis* and *E**scherichia. coli* also exhibited 100% resistance to moxifloxacin, ciprofloxacin, and gentamycin.

Originality

This pilot study examines the vaginal microbiota and antibiotic resistance in women from a region in South-South Nigeria. It provides essential data on microbial composition and resistance patterns, contributing valuable knowledge to the global understanding of vaginal health and microbiota diversity.

Research limitations

The pilot study provides valuable insights, but the small sample size limits the generalizability of the findings. Advanced genomic methods could provide a more detailed characterisation of microbial communities. Despite these limitations, the study emphasises the need for more extensive investigations to enhance our understanding of vaginal health and antibiotic resistance.

Keywords: Vaginal microbiota, antibiotic resistance, women's health, pilot study.

Introduction

The vaginal microbiota, also known as the vaginal microbiome, comprises a diverse community of microorganisms crucial for women's health and reproductive well-being. This dynamic ecosystem experiences continuous changes influenced by hormonal fluctuations, sexual activity, and overall health. These variations play a significant role in maintaining vaginal health and preventing infections throughout a woman's life (Lehtoranta et al., 2022). Hormonal variations at different stages of a woman's life can have a substantial impact on the composition and diversity of the vaginal microbiota, further emphasising its importance in overall health and reproductive well-being (Van de Wijgert and Jespers, 2017).

The microbiome plays a major part in shaping reproductive health by intricately balancing diverse microbial communities within the female reproductive organs. The vaginal microbiome, characterised by a rich collection of bacteria, fungi, and viruses, is paramount for maintaining vaginal health and preventing infections. These microorganisms' intricate interplay helps create a balanced and healthy environment within the female reproductive system, contributing to overall reproductive well-being (Chen et al., 2021). In most females, the majority of the vaginal microbial community comprises of the Lactobacillus bacterial species (Chen et al., 2021). These bacteria help protect the reproductive tract by producing hydrogen peroxide and releasing compounds like bacteriocins to prevent harmful bacteria from thriving (Gupta et al., 2019).

Lactobacillus species, especially Lactobacillus crispatus and Lactobacillus jensenii, are dominant in a healthy vagina, and their lactic acid production helps to maintain a low vaginal pH, preventing the growth of harmful microbes (Kalia et al., 2020). However, a significant percentage of asymptomatic and healthy women have vaginal microbiota that lacks a sufficient number of Lactobacillus bacteria and instead harbours a variety of strictly and

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facultative anaerobic microbes (Ma et al., 2012). This suggests that there are multiple core microbiomes in the human vagina rather than a single core microbiome (Kalia et al., 2020).

Ethnicity is a significant factor contributing to vaginal composition, and research has shown that Caucasian and Asian women have significantly higher levels of Lactobacilli than Hispanic or Black women (Feng et al., 2022). While some studies have examined the vaginal microbiome of reproductive-aged women in parts of Nigeria, we initiated a preliminary, small-scale study in a hospital in South-South Nigeria to further contribute to this research area. This study aims to provide new insights into the antibiotic resistance profiles of vaginal microbiota in reproductive-age women in South-South Nigeria, an area with limited existing research.

Methodology

Study area, ethical permission, participants and sample collection

We conducted a pilot study at the Niger Delta University Teaching Hospital Okolobiri, and a total of 55 patients were randomly selected to participate. These patients met specific inclusion criteria and expressed willingness to participate in the study. The study procedure carefully explained the research objectives to the participants, obtaining informed consent and ethical permission from the hospital's ethical board. Healthcare professionals collected the vaginal swab samples in a clinical setting to ensure standardisation and proper technique.

The samples were processed in the microbiology laboratory using standard techniques to identify and characterise the microorganisms. The strict adherence to the inclusion criteria ensured that the participants represented the hospital's patient population.

Culture

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The samples were cultured in Blood, Mac Conley, and Chocolate agar, prepared according to the manufacturer's instructions, and then sterilised using the autoclave at 121°c for 15 minutes. Different biochemical tests were performed, such as gram staining, oxidase, catalase, coagulase, citrate, indole, urease, and the Kilgler iron agar test (Cheesbrough, 2006).

