



OCCURRENCE OF *GARDNERELLA VAGINALIS* AMONG PATIENTS DIAGNOSED OF BACTERIAL VAGINOSIS IN AHMADU BELLO UNIVERSITY TEACHING HOSPITAL, SHIKA, ZARIA-NIGERIA

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ABSTRACT

Background: Bacterial vaginosis is the condition that occurs when the balance of the vaginal flora is distorted, and replaced by a high concentration of harmful bacteria. Such harmful bacteria are *Gardnerella vaginalis* and *Mycoplasma* species.

Aim: The aim of this study was to detect bacterial vaginosis (BV) among female patients presenting with suspected cases of vaginitis in Ahmadu Bello University Teaching Hospital, Shika, Zaria, determine the occurrence of the implicating *Gardnerella vaginalis* and its antibiotic susceptibility pattern.

Methods: One hundred high vaginal swab (HVS) samples of patients presenting with vaginitis in Ahmadu Bello University Teaching Hospital, Shika, Zaria-Nigeria were screened for bacterial vaginosis (BV) using Amsel's criteria. Samples positive for BV were subjected to cultural test to isolate *Gardnerella vaginalis*. The isolates were identified using biochemical test and subjected to antibiotic susceptibility using Agar disc diffusion method. The socio-demographic and risk factors associated with the infection were also determined using structural questionnaire.

Results: Fifty-two percent prevalence of BV was recorded among the consented patients. The overall occurrence of *Gardnerella vaginalis* among BV patients was 15.4%. The highest occurrence of *Gardnerella vaginalis* (50%) was recorded against ≥ 51 years age group, while age group 21-30 years had the lowest occurrence rate (8.7%). However, there was no association between the infection and age groups ($p > 0.05$). There was no significant association between level of education and the infection, despite highest occurrence (50%) recorded among non-educated patients. There was high occurrence among the business women (26.7%) compared to house wives (0.0%). Also, there was no significant association between risk factors and the occurrence of *Gardnerella vaginalis* ($P > 0.05$). Patients that have had cases of miscarriage had the highest occurrence of *Gardnerella vaginalis* (22.2%) compared to those that do not have miscarriage (14.0%). No *Gardnerella vaginalis* infection recorded against patients that use condom (0.0%). In relation to *Gardnerella vaginalis* infection and symptoms, there was no significant association. The isolates were 100%, 87.5% and 75.0% susceptible to Ceftriaxone, Gentamicin and Levofloxacin respectively. However, they were 100% resistant to ampicillin. Fifty percent of the isolates were multi-drug resistant.

Conclusion: Bacterial vaginosis was detected among patients presenting with vaginitis and *Gardnerella vaginalis* was implicated in some cases. It proper diagnosis and prompt treatment is important in preventing pregnancy complications and infertility.

Keywords: HVS; Amsel's Criteria; Bacterial Vaginosis; *Gardnerella vaginalis*; MDR

INTRODUCTION

In healthy women, the vaginal is characterised by the presence of various species of *Lactobacillus*. It is colonized by a

variety of species of harmless bacteria except under abnormal conditions (Muhammed *et al.*, 2019).

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Vaginitis is an inflammation of the vagina. It is characterized by vaginal discharge containing many white blood cells (WBC), vulva itching and irritation, vaginal odour, vaginal erythema and painful urination (Bohbot *et al.*, 2014). Nearly 5-10 million females seek gynecologic advice for vaginitis every year worldwide (Hay, 2014). Vaginal infection with bacterial vaginosis, candidiasis and trichomoniasis are a global health problem among women of reproductive age (Eligantri *et al.*, 2010).

