"ISOLATION AND IDENTIFICATION OF ORGANISMS ASSOCIATED WITH BACTERIAL VAGINOSIS IN PREGNANT WOMEN WITH PER VAGINAL DISCHARGE. A ONE YEAR CROSS SECTIONAL STUDY"

By

DR. GULNAR PARVEEN KAZI

Dissertation

Submitted to the

KLE University

Belgaum, Karnataka

In partial fulfilment

of the requirements for the degree of

DOCTOR OF MEDICINE (M.D)

in

MICROBIOLOGY

Under the Guidance of

DR. (Mrs). Sumati Hogade M.D

DEPARTMENT OF MICROBIOLOGY
J.N.MEDICAL COLLEGE, NEHRU NAGAR,
BELGAUM-590010.

MAY 2010

KLE UNIVERSITY, BELGAUM

DECLARATION BY THE CANDIDATE

I hereby declare that this dissertation entitled **"ISOLATION AND IDENTIFICATION OF ORGANISMS ASSOCIATED WITH BACTERIAL VAGINOSIS IN PREGNANT WOMEN WITH PER VAGINAL DISCHARGE. A ONE YEAR CROSS SECTIONAL STUDY"** is a bonafide and genuine research work carried out by me, under the guidance of **Dr. (Mrs). Sumati Hogade.,** Associate Professor, Department of Microbiology, J. N. Medical College, Belgaum.

Date:

Place: Belgaum. **Dr. GULNAR PARVEEN KAZI**

CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled **"ISOLATION AND IDENTIFICATION OF ORGANISMS ASSOCIATED WITH BACTERIAL VAGINOSIS IN PREGNANT WOMEN WITH PER VAGINAL DISCHARGE. A ONE YEAR CROSS SECTIONAL STUDY"** is a bonafide research work done by **Dr. Gulnar Parveen Kazi** in partial fulfilment of the requirement for the degree of M.D Microbiology.

Date:

Place: Belgaum.

Dr. (Mrs). Sumati Hogade
Associate Professor
Department of Microbiology,
J.N. Medical College,
Belgaum – 590010.

CERTIFICATE BY THE CO-GUIDE

This is to certify that the dissertation entitled **"ISOLATION AND IDENTIFICATION OF ORGANISMS ASSOCIATED WITH BACTERIAL VAGINOSIS IN PREGNANT WOMEN WITH PER VAGINAL DISCHARGE. A ONE YEAR CROSS SECTIONAL STUDY"** is a bonafide research work done by **Dr. Gulnar Parveen Kazi** in partial fulfilment of the requirement for the degree of M.D Microbiology.

Date:

Place: Belgaum.

Dr. M. B. Bellad
Professor
Department of Obstetrics & Gynaecology
J.N. Medical College,
Belgaum – 590010.

ENDORSEMENT BY THE HOD, PRINCIPAL/HEAD OF THE INSTITUTION

This is to certify that the dissertation entitled **"ISOLATION AND IDENTIFICATION OF ORGANISMS ASSOCIATED WITH BACTERIAL VAGINOSIS IN PREGNANT WOMEN WITH PER VAGINAL DISCHARGE. A ONE YEAR CROSS SECTIONAL STUDY"** is a bonafide research work done by **Dr. Gulnar Parveen Kazi** under the guidance of **Dr. (Mrs). Sumati Hogade.,** Associate Professor, Department of Microbiology, J. N. Medical College, Belgaum.

Dr. (Mrs). S. C. METGUD M.D
Professor & Head
Department of Microbiology,
J.N.Medical College,
Nehru Nagar,
Belgaum-590010

Date:
Place: Belgaum.

Dr. V. D. PATIL M.D, DCH
Principal
J.N.Medical College,
Nehru Nagar,
Belgaum-590010

Date:
Place: Belgaum.

COPYRIGHT

DECLARATION BY THE CANDIDATE

I hereby declare that the KLE University Belgaum, Karnataka shall have the rights to preserve, use and disseminate this dissertation in print or electronic format for academic/research purpose.

Date:

Place: Belgaum **Dr. Gulnar Parveen Kazi**

ACKNOWLEDGEMENT

First and foremost, my salutations to God, the ALMIGHTY, for his divine grace and blessings.

I am thankful to Dr. V. D. Patil, Principal, JNMC for allowing me to undertake this study.

It gives me great pleasure to express my gratitude to Dr. (Mrs.) S. C. Metgud, Professor and Head, Department of Microbiology. JNMC, Belgaum for her valuable advice and encouragement throughout my Postgraduate study period.

I humbly acknowledge my gratitude to my Guide Dr. (Mrs.) Sumati. Hogade, Associate Professor, Department of Microbiology. JNMC, for her constant encouragement, valuable suggestions and patience during the course of this study.

I am immensely grateful to my co guide Dr. M. B. Bellad, Professor, Department of Obstetrics and Gynaecology for his guidance and help towards the completion of my dissertation.

I thank Dr. B. R. Desai. Professor and Head, Department of OBG for permitting me to obtain samples for my work.

I am greatly indebted to Dr. S. G. Karadesai, Professor, Department of Microbiology, for kindly reviewing the progress of my study and giving helpful suggestions.

I extend my heartfelt thanks to Dr. Jyoti Nagmoti, Professor and Dr. M. B. Nagmoti, Professor, Department of Microbiology for their valuable ideas and guidance.

I am extremely thankful to Dr. Manjula Vagrali, Assistant Professor, Dr. Shashank Purwar, Assistant Professor, Dr. Sheetal Harakuni, Assistant Professor, Dr. Madhumati, Assistant Professor and Dr. Kavita, Assistant Professor for their help and advice.

My special thanks to Shri. M. D. Mallapur, Lecturer, Department of Community Medicine, JNMC for the statistical analysis.

I am grateful to Dr. M. G. Kulkarni, Retired Professor, Department of Microbiology, for his kind words of encouragement, concern and help during my study.

I am thankful to all the women who participated in this study, without whose cooperation my study would not have been possible.

I thank Dr. Amit Srivastava and Dr. Samina, Postgraduate students of Department of OBG for helping me throughout the study.

I thank all my fellow postgraduate friends Dr. Shilpa, Dr. Premalatha, Dr. SwaroopRani, Dr. Sheela, Dr. Varun, Dr. Kirtilaxmi, Mrs. Preeti, and Mrs. Shobha for their support and help. I will always cherish the beautiful moments I shared with them.

I wish to convey my special thanks to my Co-PG and friend Dr. Rashmi for standing by me always.

I would like to thank Mr. Prathik, Mr. Diparayan, Mr. Nadeem, Miss Pooja and Miss Nimisha for their help.

I wish to express my appreciation and thanks to all the non-teaching staff of my Department for their help.

I express my thanks to Mr. Satish of Sheegra Computers for his dedicated work and patience in designing and printing my dissertation.

I am deeply indebted to my father Mr. Mohammad. Yusuf and mother Mrs. Rehan for being my pillar of strength and whose cherished blessings and countless sacrifices are behind whatever success I have achieved in my life.

My special thanks to my husband Dr. Reyas and my little daughter Samah, without whose love and support, this work would not have been completed.

I would also like to thank Dr. Fayyaz and my sisters Dr. Taheen and Miss. Alphia for their support.

Dr. Gulnar Kazi.

