

Investigation of vaginal colonization of bacterial infections in women**Sadia Razzaq,^a Muhammad Imran Arshad,^a Mashkoor Mohsin Gillani,^b Sana Shahid^b**^a Allied Hospital Faisalabad^b Institute of Microbiology, Faculty of Veterinary Sciences, University of Agriculture, Faisalabad, PakistanCorrespondence: Sadia3433@gmail.com**ABSTRACT**

Background and Objectives: Vaginal microbiota is responsible for up to 70% reproductive tract infections in women. Bacterial vaginosis is a significant vaginal infection involved in replacing the normal flora of lactobacilli with aerobic and anaerobic opportunistic bacteria. This study was conducted to determine the prevalence of bacterial vaginosis in women of reproductive age with the complaint of vaginal discharge in Pakistan.

METHODOLOGY: A total of 100 sterile vaginal swab samples were collected from women aging between 15-50 years at Allied Hospital Faisalabad. The swabs were inoculated on sheep blood and MacConkey agar for bacterial isolation. Gram staining and biochemical testing were done to identify the bacterial species. All resulted isolates were subjected to antibacterial sensitivity testing by Kirby Bauer diffusion method.

RESULTS: High bacterial colonization rate (75%) was recorded in women among age group 26-35 years. Bacteria isolated in this study were *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus pyogenes*, *Streptococcus pneumoniae*, *Escherichia coli*, *Micrococcus* spp., *Acinetobacter baumannii*, *Peptostreptococcus* spp. and *Enterobacter faecalis*. The bacterial isolates showed highest resistance against tetracycline, ofloxacin and ciprofloxacin and were sensitive to meropenem, gentamycin, clindamycin and kanamycin.

CONCLUSION: : In conclusion our findings evidenced that bacterial vaginosis is prevalent in women and showed resistant patterns to clinical antibiotics.

KEYWORDS: Vaginal Microbiota, Bacterial Vaginosis, Genital Infections, Vaginal Colonization, Women's Health

INTRODUCTION

Vaginitis is an inflammation of mucosal membrane of vagina that involves both infectious and non-infectious agents. It is the common problem in most of reproductive age women and its causes involve irritation, burning, itching and discharge. Many bacteria, viruses, parasites and fungi are responsible for infective vaginitis (1) Vaginal discharge and vulvar discomfort are the distinguished features for vaginitis. Most common diseases associated with vaginal discharge are Bacterial Vaginosis, Candidiasis and Trichomoniasis(1) Lactobacilli are a gram positive bacilli which play a significant role in the healthy vaginal microflora. Some other microorganisms may be present in less number and beneficial for maintaining its microenvironment. Lactobacilli act as a probiotic and provide a host defense mechanism against pathogens. It is responsible for maintaining the vaginal pH acidic with

the production of lactic acid, bacitracin, hydrogen peroxide and other antimicrobial compounds (2)

Bacterial Vaginosis (BV), also called non-specific vaginitis is one of the leading vaginal disease that is characterized by shifting the normal vaginal flora with opportunistic pathogens. Most of the women complaint fishy smelling discharge with symptomatic bacterial vaginosis at the time of menstruation (3).

Bacterial vaginosis is a multimicrobial vaginal infection appearing as less lactobacilli number and increase number of potential pathogens including anaerobes such as *Peptostreptococcus*, *Gardnerella vaginalis* and *Mycoplasma hominis* species (Mehmood et al., 2018). The other microorganisms associated with BV are *Staphylococcus aureus*, Group B *Streptococcus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus*, *Bacteroides*, *Proteus vaginalis* and some other oppor-

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tunistic pathogens (4).

Most of cases of Bacterial vaginosis in women are asymptomatic and its prevalence rate varies from a healthy population to higher risk population. It depends on age, menstrual history, marital status, gestation age, education, employment status, recent antibiotic therapy, sexual activity and ethnicity. The higher prevalence of Bacterial vaginosis occurs among the age group of 20-30 years women (5).

Early prevention of reproductive tract infections is difficult due to increased risk of other complex reproductive diseases which includes infertility, sexually transmitted diseases, miscarriage, pelvic inflammatory disease and preterm birth. Bacterial vaginosis and vaginal candidiasis, which have been associated to both sexual and vaginal hygiene exposures (6).

Most of the bacterial vaginal infections if not treated on time, are become a major source for causing infections in neonates especially in childbearing age women (7-9).

Antibiotics such as metronidazole and clindamycin are commonly used against aerobic and anaerobic microbes in bacterial vaginosis.

METHODOLOGY

Study Design and Area

A hospital based study was conducted at Gynaecology Department of Allied Hospital Faisalabad.

Ethical Consideration

The study was carried out in accordance with Institutional Bioethics Committees of the University of Agriculture Faisalabad and concerned hospitals. All the samples were collected after taking ethical consent from the patients with full compliance. The questionnaire was based on all those important questions which were helpful to understand the expected reasons of disease. It included age, educational and marital status, and history of abortion, antibiotic therapy and previous genital tract infection. A total of 100 high vaginal swab (HVS) samples were taken from women by using sterile cotton swabs. It was distributed based on different age groups from 15 to 60 years old including pregnant, non-pregnant and 20-25 normal healthy women. Transportation

All vaginal swabs were transported immediately after collection to the Microbiology Laboratory of University of Agriculture Faisalabad.

Inoculation and Incubation

Swab samples were inoculated on Blood Agar and MacConkey Agar for isolation of both gram positive and gram-negative bacteria including Staphylococci,

Streptococci, Enterococcus, Escherichia coli, Pseudomonas, and Klebsiella. The inoculated plates were incubated at 37°C for 24-48 hours (10).