Antibiotic sensitivity testing

The disk diffusion method, the Kirby-Bauer method, was used for antimicrobial susceptibility testing. This qualitative method assesses the effectiveness of different antibiotics against bacterial isolates by measuring the inhibition of bacterial growth around antibiotic-impregnated disks. In the Kirby-Bauer Disk Diffusion method, the diameter of the zone of inhibition around an antibiotic disk is measured to determine susceptibility. Bacterial colonies were introduced into peptone water and onto a Mueller Hinton plate. The plate was swirled to ensure an even distribution of the peptone water and then discarded. A sensitivity disk was selected and placed onto the plate using sterilised forceps. The plates were then incubated, and the zone of inhibition was observed and measured after the specified incubation period (Benkova et al., 2020; eucast: EUCAST)

Results

A total of 55 women participated in this study, and their ages are illustrated in Figure 1. The vaginal microbiota examined in the study comprises both gram-negative and gram-positive

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Figure 1: Age distribution

2.

The pie chart illustrates the age distribution within the surveyed population. The age group (30-35) has the highest percentage at 32.7%, while the age group (40-45) has the lowest percentage at 3.6%.



Figure 2: Gram reaction

The bar chart shows the distribution of bacteria isolates based on gram reaction. It shows that 72.7% of the isolates are gram-positive, while 27.3% are gram-negative.

Table 1 shows the frequency and percentage of bacterial isolates. There are 55 isolates, with Lactobacilli being the most frequent at 29.1%, *Staphylococcus aureus* at 20.0% and Non-pathogenic *Staphylococcus* at 23.6%. *Gardenella vaginalis* and *Escherichia coli* have frequencies of 14.5% and 12.7%, respectively.

Table 1	Frequency	and p	per cen	t of bacter	ial isolates
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Bacteria isolates	Frequency	Percentage
Lactobacilli	16	29.1%
Staphylococcus aureus	11	20.0%
Non-pathogenic staphylococcus	13	23.6%
Gardenella vaginalis	8	14.5%
Escherichia coli	7	12.7%
Total	55	100%

Table 2: Antibiotic susceptibility and resistance of Staphylococcus aureus

Staphylococcus aureus

Percentage(Frequency)

	Resistant	Intermediate	Susceptible
Pefloxacin	100%	0%	0%
Gentamycin	0%	18.18%	81.82%
Ampiclox	0%	18.18%	81.82%
Zinnacef	100%	0%	0%
Amoxicillin	100%	0%	0%
Rocephin	100%	0%	0%
Ciprofloxacin	9.09%	18.18%	72.73%
Streptomycin	100%	0%	0%

Septrin	0%	45.45%	45.45%
Erythromycin	0%	18.18%	81.82%

Staphylococcus aureus is 100% resistant to Pefloxacin, Zinnacef, Amoxicillin, Rocephin, but susceptible to Streptomycin (100%) and 100%.

	Gardenella vaginalis Percentage(Frequency)			Escherichia coli		
				Percentage(Frequency)		
	Resistant	Intermediate	Susceptible	Resistant	Intermediate	Susceptible
Moxifloxacin	100%	0%	0%	100%	0%	0%
Levofloxacin	100%	0%	0%	0%	0%	100%
Ciprofloxacin	100%	0%	0%	14.29%	0%	85.71%
Amoxicillin	77.78%	0%	22.22%	100%	0%	0%
Augmentin	0%	88.89%	11.11%	0%	88.89%	11.11%
Gentamycin	100%	0%	0%	14.29%	0%	85.71%
Azithromycin	0%	0%	100%	0%	0%	100%
Cefotaxime	0%	11.11%	88.89%	0%	0%	100%
Cefpodoxime	0%	0%	100%	0%	0%	100%

Table 3 Antibiotic susceptibility pattern of the gram-negative isolates

Gardenella vaginalis was 100% resistant to Moxifloxacin, Levofloxacin, Ciprofloxacin, and Gentamicin. *Escherichia coli* was 100% resistant to Moxifloxacin.

Discussion

While studies have analysed the composition of the vaginal microbiota in various populations, there is limited data on antibiotic resistance patterns and microbial profiles, specifically among reproductive-age women in South-South Nigeria. This study aims to address this gap by characterising the microbiome and examining resistance patterns in this population. In line

with our research aims, we found that Lactobacillus species dominated the microbiota in reproductive-age women, similar to findings in other regions. Additionally, high resistance rates were observed in key pathogenic bacteria, with *Staphylococcus aureus* showing complete resistance to multiple antibiotics, highlighting the challenge of treating infections in this demographic.