LIST OF ABBREVIATIONS

BV	:	Bacterial Vaginosis
CBA	:	Columbia Blood Agar
HSV	:	Herpes Simplex Virus
IUD	:	Intrauterine Device
IUGR	:	Intra Uterine Growth Retardation
LBW	:	Low Birth Weight
NBV	:	No Bacterial Vaginosis
PCR	:	Polymerase Chain Reaction
PID	:	Pelvic Inflammatory Disease
PROM	:	Premature Rupture Of Membranes
Spp	:	Species
STD	:	Sexually Transmitted Diseases
UTI	:	Urinary Tract Infection

ABSTRACT

Background and Objectives : Bacterial Vaginosis (BV) is one of the important causes of vaginal discharge among women of child bearing age. It is also a risk factor for premature rupture of membranes (PROM), prematurity, chorioamnionitis, postpartum endometritis and delivery of low birth weight babies etc [1].

Objectives of the study : 1. To diagnose BV by two noncultural methods (Amsel's criteria and Nugent's Scoring and Grading of Gram stained smears) and to compare them. 2. To isolate and identify aerobic and anaerobic organisms associated with per vaginal discharge.

Materials and Methods : Samples were collected from 120 pregnant women complaining of vaginal discharge attending the OBG Out Patient Department of KLE'S Dr. Prabhakar Kore Hospital and MRC., Belgaum. Three high vaginal swabs were collected from the posterior fornix of the vagina, two were collected in 0.5ml of saline and one in 2ml of Thioglycollate medium. BV was diagnosed according to the Clinical Composite Criteria (Amsel's criteria) if at least three out of the following four criteria were met [2]. (1) .A thin homogenous white discharge, (2) vaginal pH of more than 4.5, (3) a positive Amine test, and (4) presence of clue cells on wet mount. According to Nugent's method[1], the Gram stained smears of vaginal discharge were scored and graded as BV if the score was more than 7.The organisms associated with vaginal discharge were isolated and identified according to standard microbiological methods[3]. *G.vaginalis* was cultured by incubating Columbia Blood Agar (CBA) plates in CO_2 jar and isolates were further identified by following standard methods[4].

Results : BV was diagnosed in 51 (42.5%) cases according to Clinical Composite Criteria (Amsel's) and in 42 (35%) cases by Nugent's Scoring and Grading of Gram stained vaginal smears. Using Gram's staining as standard, sensitivity, specificity, positive predictive value and negative predictive value of Amsel's criteria was 78.5%, 76.9%, 64.7% and 86.9% respectively.

Culture was positive in 40 (95.2%) cases among BV (diagnosed by Nugent's method) and 13 (27.6%) NBV cases. *Bacteroides fragilis* (*B.fragilis*) was the most common anaerobic bacteria isolated followed by *Prevotella melaninogenica* (*P. melaninogenica*) and *G. vaginalis* was the most common aerobe isolated.

Conclusion : The present study showed moderate agreement between the two non cultural methods (Amsel's criteria and Nugent's method). Polymicrobial mixed isolates were significantly raised in BV as compared to NBV cases.

Key words : Bacterial vaginosis, Amsel's criteria, Nugent's method, *Gardnerella.vaginalis*

TABLE OF CONTENTS

LIST OF TABLES

LIST OF GRAPHS

LIST OF PHOTOGRAPHS

INTRODUCTION

Bacterial vaginosis (BV) represents a complex change in the vaginal flora characterized by a marked reduction in the Lactobacilli and an increase in other organisms such as *Gardnerella vaginalis (G.vaginalis), Mycoplasma hominis* (*M.hominis), Peptostreptococcus* spp, *Mobiluncus* spp, anaerobic Gram negative bacilli belonging to the genera Prevotella, Porphyromonas, Bacteroides and Fusobacterium. Aerobic bacteria commonly implicated in BV include *Staphylococcus aureus*, Group B β hemolytic streptococci and *Escherichia coli*[4].

BV in pregnancy is associated with maternal complications like premature rupture of membranes (PROM), chorioamnionitis, post cesarean endometritis, vaginal cuff cellulitis, fetal complications like intra uterine growth retardation (IUGR) and prematurity [1].

Clinical composite criteria for the diagnosis of BV comprises of an increased homogenous greyish white vaginal discharge, vaginal pH >4.5, fishy smell on addition of 10% KOH to vaginal fluid (Whiff test) and the presence of clue cells in the wet mount preparation of vaginal discharge. The presence of three out of the above four criteria is diagnostic of BV [2].

The laboratory methods for the diagnosis of BV include Nugent's Grading and Scoring of Gram stained vaginal smears, Culture, biochemical tests for metabolic byproducts of vaginal bacteria (gas liquid chromatography), Proline aminopeptidase assay and molecular methods like PCR etc.

Early detection and treatment of BV especially in pregnancy appear to have a role in reducing neonatal morbidity and mortality. BV is a preventable cause of prematurity, early diagnosis by simple microbiological techniques help to prevent it.

The present study was undertaken to isolate and identify common organisms associated with BV and to compare the non-cultural methods for the diagnosis of BV.

OBJECTIVES

1. To diagnose BV by two noncultural methods (Amsel's criteria and Nugent's Scoring and Grading of Gram stained smears) and to compare them.
2. To isolate and identify aerobic and anaerobic organisms associated with per vaginal discharge.

REVIEW OF LITERATURE

HISTORY : In the year 1894, Doderlein described the presence of Lactobacilli in normal vaginal flora. Lactobacilli produce lactic acid which inhibits the growth of other vaginal microorganisms by maintaining a low vaginal pH and occupying a ecological niche[5].

In 1914 Curtis associated anaerobic cocci and Mobiluncus with abnormal vaginal discharge. He postulated that the anaerobic microorganisms were part of a complex bacterial milieu that caused not only an abnormal vaginal discharge but also postpartum endometritis[5].

In 1921 Schroder categorized vaginal flora using Gram stain into the least pathogenic (consisting of a predominance of Lactobacilli), intermediate stage and most pathogenic stage that is now identified as Bacterial vaginosis[5].

In 1950, Weaver et al associated Bacteroides with bacterial vaginosis and also confirmed the absence of Lactobacillus in the syndrome[5].

In 1955, Gardner and Dukes described a new microorganism which they called *Haemophilus vaginalis.* They believed that this agent caused vaginitis. They also described the clinical features of this syndrome that forms the basis of the diagnosis today. The vaginal discharge was characterized by a grey homogenous appearance, had a pH between 5.5 and 6.0, it had a malodour, an absence of Lactobacillus, and the presence of so-called vaginal epithelial clue cells[5].

The discoveries of Gardner and Duke were important in defining the clinical disease and the association of atleast one organism *H.vaginalis* with the syndrome. However, it is now apparent that a variety of anaerobic microorganisms also have a role in bacterial vaginosis[5].

Confusion existed over the taxonomy such that *H.vaginalis* was first reclassified as Corynebacterium and then classified in genus Haemophilus. Deoxyribonucleic homology studies were needed to ultimately classify this organism in a separate genus and the microorganism is now called *Gardnerella vaginalis* in honour of Dr. Gardner[5].

Advancements in anaerobic microbiology in the 1970s finally lead to the realization that several anaerobic bacteria, as well as *G.vaginalis* were present in this disease[5].

WHAT IS BV ?

