

Bacterial Profile of Vaginal Discharge among Apparently Healthy Female Students of Tansian University, Anambra State, Nigeria

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Abstract: The human vaginal environment hosts a diverse microbiota that plays an essential role in maintaining mucosal health and preventing infections. Normal vaginal discharge is part of this defense mechanism; however, deviations in its appearance or volume may indicate an underlying pathological condition. This study aims at isolation and identification of bacteria associated with vaginal discharge from apparently healthy female students of Tansian University, Umunya, Anambra State, Nigeria. Twenty (20) vaginal samples were collected from different students using sterile swab sticks. Each vaginal swab sample was inoculated onto Nutrient Agar plates using the streak plate method and incubated aerobically at 37°C for 24 hours. Colony morphology and pigmentation were recorded. The Gram reaction was performed on each isolate using Gram staining technique, and the result was recorded as Gram-positive or Gram-negative. The biochemical tests which include: catalase, coagulase, indole, citrate, oxidase, sugar fermentation, were also performed, using standard method by Cheesbrough (2012). The result was recorded as positive (+) or negative (-). The prevalence of infection was determined, and recorded in percent (%). The antibiotic susceptibility patterns of the bacterial isolates against nine commonly used antibiotics were determined. The results indicate varying levels of resistance and susceptibility. This study revealed that both commensal and opportunistic bacteria were present in the vaginal tracts of asymptomatic students. The emergence of a multidrug-resistant *Staphylococcus aureus* strain highlights the urgent need for antibiotic stewardship and routine screening, even in apparently healthy individuals.

Keywords: Bacteria, Vaginal Discharge, Female Students, Anambra, Nigeria.

Introduction

There is a growing concern over the increased prevalence of antibiotic-resistant bacteria in the female reproductive tract. As a result, treatment failures and recurrent infections are increasingly common. There is a lack of current, localized data on the bacterial agents present in vaginal discharge among apparently healthy women and their susceptibility to commonly used antibiotics, particularly among students in university communities.

The human vaginal environment hosts a diverse microbiota that plays an essential role in maintaining mucosal health and preventing infections. Normal vaginal discharge is part of this defense mechanism; however, deviations in its appearance or volume may indicate an underlying pathological condition (Sobel, 2020). While many infections are symptomatic, some individuals may remain asymptomatic carriers of potentially harmful microbes, including *Escherichia coli*, *Staphylococcus aureus*, *Streptococcus spp.*, and *Pseudomonas spp.* Antibiotic resistance among urogenital

pathogens has emerged as a major public health challenge globally. The World Health Organization (WHO, 2016) has identified antibiotic resistance as one of the top ten global health threats, with misuse and overuse of antibiotics being key drivers. In developing countries like Nigeria, the tendency for self-medication, over-the-counter access to antibiotics, and poor compliance to treatment regimens exacerbate the problem (Olayemi *et al.*, 2018).

Alteration in the normal flora, often caused by antibiotic use, hormonal fluctuations, sexual activity, or poor hygiene, can lead to a reduction in *Lactobacillus* population, paving the way for colonization by anaerobes and other potentially pathogenic organisms.

In university communities, close living quarters, limited health-seeking behavior, and poor sexual health education may facilitate the spread of drug-resistant organisms, even among apparently healthy individuals. Therefore, assessing the bacterial agents associated with vaginal discharge and their antimicrobial

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susceptibility is important for informing treatment strategies and public health policy (Iwuafor *et al.*, 2020).

Area of Study

The study was conducted at Tansian University, Umunya, located in Oyi Local Government Area of Anambra State, Nigeria. The university is a private institution with a population comprising male and female students from various parts of the country.

Sample Collection

Twenty (20) Vaginal swab samples were collected from apparently healthy consenting female students using sterile cotton swabs. Each swab was labeled appropriately and transported to the laboratory for microbial analysis.

Methods of Sample Analysis

Culture and Isolation

Each vaginal swab sample was inoculated onto Nutrient Agar plates using the streak plate method and incubated aerobically at 37°C for 24 hours. Colony morphology and pigmentation were recorded. Subcultures were made to obtain pure colonies.

Gram Staining

Gram staining was performed on the pure isolates to determine Gram reaction and cellular morphology. Gram-positive organisms appeared purple, while Gram-negative organisms appeared pink under the microscope.

Biochemical Tests

Following preliminary isolation and Gram staining, the bacterpresumptiveial isolates were subjected to a series of standard biochemical tests for identification based on their metabolic characteristics. These tests were performed in accordance with procedures outlined by Cheesbrough (2012) and included:

Catalase Test

A drop of 3% hydrogen peroxide was placed on a clean slide. A colony of the test organism was added. Immediate bubbling indicates a positive result, showing the presence of the catalase enzyme.

