Prevalence of Bacterial Vaginosis among Married Women in Kalar District, Iraqi Kurdistan Region

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ABSTRACT
Bacterial vaginosis (BV) is an inflammatory disease, caused by polymicrobial infection, including pathogenic bacteria which replace the vaginal normal flora and finally this replacement causes manifestations of several physiological and clinical symptoms among women within different ages. BV has become one of the main problems that make woman patients visit gynecological and obstetric consultant hospitals in most country. The present study is designed to determine the causative pathogen and the prevalence of bacterial vaginosis among married women patients in Kalar district. This cross-sectional study was performed from the beginning of March to the mid April-2021 among women who attended Obstetrics and Gynecological governmental hospital and out-patient clinics in Kalar City. Intra vaginal swabs have been collected in sterile Amies transport medium sticks and processed for isolation and identification of bacterial species depending on colony morphology, Gram’s stain and microbiological analysis protocols. Then socio-demographic and gynaecologic data were collected by questionnaire. Out of the 108 participant women who suffered from Gynecological diseases, 67(62.03%) of them exhibited bacterial vaginosis. From the 73 different isolated colonies, 18 bacterial species were identified; coagulase-negative staphylococci (CoNS) were the predominant cause of BV (32.84%), followed by E. coli (14.93%), Staphylococcus aureus (13.43%), Klebsiella pneumonia (8.96) and Micrococcus luteus (7.46%), while Proteus spp. and some uncommon bacteria display (1.49%) for each of them. The socio-demographic analysis between positive and negative woman patients revealed that the association between all studied risk factors and BV were statistically significant (P value < 0.05) except the age factor which was statistically non-significant meaning that the age was not associated with BV. In addition, the clinical symptom analysis showed that abnormal vaginal discharge, lower back pain, dysmenorrhea and strawberry were significantly associated with BV (P value < 0.05), while the rest of other factor did not exhibit statistically significant association.

Keywords: Bacterial Vaginosis, Prevalence, Women, Risk Factor, Iraq

1. Introduction
Generally, at all ages the healthy women have a balanced vaginal ecosystem, this due to the predominance of Lactobacillus species which is considered as a normal flora in this region. The main function of these bacteria is the inhibition of the growth and spread of the rest of other microorganisms via different mechanisms such organic acid production like lactic acid and antimicrobials ingredient including Bacteriocins and hydrogen peroxide). Bacterial vaginosis is basically associated with the diminution of the Lactobacillus species in the vaginal area. Furthermore, the pH also changes simultaneously after the unbalance that occurred in the vaginal normal flora, which give the chance of growing a variety of other microorganisms especially anaerobic bacteria [1,2,3,4]. On the other hand, the alteration of normal flora in the vaginal region with all associated symptoms caused by a polymicrobial assemblage will reflected as bacterial vaginosis [5]. Reviewing the presence and prevalence of bacterial vaginosis among all the wide world became the theme and showed widely various in different countries, also it showed different among the same region, as well as, with similar population groups, concerning the range about 8% to 75% [6]. The clinical condition characterization of bacterial vaginosis include a thin, homogenous, vaginal discharge as malodorous adherent which considered as a noticeable character after intercourse and menses. A fishy odor after adding 10% potassium hydroxide was noticed when mixed with vaginal fluid. Furthermore, clue cells (vaginal epithelial cells), a few lactobacilli and a little number of
polymorphonuclear leucocyte were found represent the future characters of bacterial vaginosis [7]. Previous studies showed the association between prevalence of bacterial vaginosis with different criteria such education level, race, age income, ethnicity, chronic stress as well as gynecologic post-operative infection, pelvic inflammatory disease and both HIV infection and papilloma virus [8,9,10,11,12]. The present study was aimed to determine the causative pathogens of bacterial vaginosis and their prevalence among women attending gynecology hospital and outpatient clinics in Kalar City and determining the associated clinical characters among them.

2. Materials and Methods

2.1 Data collection

This cross-sectional study was conducted from the beginning of March to the mid April-2021 in Obstetrics and Gynecological governmental hospital and out-patient clinics in Kalar City. Socio-demographic and clinical data was obtained from the patients by pretested questionnaire. The questionnaire was comprised of several information concerning several factors including (living place, nationality, age, education, occupation, year of marriage, chronic diseases, previous bacterial vaginosis, vaginal bath, abortion etc. Another part of the questionnaire includes several clinical symptoms such (abnormal vaginal discharge, lower back pain, burning/itching of genitalia, genital sores, dyspareunia, dysuria, dysmenorrhoea and strawberry cervix) [13, 14].

