Original Research Article

Microbial Communities and Antimicrobial Resistance Patterns in Aerobic BacteriaAssociated withthe 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 diease 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 sythesize 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 pnumoniae* 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 pnumoniae*, 58%, *Escherichiacoli*,54.1%, *Staphylocuccus aureus,* 51.0%, *Proteus mirabilis,* 51.0%. *Enterocuccusfaecalis,*48.4% and *Streptococcus agalactiae* 38.9%. This study has contributed in ascertaining the prevalence ofbacterial 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, Vaginalmicrobiota, Vaginal microbial communities, Vulvo-vaginal candidiasis, Trichomoniasis,*Lactobacilli* dominance

Introduction

Vaginal microbial communities(VMC) refer tovarious ecological populationsof pathogenic, symbiotic, mutualistic or commensal organismsincluding bacteria, viruses, fungi, archaea, protistswhich may be resident or transient and in varying compositions, classifications and populations constituting the vaginal microbiota;as exists in all otherinternal 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-ecosystemin contrast to those of some othersites especially the highly diversifiedadjacentintestinal 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 uniquenesson the vaginal micro ecosystem, andisrecognized as a key factor in the maintenance of the vaginal and generalfemale 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, antibioticusesetc.;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 ina variety of vaginal infections and conditions such as vulvo-vaginal candidiasis (VVC), bacterial vaginosis (BV), trichomonasis, cytolytic vaginosis (CV) or aerobic vaginitis (AV), abnormal leucorrhea, increased discharge, vulval itching and burning pain(Zhang *et al* 2020; Serretiello *et al* 2021; Yasin*et al* 2021; Holdcroft *et al* 2023)

The focus of this study is generally on the vaginal microbial communityand specifically on aerobic bacteria which are aetiologic 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; Yasin*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 2002buthas 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 its etiologyand suitable antimicrobial agents for the treatment.This study is therefore aimedat identifying and characterizing the etiological agents of aerobic vaginitis as well as determining the their patterns of resistance against common antimicrobial drugs used in the treatment of the condition, so as to assist in its curtailment in the area under study. Causative organisms and other conditions associated with the vaginal microbiota will also 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 outat 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), theHVS 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(20µg), Azithromycin (30 µg), Ceftriaxone(30 µg), Chloramphenicol(30 µg), Ciprofloxacin(10 µg), Levofloxacin(20µg), 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

Statistical analysis was carried out using the Microsoft Excel spreadsheet software2007

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 strainscomprises of 485 (61.4%),strains of fungi, 223 (15.0%) bacteria and 2 (0.3%) strains of protozoa. Approximately50% of the isolates were produced by 21 to 30 (20.6%) and 31 to 40 (29.1) years age group, while the 10 to 20 (8.1%), 41 to 50 (13.9%) 50 to 60 (13.0%) and the >60 (15’2%) groups trailed.Mixed microbial growths accounting for 81 **(13.6%)** of all the growths, produced 193 (32.4%) of the 710 isolatesincluding 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 negativebacteria 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 pnumoniae* 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 pnumoniae* 58%, *Escherichiacoli*,54.1%, *Staphylococcus aureus,* 51.0%, *Proteus mirabilis,* 51.0%. *Enterococcusfaecalis,*48.4% and *Streptococcus agalactiae* 38.9%. The fluoroquinolone norfloxacinrecorded 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)

Table 1: Frequency and Distribution of High Vaginal Swabs andMicrobial GrowthsAssociated 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**	**61.4**	**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**	
Bacterial Isolates	**n**	**Percent**	**n**	**Percent**	**n**	**Percent**	**n**	**Percent**
Escherichia coli	41	54.0	38	47.5	19	28.3	98	44.0
Enterococcus faecalis	28	36.8	15	18.8	28	41.8	55	31.8
Staphylococcus aureus	7	9.2	22	27.5	12	17.9	41	18.4
Proteus mirabilis	0	0	4	5.0	1	1.5	10	2.2
Streptococcus agalactiae	0	0	1	1.3	3	4.5	9	1.8
Klebsiella pnumoniae	0	0	0	0	2	3.0	5	0.9
Pseudomonas aeruginosa	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								
*Candida*spp	143	29.5	172	35.5	170	35.1	485	68.3
Protozoa								
Trichomonas vaginalis	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

