

Microbial Communities and Antimicrobial Resistance Patterns in Aerobic Bacteria Associated with the Vaginal Microbiota: A Retrospective Study in Port Harcourt, Nigeria

Abstract

The vaginal microbiota, unlike some other sites such as the intestinal microbiota is a less diversified micro-ecosystem; dominated normally by *Lactobacilli* which play specific important roles in the wellbeing of the vagina and the female person. Dysbiosis or the loss of *Lactobacilli* dominance results in ecological shift that leads to a number of conditions such as proliferation of intestinal aerobic bacteria leading to aerobic vaginitis and such other disease conditions. In this retrospective cross sectional descriptive study, laboratory records of microscopy, culture and susceptibility analysis of high vaginal swabs (HVS) and other relevant data were reviewed to synthesize information relating to the microbial community and antimicrobial resistance among aerobic bacteria. Out of 790 specimens examined, microbial growths were observed in 596 (75.4%); dominated by fungi (*Candida* spp), 485 (68.3%), followed by aerobic bacteria, 223(31.4%) and protozoa (*Trichomonas*), 2 (0.3%). The bacterial population was dominated by *Escherichia coli* 98 (44.0%), followed by *Enterococcus faecalis* 55 (24.7%), *Staphylococcus aureus* 41(18.4%), *Proteus mirabilis* 10(4.5%), *Streptococcus agalactiae* 9 (4.0%), *Klebsiella pneumoniae* 5 (2.2%) and *Pseudomonas aeruginosa* 10(4.5%). Gram negative bacteria constitutes 118 (52.9%), while gram positive are 105(47.1%) The cumulative antimicrobial resistance profile of the 223 bacterial strains tested against 10 antimicrobial agents was 55.5%. *Pseudomonas aeruginosa* was the most resistant being resistant to by 62% of the test antimicrobial agents, followed by *Klebsiella pneumoniae*, 58%, *Escherichia coli*, 54.1%, *Staphylococcus aureus*, 51.0%, *Proteus mirabilis*, 51.0%. *Enterococcus faecalis*, 48.4% and *Streptococcus agalactiae* 38.9%. This study has contributed in ascertaining the prevalence of bacterial and other associated organisms of the vaginal microbiota, and also unraveled the aerobic bacterial components together with their antimicrobial resistance patterns which will be useful in treatment of associated infections.

Key Words: Antimicrobial Resistance, Aerobic vaginitis, Vaginal microbiota, Vaginal microbial communities, Vulvo-vaginal candidiasis, Trichomoniasis, *Lactobacilli* dominance

Introduction

Vaginal microbial communities (VMC) refer to various ecological populations of pathogenic, symbiotic, mutualistic or commensal organisms including bacteria, viruses, fungi, archaea, protists which may be resident or transient and in varying compositions, classifications and populations constituting the vaginal microbiota; as exists in all other internal and external parts of the body such as intestinal and skin microbiota, among others. (Barrientos-Durán *et al.*, 2020) The vaginal microbiota is a less diversified micro-ecosystem in contrast to those of some other sites especially the highly diversified adjacent intestinal microbiota and plays specific important roles in the wellbeing of the vagina and the female human body. (Baud *et al.*, 2023) The VMC is normally populated by facultative and obligate anaerobes, with *Lactobacilli*, as a dominant native of the community. The *Lactobacilli* dominance confers uniqueness on the vaginal micro ecosystem, and is recognized as a key factor in the maintenance of the vaginal and general female health, given that lactic acid, an end product of *Lactobacilli* fermentation

process, maintains the pH of the vagina below 4.0; which makes it hostile for the proliferation of many pathogenic and opportunistic bacteria. (France *et al.*, 2022)

As part of a dynamically sensitive ecosystem, the vaginal microbiota responds adaptively to physiochemical changes occasioned by pregnancy, menstruation, ovulation, diets, drugs, stress, ill-health, contraceptive use, and so on. As a self-cleansing organ, the vagina secretes a white or clear slightly smelling, but not offensive discharge consisting of desquamated vaginal epithelial cells, microbiota bacteria, and glandular secretions which serves to confer protection against vulvo-vaginal and related infections (Saraf *et al.*, 2021; Bitew *et al.*, 2021; Holdcroft *et al.*, 2023)

