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Determining the Prevalence of Bacterial Vaginosis & Vulvovaginal Candidiasis among Married and Unmarried Women & Evaluating the Association Socio-Demographic Risk Factors & Symptoms-Related Variables in Women Attending Gynecology Clinic in Hargeisa Group Hospital, Hargeisa City, Somaliland

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Abstract

Background: Vaginitis refers to any inflammation or infection of the vagina. This is a common gynecological problem found in women of all ages, with one-third of women having at least one form of vaginitis at some time during their lives. The vagina is the muscular passageway between the uterus and the external genital area. When the walls of the vagina become inflamed, because some irritant has disturbed the balance of the vaginal area, vaginitis can occur. The most common types of vaginitis are: Candida or "yeast" infection, Bacterial vaginosis, Trichomoniasis vaginitis. **Objectives of Study:** Therefore, the present study was carried out to determine the prevalence of bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) among married and unmarried women and evaluate the association socio-demographic risk factors and symptoms-related variables in women attending gynecology clinic in Hargeisa group hospital. **Methodology:** A total of 150 married and unmarried women were investigated & diagnosed by a researcher in Hargeisa Group Hospital, Hargeisa City, Somaliland for determining of VVI prevalence in the present study. Vaginal swabs from these

patients were processed for detection of bacterial vaginosis (BV), VVC and trichomoniasis based on guidelines of management of vagina infection. Species specific distribution of VVC was assessed by cultured of *Candida* on differential agar media, Germ tube test, rice meal agar and carbohydrates fermentation test and BV diagnosed by using gram staining test and biochemical testes. The nurse interviewers performed a comprehensive review of patients and completed the standardized baseline questionnaire containing information regarding the association socio-demographic risk factors and symptoms-related. **Results & Discussion:** The findings of the present study indicate that VVC was the most prevalent infection with 68 (45%) followed by BV 43 (29%). However, no case of trichomoniasis was detected. *Candida albicans* was found to be the most prevalent species with 47 (60.3%). Out of non-albicans *Candida* (NAC) species, *C. tropicalis* was found to be 9 (9.9%) and *C. glabrata* 7 (12.2%). On the other hand, the results of present study indicated that BV species *Gardnerella vaginalis* was most causative with 22 (19.5) and *Lactobacillus spp*. was 14 (9.3%). These results were also confirmed by gram staining test and biochemical testes. When VVI was compared among married and unmarried women, VVC was more prevalent in married women 47 (31.96) while, BV was more prevalent in unmarried women with 32 (13.76), discussed these results detected that prevalent found most highly with VVB and BV while, no case of trichomoniasis was detected. These findings are in consonance with various previous studies which have indicated VVC & BV to be the most prevalent in VI. Furthermore, the study showed statistical significant difference ($P < 0.005$) & relationship among prevalence of VVV & BV and some socio-demographic risk factors and some symptoms which have been identified as causes of variation in the prevalence rates of bacterial vaginosis & vagina candidasis. **Conclusion:** VVC was the most prevalent VVI followed by BV in Hargeisa City. *C. albicans* was the most prevalent species in VVC while among BV species, *Gardnerella vaginalis was* found to occur at highest frequency. However, further studies are needed to assess specific diagnosis and role of clinical risk factors. Urgent action is required to improve vagina infection control measures to reduce the prevalence and make new policies for treatment of vagina infection in HGH.

Keywords

Vulvovaginal Candidiasis, Bacterial Vaginosis, Women, Prevalence

1. Introduction

Vaginitis refers to any inflammation or infection of the vagina. This is a common gynecological problem found in women of all ages, with one-third of women having at least one form of vaginitis at some time during their lives [1]. The vagina is the muscular passageway between the uterus and the external ge-

nital area. When the walls of the vagina become inflamed, because some irritant has disturbed the balance of the vaginal area, vaginitis can occur. The most common types of vaginitis are: Candida or "yeast" infection, Bacterial vaginosis, Trichomoniasis vaginitis [2] [3]. *Candida* genital infection is the leading cause of fungal vulvovaginitis. Pregnancy, broad-spectrum antibiotic use, diabetes mellitus, and immunodeficiency have been described as important risk factors for *Candida* genital infection; however, asymptomatic microorganism colonization can occur in 25% to 50% of the cases [4] [5] [6].