Coagulase Test

A loopful of the bacterial isolate was mixed with a drop of rabbit plasma on a clean slide. Clotting or clumping of the plasma indicates a positive coagulase result, typical of *Staphylococcus aureus*.

Indole Test

The test organism was inoculated into sterile peptone water and incubated at 37°C for 24–48 hours. After incubation, a few drops of Kovac's reagent were added. The appearance of a red or pink layer on the surface indicates a positive result, showing the organism can produce indole from tryptophan.

Citrate Utilization Test

The test organism was streaked on Simmon's citrate agar slant and incubated at 37°C for 24–48 hours. A color change from green to blue indicates a positive result, showing citrate utilization.

Oxidase Test

Oxidase test was performed to determine the presence of cytochrome c oxidase enzyme in the bacterial isolates. A colony of each isolate was exposed to oxidase reagent, and the appearance of a purple coloration within a few seconds was recorded as a positive result, while no color change indicated a negative reaction.

Sugar Fermentation

To assess the ability of the isolates to ferment sugars, peptone water broth containing either glucose or lactose, along with phenol red as a pH indicator and an inverted Durham tube to detect gas production. After inoculation and incubation, a color change from red to yellow indicated acid production (positive fermentation), while the presence of gas bubbles in the Durham tube confirmed gas production. No color change indicated a negative fermentation result.

Antibiotic Susceptibility Testing

The Kirby-Bauer disk diffusion method was employed to test bacterial susceptibility against the following antibiotics:

Cefoxacin, Sparfloxacin, Levofloxacin, Ciprofloxacin, Amoxicillin, Augmentin, Gentamicin, Pefloxacin, Azithromycin.

Mueller-Hinton agar plates were inoculated with standardized bacterial suspensions (0.5 McFarland standard), and antibiotic discs were placed on the surface. Plates were incubated at 37°C for 18–24 hours, and zones of inhibition were measured in millimeters. Results were interpreted using the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Methods of Data Analysis

The data obtained were compiled, tabulated, and analyzed descriptively. Results were expressed as frequencies, percentages, and mean zone diameters of inhibition. Charts and tables were used to present antibiotic susceptibility patterns.

Informed Consent:

Consent was obtained from all participants prior to sample collection. Each participant was clearly informed about the purpose of the study, the type of sample to be collected (vaginal swab), and the procedures involved. Participation was entirely voluntary, and confidentiality of personal information was assured. Only individuals who willingly agreed and provided verbal or written consent were included in the study.

Illustrations and Supporting Media : Relevant illustrations such as images of culture plates, Gram stain microscopic fields, and photographs of the antibiotic testing procedure were captured and included in the appendices section where necessary.

Results

Table 1: Bacterial Growth, Colony Characteristics, and Identified Organisms from the Vaginal discharge samples.

Sample	Growth	Colour	Elevation	Edge	Surface Opacity	Texture	Organism
TU1	G	Creamy	Raised	Entire	Opaque	Smooth	<i>Staphylococcus</i> spp
TU2	NG						
TU3	G	Grayish	Flat	Irregular	Translucent	Moist	<i>Escherichia coli</i>
TU4	NG						
TU5	G	Yellowish	Raised	Entire	Opaque	Mucoid	<i>Streptococcus</i> spp.
TU6	NG						
TU7	NG						
TU8	G	Creamy	Convex	Entire	Translucent	Mucoid	<i>Lactobacillus</i> spp
TU9	G	Creamy	Convex	Entire	Translucent	Mucoid	<i>Lactobacillus</i> spp
TU10	NG						
TU11	NG						
TU12	NG						
TU13	NG						
TU14	G	Yellowish	Raised	Entire	Opaque	Mucoid	<i>Streptococcus</i> spp.
TU15	NG						
TU16	G	Creamy	Raised	Entire	Opaque	Smooth	<i>Staphylococcus</i> spp.
TU17	G	Yellowish	Raised	Entire	Opaque	Mucoid	<i>Streptococcus</i> spp.
TU18	NG						
TU19	NG						
TU20	G	Grayish	Flat	Irregular	Translucent	Moist	<i>Escherichia coli</i>

Key: TU1=Tansian University sample 1, TU2= Tansian University sample 2, TU3= Tansian University sample 3 ... TU 20= Tansian University sample 20, G= growth, NG= No Growth

Table 2: shows the Gram staining characteristics of the bacterial isolates obtained from vaginal swab samples. Out of the 9 positive samples, 2 were identified as *Escherichia coli*, which showed Gram-negative reactions and appeared as rod-shaped cells. *Staphylococcus* spp. was identified in 2 samples as Gram-positive cocci occurring in clusters. *Lactobacillus* spp. was isolated in 2 samples and presented as Gram-positive rods, *Streptococcus* spp was identified in 3 samples as gram-positive cocci in chains. The staining results, in combination with colonial morphology and biochemical characteristics, aided in the classification of the bacterial species.