2.2 Sample Collection and Processing

Amies transport medium was used to collect samples by specialized gynaecologist from the lateral posterior vaginal fornix. Then, the samples were directly transported to the microbiology laboratory in the Garmian university following the standard laboratory procedures. Freshly prepared media (Nutrient agar, MacConkey agar, Blood agar, Chocolate agar and mannitol salt agar) were prepared for isolation and identification of bacteria; the medium was inoculated with the swab and incubated aerobically at 37°C for 24 hours. The plates with no growth were further re-incubated for additional 24 hours before being reported as negative. All isolates were purified by sub-culturing and then Gram stain was applied for differentiation between isolated bacteria under oil immersion (100X), the isolated and purified bacteria was identified according to the bacterial morphology and colony characters. Furthermore, the rest of all unknown isolated bacteria were identified by the VITEK-2 Compact bioMérieux, VITEK-2 cards were inoculated following the manufacturer’s instructions and the freshly bacterial culture were used which have less than 18hrs age [15,16,17,18].

2.3 Ethical approval:

Ethical statement was approved by the ethical committee of the college of medicine, University of Garman. Furthermore, unwritten consent of each of the participant was achieved. Obtained information from the participant will be kept strictly confidential, including all questions in the socio-demographic, reproductive, sexual history, behavioural character and clinical features.

2.4 Data analysis:

Collected data were attained for displaying bacterial vaginosis prevalence and microorganisms via descriptive analysis. The categorical variables were presented in frequency and percentages using the IBM statistical Package for Social Sciences version 25 (SPSS - 25). Paired Samples T Test (correlated pairs t-test) were calculated for testing the significance at the 95% confidences (p value < 0.05) level of significant and discovering the correlation coefficients (CoR) among the correlated criteria.

3. Results and Discussion

Among 108 vaginal swabs, were collected from women patients who attend Obstetrics and Gynecological governmental hospital and out-patient clinics, 67 (62.03%) of them showed positive bacterial vaginosis according to the microbiological analysis as presented in (Figure 1), while the rest 41 (37.97%) of the samples represented as negative. Depending of the obtained results in our study and compared with the published articles, the bacterial vaginosis occurrence in this area confirmed the prevalence’s of it. Our results is in agreement with the principle of prevalence and in higher prevalence rate with that obtained in Nepal by Ranjit et.al., (2018) [19] in India by Modak et.al., (2011) [20] and in Egypt by Gad et.al., (2014) [21] with a value of (24.4%, 24% and 33% respectively). The variation in the obtained results among published articles considered as a normal event, this may due to several characters such geographic distribution, population size, data analysis, behavioural differences and socioeconomic status as described by Ranjit et.al., (2018) [19].

Figure 1: Total presences of bacterial vaginosis among all (108) woman were tested

The bacteria were isolated include both Gram positive and negative bacteria. A total of 73 bacteria species were isolated belong to 18 bacterial species from all 67 positive cases were gave the positive results according to the microbiological investigation. Coagulase-negative staphylococci (CoNS) were the predominant cause of BV 22/67 (32.84%), followed by E. coli 10/67 (14.93%), Staphylococcus aureus 9/67 (13.43%), Klebsiella pneumonia 6/67 (8.96) and Micrococcus luteus 5 (7.46%), while Proteus spp. and some uncommon bacteria showed the lowest rate of infection, 1/67 (1.49%) for each of them as summarized in (Figure 2). Our results were similar with
those obtained by all of these published articles Ranjit et al., (2018) [19], Tiyyagura et al., (2012) [22], Maghsoudi et al., (2014) [23], regarding the appearance of the most common Gram positive cocci include both Staphylococcus aureus and Streptococcus agalactiae (12.33% and 8.22% respectively). The most common gram negative bacteria were isolated include E. coli followed by klebsiella pneumonia and pseudomonas spp as follow (13.70%, 8.22% and 2.74% respectively). Our finding results were contradicted with the studies carried out by Marrazzo et al., (2008) [24] in Seattle, Razzak et al., (2011) [25] in Iraq, and Larsen and Monif (2001) [26] in Omaha and Ranjit et al., (2018) [19], in case of that the most predominant was pseudomonas spp., while in our study E. coli became the alternative predominant of the pseudomonas spp. In general, the appearance of the faecal flora within the vaginal discharge may accredited to the unhealthy bowel practices as mentioned by Mumtaz et al., (2008) [27].