Bacterial vaginosis is described as a shift in the balance of the vaginal microflora characterized by an increase in the vaginal pH, a reduction in lactobacilli, predominantly hydrogen peroxide producing species, and an increase in facultative and anaerobic bacteria in number and/or type [7]. Although the prevalence of bacterial vaginosis differs widely from country to country within the same region and even within similar population groups, it has been estimated to be in the range of 8% to 75% [2]. Bacterial vaginosis can occur in any age group, but globally it is more prevalent in females of reproductive age [8]. Furthermore, bacterial vaginosis & vagina candidiasis have been associated with increased susceptibility to HIV-AIDS and other STD [9].

Symptoms and signs of vaginitis may include the change in color, odor, or amount of vaginal secretions, pain during intercourse, painful urination, and light vaginal bleeding or spotting. The characteristics of vaginal discharge may indicate the type of vaginitis [10]. Previous data from epidemiological surveys show that within countries and between countries in the same region, the prevalence and incidence of genital tract infections may vary widely even in similar population groups [3]. In developing countries, patients with genital tract infections often seek care through formal healthcare services and in the informal sector [2] [11]. Diverse studies performed, with the objective of establishing the frequency of the most common infectious agents for vaginitis, have shown varying results. The prevalence found for BV has varied, ranging between 8% and 75%; VVC has presented 2.2% - 30% and trichomoniasis between 0% and 34% [12]. VVC is an extremely common infection in women of childbearing age of all strata of society. Since it has now been excluded from the ranks of sexually transmitted diseases and is also not a notifiable disease, not much information regarding its incidence and epidemiology is available [9]. VVC is the second most common cause of vaginitis in the United States and the most common cause in Europe [6] Infection of the female genital tract can result in vaginitis, cervicitis, and urethritis, and trichomoniasis has been associated with adverse pregnancy outcomes [13].

Therefore, the present study was carried out as first study to determine the prevalence of bacterial vaginosis (BV), vulvovaginal candidiasis (VVC) and evaluating the association socio-demographic risk factors and hygiene-related variables in women attending gynecology clinic in Hargeisa group hospitals, Somaliland.

2. Material & Methods

A hospital-based cross-sectional study was conducted from June 2019 to December 2019 at Department of Obstetrics and Gynecology, in Hargeisa group hospital, Somaliland. This is a cross-sectional study was involved 150 patients married & unmarried women according to exclusion criteria such as pregnant and non pregnant females, Postmenopausal women, Vaginal discharge due to malignancies or due to fibroid polyp, A thorough history taking including name, age, presenting complaints, obstetric history & menstrual history. General examination done & local examination including inspection of vulva, vagina and cervix done and findings recorded.

Sociodemographic characteristics data's and reproductive health characteristics such as age, name, educational level and marital status, residency, monthly income, frequency of vaginal bathing, and pant change were collected by face-to-face interviews used a structured questionnaire.

Upon admission to the study, physicians performed clinical examination of each patient and recorded signs of vaginal abnormalities. During examinations, vaginal specimens were collected aseptically from the study patients by using sterile rayon-tipped applicator stick swabs through experienced nurses. All vaginal swabs were then transferred without delay to the microbiology laboratory in Hargeisa University & HGH. In order to, diagnosis of bacterial vaginosis & vagina candidiasis, slides smears were prepared from vaginal swabs, and the slides were heat-fixed, Gram-stained was examined under microscope lenses. Each slide was then graded as per the standardized quantitative morphological classification method developed by Nugent *et al*. [14].

The diagnosis methods was done based on the following various bacterial & *Candida* characteristics such as morph types: large Gram-positive rods (*Lactobacillus* morphotypes), small Gram-variable rods (*G. vaginalis* morphotypes), small Gram-negative rods (*Bacteroides* spp. morphotypes), curved Gram-variable rods (*Mobiluncus* spp. morphotypes), and Gram-positive cocci. On the other hand, budding shape in case of *Candida*. To analyze the growth of *Candida* species, samples were collected using a vaginal swab dampened with sterile saline, plated aseptically streaked on Sabouraud Dextrose Agar plates and incubated at 35˚C for 24 hours. White, creamy, circular, medium-sized, and catalase positive colonies, observed as Gram-positive yeast in Gram staining, then were tested for identification of *Candida albicans* if positive germ tube test, green stain in CHROM agar test, carbohydrates fermentation test *Candida* medium were indicative of these species. The species that have been tested negative for the germ tube test and have not stained green in the CHROM agar medium were identified as other *Candida spp*.