When the healthy state of the microbiota becomes displaced due to a compromised immunity, ill-health, antibiotic use etc.; the unique low microbial diversity, the *Lactobacilli* predominance status and lactic acid protection are lost, with resultant overgrowth of opportunistic bacteria. This condition is known as dysbiosis and results in a variety of vaginal infections and conditions such as vulvo-vaginal candidiasis (VVC), bacterial vaginosis (BV), trichomoniasis, cytolytic vaginosis (CV) or aerobic vaginitis (AV), abnormal leucorrhoea, increased discharge, vulval itching and burning pain (Zhan *et al.*, 2020; Serretiello *et al.*, 2021; Yasinet *et al.*, 2021; Holdcroft *et al.*, 2023)

The focus of this study is generally on the vaginal microbial community and specifically on aerobic bacteria which are aetiological agents of aerobic vaginitis, a bacterial infection of the vagina arising from displacement of the vaginal *Lactobacilli* with the attendant proliferation of aerobic bacteria mostly from the intestinal tracts. It is characterized by purulent vaginal discharge of abnormally high pH ranging from 5.0 to 8.0 as against the normal acidic vaginal pH of between 3.8 and 4.5, significant inflammatory changes, vaginal epithelial atrophy, slight erosions or ulcerations, vaginal/vulval pruritus, erythema and burning pain. (Zhang *et al.* 2020; Oerlemans *et al.*, 2020; Serretiello *et al.*, 2021; Yasinet *et al.*, 2021; Holdcroft *et al.*, 2023) AV has been attributed with risky situations in pregnancy such as preterm birth, premature membranes rupture, negative effects on neonates, neurological injury, fetal infections etc (Donders & Bellen 2011; Serretiello *et al.*, 2021)

AV was first described by Donders and colleagues in 2002 but has not been adequately studied or unravelled till date. (Zhang *et al.*, 2020; Oerlemans *et al.*, 2020) It has remained beclouded with difficulties in diagnosis and choice of antibiotics due to increasing incidence of antimicrobial resistance, and these have adversely affected its treatment for long. (Kareem & Abdulhamid, 2023) The need thus arises for more clarity on the identity of AV as an infection with regards to

etiology and suitable antimicrobial agents for the treatment. This study is therefore aimed at identifying and characterizing the etiological agents of aerobic vaginitis as well as determining the patterns of resistance against common antimicrobial drugs used in the treatment of the condition, so as to contribute in the curtailment, particularly in the area under study. Causative organisms of some other conditions associated with the vaginal microbiota are also to be ascertained.

Methodology

Study Design

This is a retrospective cross sectional descriptive study involving a review of laboratory records of microscopy, culture and susceptibility analysis of high vaginal swab (HVS) specimens carried out at Diagnostix and Scientifique Research Laboratories, Port Harcourt, Nigeria. The analysis involved female patients from public and private healthcare facilities and walk-in patients. The specimens included in the study were those with complete records of the age of subjects, isolated organisms, resistant and susceptible antimicrobial drugs.

Records of Isolation and identification of organisms

As contained in the standard operating procedure (SOP), the HVS specimens were cultured on blood agar and MacConkey agar (Oxoid, Hampshire, England); then incubated under aerobic conditions at 37 °C for 18 to 24 hours. The culture plates were examined visually for growths and the colonial morphologies were recorded; followed by gram-staining and biochemical testing. The morphological, biochemical, and physiological data were inputted into the ABIS online bacterial identification software, and the organisms were identified by the best match (Costin & Ionut 2017; Islam *et al* 2018; Ndukwu *et al* 2021a; Ndukwu *et al* 2021b).