The term, “Bacterial vaginosis” was coined to describe abnormal bacterial flora of the vagina characterized by an overgrowth of anaerobic bacteria and *G.vaginilis* with a marked decrease in the presence of Lactobacillus. The term was chosen to replace nonspecific vaginitis, with the suffix “-osis” specifically chosen to indicate the absence of an inflammatory reaction that is the presence of neutrophils. BV is defined as abnormal microflora of the vagina characterized by a significant reduction of the dominant bacterium Lactobacillus to extremely low levels, fewer than 10,000 col/ml of vaginal fluid and a marked increase in the

anaerobic bacterial population. Associated with this change is an increased colonization of *G. vaginalis*[6].

Clinically and microscopically BV is defined as follows : vaginal pH >4.5, the liberation of amines when vaginal discharge is mixed with 10% potassium hydroxide solution and the presence of clue cells. Important to this definition is the absence of inflammatory cells[6].

In the healthy vagina, Lactobacillus predominates whereas in BV, Lactobacillus is significantly reduced in number and obligate anaerobes, both gram-positive and gram-negative, make up the dominant flora[6].

NORMAL FLORA OF THE GENITOURINARY TRACT :

The mucosal surfaces of the genitourinary tract are colonized by a complex mixture of bacterial species.

Vaginal microflora :

Although the vaginal flora is generally considered to be a single entity, the vagina does not provide a uniform environment throughout its length from the introitus to the cervix and the balance of species differs with these different environments. The flora of the lower vagina is a mixture of organisms from the upper vaginal mucosa and from the cervix and organisms from the vulva and perineum. In the lower vaginal flora there are Staphylococci, Propionibacteria, Diphtheroids, *E.coli* and other enterobacteria from the faeces[7].

However the vaginal environment is far from constant. It changes markedly with the various hormonal changes of the human reproductive cycle[7].

The vaginal flora is predominantly anaerobic and the pH is low. Some aerobic / facultative species are present, mostly *Staphylococcus aureus(S.aureus),* Viridans Streptococci, Enterococci, Coryneforms, *S.epidermidis,* Group B β haemolytic Streptococci and Peptostreptococci.

The species most commonly present in the vagina is *Lactobacillus acidophilus*, a name that reflects its acid producing role in the normal vaginal flora. The vaginal epithelial cells contain glycogen, and it has been generally accepted since the mid 1930s that this is metabolized, first to glucose and then to lactic acid, by the vaginal Lactobacilli, maintaining the low pH and selectively favouring colonization by acid tolerant organisms such as Lactobacilli themselves[7].

The CO_2 dependent microaerophilic species, *G.vaginalis* is normally present in the vaginal microbiota. It has an important place in BV, a condition in which by their metabolic activity, a mixture of anaerobic bacteria produce an altered vaginal environment which manifests as a profuse, offensive discharge.

Members of the Bacteroides Melaninogenicus-oralis group are the commonest bacteriodes of the vagina. *Bacteriodes asaccharolyticus (B. asaccharolyticus*) is present in small numbers in specimens from normal subjects.

Anaerobic gram-positive cocci like Peptostreptococci are also a part of vaginal flora. Most common species being *P.magnus, P.asaccharolyticus.*

The physiologic changes in the vagina in pregnancy are dramatic, as are the changes in the bacterial flora. The amount of glycogen in the mucosa is much lower, the pH of secretions much less acidic and normally anaerobic flora is replaced by a preponderance of aerobic / facultative species[7].

The vaginal secretions of prepubertal children and post menopausal women are more alkaline and contain a varied bacterial flora dominated by coagulase negative staphylococci, Diphtheroids, and α hemolytic streptococci, anaerobic gram negative rods and *E. coli*[7].

MICROBIOLOGY OF BV :

Bacterial vaginosis is not a specific, monobacterial infection, but a synergic mixture of anaerobic, microaerophilic and CO_2 dependent species that are present in small numbers in many normal asymptomatic women but in large numbers in vaginosis[7].

The normal Lactobacillary flora is replaced by a mixture of small bacilli normally inhibited by the Lactobacilli, CO_2 dependent *G.vaginalis* and two anaerobic gram negative groups – Bacteroides spp. of the melaninogenicus-oralis group (principally *B.bivius* and *B.disiencs*) and curved motile rods of Mobiluncus spp. *G.vaginalis* and Bacteroides spp. are present in most cases. Mobiluncus are curved, motile gram-negative rods. They were first described in vaginal discharge by Curtis. Two species have been described and named – *M. curtisii* and *M.mulieris*[7].

Mycoplasma species are also associated with bacterial vaginosis, but their role is uncertain. The vaginal pH, normally ≤ 4.0 rises to ≥ 5.5. The lactate concentration is reduced and the amount of succinate, acetate, propionate and butyrate (all principally produced by Bacteroides spp.) increase. The secretions also contain volatile amines, eg. putresine, methylamine, cadaverine etc which are products of anaerobic metabolism and cause the fishy smell[7].

Bacterial relationships in the pathogenesis of BV are not clear, but metabolic interactions may generate active products that cause excessive secretion, eg. Pyruvate and aminoacids secreted by *G.vaginalis* may be decarboxylated to amines by Bacteroides spp[7].

Lactobacilli predominate in the normal vagina, the pH is low and the principal fatty acid product of metabolism is lactate. The vaginosis-associated organisms that are present in relatively small numbers, particularly the Bacteroides spp. and *G.vaginalis* are inhibited in vitro by lactic acid and low pH.

In susceptible women, the natural protective mechanism is lost by a combination of inhibition of the Lactobacilli, increase in pH and buffering of the lactate, and allows the proliferation of *G.vaginalis* and Bacteroides spp. The metabolic interactions of these synergic mixtures may then produce active metabolites which induce secretion from the vaginal mucosa while endowing the discharge with its offensive character[8].

Anaerobic gram negative bacilli particularly Prevotella, Porphyromonas and Fusobacterium are also associated, anaerobic gram positive bacilli include

Eubacterium species and Propionibacterium spp. The most common anaerobic gram positive cocci include *P. asccharolyticus, P.prevotii* and *P.anaerobius*[8].

Aerobic bacteria commonly implicated in BV include *S. aureus*, Group B β hemolytic streptococci and *E.coli* have been noted in 25% or fewer of women with BV[8] .

In a study conducted by P. S. Rao et al, the prevalence of BV was 20.5% by smear and 17.42% by culture. The organism most commonly isolated was Gardnerella spp. followed by Prevotella spp and Peptostreptococci[9].

In another study conducted by A. Aggarwal et al, the prevalence of BV in pregnant women was 36.4% and anaerobes most commonly isolated were Peptostreptococci. Spp (40%)[2].

RISK FACTORS AND CLINICAL FEATURES:

Approximately 50 to 75% of women with BV are asymptomatic. In symptomatic women the manifestations vary from increased greyish white vaginal discharge, which may have an offensive odour which is intensified after intercourse and during menstruation. Other symptoms are pruritis, lower abdominal pain, pain during coitus etc[9].

Risk factors for acquisition of BV include multiple or new sexual partners, douching and cigarette smoking. Women of lower socioeconomic status and women with higher levels of psychological stress also have increased rates of BV[10].

Culhane et al, assessed the role of chronic maternal stress, as measured by Cohen perceived stress scale and found that independent of sociodemographic and behavioral factors, chronic maternal stress remained a significant predictor of BV among pregnant women[11].

Epidemiological studies have found that early sexual activity, a high number of lifetime sexual partners, women with a new sexual partner, and women with a prior sexually transmitted disease are also at increased risk of BV[11].

Some behaviors such as vaginal douching, use of diaphragms are potential risk factors for BV. Vaginal douching may change the vaginal flora, reduce the amount of Lactobacillus, and create an environment promoting excessive anaerobic growth.There is high occurrence of BV and concordance of flora in lesbians, suggesting sexual transmission is important in this setting[11].