In order to bacterial identification pure isolates of bacterial pathogen were preliminarily characterized by colony morphology, Gram stain, and hemolytic reactions on blood agar plates. Identification of bacteria to genus and/or species level was done by employing an array of routine biochemical tests such as cata-

lase, bacitracin and bile-esculin tests for Gram-positive bacteria and H_2S production, gas production, urease, citrate utilization tests, and fermentation of various carbohydrates for Gram-negative bacteria. Descriptive analysis of all variables was performed, including frequency distributions for groups, family income, conjugal status, educational level,, age at first sexual intercourse, residence rural or urban & use of contraceptives. The prevalence was calculated as percentage with a confidence interval of 95%. Chi-square test was used for the analysis of categorical variables. Students t-test was used to analyze the numeric data. A value of $P \leq 0.05$ was considered statistically significant.

3. Results Analysis

In the current study a total of one hundred fifty (150) high vaginal swabs samples were investigated from women the results in Table 1 shown that 111 out of 150 with percentage (74%) was positive patients. The highest prevalence of vaginal infection was seen in the Vulvovaginal Candidiasis 68 with (45%) while, 43 with (29%) was prevalence of bacterial vaginosis. On the other hand, the negative patients in this study were 39 (26%) patients.

Table 1. Prevalence of bacterial vaginosis & vulvovaginal candidiasis among women in Hargeisa, Somaliland.

Vaginal Infection	Prevalence of Vulvovaginal Candidiasis (VVC)	Prevalence of Bacterial Vaginosis (BV).	Total of Positive & Negative Patients.
Positive patients	68 (45%)	43 (29%)	111 (74%)
Negative patients	39 (14%)	39 (12%)	39 (26%)
Total of Patients			150 (100%)

Analysis of vaginal swabs had indicated the presence of BV and VVC while no case of trichomoniasis was detected. The results in (Table 2) showed that the prevalence of bacterial vaginosis (BV) was found to be 43 with (29%) and genital infection by *Candida albicans* & *Candida* spp. was 68 with (45%). Vulvovaginal Candidiasis (VVC) was found to be the most prevalent infection than prevalent of bacterial vaginosis. The causative agents such as *Candida* species and bacterial vaginosis were observed among married and unmarried women in Hargeisa City, Somaliland.

Table 2. Distribution of vagina infection patients investigated according to the causative organism.

Causative agent	Frequency of Patients Studded	Percentage
Bacterial vaginosis	43	(29%)
Vulvovaginal candidiasis	68	(45%)
Trichomoniasis	No case of was detected.	0.0
Total of positive patients	111	74%

The results in present study indicated in the (Table 3) that BV to be the most common cause are *Gardnerella vaginalis* 22 (19.8%) followed by *Lactobacillus spp.* 11 (9.9%) and *Streptococcus agalactiae* 7 (6.3%). While the distribution of VVC species the results indicated that *Candida albicans* species most common with 47 (42.3%) followed by *C. tropicalis* with 9 (8.1%) and *C. glabrata* 7 (6.3%). All species were further confirmed by germ tube test and morphological characteristics such as Saboraud Dextrose Agar (SDA) culture, rice meal agar test, CHROM agar medium and sugar fermentation test as showed also in (Figures 1-3 and Table 4).

Table 3. Distribution of diagnosed of microorganisms which isolated from vaginal infection.