Table 2: Gram Staining Reactions of Bacterial Isolates from the Vaginal discharge samples from female students of Tansian University, Umunya, Anambra State.

Sample Code	Identified Organism	Gram Reaction	Morphology
Tu1	<i>Staphylococcus</i> spp.	+	Cocci in clusters
Tu3	<i>Escherichia coli</i>	-	Rods
Tu5	<i>Streptococcus</i> spp.	+	Cocci in chains
Tu8	<i>Lactobacillus</i> spp.	+	Rods
Tu9	<i>Lactobacillus</i> spp.	+	Rods
Tu14	<i>Streptococcus</i> spp.	+	Cocci in chains
Tu16	<i>Staphylococcus</i> spp.	+	Cocci in clusters
Tu17	<i>Streptococcus</i> spp.	+	Cocci in chains
Tu20	<i>Escherichia coli</i>	-	Rods

Key: TU1=Tansian University sample 1, TU2=Tansian University sample 2...TU20=Tansian University sample 20.+ = Positive, - Negative.

Table 3: shows the biochemical reactions of the bacterial isolates, including catalase, coagulase, indole, citrate, oxidase, and sugar fermentation tests. Twenty percent (20%) of the total samples were catalase positive, Ten percent (10%) were indole positive and one hundred percent (100%) were oxidase and citrate negative. Ninety percent (90%) were glucose positive, lactose positive, while 10% (ten percent) were Glucose positive and Lactose negative.

Table 3: Biochemical Test Results of Bacterial Isolates from the Vaginal discharge samples from female students of Tansian University, umunya, Anambra State.

Sample Code	Organism	Catalase	Coagulase	Indole	Citrate	Oxidase	Sugar Fermentation
Tu1	<i>Staphylococcus spp.</i>	+	+	-	-	-	Glucose +, Lactose -
Tu3	<i>Escherichia coli</i>	+	-	+	-	-	Glucose +, Lactose +
Tu5	<i>Streptococcus spp.</i>	-	-	-	-	-	Glucose +, Lactose +
Tu8	<i>Lactobacillus spp.</i>	-	-	-	-	-	Glucose +, Lactose +
Tu9	<i>Lactobacillus spp.</i>	-	-	-	-	-	Glucose +, Lactose +
Tu14	<i>Streptococcus spp.</i>	-	-	-	-	-	Glucose +, Lactose +
Tu16	<i>Staphylococcus spp.</i>	+	+	-	-	-	Glucose +, Lactose +
Tu17	<i>Streptococcus spp.</i>	-	-	-	-	-	Glucose +, Lactose -
Tu20	<i>Escherichia coli</i>	+	-	+	-	-	Glucose +, Lactose +

Key: += positive, - = Negative, TU1=Tansian University sample 1, TU2= Tansian University sample...TU20=Tansian University sample 20.

Table 4: shows the distribution of participants across different age groups, the number of individuals in each group, the number of those who tested positive for bacterial infection, and the corresponding prevalence in percentage. The age range is between 18 (eighteen) and (thirty) years, with the total number of participants equal to 20 (twenty), those infected= 9(nine), The prevalence of infection is ranged between 57% and 33% with the age group 18 to 21 years having the highest prevalence of infection

Table 4: The Age Group Distribution and Infection Rate Of The Formal Participants from Tansian, University Umunya, Anambra State

Age Group	Number of Participants	Number Infected	Prevalence of Infection (%)
18-21	7	4	57.1
22-25	10	4	40.0
26-30	3	1	33.3
Total	20	9	100

Key:% = Percentage.

Table 5: Shows the antibiotic susceptibility patterns of the nine bacterial isolates against nine commonly used antibiotics. The results indicate varying levels of zones of inhibition. *Lactobacillus spp.* were sensitive to all antibiotics tested. In contrast, *Staphylococcus aureus* isolate from TU16, showed complete resistance to all antibiotics, suggesting multidrug resistance. *Escherichia coli* and *Streptococcus spp.* showed high resistance to amoxicillin and augmentin but remained sensitive to gentamicin, ciprofloxacin, and levofloxacin. These findings highlight differences in antibiotic effectiveness across bacterial species.