COMPLICATIONS :

BV in pregnancy is associated with PROM, post cesarean endometritis, amniotic fluid infection, vaginal cuff cellulitis, post abortal infection, foetal complications include prematurity and intrauterine growth retardation [12].

BV is a risk factor for HIV acquisition and transmission. BV increases the risk of HIV transmission by 2-4 fold and has been estimated to contribute 23% to antenatal HIV seroconversion in a high prevalence population of pregnant women in Malawi [13]. BV is also a risk factor for HSV-2, gonorrhoea and chlamydial infections. BV is more common among women with pelvic inflammatory disease, but it is not clear if it is an independent risk factor for this disease [11].

The bacterial flora that characterizes BV have been recovered from the endometria and salpinges of women who have pelvic inflammatory disease (PID). BV has also been associated with endometritis and vaginal cuff cellulitis after invasive pelvic procedures like endometrial biopsy, hysterectomy, hysterosalpingography, insertion of IUD, Cesarean section and uterine curettage[14].

There are reports suggesting women suffering from BV are at greatest risk of acquiring urinary tract infection (UTI) than others. Harmanli et al conducted a study and found that 15 out of 67 women (22.4%) had both BV and UTI whereas only 6 (9.7%) had UTI without BV[15]. Hillebrand et al in a cross sectional study examined 503 pregnant women from the viewpoint of UTI and BV and reported that 13.6% percent of 140 women suffering from BV also had UTI whereas only 6.6 percent of 363 women without BV had UTI. He concluded that BV in pregnancy increases the risk of UTI [16] .

Some studies have found that the presence of *G.vaginalis* on the cervix as detected on Papanicolaou smear is associated with high grade squamous intraepithelial neoplasia (HSIL); however, a causal relationship has not been proven and others have not reported this association [11].

PATHOGENESIS :

The association between BV and its attendent microbes and serious infections in the female pelvis has led to understand this entity more completely to facilitate development of improved modalities for prevention and treatment.

BV is related to :

1. An increased potential for other vaginal pathogens to gain access to the upper genital tract.
2. The presence of enzymes that reduce the ability of leucocytes to reduce infection.
3. An increased level of endotoxins stimulating cytokine and prostaglandin production.

Infact, Imseis et al, reported higher vaginal levels of interleukin-1 beta, an inflammatory cytokine, among pregnant women with BV and Spandorfer et al, found higher levels of both cervical interleukin-1 beta and interleukin-8 cytokine levels among non-pregnant women with BV [11].

Hydrogen peroxide producing Lactobacilli are important in preventing overgrowth of the anaerobes which are normally present in the vaginal flora. With the loss of Lactobacilli, pH rises and massive overgrowth of vaginal anaerobes occurs. These anaerobes produce large amounts of proteolytic carboxylase enzymes, which break down vaginal peptides into a variety of amines that are volatile, malodorous and associated with increased vaginal transudation and squamous epithelial cell exfoliation, resulting in the typical clinical features

observed in patients with BV. The rise in pH also facilities adherence of *G.vaginalis* and other organisms to the exfoliating epithelial cells thereby creating the "clue cells" that are diagnostic of the disorder [10].

PROM :

Knox and Hoerner in 1950, suggested that infection of the cervix could extend to the membranes overlying the internal OS. This infection causes membranes to become inflamed and "friable" and thus liable to give away under the stress of Braxton-Hicks contractions [17].

In 1962, Benirschke again suggested that the fetal membranes become inflamed because of ascending vaginal bacteria predisposing to PROM [17].

Subsequently in 1969, Dukes and Gardner noted that growth of abnormal bacterial agents, including *G.vaginalis* could replace normally predominant vaginal Lactobacilli and Diptheroids and mediate obstetric morbidity[17].

Minkoff et al were the first to correlate clinical findings of BV and characteristics of vaginal microflora with PROM and preterm birth[17].

Kurki et al performed a Cohort study of 790 nulliparous pregnant women. They demonstrated a 7.3 fold risk for PROM for women with BV. Purwar et al, found that BV diagnosed in the second trimester was associated with an increased risk of PROM and preterm delivery and that BV accounted for 83 percent of the attributable risk for preterm birth[18].

Bacteria associated with BV produce proteases that may destroy or weaken collagen. These microorganisms also produce mucinase that may hydrolyse protective cervical mucin as well as immunoglobin A (IgA) proteases which can destroy mucosal membrane IgA, an important element of reproductive tract host defence. The short chain fatty acid salts butyrate and propionate, which are increased in BV, are inhibitory to fibroblasts. These or other cytotoxic substances may cause fetal weakening or necrosis of fetal membranes. Increased concentrations of succinate which are characteristic of BV, reduce polymorphonuclear cell chemotaxis and impair host responses to BV associated microorganisms [17].

CHORIOAMNIONITIS :

Silver et al, concluded that in intraamniotic infections organisms arise concurrently from bacterial vaginosis. Isolation of bacterial vaginosis organisms from the amniotic fluid provide additional data to suggest a causal relationship between BV and intraamniotic infection [19].

Hillier et al studied demographic and obstetric characteristics as well as placental histologic findings and carefully collected chorioamniotic cultures of women who delivered prematurely versus women who delivered at term. This study provides strong evidence of a relationship between low birth weight (LBW), histologic chorioamnionitis and positive culture from membranes [19].

DIAGNOSIS OF BACTERIAL VAGINOSIS :

I. CLINICAL COMPOSITE CRITERIA (AMSEL'S CRITERIA) :

Patients were diagnosed as having bacterial vaginosis if they fulfilled 3 of the following 4 criteria [1].

1. Homogenous greyish white vaginal discharge.
2. Vaginal pH above 4.5
3. A fishy smell on addition of 10% KOH to vaginal fluid (Whiff test)
4. Presence of clue cells.

II. GRAM STAIN:

Gram stain of vaginal fluid has been used for laboratory confirmation of bacterial vaginosis since 1965.

In 1983 Spiegel et al, modified the Gram stain criteria for BV to include those smears with the Gardnerella morphotype plus other bacteria (Cocci, fusiforms and curved rods) and fewer than five Lactobacillus morphotypes per oil immersion field [20].

Nugent et al, reported that the three bacterial morphotypes that were recognized with the highest degree of reproducibility were Lactobacillus (large gram positive bacillus), Gardnerella and Bacteriodes (small gram negative or gram variable rods) and Mobiluncus (curved gram-negative or gram variable rods). These three bacterial morphotypes were used to develop a 0-10 point scoring system for the diagnosis of BV [20].

A score of 0-3 is normal, 4-6 is considered intermediate and 7-10 is diagnostic of BV.

III. VAGINAL CULTURES :

Krohn et al, evaluated the sensitivity and specificity of vaginal cultures for anaerobic bacteria. She found that the presence of these organisms was a more specific indicator of bacterial vaginosis than was the presence of *G.vaginalis*.

Mobiluncus species, another group of anaerobic bacteria highly associated with BV are very difficult to recover with culture methods [20].

In a study conducted by Rao P. S. et al, prevalence of 17.42% was detected by culture methods. Among the culture isolates, Gardnerella spp was the most common organism isolated followed by Prevotella and Peptostreptococci [9].

IV. PAPANICOLAOU SMEARS :

Pap smears were also used for the diagnosis of BV. But, recent studies suggest that Pap smears are less specific because standardized criteria for the evaluation of Papanicolaou smears have not been routinely applied [20].