| Microorganisms isolated with bacterial vaginosis	Total of positive patients 111(%)	Microorganisms isolated with vulvovaginal candidiasis	Total of positive patients 111(%)
Lactobacillus spp.	11 (9.9%)	*Candida albicans.*	47 (42.3%)
Staphylococcus aureus.	3 (2.7%)	*C. tropicalis*	9 (8.1%)
Streptococcus agalactiae	7 (6.3%)	*C. glabrata.*	7 (6.3%)
Gardnerella vaginalis.	22 (19.8%)	*C. krusei*	5 (4.5%)
Total of (BV)	43 (38.7%)	Total (VVC)	68 (61.2%)
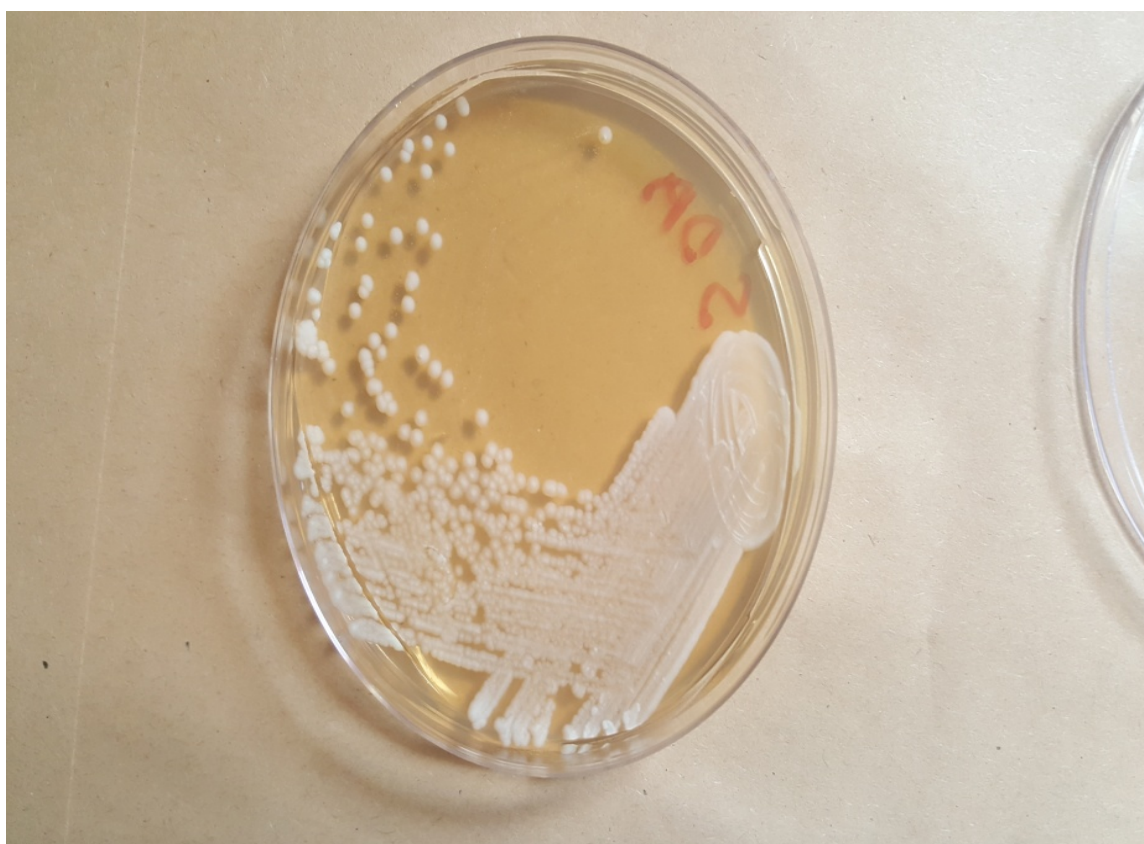
Figure 1. 24-h-pure culture of *Candida albicans* isolated on SDA plate from vagina positive patient in Hargeisa Group Hospital.
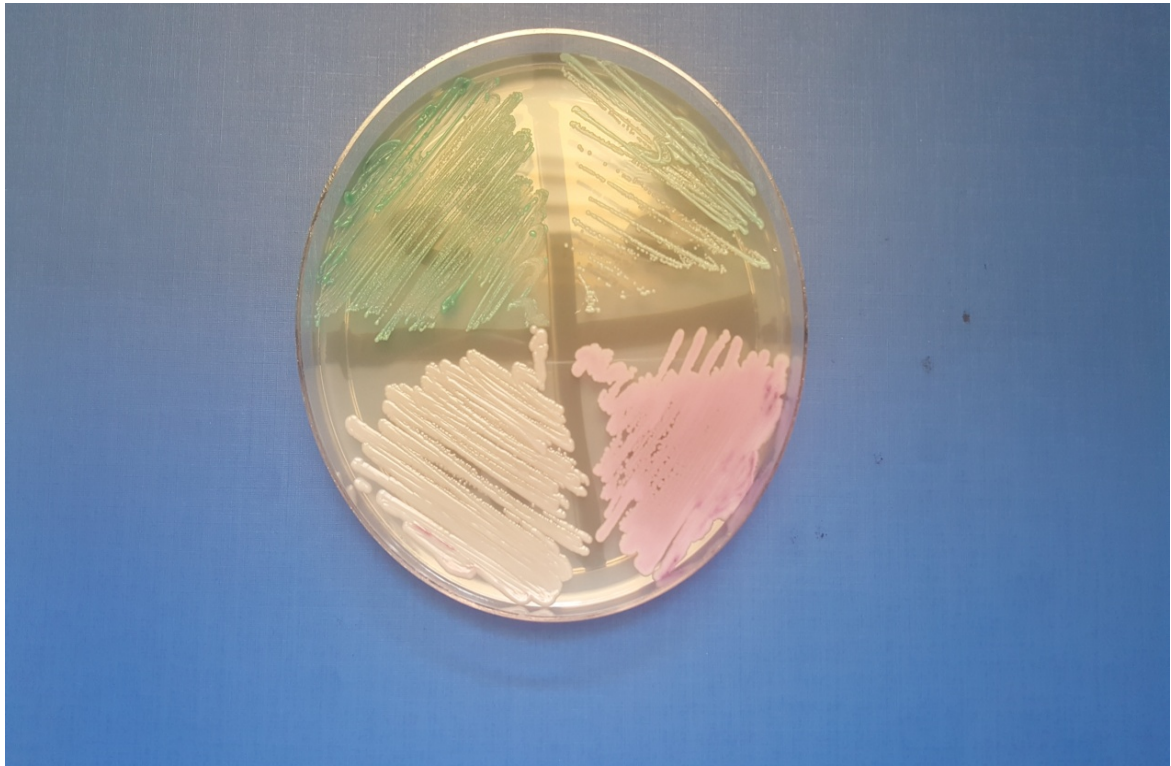
Figure 2. Different colors of colony and morphologies of various Candida Species on CHROM agar medium. *C. albicans* (light-green), *C. krusei* (pale-pink), *C. glabrata* (white-pink).
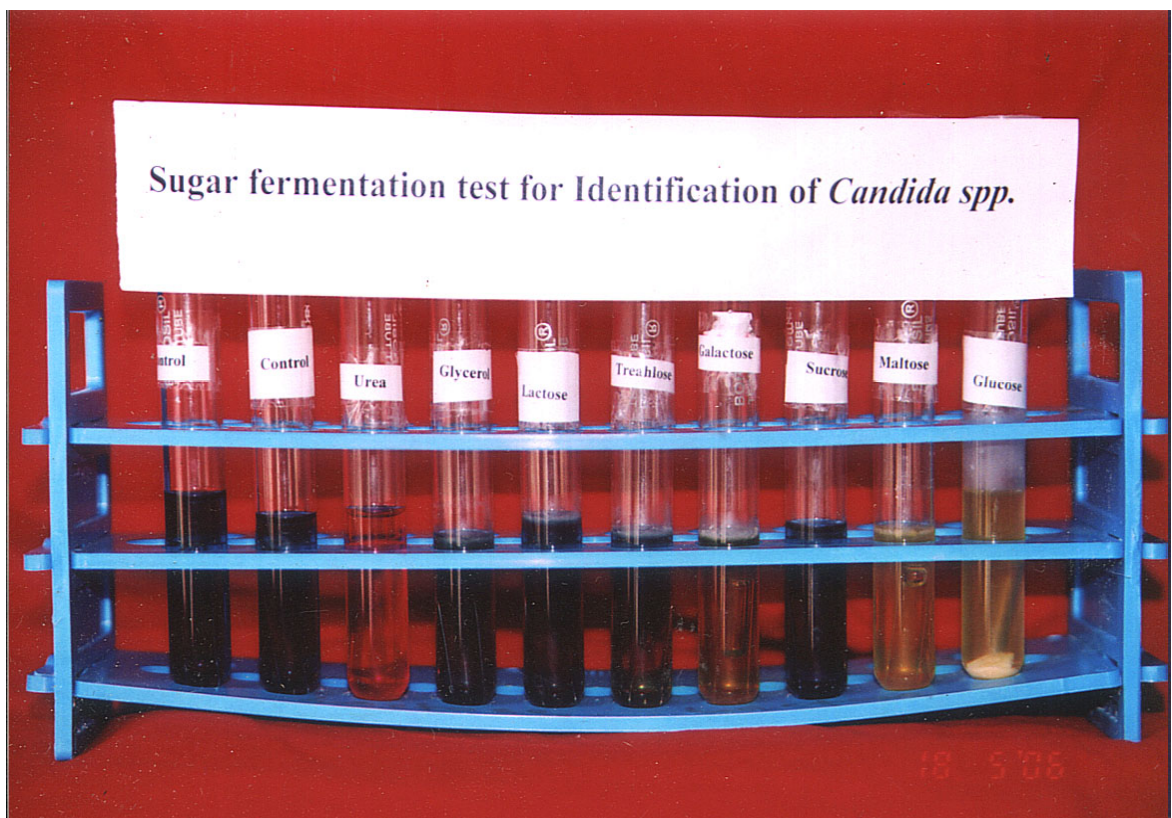