Bacterial vaginosis (BV) is the condition that occurs when the balance of the vaginal flora (*Lactobacillus* species) is depleted, and replaced by a high concentration of harmful bacteria in the vagina like *Gardnerella vaginalis*, *Prevotella*, *Ureaplasma urealyticum*, and *Bacteroides* species (Yudin *et al.*, 2008; Bastani *et al.*, 2012; Afolabi *et al.*, 2018; Bitew *et al.*, 2021). *Gardnerella vaginalis* is one of the bacteria that cause bacterial vaginosis. It is an anaerobic, catalase and oxidase negative as well as Gram variable bacteria (Hashemi *et al.*, 2017). The risk factors associated with the infection include; pregnancy, vaginal douching, use of contraceptives (Muzny *et al.*, 2013 and Ranjit *et al.*, 2018) miscarriage, diabetes and sexual activities (Awoniyi *et al.*, 2015). The most typical symptoms of bacterial vaginosis include watery, gray discharge and typical fishy odor which can be diagnosed by checking p^H of the vaginal and also presence of clue cells on wet mount microscopy (Donders *et al.*, 2014).

Vaginal infections are common among women of reproductive age. Bacterial vaginosis increases women's risk of getting other sexually transmitted diseases including HIV (Spear *et al.*, 2007; Martin *et al.*, 2016). It is associated with pelvic inflammatory diseases, a serious disease that can harm a woman's reproductive organ and thereby cause infertility (Bilardi *et al.*, 2013; Chenicheri *et al.*, 2017).

In developing countries, cases of genital tract infection are uncommon, hence prevalence data is restricted to vaginitis such

as BV, Candidiasis and Trichomoniasis. Although some work has been reported, very few has focused attention on detection of *Gardnerella vaginalis* from female of reproductive age despite the serious outcome of the infection among young females such as vagina itching, painful urination, and fishy odour (William *et al.*, 2022).

Early diagnosis and treatment are used for prevention and control of bacterial vaginosis. The treatment of bacterial vaginosis involves using systemic antibiotics which is in accordance with the CDC standard guidelines, alternative strategies involve use of antiseptics and probiotics preparations (Donders *et al.*, 2014). Recurrence of bacterial vaginosis is common. Women should be advised to return for treatment if symptoms persist.

The aim of this study was to detect bacterial vaginosis (BV) among female patients presenting with suspected cases of vaginitis in Ahmadu Bello University Teaching Hospital, Shika, Zaria, determine the occurrence of the implicating *Gardnerella vaginalis* and its antibiotic susceptibility pattern. The socio-demographic and risk factors associated with the infection were also determined.

METHODOLOGY

Study Area

The study was carried out at Ahmadu Bello University Teaching Hospital (ABUTH), Zaria.

Ethical Clearance and Consent

The study was conducted after securing approval from the Management of Ahmadu Bello University Teaching Hospital, Shika, Zaria-Nigeria, with reference number NHREC/TR/ABUTH-NHREC/01/02/23.

Patients' consents were sought for before collection of sample.

Study Population

A cross-sectional study was conducted involving females of reproductive age between 13 to ≥ 51 years who are attending the gynecology clinic of Ahmadu Bello University Teaching Hospital (ABUTH), Zaria.

Sample Size

The sample size was calculated by using previously reported 6.5% prevalence of *Gardnerella vaginalis* by Adinma *et al.* (2001) and sample size formula was employed to arrive at 92.77. However, for easier and convenient collection of high vaginal swab (HVS), the size was rounded up to 100. The collected HVS samples were transported to the Department of Microbiology Laboratory, Ahmadu Bello University, for microbiological analysis.

Inclusion and Exclusion criteria

Females who consented to participate in the study and clinically diagnosed of vaginitis without history of antibacterial therapy within two weeks prior to the clinic attendance constituted the inclusion criteria. Those that were not diagnosed of vaginitis or not consented to participate were excluded.

Determination of Risk Factors

A standardized questionnaire was used in collecting the following risk and socio-demographic factors associated with bacterial vaginosis such as diabetes, pregnancy, miscarriage, age, occupation, level of education. Information obtained from the participant were kept strictly confidential.