The Pap smear is used commonly as cytologic screening test for eradication of precancerous lesions. It has also been evaluated as a diagnostic test for bacterial vaginosis, but the results of these studies are contradictory.

Smears performed with endocervical brush, are fixed in 95% ethanol and stained by Papanicolaou method. If there is a filmy background of small coccobacilli, individual squamous cells with a layer of coccobacilli along the margins of the cell membranes and conspicuious absence of Lactobacilli, the smear is evaluated as positive for BV [21].

Schnadig et al reported a high correlation between Pap smears and Gram smears for the diagnosis of BV. Davis et al reported that compared to Gram stain, cervical cytologic test results had a sensitivity of 55%, specificity of 98%, positive predictive value of 96% and a negative predictive value of 78% [21].

But recent studies suggest that Pap smears are less specific because standardized criteria for the evaluation of Pap smears have not been routinely applied.

VI. OLIGONUCLEOTIDE PROBES :

Oligonucleotide probe tests have the advantage of being specific and can be adjusted in sensitivity to detect either low or high concentrations of bacteria. One such application had been applied to *G.vaginalis* with use of rapid, non-isotopic assay for high concentrations of this microorganism. Sheiness et al, reported that detection of greater than 10^7 colony forming units of *G.vaginalis* per milliliter of vaginal fluid was 95% sensitive and 79% specific for the diagnosis of BV [20].

VII. GAS LIQUID CHROMATOGRPHY :

Succinic acid, a metabolic product of anaerobic bacteria is present more frequently and at a higher concentration among women with BV. When Lactobacilli are the predominant members of the vaginal flora, lactic acid is the predominant acid present. Among women with BV, succinate, acetate and other short chain organic acids can be detected [20].

Spiegel et al, reported that a succinate/lactate ratio of >0.4, based on peak heights on gas chromatographic analysis of vaginal fluid was correlated with clinical diagnosis of BV [20].

This method has been evaluated in several case-control and Cohort studies and is reported to be 54% to 89% sensitive and 80%-96% specific for the diagnosis of this syndrome[20].

This method is probably not adaptable for wider use because it relies on laboratory equipment that is not widely available.

VIII. PROLINE AMINOPEPTIDASE ASSAY :

This test is based on the detection of enzymatic activity. Proline aminopeptidase cleaves the substitute material, proline β-naphthalamide, yielding proline and β-naphthalamine. The naphthalamine can react with many aniline dyes to form various coloured complexes. It can also be combined with nitrite to form a diazo complex or it can be measured directly flourometrically.

This test requires no sophisticated instruments and has greater than 80% sensitivity. Another advantage is that up to 90 specimens can be run concurrently on a single microtitre plate in 1-4 hour period in contrast to Gas Liquid Chromatography which requires at least 30 minutes / specimen [22].

MOLECULAR METHODS :

Many researchers are exploring a genetic basis for exploration of the complex microbial flora associated with BV. The drawback of these techniques is the complexity and cost.

David N. Fredrick et al developed a series of 16S rRNA gene PCR assays for more sensitive detection of key vaginal bacteria. According to their study *Leptotrichia amnionii / sneathia*, *Atopobium vaginae*, an Eggerthella like bacterium, Megasphaera species, and three novel bacteria in the order Clostridiales were among the bacterial species significantly associated with BV.

Flourescent Insitu Hybridisation(FISH) confirmed that newly recognized bacteria detected by PCR corresponded to specific bacterial morphotypes visible in vaginal fluid [23].

Thies F et al, analysed vaginal flora just within 12 hours using T-RFLP (terminal restriction fragment length polymorphism). T-RFLP analysis is based on the restriction endonuclease digestion of fluorescently end-labelled PCR amplificates (derived from the 16S rRNA gene). *Atopobium vaginae* and *G.vaginalis* proved to be the predominant species [24].

Jean Pierre Menard et al quantitatively analyzed vaginal flora. A quantitative molecular tool targeting 8 BV related microorganisms and a human gene was developed using a specific real time PCR assay and serial dilutions of plasmid suspension. The targeted microorganisms were *G.vaginalis*, Lactobacillus species, *M. curtisii*, *M.culieris* and *C. albicans* as well as *Atopobium vaginae, Mycoplasma hominis* and *Ureaplasma urealyticum* [25].

Estelle Devillard et al used PCR denaturing gradient gel electrophoresis (D GGE), for the analysis of organisms associated with BV [26].

Other methods : Diagnostic cards (e.g. Femcard, Quick Vue, Pig Activity and test card) are other rapid tests for confirming the clinical suspicion of BV. These cards are particularly useful for practitioners not able to perform microscopy. One group reported these tests detected the presence of elevated vaginal pH and increased amines with sensitivity and specificity of 87 and 92 percent, respectively, although others have reported lower values [10] .

Treatment : The established benefits of therapy in non-pregnant women are to 1) relieve vaginal symptoms and signs of infections and 2) reduce the risk of infectious complications after abortion or hysterectomy. Other potential benefits include a reduction in risk for other infections (e.g. HIV and other STDs). All women who have symptomatic disease require treatment [14] .

Recommended regimens :

Metronidazole 500mg orally twice a day for 7 days

OR

Metronidazole gel, 0.75%, one full applicator (5g) intravaginally, once a day for 5 days

OR

Clindamycin cream, 2%, one full applicator (5g) intravaginally at bedtime for 7 days.

Patients should be advised to avoid consuming alcohol during treatment with Metronidazole and for 24 hours thereafter.

Management of sex partners :

The results of clinical trials indicate that a women's response to therapy and the likelihood of relapse or recurrence are not affected by treatment of her sex partner (s). Therefore routine treatment of sex partners is not recommended [14].

Pregnancy :

All pregnant women who have symptomatic disease require treatment. Treatment of BV in asymptomatic pregnant women at high risk of preterm delivery (i.e. those who have previously delivered a premature infant) with a recommended oral regimen has reduced preterm delivery in three of four randomized controlled trials; some specialists recommend screening and oral treatment of those women.

For the treatment of BV in pregnancy, Metronidazole is no longer recommended. A metaanalysis evaluated the effects of three antibiotics, administered during the second trimester of pregnancy, on the rate of preterm delivery. Although mid trimester administration of Macrolides or Clindamycin was associated with reduction of delivery prior to 37 weeks gestation. Midtrimester Metronidazole was not linked with significant improvement. Clindamycin 300mg orally twice a day for 7 days is the recommended regimen for the treatment of BV in pregnancy [27].

Followup of pregnant women :

Treatment of BV in asymptomatic pregnant women who are at high risk for preterm delivery might prevent adverse pregnancy outcomes. Therefore, a follow-up evaluation 1month after completion of treatment should be considered to evaluate whether therapy was effective.

HIV infection : Patients who have BV and also are infected with HIV should receive the same treatment regimen as those who are HIV negative. BV appears to be more persistent in HIV–positive women [14].

Role of Probiotics : There is now extensive evidence to support the claim that Lactobacilli can be critical in the host's defence against urogenital infection.

A European based product, Gynoflor, reported to contain 50mg of viable H_2O_2 producing *L.acidophilus* and 0.03mg oestriol has been tested on non-menopausal women with BV. 6 days of therapy with 1-2 vaginal suppositories per day gave a cure rate in two weeks out of 77% in the treatment arm and 25% in placebo [28].