The results of Socio-demographic characters of the studied woman enlisted according to the presence or absences of bacterial vaginosis with its frequency, P value and correlation summarized in (Table 1). A total of 108 participated patient woman 62.03% of them represent positive bacterial vaginosis outcomes. Furthermore, all studied parameters after analyzing the results exhibited significant association with BV at the P value < 0.05 except the age feature which did not show significant association between the positive and negative woman patients. Regarding the correlation coefficient results all characters exhibit the positive correlation except the data associated with age, chronic diseases and years married depicted negatively correlation coefficient with (-0.072,-0.104 and -0.127 respectively). The study of Allsworth et al., (2007) [28] mentioned that the bacterial vaginos is prevalence will increases with age, which measured in line with high prevalence detected with the age range between 21-25 years, our results was disagreement with this outcome. Furthermore, our results were in agreements with that obtained by Bhalla et al., (2007) [29], Bitew et al., (2017) [30], Bhakta et al., (2021) [31], concerning that the presence of the bacterial vaginosis was not significantly associated with age. The results for education was exhibited significantly association among education level (p<0.001), our result was in agreements with that obtained by Bahram et al., (2009)35 and Nayak et al., (2020) [34], while it was contradicted with that obtained by Xueqiang et al., (2007) [32] and Bitew et al., (2017) [30]. Woman’s occupation another feature that exhibit the statistically significant differences (p<0.001), our results was disagreement with that obtained by both Aduloju et al., (2019) [33] and Nayak et al., (2020) [34], in which they achieved there was no significantly association between woman occupation at different skill and jobs.

The next part of Socio-demographic characters’ results which related to numerous clinical symptoms with the prevalence of bacterial vaginosis include (abnormal vaginal discharge, burning/itching genitalia, genital sores, lower back pain, dyspareunia, dysuria, dysmenorrhea, and strawberry cervix) are tabulated in (Table 2). The analyzed data indicated that only abnormal vaginal discharge, lower back pain, dysmenorrhea and strawberry cervix showed the statistically significant association at (P value < 0.05) among all clinically studied characters, while the rest of other characters don’t exhibit statistically significant association between the presence and absences of bacterial vaginosis. The results of correlation coefficient between all studied parameters displayed negative correlation except (dysmenorrhea, and strawberry cervix) with r² positive results (0.152 and 0.029 respectively). The obtained results for abnormal vaginal discharge, lower back pain, and strawberry cervix were in agreement with that obtained by Nayak et al., (2020) [34], Bhakta et al., (2021) [31]. Concerning the woman reproductive age each of abnormal vaginal discharge, itching of genitalia and burning

![Figure 2: Prevalence of bacterial vaginosis isolates from all positive samples in the study](image)
remain the most common problems which related with bacterial vaginosis as documented by Rao et al., (2004) [36] and Valsangkar et al., (2014) [37]. The studies of Schwebke et al., (1996) [38] Afolabi et al., (2016) [39]; Aduloju et al., (2019) [33] they revealed that most of clinical diagnostic feature somewhat exhibit the good correlation with the gram staining for vaginal discharge in subsequent studies of bacterial vaginosis diagnosis, our outcome data were in agree with overall criteria finding in them previous published articles.

Table 1: Socio-demographic parameters association with the prevalence of bacterial vaginosis demonstrating both positive and negative results with P value and correlation