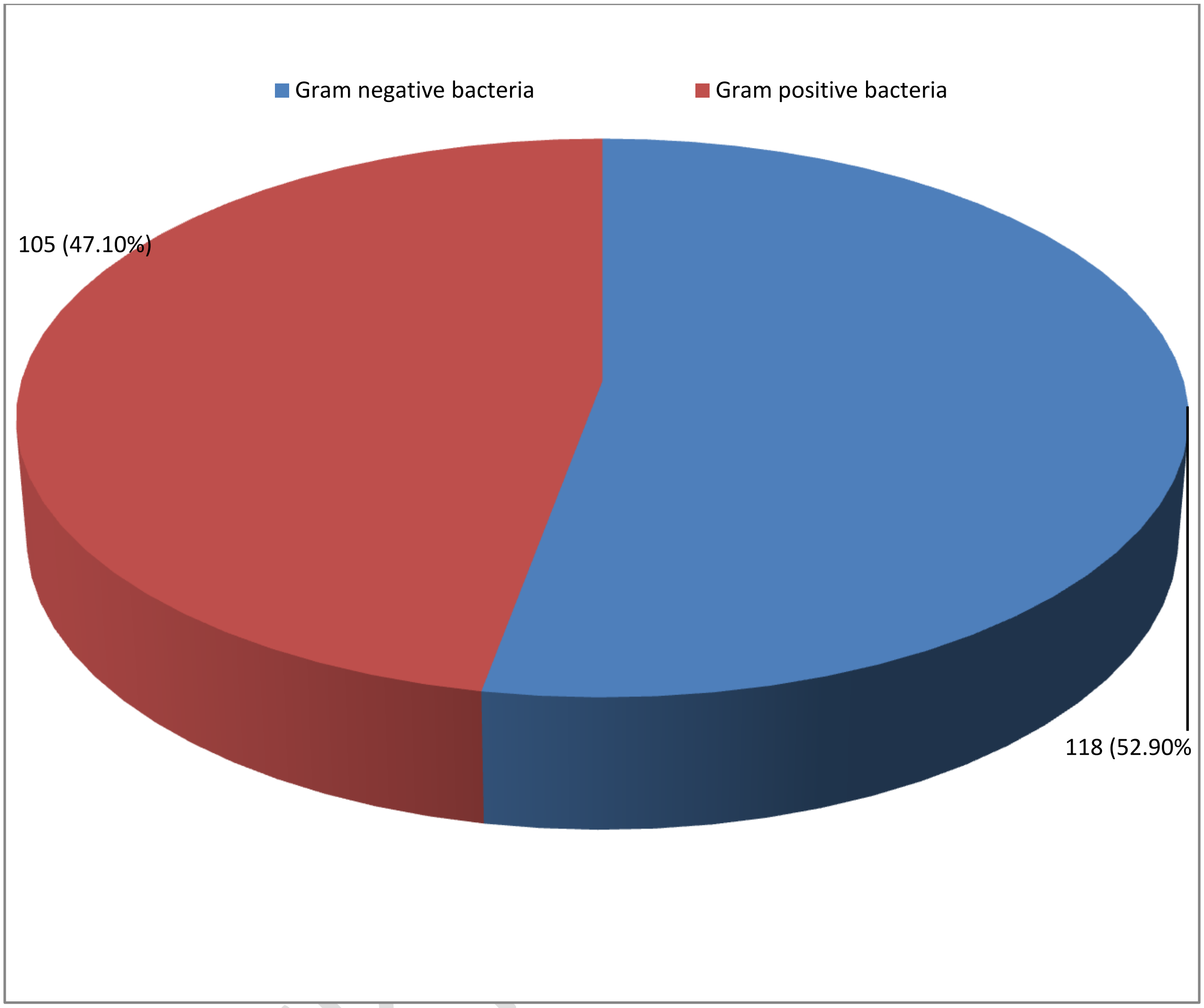

Figure 1: Prevalence of Gram Negative and Gram Positive Aerobic Bacteria Associated with Vaginal Microbiota

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

	n	**nx15**	**APX**	**AZT**	**CTX**	**CHL**	**CPX**	**LV**	**CN**	**NB**	**RD**	**STR**	**Tota**
Escherichiacoli	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
Enterocuccusfaecalis	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
Staphylocuccus aureus	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
Proteus mirabilis	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
Streptococcus agalactiae	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
Klebsiella pnumoniae	5	50	4 (80.0)	3 (60.0)	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
Pseudomonas aeruginosa	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 hascontributed 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 collegues 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 inIndia (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 as65.49% (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 endogenic 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 *Escherichiacoli* 44.0 %, *Enterocuccusfaecalis* 24.7% and *Staphylocuccus aureus* 18.4%, which like the other isolates, are common uropathogenic bacteria that 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 *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%, trichomonasis 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 pnumoniae* 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 likenorfloxacin, amoxicillin/cloxacillin, chloramphenicol, rifampicin, azithromycin, ceftriaxone and gentamicin as observed in this studyagrees 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 microbiata, 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 dtysbiosis 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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