Sample Collection

High vaginal swabs specimen were collected aseptically using a sterile swab sticks. Samples collected were transported in an ice pack to the Department of Microbiology Laboratory, Ahmadu Bello University, Zaria, for immediate analysis (Cheesbrough, 2006).

Determination of Bacterial Vaginosis

Amsel's criteria were used to determine bacterial vaginosis.

In Amsel's criteria, there are four parameters used in determining the presence or absence of bacterial vaginosis. They include:

- Presence of thin greyish homogenous discharge.
- Test for p^H; vaginal p^H was measured by rolling the swab over a p^H strip immediately after swabbing and observed for color

change to record the p^H of the subject. The pH of the vaginal must not be greater than 4.5 (Paladine *et al.*, 2018).

Whiff's Test; a drop of 10% potassium hydroxide (KOH) was added on a glass slide, followed by rolling the swab on it. It was whiffed for liberation of a fishy odor which indicates the presence of volatile amines such as trimethylamine. The whiff result was recorded as either positive or negative (William *et al.*, 2022; Udeogu *et al.*, 2022).

Wet film preparation for clue cells; a sample of vaginal fluid from a swab stick was mixed with normal saline on a glass slide and cover slipped. The wet preparation was examined using 40X objectives for the presence of clue cells (Nwadioha *et al.*, 2011; William *et al.*, 2022).

However, three of the four above criteria must be present before it will be confirmed positive for bacterial vaginosis.

Isolation of *Gardnerella vaginalis* Implicated in Bacterial Vaginosis

Samples that were positive for Amsel's criteria were aseptically cultured by streaking on the surface of blood agar and chocolate agar plate, incubated anaerobically in a candle jar at 37⁰C for 72 hours. After incubation period, bacterial colonies were examined for appearance based on shape, elevation, edge and pigmentation. *Gardnerella vaginalis* form small round smooth grey colonies that are pinpoint in size with β-hemolysis on blood agar and chocolate agar (Ara *et al.*, 2017).

Gram Staining

Smear of the suspected isolates were prepared on a clean grease-free slide, air dried, heat fixed and Gram stained as described by Cheesbrough (2006). *Gardnerella vaginalis* appear Gram-positive or Gram variable coccobacilli or small rods.

Biochemical Characterization of the Isolates

The isolated bacteria were confirmed using the biochemical test such as catalase, oxidase, urease, indole, methyl red and Voges-Proskauer (Cheesbrough 2006).

Antibiotic Susceptibility Test

A total of five (5) antibiotics were tested using modified Kirby-Bauer disc diffusion method. The antibiotics used include Amoxicillin, Gentamicin, Levofloxacin, Ceftriaxon and Chloramphenicol.

Muller Hinton media was prepared according to manufacturer's instruction and stored at 4°C. A sterile swab stick was dipped into a standardized bacterial isolates suspension; the swab stick was pressed against the side of the test tube to get rid of excess inoculum after which it was swabbed evenly across the surface of Muller Hinton Agar Plates. The plates were allowed to dry briefly and using a sterile forcep, impregnated single antibiotic discs containing designated concentrations were placed on the inoculated plates. The discs were placed at least 15mm from the edge of the plate and not closer than 25mm from each other. The discs were firmly pressed down, allowed to diffuse and incubated at 35°C for 18 hours. After the incubation period, the plates were examined and zone of inhibitions were measured in diameter (mm) using a transparent ruler. The results were interpreted as susceptible, resistant or intermediate according to Clinical and Laboratory Standard institute (CLSI, 2021).

RESULTS

The Prevalence of Bacterial Vaginosis among patients presenting with vaginitis

using the Amsel's clinical criteria was 52% and the occurrence of *Gardnerella vaginalis* among patients with Bacterial vaginosis was found to be 15.4% (fig. 1 and fig. 2).

The occurrence of *Gardnerella vaginalis* based on age was found to be higher among age group ≥51-year (50.0%) while the least was among age group 21-30 years (8.7%). There was high occurrence of *Gardnerella vaginalis* among the non-educated patients (50.0%) but no infection was recorded among those with primary level of education. *Gardnerella vaginalis* infection was higher among the businesswomen (26.7%) compared to house wives where no infection was recorded. However, the difference was not statistically significant ($p>0.05$).