The arrangement and composition of the vaginal microbiota have been well described in the literature, from analyses using light microscopy to high-throughput sequencing techniques. The vaginal microbiota of healthy reproductive-aged women has been defined as a Lactobacillus-dominated microflora that produces a significant amount of lactic acid, resulting in a pH of less than 4.5 (Kalia et al., 2020). Lactobacillus bacteria in the vagina help maintain a healthy pH, protect against infection, and regulate the immune system. They also offer additional protection against pregnancy-related issues (Chen et al., 2021)

Our study also indicates that the predominant vaginal bacteria was Lactobacillus species, consistent with the findings of (Anukam et al., 2019) in Anambra, where Lactobacillus genera were identified as the primary microbiota. Our research findings differ from a study in Port Harcourt, where *Escherichia coli* was identified as the most common bacterial strain among female participants aged 24-26 (Sampson et al., 2021). Similarly, in another retrospective study in Port Harcourt, Escherichia coli also dominated the bacterial population (Ndukwu et al., 2024). These differences could be attributed to regional variations in antibiotic use, hygiene practices, or genetic factors.

Recurrent spontaneous abortion may be linked to low Lactobacillus levels and high bacterial diversity in the vagina, as found in a study in Eastern Nigeria. The study showed higher bacterial diversity in women with a history of recurring spontaneous abortion compared to

those without such a history. Additionally, Lactobacillus was more abundant in nonspontaneous abortion cases (83%) than in cases of spontaneous abortion (Emele et al., 2023).

It is important to study the vaginal microbiome because it is a complex and ever-changing ecosystem that goes through fluctuations during the menstrual cycle and throughout a woman's life. When there is a decrease in the levels of Lactobacillus bacteria and an increase in anaerobic microbes, it indicates bacterial vaginosis (Chen et al., 2021). Following puberty, increased estrogen levels in women promote Lactobacillus colonisation, thickening the epithelial lining and lowering vaginal pH to \leq 4.5 through lactic acid and H2O2 production. Other common microorganisms that can be found include *Candida albicans, Staphylococcus aureus*, and *Streptococcus agalactiae*, which can cause discharge changes if they overgrow (Sangeetha, 2014).

Most commensal microbiotas coexist in mutualistic relationships with their human hosts, meaning they benefit each other. However, a few of these microorganisms are considered opportunistic pathogens, which can potentially cause chronic infections under certain circumstances (Chen et al., 2021).

Changes in the vaginal environs can lead to fluctuations in the vaginal microbiota, potentially resulting in bacterial vaginosis (BV) or aerobic vaginitis. The prevalence of aerobic vaginitis is between 12% and 23.7% in symptomatic non-pregnant women and between 4% and 8% during pregnancy. This state is associated with an increased risk of sexually transmitted diseases. The bacteria that can cause aerobic vaginitis include *Enterococcus faecalis* (*E. faecalis*), *Escherichia coli*, group B streptococcus, and *Staphylococcus aureus* (Donders et al., 2017; Plisko et al., 2021; Jahic, 2021). Our research revealed that 23.6% of the isolates were non-pathogenic *Staphylococcus aureus*, which can also contribute to aerobic vaginitis.

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Research has also shown that menstrual toxic shock syndrome (TSS), a severe illness that affects premenopausal women worldwide, results from vaginal infection by *Staphylococcus aureus* and parallel production of toxic shock syndrome toxin-1 (MacPhee et al., 2013). In our study, 20% of the isolates were *Staphylococcus aureus*. This indicates a significant risk for TSS among this population studied, highlighting the need for monitoring and preventive methods to manage and reduce the potential for TSS development.

In 2011, a research study explored the relationship between aerobic vaginitis and pregnancy. The study found that aerobic vaginitis can lead to fetal infections, premature rupture of the membranes, preterm birth and neurologic injury. Bacteria that cause BV are from the intestinal flora, mainly group B Streptococci (GBS), followed by Enterobacteriaceae. GBS are important pathogens in reproductive tract infections among pregnant women, increasing the frequency of premature labour and perinatal transmission of bacterial organisms (Donders et al., 2011).

The population of *Escherichia coli* in this study was the lowest, at 12.7%, which differs from other studies conducted in Port Harcourt (Sampson et al., 2021; Nduke#we, 2024). The presence of *E. coli* in the vaginal microbiome can lead to a heightened risk of urinary tract infections, indicate an imbalance in the microbiome, raise the risk of vaginal infections, pose risks for pregnant women, and present concerns about antibiotic resistance (Lewis et al., 2020).

Understanding the presence of *Gardnerella vaginalis* in the vaginal microbiome is important for diagnosing and managing bacterial vaginosis and related health issues. Our study identified seven isolates representing 14.5% as *Gardnerella vaginalis*. This bacterium is commonly associated with bacterial vaginosis, a condition characterised by an imbalance of the vaginal microbiota, where the normal Lactobacillus-dominated flora is disrupted. While

Gardnerella vaginalis is a natural component of the normal vaginal flora, an excessive proliferation of this bacterium can lead to BV. Bacterial vaginosis can cause symptoms such as unusual discharge, odour, irritation, and itching (Kairys et al., 2023).