Figure 3. Sugar fermentation test for identification of *Candida albicans* isolated from vagina.

Table 4. Morphological features and biochemical test results of selected pathogenic species for identification of *Candida*.

Name of species.	Microscopic characteristic			Growh on SDA	Growth on rice meal agar			Carbohydrate fermentation test.					CHROM Agar test
	Morphology	Gram's stain	Germ tube test		Presence of blasto-conidia	Presence of pseudo-hyphe	Chlymydo spores	Glu	Mal	Suc	Lac	Gal	According to color
C. albicans	Spherical to budding	+	+	+	+	+	+	+	+	−	−	+	Light-green
C. tropicals	Spherical to budding	+	−	+	+	+	−	+	+	±	-	+	Dark-blue
C. krusei	Small to ovoid	+	−	+	+	+	−	+	−	−	−	−	Pale-pink
C. glabrata	Ovoid to bud	+	−	+	+	−	−	+	−	−	−	−	White-pink

Figure 4 shows the socio-demographic characteristics of patients. The age range of the married & unmarried women was between 18 years and 60 years, with a mean age of 30.55 ± 6.171. The prevalence of BV decreased with VVC age from 18 years up to the age of 20 while, the prevalence of BV & VVC it was increasing with age from 21 years up to 35 years. The results also showed more married women aged 21 - 35 years had BV & VVC than those in other age groups. On the other hand, the results showed the residency were increasing with BV in the urban area it was 32 (74.4%) & VVC it was with rural 47 (69.1%). The study also showed statistical significant difference ($P < 0.5$) between patients which belonged to the low monthly income class, housewife's occupation and illiterates educational level.
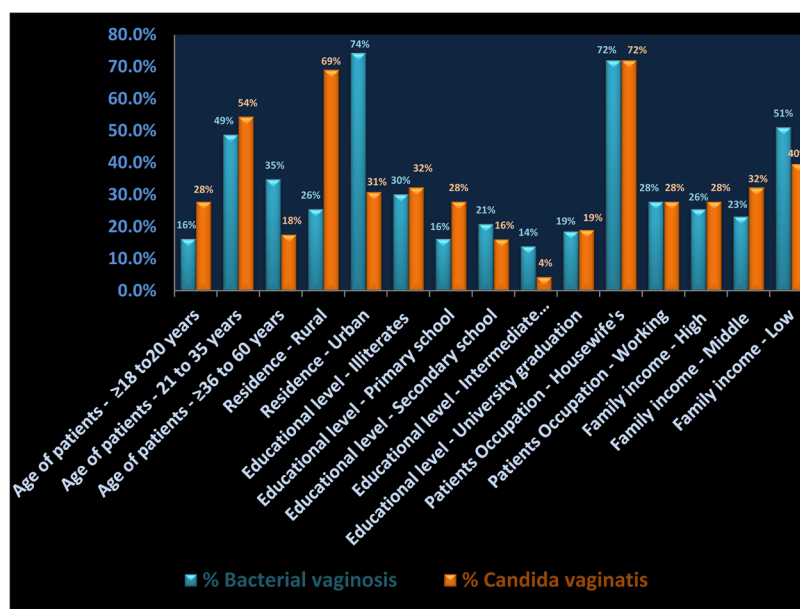
Figure 4. Distribution sociodemographic of the study patients, on the bacterial vaginosis genital *Candida* infection.

The results in the present study as showed in (Table 5) revealed that the proportion of Candida vaginitis was the highest among married women with 47 (69.1%) while, bacterial vaginosis was highest among unmarried females 32 (74.4%), while, VVC was low with 21 (14.28 among unmarried women.

Table 5. Prevalence of vaginal infections among married and unmarried women in Hargeisa City.

Marital Status	Bacterial vaginosis (positive n = 43)	Candida vaginitis (Positive n = 68)
Married women	11 (25.5%)	47 (69.1%)
Unmarried women	32 (74.4%)	21 (30.8%)
Total of positive patients	43 (100%)	68 (100%)