Table 5: Antibiotic Susceptibility Profile (mm) of Bacterial Isolates from the samples.

Sample Code	Organism	CX	SFX	LFX	CPX	AMX	AUG	GEN	PEF	AZM
Tu1	<i>Staphylococcus</i> spp.	10	16	18	17	00	18	18	14	10
Tu3	<i>Escherichia coli</i>	10	15	17	18	00	11	16	17	14
Tu5	<i>Streptococcus</i> spp.	14	13	16	14	00	10	18	16	17
Tu8	<i>Lactobacillus</i> spp.	16	17	18	17	14	15	19	18	17
Tu9	<i>Lactobacillus</i> spp.	15	16	17	16	13	13	18	18	16
Tu14	<i>Streptococcus</i> spp.	12	14	16	15	8	10	17	15	16
Tu16	<i>Staphylococcus</i> spp.	10	16	18	17	12	00	18	14	10
Tu17	<i>Streptococcus</i> spp.	11	12	15	14	9	10	17	15	15
Tu20	<i>Escherichia coli</i>	13	14	16	17	9	12	18	17	15

Key: mm=millimeter, CX =Cefoxitin,SFX=Sparfloxacin,LFX =Levofloxacin,CPX =Ciprofloxacin,AMX =Amoxicillin,AUG =Augmentin,GEN =Gentamicin,PEF =Pefloxacin,AZM =Azithromycin. TU 1=Tansian University sample 1, TU2=Tansian University sample 2....TU20=Tansian University sample 20.

Discussion and Conclusion

5.1 Discussion

This study aimed to isolate, identify, and investigate the antibiotic susceptibility profile of bacteria associated with vaginal discharge among apparently healthy female students of Tansian University. Out of the 20 samples analyzed, 9 (45%) yielded bacterial growth, indicating the presence of both normal flora and potential pathogens in asymptomatic individuals.

The most frequently isolated organism was *Streptococcus* spp. (33.3%), followed by *Lactobacillus* spp. (22.2%), *Echerichia coli* (22.2%), and *Staphylococcus aureus* (22.2%). These findings align with previous studies that reported *Streptococcus* spp. and *Lactobacillus* spp. as common constituents of the vaginal microbiota (Olowe *et al.*, 2014; Onemu & Idemudia, 2010). The presence of *E. coli* and *Staphylococcus* Spp, though often opportunistic, may represent contamination or early stages of infection.

Lactobacillus spp., which play a protective role in maintaining acidic vaginal pH and inhibiting pathogen growth, were found in 2 of the samples (22.2%) and showed complete sensitivity to all antibiotics tested, confirming their non-pathogenic role. This finding is similar to report by Motevaseli *et al.*, 2013.

The antibiotic susceptibility tests revealed varying resistance patterns among the isolates. *Escherichia coli* and *Streptococcus* spp. exhibited notable resistance to amoxicillin and augmentin, in line with findings by Akinyemi *et al.* (2016), who observed high β -lactam resistance in vaginal and urinary tract isolates. *Staphylococcus* Spp.(TU1) showed susceptibility to ciprofloxacin, levofloxacin, and gentamicin but was resistant to amoxicillin and augmentin.

Of particular concern was TU16, identified as *Staphylococcus* Spp. which showed complete resistance to all antibiotics tested, indicating possible multidrug-resistant strain (MDR), potentially methicillin-resistant *Staphylococcus aureus* (MRSA). Similar multidrug resistance among vaginal isolates has been reported in previous works (Anukam *et al.*, 2017; Sharma *et al.*, 2019). This

poses a significant risk, especially in immunocompromised individuals or if left untreated.

The detection of antibiotic-resistant organisms in apparently healthy individuals underscores the need for continuous surveillance, education on personal hygiene, and rational use of antibiotics to reduce the risk of resistance development and transmission.

5.2 Conclusion

This study revealed that both commensal and opportunistic bacteria were present in the vaginal tracts of asymptomatic students. The dominant isolates were *Streptococcus* spp., followed by *Lactobacillus* spp., *Escherichia coli*, and *Staphylococcus* Spp. While *Lactobacillus* spp. exhibited no resistance, *Streptococcus* spp., *E. coli*, and *Staphylococcus* Spp. showed varying degrees of resistance to commonly used antibiotics, particularly β -lactams. The emergence of a multidrug-resistant *Staphylococcus aureus* strain highlights the urgent need for antibiotic stewardship and routine screening, even in apparently healthy individuals.

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