There was no association between the infection and risk factors despite the high prevalence among patients who have had cases of miscarriage (22.2%) followed by those with diabetes (21.4%). There was no infection recorded for patients who are HIV positive and those who uses condom.

The isolates were 100%, 87.5% and 75.0% susceptible to Ceftriaxone, Gentamicin and Levofloxacin respectively. However, they were 100% resistant to ampicillin. Occurrence of Multi-Drug Resistant *Gardnerella vaginalis* was found to be 50% among the isolates.

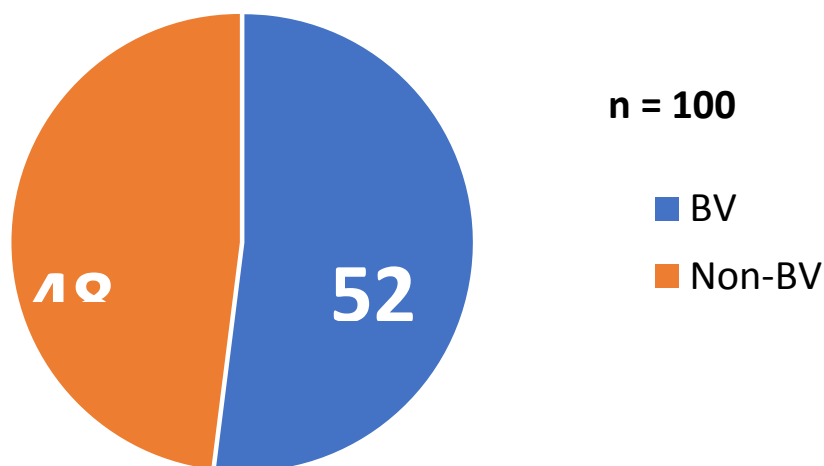


Figure 1: Prevalence of Bacterial Vaginosis among Patients Presenting with Vaginitis

Key: BV =Bacterial vaginosis, n=number of sample

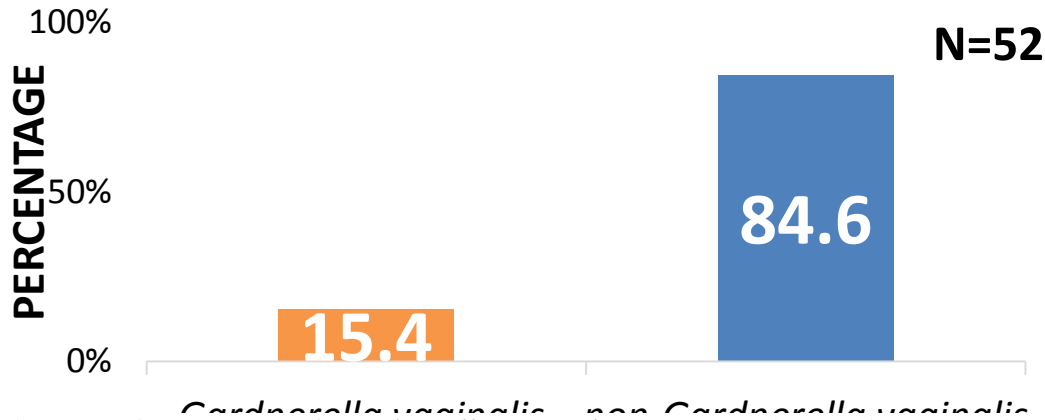


Figure 2: Occurrence of *Gardnerella vaginalis* among Patients with Bacterial Vaginosis
 Key: N = Number of Positive Bacterial Vaginosis (BV)