The results in (Figure 5) showed that genital itching was the clinical symptom most frequently observed in patients with bacterial vaginosis 32 (74.4% and VVC 51 (75%). On the other hand, Vaginal discharge was the clinical symptom most frequently observed in patients with bacterial vaginosis 31 (72.1%) and VVC was 52 (76.5%). Change in color, change in odor, pain during intercourse and pain during urination were also found in different percentage of positive patients and related with bacterial vaginosis & vulvovaginal candidiasis.
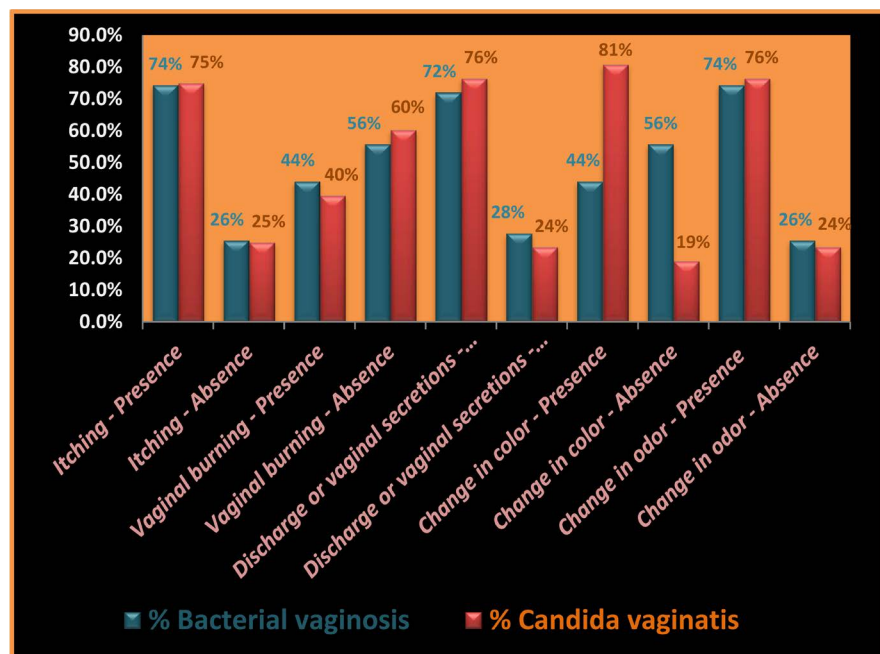

Figure 5. Association between signs & symptoms of the study patients & laboratory diagnosis confirmed of vaginal infection.

4. Discussion

Diagnosis & analysis of vaginal swabs had indicated the presence of BV and VVC while no case of trichomoniasis was detected. VVC was found to be the

most prevalent infection 68 (45%) followed by BV 43(29%) (Table 1). This finding is in consonance with various earlier studies which have indicated VVC to be the most prevalent cause of BV. However, percentage prevalence of BV in the present study was found to be comparatively very similar with other reported in earlier studies whereas bacterial vaginosis was found in 38(38%) of study participants [15]. BV percentage in our study also compared with that (1.4%) reported by [4] among women attending antenatal and gynaecological clinic in Accra. However, it was comparable to a prevalence of (28.0%) reported among non-pregnant women in the coastal area of Ghana [2]. Another study reported a prevalence of 47.0% among women with incomplete abortion in Accra [16]. This high rate was probably due to the complication of the abortion which could alter the normal flora of the vagina. Other reported an aggregate rate of 54% on women presenting with vaginal discharge in five West African states including Ghana [17]. In other West African states, the prevalence of BV of 64.3, 17.3 and 6.4% were reported in Southwestern-Nigeria, Northeastern-Nigeria and Burkina Faso respectively in pregnant women [18]. Higher prevalence rates of BV than those in the present study were also reported by [19] in Egypt (33%). The variation in the findings might be due to population size, methods of analysis, geographic distribution, and socioeconomic and behavioral differences in the studied population. On the other hand, prevalence of VVC in our study 68 (45%) found to be comparatively highly similar 39 (48%) with other studies from India and some other countries [20] [21]. The VVC also reported rate among women compares well with (34.2%) in Accra, (36.0%) in Southwestern Nigeria, (37.4%) in Turkey [4] [22] [23]. No case of trichomoniasis was found in our study groups. This finding is in agreement with some earlier reports which have documented very low prevalence (2%) of trichomoniasis [17] [20]. However, the prevalence of trichomoniasis appears to be relatively lower than the other vaginal infections and seems to have declined when compared with earlier reports [24]. These differences in the percentage of prevalence of among types of VVI can be attributed to environmental, behavioral, socioeconomic status and stressor differences in the geographical variation [25]. Recall, this disparity in the prevalence also might be due lack of proper sanitary conditions in many rural communities such as Hargiesa compared to other developing countries. Risk factors for the infection and local population also play dynamics accounts for the huge disparities in the prevalence rate across countries. In addition to, geographical distribution, vaginal hygiene practices and systematic difference in the various populations sampled could account for the variation in the prevalence values. All of the Candidiasis morphotypes were indicated by creamy white smooth colonies on SDA culture (Figure 1) For identification of Candida species in the positive patients for VVC including were further examined by positive germ tube test, green stain in CHROMagar Candida medium (Figure 2), carbohydrates fermentation test (Figure 3) and rice meal agar test (Table 4). C. albicans found in our study as showed (Table 3) to be the single most prevalent species with 47 (42.3%) followed by C. tropicalis 9 (8.1%) in VVC. This finding

of the present study was similar to the previous studies Int. J. Curr. Microbiol. [20] conducted in Nigeria, USA, Kenya, India, UAE, Iran [17]. However, this is in contrast to a single study reported in India where *C. tropicalis* was found to be more prevalent than *C. albicans* [26].