Likewise in a Japanese study[29] of 11 women aged 20 to 60 years, intravaginal treatment with 5ml commercial Yoghurt caused a bacteriological cure. Further evaluation of these products are needed.

METHODOLOGY

The present study was conducted at the Department of Microbiology, JNMC, Belgaum.

Source of Data : Samples were collected from pregnant women complaining of vaginal discharge attending out patient department of Obstetrics and Gynaecology at KLE's Dr. Prabhakar Kore Hospital and MRC, Belgaum and were processed in the Department of Microbiology.

Study design : A one year cross sectional study.

Sample size : 120 (Calculated based on an earlier study which showed an incidence rate of 5.15% of BV) [30].

The formula used was $n = 4pq/d^2$; where $p = 5.15\%$

$q = 94.8\%$

$d = \text{error} = 3\%$

Statistical methods used for analysing the data : Results were analysed using kappa statistics for showing degree of agreement between Nugent's method and Amsel's criteria. Sensitivity, specificity, positive predictive value and negative predictive value were calculated to evaluate the Amsel's criteria against the standard Nugent's method. The prevalence of different organisms in BV and NBV groups were compared using Z test.

Inclusion criteria : Pregnant women upto 24 weeks of gestation with per vaginal discharge.

Exclusion criteria :

1. Pregnant women who have undergone cervical encirclage and any other pelvic surgical procedures.
2. Pregnant women on antibiotic therapy.
3. Pregnant women with white curdy discharge (suggestive of Candida vaginitis) and greenish yellow discharge (suggestive of Trichomonas infection).
4. Not willing to provide informed consent.

METHODOLOGY :

Detailed information regarding age, complaints and Obstetric history were obtained.Three high vaginal swabs, two in 0.5ml normal saline and one in 2ml of Thiogycollate medium were collected from the posterior fornix of the vagina. A smear was prepared by rolling the swab on a glass slide before placing it in 0.5ml physiological saline.

NON-CULTURAL METHODS:

1. Clinical Composite Criteria : (Amsel's Criteria) [2]

The nature of the discharge was evaluated. The pH of the vaginal discharge was measured using indicator paper for pH range 4-6.

Whiff test was done by adding few drops of 10 % KOH to the discharge on a glass slide and sniffed for detection of fishy odour.

Wet mount was done by adding 0.2ml of physiological saline to vaginal discharge on a glass slide. It was covered with a coverslip and examined under low power of light microscope. Clue cells were identified as vaginal epithelial cells studded with bacteria so that the borders are obscured.

If three of the following four criteria were met, then a clinical diagnosis of BV was made.

1. Thin greyish white homogenous discharge adherent to the vaginal wall.
2. A vaginal pH of >4.5.
3. Whiff test positive.
4. Presence of clue cells on wet mount.

2. Grams staining of vaginal smears (Nugent's Method) [1] :

The smear was heat fixed and Gram-stained using saffranine as the counter stain. The smear was then evaluated for the following morphotypes for the scoring and grading of BV. Large gram positive rods (Lactobacillus morphotypes), small gram variable rods (G.*vaginalis* morphotypes), small gram negative rods (Bacteroides species morphotypes) and curved Gram variable rods(Mobiluncus morphotypes). A score of 0 to 3 is considered as normal flora, 4 to 6 as intermediate and 7 to 10 as BV.

Scoring and Grading of Gram-stained smears (Nugent et al)

Bacterial Morphotype	**Score**				
	None	**1+**	**2+**	**3+**	**4+**
Large GPB	4	3	2	1	0
Small GN / G variable bacilli	0	1	2	3	4
Curved GNB	0	1	2	3	4

<1 / oil immersion field	– 1+	0-3	Normal
1-5 / oil immersion field	– 2+	4-6	Intermediate
6-30 / oil immersion field	– 3+	7-10	Bacterial vaginosis
>30 / oil immersion field	– 4+		

CULTURAL METHODS :

Aerobic Culture Methods

The culture swab was inoculated onto.

1) 5% Sheep blood agar.
2) Mac Conkey agar plate.

The plates were incubated at 37^0C for 24 hrs aerobically.

All the isolates were identified biochemically by standard procedures as described by Bailey and Scott's diagnostic microbiology [3].

Antibiotic Sensitivity Testing

The antimicrobial susceptibility testing was done for aerobic isolates by disc diffusion method as described by Kirby Bauer, on Mueller Hinton Agar.

Different antibiotics and concentration of discs used were as follows:

Antibiotics	**Concentration per disc**
1) Ampicillin	10µg
2) Ceftazidime	30µg
3) Amoxycillin / Clavulanic acid (Ac)	30µg
4) Ciprofloxacin	05µg
5) Oxacillin(Ox)	01µg

For antimicrobial sensitivity testing a single colony was inoculated in peptone water and incubated at 37^0C for 4 to 6hrs and turbidity was adjusted to Mac Farland's 0.5. Muellor Hinton Agar plate was inoculated with broth culture by means of cotton swab and antibiotic discs were applied and incubated over night at 37^0C. Zone of inhibition was measured. Interpretation was made according to the Kirby Bauer chart [3].

2. Microaerophilic incubation (Isolation and identification of *G.vaginalis*) :

One swab of vaginal discharge from 0.5ml saline was inoculated onto Columbia Blood agar (CBA) plate which was prepared using Columbia Blood agar base. Human blood was used to prepare CBA media.

The presumptive identification of *G.vaginalis* was made by [4]:

1. Detection of β-hemolytic colonies with diffuse edges around 0.3 to 0.5mm in diameter after 48 hours of incubation in CO_2 jar.
2. Non motile
3. A negative catalase reaction
4. A negative oxidase reaction
5. Sensitivity to high content metronidazole discs (50μg)

3. Culture for anaerobic organisms

Each sample was inoculated onto

1) Blood agar supplemented with Haemin and Vit K.

↓

Single colony of each distinct type

↓

Aerotolerance testing

↓

-ve → Aerobic (CA) incubation in 5-10% candle jar

↓

Purity blood agar plate

↓

Add antibiotic Identification discs	**Perform**	**Record**
- Sodium polyanethol Sulphonate (SPS) disc	1. Gram stain	1. Colony Morphology
- Nitrate disc	2. Spot indole test	2. Pigment
- Susceptibility to Kanamycin (1mg) Vancomycin (5μg), Colistin (10μg)	3. Catalase test	3. Haemolysis 4. Fluorescence 5. Pitting

Blood agar was supplemented with Haemin (5mcg/ml) and Vit K (10mcg/ml). Blood agar plates used for anaerobic isolation were prepared with Brucella agar base.

The method used for obtaining anaerobiosis in the jar was "internal gas generating system" described by Lakshminarayana and Vaidhyalingam [31].

After 72 hours of incubation at 37^0C anaerobic jar was opened. The plates were examined for the presence of colonies. When the colonies appeared on the anaerobic plates each predominant distinct colony was subcultured to purity blood agar plate (BAP). From a pure culture on a BAP, following was recorded,

- Colony morphology, including size of colony, shape, color, internal appearance (such as speckling) and general appearance (eg: mucoid transparent, opaque)
- Pigment
- Haemolysis
- Fluorescence
- Pitting

Single colony of each distinct type was plated on to blood agar plates with antibiotic identification discs.

- Sodium polyanethol sulphonate (SPS) disc for rapid presumptive identification of Peptostreptococcus anaerobius.