Table 1: Occurrence of *Gardnerella vaginalis* in Relation to Age of Patients

Age Group (Years)	Number Examined	Number Positive (%)	Number Negative (%)
13-20	7	1(14.3)	6(85.7)
21-30	23	2(8.7)	21(91.3)
31-50	10	1(10.0)	9(90.0)
41-50	8	2(25.0)	6(75.0)
≥51	4	2(50.0)	2(50.0)
Total	52	8(15.4)	44(84.6)

$\chi^2 = 5.270$ P-value = 0.261

Table 2: Occurrence of *Gardnerella vaginalis* based on Socio-Demographic Factors

Factors	Number Examined	Number Positive (%)	Number Negative (%)	χ^2	P-value
Level of Education				2.229	0.526
Primary	2	0(0.0)	2(100.0)		
Secondary	7	1(14.3)	6(85.7)		
Tertiary	41	6(14.6)	35(85.7)		
Non-Educated	2	1(50.0)	1(50.0)		
Occupation				2.843	0.296
Civil Servant	7	1(14.3)	6(85.7)		
House Wife	7	0(0.0)	7(100.0)		
Business Woman	14	4(26.7)	11(73.3)		
Student	23	3(13.0)	20(87.0)		

Occurrence of Gardnerella vaginalis

Table 3: Occurrence of *Gardnerella vaginalis* Based on Risk Factors

Risk Factors	Number Examined	Number Positive (%)	Number Negative (%)	χ^2	<i>p-value</i>
Diabetes				0.538	0.463
Yes	14	3(21.4)	11(78.6)		
No	38	5(13.2)	33(86.8)		
HIV Positive				0.185	0.667
Yes	1	0(0.0)	1(100.0)		
No	51	8(15.7)	43(84.3)		
Use of Condom				1.233	0.267
Yes	6	0(0.0)	6(100.0)		
No	46	8(15.4)	44(84.6)		
Vaginal Douching				0.084	0.772
Yes	11	2(18.2)	9(81.8)		
No	41	6(14.6)	35(85.4)		
Miscarriage				0.391	0.532
Yes	9	2(22.2)	7(77.8)		
No	43	6(14.0)	37(86.0)		
Pregnancy				0.855	0.652
Yes	19	4(21.1)	15(78.9)		
No	32	4(12.5)	28(87.5)		
No Response	1	0(0.0)	1(100.0)		

Table 4: Occurrence of *Gardnerella vaginalis* in Relation to Symptoms

Symptoms	Number Examined	Number Positive (%)	Number Negative (%)	χ^2	<i>p-value</i>
Painful Urination	6	1(16.7)	5(83.3)	1.835	0.607
Vaginal Itching	33	4(12.1)	29(87.9)		
Vaginal Odour	11	3(27.3)	8(72.7)		
No Response	2	0(0.0)	2(100.0)		

Table 5: Susceptibility Profile of the Isolated *Gardnerella vaginalis*

Antibiotics (μ g)	N=8	Number Susceptible (%)	Number Intermediate (%)	Number Resistant (%)
Ampicillin (10)		0(0.0)	0(0.0)	8(100.0)
Gentamicin (10)		7(87.5)	0(0.0)	1(12.5)
Ceftriaxone (30)		8(100.0)	0(0.0)	0(0.0)
Levofloxacin (5)		6(75.0)	1(12.5)	1(12.5)
Chloramphenicol (30)		5(62.5)	1(12.5)	2(25.0)

Key: N = Number of Isolates

Table 6: Resistance Patterns of *Gardnerella vaginalis*

Isolate code	Susceptible	Intermediate	Resistant	MDR
G6	LEV, C, CN CRO		AM	-
G18	C, CRO	LEV	CN, AM	+
G29	LEV, C, CN, CRO		AM	-
G36	LEV, CN, CRO		C, AM	+
G37	C, CN,CRO		LEV,AM	+
G56	LEV, C, CN, CRO		AM	-
G66	LEV, CN, CRO	C	AM	-
G82	LEV, CN, CRO		C, AM	+