The high *Candida spp* colonization/infection of current study patients' vagina could have been due to their pregnancy & marital status which plays a major role in colonization and infection. Study demonstrates frequent colonization of the VVC of married women with *Candida spp* compared to unmarried women (Table 3). This is as a result of the high concentration of estrogen during married which provides a favorable environment for the growth of *Candida spp* [27]. VVC could also be an indication of an underlying infection such as diabetes mellitus. On the other hand, *Gardnerella vaginalis* was 22 (19.8%) as hazardous in BV pathogenesis and symptomatology followed by *Lactobacillus spp.*, recorded also high 11 (9.9%) among BV positive married and unmarried women, was found to be one of most prevalent natural micro biota of the lower genital tract in women. While, *Streptococcus agalactiae* was 7 (6.3%). Elevation alters of pH in vagina women above age of 45 years has been identified as a cause of a decline in the level of estrogen, which in turn creates an optimal condition for the growth of bacteria other than lactobacilli & depletion of lactic acid also producing lactobacillus. On the other hand, the presence of *lactobacilli* together with other opportunistic pathogens may be due to several factors like effects of antibiotics, type of incubation (as some *Lactobacillus spp.* are unable to produce some defense factors under anaerobic incubation), and antagonism among *lactobacilli* species to maintain dominance [28]. According to our study the results found a high prevalence of vaginal infections (BV) among married women 32 (74.4%) while, vulvovaginal candidiasis VVC) was higher among unmarried women 47 (69.1%) (Table 5). These findings among married women and unmarried women are in consonance with previous studies [4] [22]. The finding of the study also was contradicted by [19] in Egypt. However, several studies have documented the occurrence of BV in sexually inactive females or virgins [14] [29]. This provides evidence that sexual activity is not a prerequisite for BV. The change in lifestyle, improper perineal care, food habits, tight clothing, lack of attention towards menstrual hygiene, and sedentary factor might be the reasons for the acquisition of BV in unmarried women.

The incidence of vagina infections has greatly increased more especially with the married women aged 21 - 35 years among both BV & VVC patients than those were least for 18 - 20 and 60 years' age groups 7 (16.3%) 15 (34.8%) respectively. The result also similar with [21] in Nigeria who found BV to be most prevalent among 26 - 30 age group (35.8%).The highest prevalence in the age group 21 - 35 years might be due to the age being the most reproductively active age group and high sexual exposure at this age. According to, the results showed the residency were increasing with BV in the urban area it was 32 (74.4%) & VVC it was increasing among rural patients 47 (69.1%). The study also showed statistical significant difference between patients which belonged to the low

monthly income class, housewife's, illiterates educational level and menstrual hygienic pads (**Figure 4**). However, sociodemographic characteristics, sexual activity, reproductive health information, and behavioral and genital hygiene have been identified as causes of variation in the prevalence rates of BV& VVC [30] [31]. In this investigation, the most common clinical sign and symptoms such as itching, odor, and amount of vaginal discharge were found to be statistically significant relationship (*P* value < 0.05) with BV & VVC (**Figure 5**). This finding corresponds with previous study conducted by [21] in Nigeria and other studies done in different countries [22] [26] [32].

5. Conclusion

The overall prevalence of bacterial vaginosis and vulvovaginal candidiasis among married and unmarried women in this study indicated that VVC was more prevalent among married women while, BV was more prevalence among unmarried women. Therefore, further studies for investigating bacterial vaginosis and vulvovaginal candidiasis among married and unmarried women for evaluating the association socio-demographic risk factors and hygiene-related variables in women attending gynecology clinic in Hargeisa group hospitals are required. In addition, differential Bacteria & Candida species can be helpful in better diagnosis of VVI and provide better antenatal care. Particularly, there are no published studies that have been conducted in Somaliland to describe the prevalence of BV & VVC among women.

Acknowledgements

Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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