Bacterial Identification

Pure culture of bacteria was characterized by colony morphology, haemolytic reactions on blood agar plates and fermentation reaction on MacConkey agar plates. Bacterial identification was done by using different routine biochemical tests such as catalase, coagulase, Triple sugar iron (TSI), indole, motility, citrate utilization tests (11).

Antibiotic Susceptibility Pattern

The antibacterial susceptibility testing of bacterial isolates was done by using Kirby-Bauer disc diffusion method (Mulu et al., 2015). Sensitivity pattern of all isolated bacteria were investigated for amikacin (30µg), imipenam (20µg), cefotaxime (30µg), cefazolin (30µg), ciprofloxacin (5µg), Norfloxacin (10µg), gentamycin (10µg), amoxicillin (10µg) and tetracycline (25µg) (12).

Statistical Analysis

The data was analyzed with person positivity.

RESULTS

More than half population of women were have bacterial infection by culture positivity (52%). But 33 patients out of 52 patients were fulfill the Amsel's criteria (63.4%) by showing homogeneous vaginal discharge, positive whiff amine test, basic vaginal pH. Only 5 cases were positive for Candida albican infection among women of age group 15-25 years. Trichomoniasis infection was not reported in this study (Table 1).

Table 1. Demographic distribution of etiological agents of vaginal infections.

Types of infections	No. of patients (n=100)	%age
Bacterial vaginosis by culture	52 (n=100)	52%
BV Positive by Amsel's criteria	33 (n=52)	63.4%
Candidiasis	5 (n=100)	5%
Trichomoniasis	0 (n=100)	0%

In current study, age limit among the women was 15-50 years. Majority of bacterial vaginosis (BV) was present among women of age group 31-35 years (63.3%), followed by 55.6% prevalence rate among age group 41-45 years, 48.1% BV positive results in age group 26-30 years, 46.2% among 36-40 years age group, 45.4% among 21-25 years women, 42.9% prevalence in 45-50 years age women and 33.3% BV was found among 15-20 years of age group women (graph 3.1).

In this study, the most prevalent pathogenic bacteria for BV was *Staphylococcus aureus* with 28.8%, followed by *Escherichia coli* (17.3%), *Staphylococcus epidermidis* (13.4%), *Klebsiella oxytoca* and *Enterococcus faecalis* were present (9.61%), *Streptococcus pyogenes* and *peptostreptococcus spp.* (5.78%), *Acinetobacter baumannii* (3.85%), *Streptococcus pneumonia*, *Klebsiella pneumonia* and *Pseudomonas aeruginosa* (1.9%).

DISCUSSION

Vaginal infections are the most common and serious reproductive health hazards among all age groups of women throughout the world. These vaginal infections are categorized into Bacterial vaginosis, candidiasis and trichomoniasis. Bacterial vaginosis is an abnormal condition of vagina by replacing the normal lactobacilli with opportunistic pathogens and responsible for raising the vaginal pH. It is the most prevailing lower genital tract infection in sexually active women and its prevalence varies from 7-85% in women of different populations (13-14). It is a need of the present era to complete understand the composition of vaginal microbiota and its mechanism for pathogenesis provide a base for understanding the associated risk factors and for the prevention and control of the Bacterial vaginosis.

The aim of present study was to investigate the frequency of opportunistic pathogens in vaginal colonization of bacterial infections in symptomatic women. Additionally, several associated risk factors also assessed for BV conditions which included marital status, abnormal discharge, educational level, use of antibiotics, number of abortions. Marconi et al. found that these risk factors provide an evidence for transmission of sexually transmitted diseases.

In this study, the prevalence rate of BV was 33% by using Amsel's criteria. Prasad et al. detected the Bacterial vaginosis by Nugent score system from 200 women with complaint of vaginal discharge. They were reported as 39 women had BV intermediate score with 19.7% prevalence rate (15-16). Our study showed the chances of infections are more common among women of age group 31-35 years and low rate of infection was observed by declining the age of women among 15-20 years. Our study correlates with the study of Gopalan et al. who reported the prevalence of positive bacterial culture was 39%. This study also resembles the study conducted by Krauss-silva et al. reported the prevalence for BV as 29%. Gram's stain Nugent scoring system was used by Bitew et al. to

estimate the prevalence rate that was 48.7% that is higher than the prevalence rate of our study. (17-20)

In our study, the highest colonization rate for BV (63%) among age group 31-35 years of women and less colonization (33.3%) was seen among age 15-20 years of patients. Similar results were reported by Ranjit et al. (8.8%) and Reddy, from India that showed that the more prevalence of BV about 34.2% was found among age 31-40 and our study also contradict from Reddy's study at that result by increasing the age factor chances of BV infection decline. They reported the less prevalence of BV (14.5%) among age 51-60 years of women.

CONCLUSION

Our research emphasizes how important it is to look into vaginal bacterial colonization in order to comprehend the frequency and consequences of bacterial infections in women. The results highlight the necessity of early identification and routine screening in order to avoid consequences related to pathogenic colonization. By determining the microbial makeup of vaginal infections, specific treatment plans can be created, benefiting women's reproductive and general health. In order to improve women's healthcare outcomes, future studies should concentrate on the function of host variables, patterns of antibiotic resistance, and the effectiveness of preventive treatments.

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Sadia Razzaq, Muhammad Imran Arshad: Substantial contribution to the conception, design of the work.

Mashkoor Mohsin Gillani: Survey and design of the work. Data collection. SPSS computing tool.

Sana Shahid: Drafting for approval of the final version to be published

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