Key: AM – Ampicillin, C – Chloramphenicol, CRO – Ceftriaxone, LEV – Levofloxacin, CN – Gentamicin, + = Positive, - = Negative

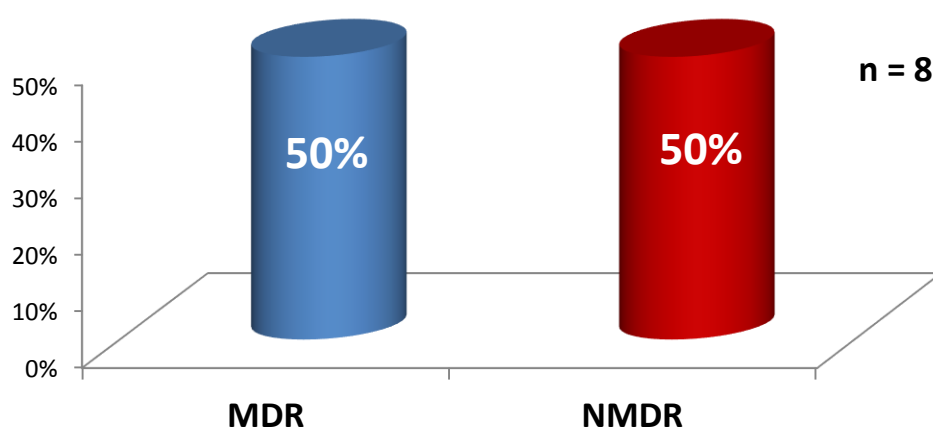


Figure 4.3: Occurrence of Multi-Drug Resistant *Gardnerella vaginalis*

Key: MDR = Multi-Drug Resistant; NMDR = Non-Multi-Drug Resistant; n = Number of Isolates

DISCUSSION

Out of 100 consenting females presenting with suspected cases of vaginitis in Ahmadu Bello University Teaching Hospital, Shika-Zaria, 52% were positive for bacterial vaginosis (BV). The prevalence was higher compared to 40.1% documented by Abdullateef *et al.* (2017) and 25.30% by Ara *et al.* (2017). This might be due to differences in study population.

Occurrence of *Gardnerella vaginalis* in BV patients was found to be 15.4% which was lower compared to 22.3% reported by Ara *et al.* (2017) in Chittagong Medical College Hospital. However, it was similar to 17.0% reported by Adinma *et al.* (2001).

There was no significant association between the risk factors, socio-demographic factors and the infection. Higher occurrence was recorded among age group ≥ 51 year

(50.0%) which can be attributed to the fact that some patients might have an underlying ailment like diabetes, and it was lower among 21-30 years (8.7%). Contradicting these findings was that of Mohammed *et al.* (2019) who reported 71.4% occurrence of *Gardnerella vaginalis* in BV and more common among younger women than older. *Gardnerella vaginalis* was not isolated from patients with primary level of education. This might be due to the fact that most patients at this level may not have been exposed to sexual activity compared to the patients with tertiary level of education (14.6%). Non-educated patients had higher occurrence (50.0%) and this might be as a result of their low socio-economic status and lack of awareness on the dangers of the infection.

Business women had higher occurrence of the infection (26.7%) compared to house wives who had 0% occurrence of the infection. A combination of environmental and stress may contribute to the higher prevalence among the business women.

An occurrence of 21.4% of *Gardnerella vaginalis* was recorded among diabetes patients, and this finding was similar with the 25.20% recorded by Makhlof *et al.* (2019). This might be as a result of high production of blood sugar that supports the growth of *Gardnerella vaginalis* (Catalano *et al.*, 2011; Makhlof *et al.*, 2019).

No *Gardnerella vaginalis* infection was recorded among HIV positive patients and those that uses condom. This might be as a result of restrain of HIV positive patients from sexual intercourse or as a result of the used of condom during the intercourse.