Antimicrobial Susceptibility Testing

Antimicrobial Susceptibility Testing, (as stated in the SOP) was performed on the bacterial isolates by employing the Kirby Bauer disk diffusion method using Mueller-Hinton agar (Oxoid, Hampshire, England). (Kowalczyk *et al*, 2022) The following antimicrobial agents tested: Amoxicillin/cloxacillin (20 µg), Azithromycin (30 µg), Ceftriaxone (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (10 µg), Levofloxacin (20 µg), Gentamicin (10 µg), Norfloxacin (10 µg), Rifampicin (20 µg), Streptomycin (30 µg) (Oxoid, England) Resistance data were read and interpreted in

accordance with the standards of the Clinical Laboratory Standards Institute (CLSI). (Kowalczyk *et al.*, 2022; Ndukwu & Akani 2023)

Data Analysis

Descriptive statistical analysis was carried out using the Microsoft Excel spreadsheet software 2007

Results

The results obtained from this study indicate that out of a total of 790 specimens of high vaginal swabs examined within the study period, microbial growths were observed in 596 specimens representing a prevalence of (75.4%), while no microbial growths appeared in 194 (24.6%) of the specimens. The 596 specimens that produced growths include 515 (86.4%) single growths and 81 (13.6%) mixed growths; which altogether yielded 708 strains of fungi and bacteria, which together with two strains of *Trichomonas vaginalis* made a total of 710 (89.9%) strains recovered from the 790 specimens under review. The 710 strains comprise of 485 (68.3%), strains of fungi, 223 (31.4%) bacteria and 2 (0.3%) strains of protozoa. Approximately 50% of the isolates were produced by the age groups 21 to 30 years (20.6%) and 31 to 40 years (29.1), while the 10 to 20 years (8.1%), 41 to 50 years (13.9%) 50 to 60 years (13.0%) and the >60 years (15.2%) groups trailed. Mixed microbial growths accounted for 81 (13.6%) of all the growths, produced 193 (32.4%) of the 710 isolates including of 8 (4.1%) from bacteria + bacteria growths, 92 (47.7%) from bacteria to fungi growths and (48.1) from bacteria + bacteria + fungi growths. (Tables 1 & 2)

The gram negative bacteria were dominant with 118 (52.9%) strains over the gram positive bacteria 105 (47.1%) as shown in figure 1. Two hundred and twenty two aerobic bacterial strains belonging to seven species were recorded; *Escherichia coli* is the most prevalent with 98 (44.0%), followed by *Enterococcus faecalis* 55 (24.7%), *Staphylococcus aureus* 41 (18.4%), *Proteus mirabilis* 10 (4.5%), *Streptococcus agalactiae* 9 (4.0%), *Klebsiella pneumoniae* 5 (2.2%) and *Pseudomonas aeruginosa* 10 (4.5%). Fungi (*Candida* spp) was the most prevalent microbial group consisting of 485 (68.3%) strains, while protozoa (*Trichomonas vaginalis*) had 2 (0.3%) strains. (Table 3)

The cumulative antimicrobial resistance profile of the 223 bacterial strains tested against 10 antimicrobial agents was 55.5%. *Pseudomonas aeruginosa* was the most resistant with 62%, followed by *Klebsiella pneumoniae* 58%, *Escherichia coli*, 54.1%, *Staphylococcus aureus*, 51.0%,

Proteus mirabilis, 51.0%. *Enterococcus faecalis*, 48.4% and *Streptococcus agalactiae* 38.9%. The fluoroquinolone norfloxacin recorded the least effect against the bacterial isolates with a resistance profile of 80.7%, followed by amoxicillin clavulanate by (71.7%), chloramphenicol (61.9%), rifampicin (52.9%), azithromycin (48.0%), ceftriaxone (31.8%), gentamicin (30.9%) streptomycin (27.4%), ciprofloxacin (25.6%) and levofloxacin (17.9%) (Table 4)

UNDER PEER REVIEW

Table 1: Frequency and Distribution of High Vaginal Swabs and Microbial Growths Associated with Vaginal Microbiota