- The 3 antibiotic discs Kanamycin 1 mg, Colistin 10µg and Vancomycin 5µg were placed on the first quadrant of the purity BAP, which aid in preliminary grouping of anaerobes and serve to verify the Gram's stain.
- A nitrate disc was placed on the 2nd quadrant for subsequent determination of nitrate reduction.

Chocolate agar plate was inoculated for incubation in candle jar at 37^0C to test for aerotolerance.

If there was no growth on plates after 72 hours of anaerobic incubation, the plates were reincubated for an additional period of 48 hours and for a maximum period of 1 week.

The following tests were done from the purity plate.

Catalase test : Growth was removed from blood agar plate to a drop of 15 % hydrogen peroxide on a glass slide and observed for evolution of bubbles.

Spot Indole test : A loopful of growth from a pure culture on a blood agar plate was removed and this growth was smeared on filter paper that has been saturated with 1 % paradimethylaminocinnamaldehyde in 10 % (V/V) concentrated hydrochloric acid. A positive reaction was indicated by the rapid development of blue colour around the growth. Negative reaction gave no color change or a pinkish color.

Nitrate test : This test was done using nitrate discs. The disc was removed from surface of plate and placed in a clean petridish. One drop each of reagents A and B were added. Development of pink to red color indicated nitrate had been reduced to nitrite. If no colour developed in few minutes, a small amount of zinc dust was added and waited for 5 minutes. Development of red colour indicated that nitrate was not reduced. If no colour developed it was taken as positive test [32].

Nitrate reagents

Solution A

Sulfanilic acid 0.5g

Glacial acetic acid 30.0ml

Distilled water 120.0ml

Solution B

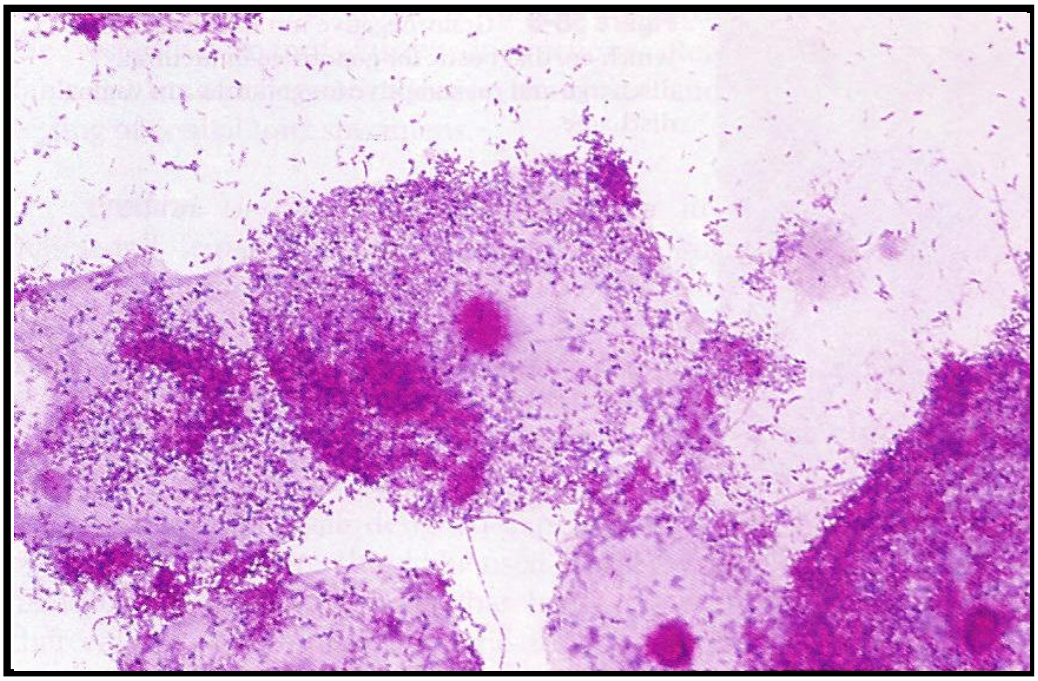
1,6-Cleve's acid 0.2g

(5-amino-2-naphthalenesulfonic acid)