Gardnerella vaginalis produces biofilms that protect it and other pathogenic bacteria from the immune system and antibiotics, making infections more challenging to treat. It is also linked to adverse pregnancy results such as preterm birth, reduced birth weight, and an increased risk of miscarriage (Chen et al., 2021).

The presence of antibiotic-resistant bacteria in the vaginal microbiome has significant implications for women's health. The results of our sensitivity testing reveal worrying resistance patterns in commonly isolated pathogens. For instance, *Staphylococcus aureus* was entirely resistant to Pefloxacin, Zinnacef, Amoxicillin, Rocephin, and Streptomycin. This suggests that current first-line antibiotics may be ineffective for treating infections in this population, necessitating revised guidelines and heightened surveillance of antibiotic resistance. *Gardnarella vaginalis* was resistant to Moxifloxacin, Levofloxacin, Ciprofloxacin, and Gentamicin. E. coli was resistant to Moxifloxacin and Amoxicillin. This resistant pattern is similar to the findings of a study in Port Harcourt (Ndukwu, 2024), where E. coli and Staph aureus were resistant to 30% of the antibiotics tested. At the same time, *Klebsiella pneumoniae* was resistant to all antibiotics.

The presence of antibiotic-resistant bacteria in the vaginal microbiome is a significant health concern that could complicate treatment, increases the risk of other infections, and poses serious public health challenges.

Clinical implications

The presence of antibiotic-resistant bacteria in the vaginal microbiota poses significant health risks for reproductive-age women. High resistance rates complicate the treatment of common

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vaginal infections, making infections persistent or recurrent. Persistent infections can lead to disruptions in the vaginal microbiome, which plays a vital role in maintaining vaginal health by preventing overgrowth of pathogenic organisms and reducing the risk of infection (van de Wijgert et al., 2017; Holdcroft et al., 2023).

Antibiotic-resistant bacteria in the vaginal environment, such as resistant strains of *Staphylococcus aureus* or *Escherichia coli*, may increase susceptibility to severe infections, including sexually transmitted infections (STIs) and pelvic inflammatory disease (PID). For instance, studies have shown that infections caused by resistant *E. coli* can lead to urinary tract infections, common in women and can cause untreated kidney infections (Lewis et al., 2020). Additionally, the presence of antibiotic-resistant *Staphylococcus aureus* could increase the risk of menstrual toxic shock syndrome (TSS), a life-threatening condition associated with toxin-producing strains of this bacterium (MacPhee et al., 2013).

For pregnant women, antibiotic resistance in the vaginal microbiota introduces additional risks. Infections that are difficult to treat due to resistance may result in adverse pregnancy outcomes, such as preterm birth, low birth weight, and increased risk of neonatal infections (Donders et al., 2011; Chen et al., 2021). Group B Streptococcus (GBS) is particularly concerning in this context, as it can be transmitted from mother to child during childbirth, leading to severe infections in newborns, including sepsis and meningitis (Holdcroft et al., 2023).

Furthermore, the spread of antibiotic-resistant bacteria in the vaginal microbiome has broader public health implications. Resistant bacteria can act as reservoirs of resistance genes, which may be transferred to other pathogens, exacerbating the problem of antimicrobial resistance on a community and global scale (Kairys et al., 2024). Effective public health strategies and

antibiotic stewardship are crucial to addressing this growing concern and ensuring that infections remain manageable with available treatments.

Conclusion

This research provides important information about the microbial composition of the vaginal environment in this specific demographic. It serves as a baseline with the hope that similar studies on women in other regions of Nigeria and Africa will help in the easier identification of imbalances in the vaginal microbiome and its potential impact on women's overall health and well-being. This, in turn, will enable personalised interventions to restore a microbiome that optimises reproductive health.

Limitations

The study involved only 55 participants, which may limit the findings to the broader population. More extensive studies are needed to confirm these results and provide more robust data. The study relied on culture-based methods for identifying bacteria, which may miss non-culturable organisms. Molecular techniques like 16S rRNA sequencing could provide a more comprehensive view of the microbiome.

Declarations

Ethics approval and consent to participate

Permission was obtained from the hospital's ethical committee.

Competing interests

The authors declare that they have no competing interests

Authors' contributions

TTN collected and analysed the samples, ELL conceptualised the idea, performed the data analysis, and wrote the manuscript. All authors read and approved the final manuscript.

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