Years	Total samples		No Growths		Total Growths		Single growths		Mixed Growths		Bacteria + Bacteria Isolates		Bacteria +Fungi Isolates		Bacteria +Bacteria +Fungi Isolates		Total Mixed Growth Isolates	
	n	n%	n	n%	n	n%	n	n%	n	n%	nx2	n%	nx2	n%	nx3	n%	n	n%
2020	295	37.3	101	34.2	194	65.8	174	89.7	20	10.3	2	25.0	26	28.3	18	19.3	46	23.8
2021	258	32.7	45	17.4	213	82.6	173	81.2	40	18.8	6	75.0	42	45.6	48	51.6	96	49.7
2022	237	30.0	48	20.3	189	79.7	168	88.9	21	11.1	0	0	24	26.1	27	29.0	51	26.4
Total	790	100	194	24.6	596	75.4	515	86.4	81	13.6	8	4.1	92	47.7	93	48.1	193	32.4
Age Group (Years)																		
10–20	69	8.7	16	23.2	53	76.8	53	100	0	0	0	0	0	0	0	0	0	0
21–30	166	21.0	48	28.9	118	71.1	99	83.9	19	23.5	4	50.0	16	17.4	27	29.0	47	23.3
31–40	213	27.0	58	27.2	155	72.8	129	83.2	26	32.1	4	50.0	26	26.3	33	35.5	63	32.6
41–50	145	18.4	37	25.5	108	74.5	92	90.2	16	19.8	0	0	22	23.9	15	16.1	37	19.2
51–60	92	11.6	15	16.3	77	83.7	68	88.3	9	11.1	0	0	14	15.2	6	30.0	20	10.4
>60	105	13.3	20	19.0	85	81.0	74	87.1	11	13.6	0	0	14	15.2	12	12.9	26	13.5
Total	790	100	194	24.6	596	75.4	515	86.4	81	10.3	8	4.1	92	47.7	93	48.1	193	32.4

Table 2: Frequency and Distributions of Different Organisms Associated with Vaginal Microbiota

	Bacteria						Fungi						Protozoa		Total Isolates					
	S		M		T		S		M		T				S		M		T	
	n	n%	n	n%	n	n%	n	n%	n	n%	n	n%	n	n%	n	n%	n	n%	n	n%
2020	49	45.8	27	23.2	76	34.1	124	30.4	19	24.7	143	29.5	1	0.5	174	33.8	46	23.8	220	62.4
2021	21	19.6	59	50.9	80	35.9	135	33.1	37	48.1	172	35.5	10	0	156	30.3	96	49.7	252	79.5
2022	37	34.6	30	25.9	67	30.0	149	36.5	21	27.3	170	35.0	1	0.5	187	36.3	51	26.4	238	71.3
Total	107	15.1	116	16.3	223	31.4	408	57.5	77	10.8	485	68.3	2	0.3	515	72.5	193	27.2	710	89.9
Age Group (Years)																				
10–20	18	16.8	0	0	18	8.1	45	11.0	0	0	45	9.3	0	0	65	12.6	0	0	65	9.2
21–30	16	15.0	30	25.9	46	20.6	79	19.4	17	22.1	96	19.8	0	0	94	18.3	47	24.3	141	19.9
31–40	26	24.3	39	33.6	65	29.1	96	23.5	24	31.2	120	24.7	2	100	117	22.7	63	32.6	182	25.6
41–50	10	9.3	21	18.1	31	13.9	77	18.9	16	20.8	93	19.2	0	0	93	18.1	37	19.2	130	18.3
51–60	18	16.8	11	9.5	29	13.0	56	13.7	9	11.7	65	13.4	0	0	74	14.4	20	10.4	94	13.2
>60	19	17.8	15	12.9	34	15.2	55	13.5	11	14.3	66	13.6	0	0	72	14.0	26	13.5	98	13.8
Total	107	15.1	116	16.3	223	31.4	408	57.5	77	10.8	485	68.3	2	0.3	515	72.5	193	27.2	710	100

S: Single growths, **M:** Mixed Growths, **T:** Total Growths, **n:** Number of isolates

Table 3: Prevalence of Species of Aerobic Bacteria and other organisms Associated with Vaginal Microbiota