Women who practice vaginal douching had higher occurrence (18.2%) compare to those that do not practice it (14.6%). However, there was no association between douching and the infection. Other researchers also observed that despite been one of the common risk factors, douching has no association with *Gardnerella vaginalis* infection (Achondou *et al.*, 2016; Abdullateef *et al.*, 2017)). The agent or substance used for douching might distort the ecology of the vaginal and eventually leading to colonization of the vagina by *Gardnerella vaginalis*.

High occurrence of *Gardnerella vaginalis* was recorded among patients with cases of miscarriage (22.2%) compared to those without any case (14.0%). It was not surprising because it had been reported earlier that miscarriage is one of the risk factors associated with the infection (Awoniyi *et al.*, 2015) and also once miscarriage occurs, the patient is likely to be exposed to infections.

The occurrence of *Gardnerella vaginalis* in pregnant women was higher (21.1%) compared to non-pregnant women (12.5%). This might be as a result of physicochemical changes in normal flora of the vagina during pregnancy. The pH of the vagina becomes

depleted which changes from acidic to alkaline thereby giving room for infections.

Patients with vaginal odour had lower occurrence of *Gardnerella vaginalis* (27.3%) which was similar to the 30.4% recorded by Mohammed *et al.* (2019). Occurrence *Gardnerella vaginalis* among patients with vaginal itching was 12.1%. Perhaps, this might be due to lack of proper hygiene. The occurrence was higher compare to the 8.3% recorded by Mohammed *et al.* (2019).

The isolates were 100%, 87.5%, 62.5% and 75% susceptible to ceftriaxone, gentamicin, levofloxacin, chloramphenicol respectively. A study conducted by Ara *et al.* (2017), shows the sensitivity of ceftriaxone to be 86.84% which was lower compared to this present study. Despite the high sensitivity of chloramphenicol, it is not commonly use because of its adverse side effects. They were however, 100% resistant to ampicillin. In comparison with Ara *et al.* (2017) findings, 78.95% of *Gardnerella vaginalis* were susceptible to ampicillin. This sharply contradicts our findings. Over the last two decades, multi-drug resistant bacteria have been a serious threat to human health and an alarming issue for the health personnel especially in resource limited areas. Fifty per cent of the isolates were multi-drug resistant. The world health organization has a future plan by focusing on the discovery and development of alternative drugs to antibiotics against multi-drug resistant bacteria.

CONCLUSION

The study shows a high prevalence of bacterial vaginosis (BV) (52%). While on the other hand, the occurrence of *Gardnerella vaginalis* among patients diagnosed of BV was 15.4%.

There was no significant association between the infection and risk factors. Higher occurrence (50.0%) was recorded among age group ≥ 51 years, while a lower occurrence (8.7%) was recorded among 21-30 years.

Business women had higher occurrence (26.7%) compared to house wives who had no *Gardnerella vaginalis* infection recorded against them. Occurrence of *Gardnerella vaginalis* infection among BV patients with pregnancy was 21.1%; those that had miscarriage have 22.2% and those with diabetes had 21.4%. This study showed that *Gardnerella vaginalis* was more common among the non-educated patients.

The high occurrence of *Gardnerella vaginalis* recorded in this study calls for public awareness on the risk of the infections such as infertility and pregnancy complications.

Fifty per cent of the isolates were Multi-drug resistant. The isolates were highly susceptible to ceftriaxone (100%) and gentamicin (87.5%) while they were 100% resistant to ampicillin.

RECOMMENDATION

1. Early detection of factors responsible for the growth of various type of bacteria in the vaginal and selecting appropriate drugs for the specific

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treatment of bacterial infection are very important for preventing and controlling the infection among women of reproductive age.

2. There should be a regular check-up of the vaginal health to reduce complications that may arise as a result of *Gardnerella vaginalis* infection.
3. Females should always clean from front to back after using toilet so as to prevent transfer of microorganisms to the vagina. This will help reduce rate of infections among females.
4. Creation of awareness on the dangers of the substance use for douching should be made known to female of reproductive ages in order to prevent destruction of the vaginal flora.

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