| Characters            | Frequency | Bacterial vaginosis | P value | r^2      |
|-----------------------|-----------|---------------------|---------|----------|
|                       |           | Positive 67(62.03%) |         | -0.072   |
|                       |           | Negative 41 (37.97%)|         |          |
| Age                   |           |                     |         |          |
| 16-25                 | 31(28.7%) | 17(15.74%)          | 14(12.96%) | 0.460     |
| 26-35                 | 36(33.3%) | 22(25%)             | 14(12.96%) |          |
| 36-45                 | 31(28.7%) | 23(21.29%)          | 8(7.40%)  |          |
| >46                   | 10(9.3%)  | 5(4.62%)            | 5(4.62%)  |          |
| Nationality           |           |                     |         | 0.014     |
| Kurdish               | 92 (85.2%)| 59(54.62%)          | 33 (30.55%) | 0.055     |
| Arab                  | 13(12.05%)| 6(5.55%)            | 7(6.48%)  |          |
| Other                 | 3 (2.8%)  | 2(1.85%)            | 1(0.92%)  |          |
| Living Place          |           |                     |         | 0.001     |
| Urban                 | 95(88.0%) | 59(54.62%)          | 36 (33.33%) | 0.004     |
| Rural                 | 13(12.05%)| 8(7.40%)            | 5(4.62%)  |          |
| Education             |           |                     |         | 0.001     |
| illiterate            | 13(12.0%) | 10 (9.25%)          | 3(2.77%)  | 0.060     |
| Just literate         | 6(5.6%)   | 3(2.77%)            | 3(2.77%)  |          |
| Primary school        | 36(33.3%) | 22(20.37%)          | 14(12.96%) |          |
| Secondary school      | 24(22.2%) | 14 (12.96%)         | 10 (9.25%) |          |
| College               | 29(26.9%) | 18 (16.66%)         | 11 (10.18%) |          |
| Occupation            |           |                     |         | 0.001     |
| Unemployed            | 2(1.9%)   | 2(1.85%)            | 0(0%)    | 0.080     |
| Employed              | 15(13.9%) | 10 (9.25%)          | 5(4.62%)  |          |
| Student               | 7(6.5%)   | 4 (3.70%)           | 3(2.77%)  |          |
| Housewife             | 84 (77.8%)| 51 (47.22%)         | 33 (30.55%) |          |
| Years married         |           |                     |         | 0.001     |
| 1 to 5                | 33(30.65%)| 16 (14.81%)         | 17 (15.74%) | -0.127    |
| 6 to 10               | 25 (23.1%)| 16 (14.81%)         | 9 (8.33%)  |          |
| 11 to 15              | 26 (24.1%)| 21 (19.44%)         | 5 (4.62%)  |          |
| >15                   | 24(22.2%) | 14 (12.96%)         | 10 (9.25%) |          |
| Previous BV/GTI       |           |                     |         | 0.015     |
| Yes                   | 83(76.9%) | 53 (49.07%)         | 30 (30.55%) | 0.068     |
| No                    | 25 (23.1%)| 14 (12.96%)         | 11 (10.18%) |          |
| Chronic diseases      |           |                     |         |          |
| Yes                   | 7(6.5%)   | 3 (2.77%)           | 4 (3.70%)  | 0.001     |
| No                    |           |                     |         | -0.104    |
Table 2: Various clinical symptoms association with the prevalence of bacterial vaginosis representing both positive and negative results with P value and correlation

| Characters                  | Frequency | Bacterial vaginosis          | P value | r^2  |
|-----------------------------|-----------|------------------------------|---------|------|
|                             |           | Positive 67 (62.03%)         |         |      |
|                             |           | Negative 41 (37.97%)         |         |      |
| Abnormal Vaginal Discharge  | yes       | 58 (53.70%)                 | 36 (33.33%) | 0.001 | -0.018 |
|                             | no        | 9 (8.33%)                   | 5 (4.62%)  |      |
| Burning\itching genitalia  | yes       | 44 (40.74%)                 | 28 (25.92%) | 0.486 | -0.027 |
|                             | no        | 23 (21.29%)                 | 13 (12.03%) |      |
| Genital sores               | yes       | 35 (32.40%)                 | 26 (24.07%) | 0.433 | -0.109 |
|                             | no        | 32 (29.62%)                 | 15 (13.88%) |      |
| Lower back pain             | yes       | 57 (52.77%)                 | 35 (32.40%) | 0.001 | -0.004 |
|                             | no        | 10 (9.25%)                  | 6 (5.55%)  |      |
| Dyspareunia                 | yes       | 36 (33.33%)                 | 26 (24.07%) | 0.510 | -0.095 |
|                             | no        | 31 (28.70%)                 | 15 (13.88%) |      |
| Dysuria                     | yes       | 36 (33.33%)                 | 23 (21.29%) | 0.278 | -0.023 |
|                             | no        | 31 (28.70%)                 | 18 (16.66%) |      |
| Dysmenorrhea                | yes       | 28 (25.92%)                 | 11 (10.18%) | 0.001 | 0.151  |
|                             | no        | 39 (36.11%)                 | 30 (30.55%) |      |
| Strawberry cervix           | yes       | 6 (5.55%)                   | 3 (2.77%)  | 0.001 | 0.029  |
|                             | no        | 61 (56.48%)                 | 38 (35.18%) |      |

4. Conclusion
This study can be considered as the first article carried out for studying and determining the bacterial vaginosis among woman in Garmian area. Isolating different bacterial species in the obtaining swap sustain highly association with different clinical symptoms were studied by socio-demographic features. So, inclusive healthcare learning for falling the bacterial vaginosis is required. In addition, it is necessary to declare the risk of infection with BV and the serious consequences, this may lead to the occurrence of occasional cases of disease, in order to evade the spread of BV to the level of prevalence among all ages and marital status.

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Conflict of interests
None.

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