Isolates	2020		2021		2022		Total	
	n	Percent	n	Percent	n	Percent	n	Percent
Bacterial Isolates								
<i>Escherichia coli</i>	41	54.0	38	47.5	19	28.3	98	44.0
<i>Enterococcus faecalis</i>	28	36.8	15	18.8	28	41.8	55	31.8
<i>Staphylococcus aureus</i>	7	9.2	22	27.5	12	17.9	41	18.4
<i>Proteus mirabilis</i>	0	0	4	5.0	1	1.5	10	2.2
<i>Streptococcus agalactiae</i>	0	0	1	1.3	3	4.5	9	1.8
<i>Klebsiella pneumoniae</i>	0	0	0	0	2	3.0	5	0.9
<i>Pseudomonas aeruginosa</i>	0	0	0	0	2	3.0	5	0.9
Total	76	34.1	80	35.9	67	30.0	223	31.4
Fungal Isolates								
<i>Candidasp</i>	143	29.5	172	35.5	170	35.1	485	68.3
Protozoa								
<i>Trichomonas vaginalis</i>	1	50.0	0	0	1	50.0	2	0.3
Total	220	31.0	252	35.5	238	33.5	710	100

n: Number of isolates

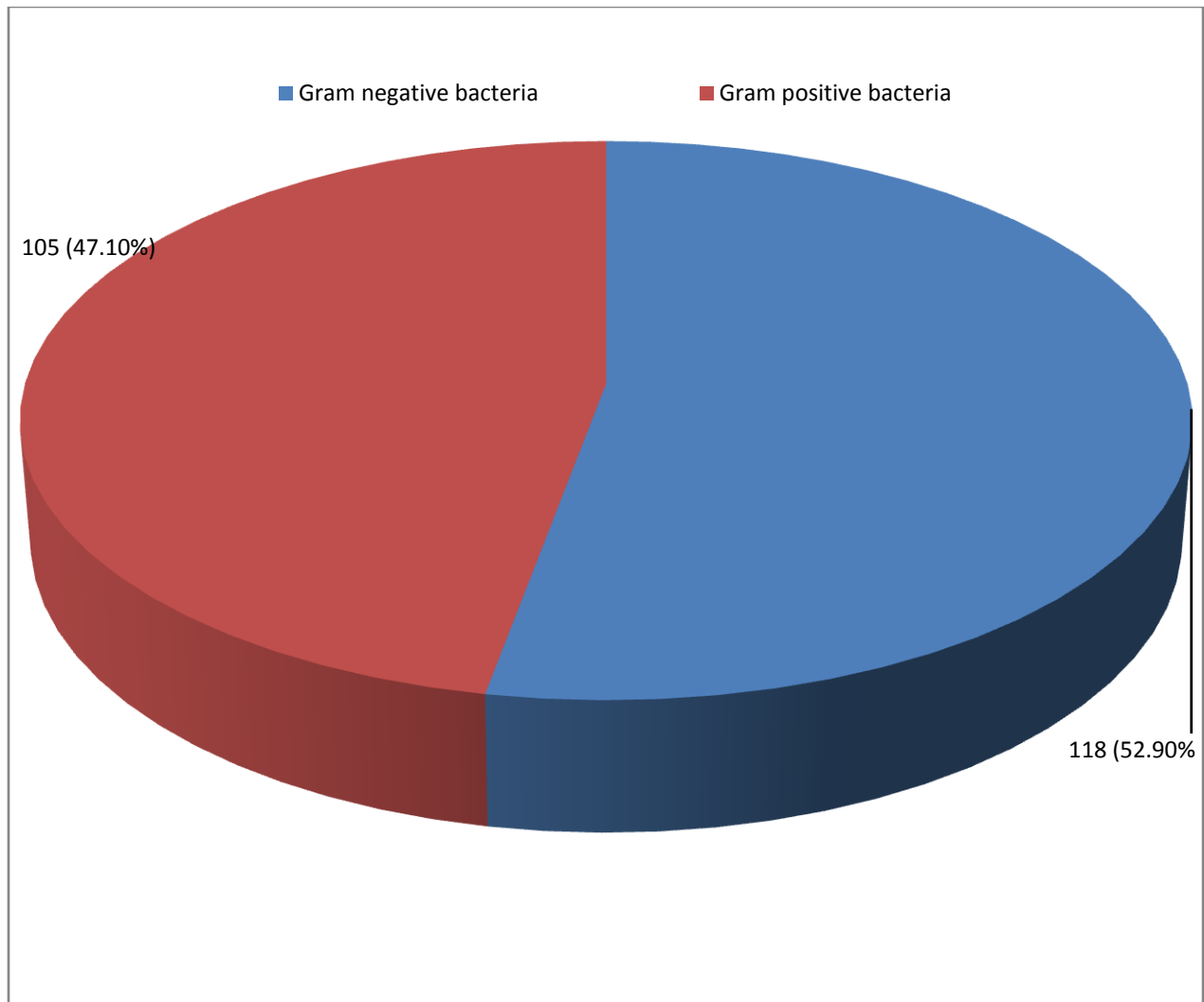


Figure 1: Prevalence of Gram Negative and Gram Positive Aerobic Bacteria Associated with Vaginal Microbiota

Table 4: Antimicrobial Resistance Patterns of Aerobic Bacteria Associated with Vaginal Microbiota

	n	nx15	APX	AZT	CTX	CHL	CPX	LV	CN	NB	RD	STR	Total
<i>Escherichiacoli</i>	98	980	76 (77.6)	57 (58.2)	34 (34.7)	53 (54.1)	21 (21.4)	15 (15.3)	28 (28.6)	74 (75.5)	46 (47.0)	28 (28.6)	530 (54.1)
<i>Enterococcusfaecalis</i>	55	550	32 (58.2)	17 (30.9)	14 (25.5)	29 (52.7)	17 (30.9)	10 (18.2)	16 (29.1)	38 (69.1)	25 (25.5)	13 (13.3)	266 (48.4)
<i>Staphylococcus aureus</i>	41	410	25 (61.0)	15 (36.6)	13 (31.7)	23 (56.1)	12 (29.3)	9 (22.0)	16 (39.0)	31 (75.6)	17 (41.5)	7 (17.1)	209 (51.0)
<i>Proteus mirabilis</i>	10	100	7 (70)	6 (60)	4 (40)	9 (90)	2 (20)	1 (10)	3 (30)	8 (80)	8 (80)	4 (40)	51 (51.0)
<i>Streptococcus agalactiae</i>	9	90	4 (44.4)	2 (22.2)	3 (33.3)	6 (66.7)	2 (22.2)	1 (11.1)	2 (22.2)	8 (88.7)	5 (55.5)	2 (22.2)	35 (38.9)
<i>Klebsiella pneumoniae</i>	5	50	4 (80.0)	3 (60.0)	0	4 (80.0)	2 (40.0)	1 (20.0)	2 (40.0)	4 (80.0)	3 (60.0)	2 (40.0)	29 (58.0)
<i>Pseudomonas aeruginosa</i>	5	50	4 (80.0)	4 (80.0)	2 (40.0)	5 (100)	1 (20.0)	2 (40.0)	2 (40.0)	4 (80.0)	4 (80.0)	3 (60.0)	31 (62.0)
Total	223	2230	160 (71.7)	107 (48.0)	71 (31.8)	138 (61.9)	57 (25.6)	40 (17.9)	69 (30.9)	180 (80.7)	118 (52.9)	61 (27.4)	1238 (55.5)

APX:Ampicillin/cloxacillin, AZT:Azithromycin, CTX: Ceftriaxone, CHL: Chloramphenicol, CPX:Ciprofloxacin, LV:Levofloxacin, CN: Gentamycin, NB: Norfloxacin, RD: Rifampicin, STR: Streptomycin

Discussion

This study has contributed in ascertaining the prevalence of aerobic bacteria (AV), associated with vaginal microbiota, and unraveled the bacterial and other associated organisms as well as the antimicrobial patterns of the AV. Since the condition known as aerobic vaginitis was described by Donders and colleagues in 2002, a lot of studies have been conducted on the condition by researchers across the world. (Zhang *et al.*, 2020) The prevalence of 31.4% obtained in this study is higher than the figures reported in a number of studies such as the range of 7.9–23.7%, (Ma *et al.* 2022), 8.1% (Yalew *et al.*, 2022), 15% reported in India (Prasad *et al.*, 2021), 15.53%, also in India (Donders *et al.*, 2002), 22.9% reported in Ethiopia (Yasin *et al.*, 2021), however higher prevalence have been recorded elsewhere such as 65.49% (Zhang *et al.*, 2020) and 51.6% (Salinas *et al.*, 2020); The prevalence of aerobic vaginitis has been reported to vary from 5 and 10.5% among symptomatic non-pregnant females subjects, while pregnant subjects range from 4 to 8%. (Donders *et al.*, 2017; Juliana *et al.*, 2020) On the other hand, there has been report that put the incidence of AV between 5 and 80%; this has been attributed to a variety of socio-demographic and related factors such as antibiotic use and abuse, nature and availability of healthcare facilities, geographic and climatic factors etc. (Vidyasagar 2021; Serretiello *et al.*, 2021) The highest prevalence of AV in this study was observed within the most active reproductive age of 20 to 40. Though not sexually transmitted, AV is sexually associated given that sexual activity serves in altering the vaginal microenvironment leading to depletion of Lactobacilli and proliferation on endogenous opportunistic infections. This could explain the high prevalence in the most active reproductive age brackets as also found in studies elsewhere.

The commonest isolates obtained in this study were *Escherichia coli* 44.0 %, *Enterococcus faecalis* 24.7% and *Staphylococcus aureus* 18.4%, which like the other isolates, are common uropathogenic bacteria that may have crossed from the urinary tract to the genital tracts. These appear to be the trend in vaginal infections such that the loss of the *Lactobacilli* protection opens the way for the invasion of the vagina by uropathogens. The results aligns closely with the predominance of *E. faecalis* (32.26%), followed by *E. coli* (25.8%), *S. aureus* (19.35%) reported in India (Sangeetha Saroj 2015; Serretiello *et al.* 2021) in several other studies. (Yasin *et al.*, 2021; Prasad *et al.*, 2021; Yalew *et al.*, 2022; Ma *et al.*, 2022) The prevalence of other vaginal infections were observed in the study with Vaginal candidiasis being the most prevalent, recording 68.3%, trichomoniasis was 0.3%, while mixed infections had a prevalence of 10.3% and contributed to 27.2 % of the pathogens.

The cumulative antimicrobial resistance profile of 55.5% the isolates corroborates the assertion of many researchers on the high antimicrobial resistance among aerobic bacteria associated with the vaginal microbiota. Five of the seven bacterial isolates have resistance profiles above 50%, four of these, namely *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* belong to the ESKAPE pathogens notorious for high levels of multidrug resistance. The way antimicrobial resistance has taken a toll on many commonly used antibiotics like norfloxacin, amoxicillin/cloxacillin, chloramphenicol, rifampicin, azithromycin, ceftriaxone and gentamicin as observed in this study agrees with a number of previous reports. (Yasin *et al.*, 2021; Prasad *et al.*, 2021; Yalew *et al.*, 2022; Ma *et al.*, 2022; Ndukwu & Akani 2023)

As a retrospective study, a major limitation of this study was that the laboratory analysis was concluded without having the study in contemplation with regards to certain important decisions such as socio-demographics variables, number and choice of antimicrobial amongst others. Also, molecular analysis would have been of great benefit to identification of the isolated organisms but could not be utilized. It is advised that corrections for these limitations be factored into future studies. Also there is need for studies on specific populations such as pregnant women, women undergoing infertility investigations and treatment; and also cohort studies to determine variation in time and conditions and also address specific conditions.

Conclusion

This study has been able to assist in the identification and ascertainment of organisms associated with the human vaginal microbiota, as well as the determination of the antimicrobial resistance patterns of the aerobic bacteria. This will serve useful purposes in the diagnosis and treatment of the various condition related to dysbiosis in the vaginal micro-ecosystem and save the patients time, finance and other resources associated with such conditions, in addition to the trauma and uncertainty that comes with complications and elongated stay in healthcare facilities.

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