Glacial acetic acid 30.0ml

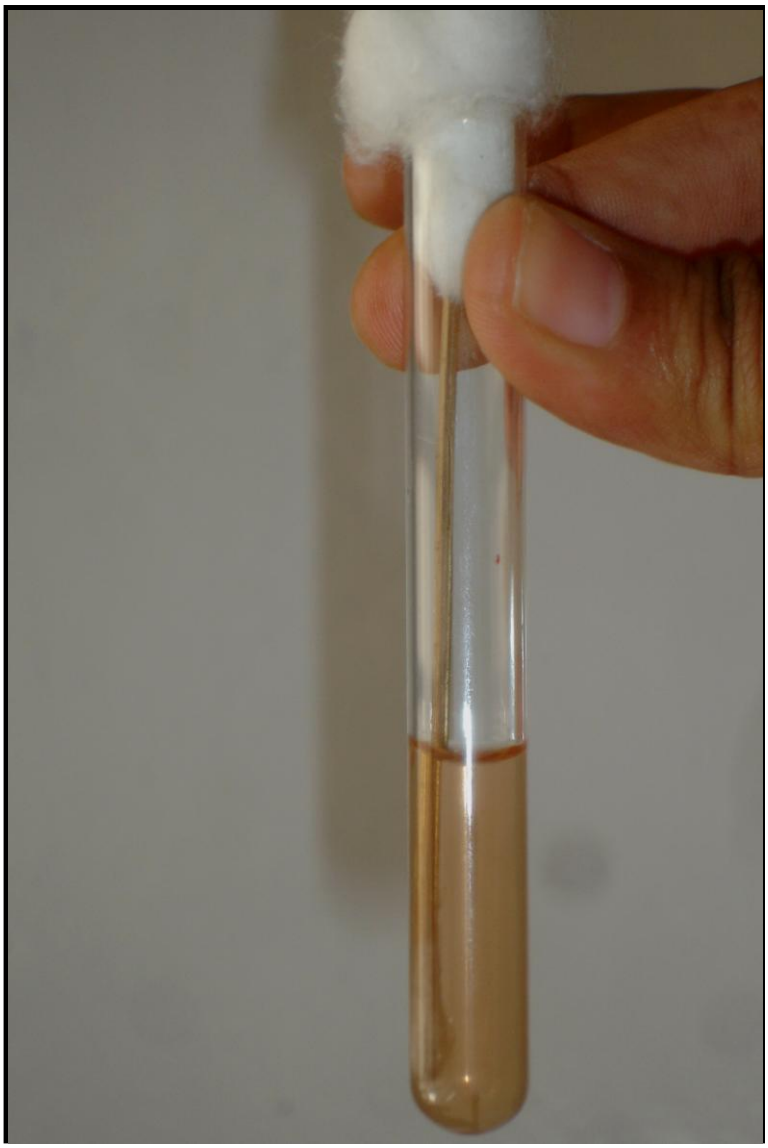
Distilled water 120.0ml

Photograph 1 : Clue cells as seen on Gram's stained smear

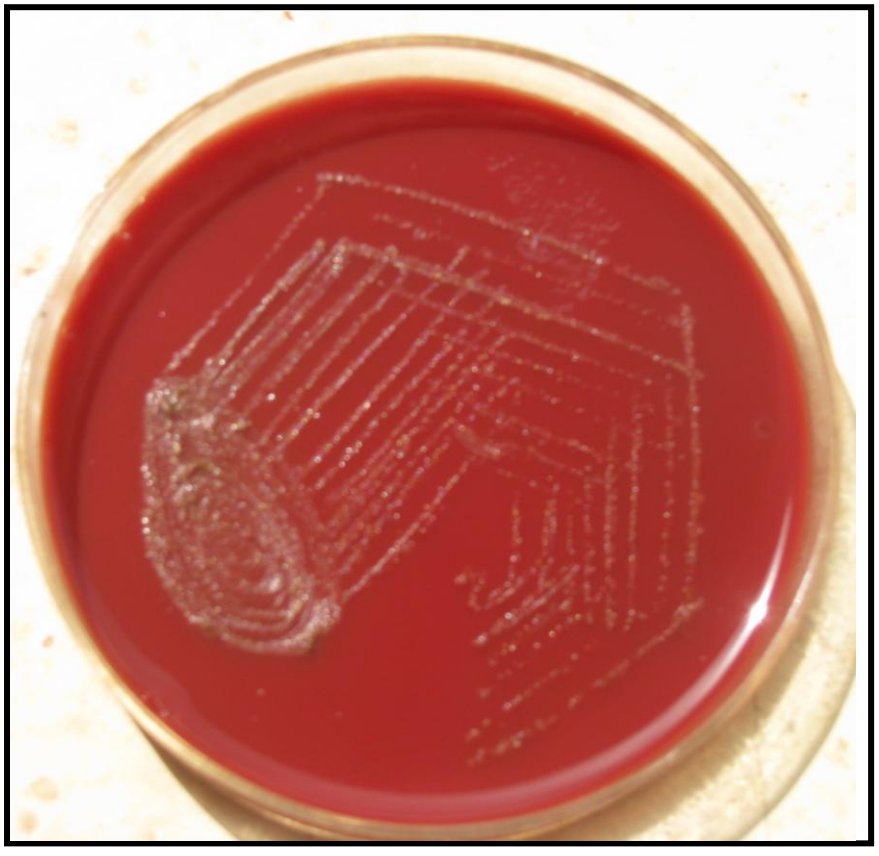


Photograph No. 2 : McIntosh Fildes Jar

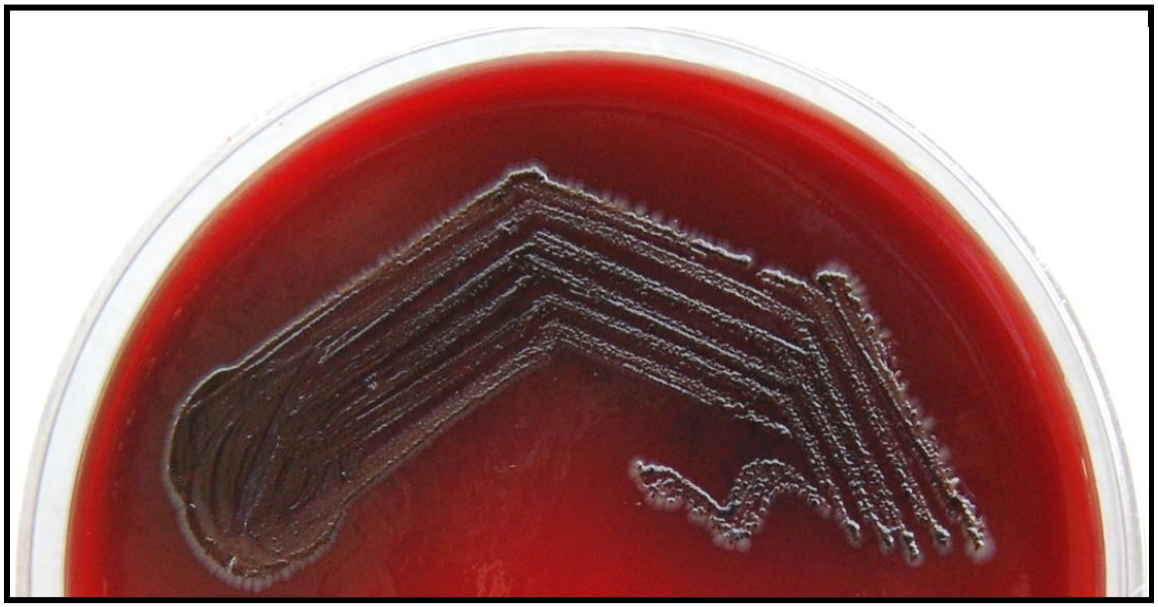
Photograph 3 : Fluid thioglycollate medium used for the collection of samples



Photograph No. 4 : Primary blood agar plate showing growth of anaerobic organisms

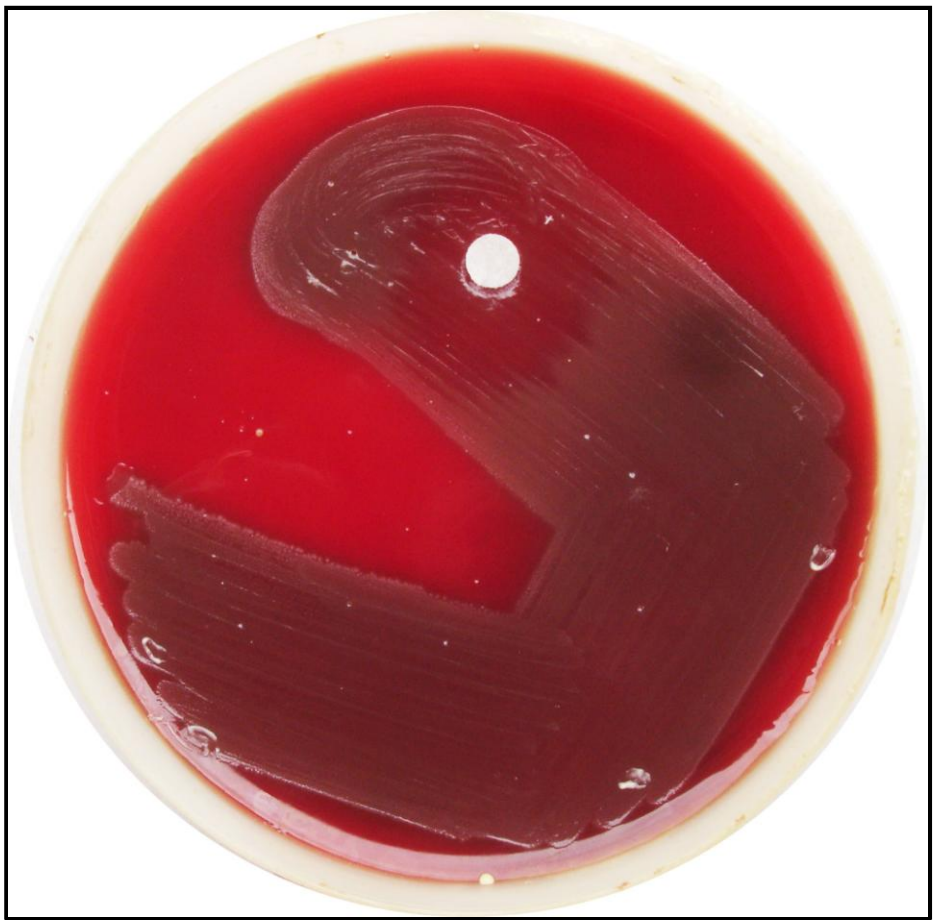


Photograph No. 5 : Growth on purity blood agar plate showing Prevotella spp.

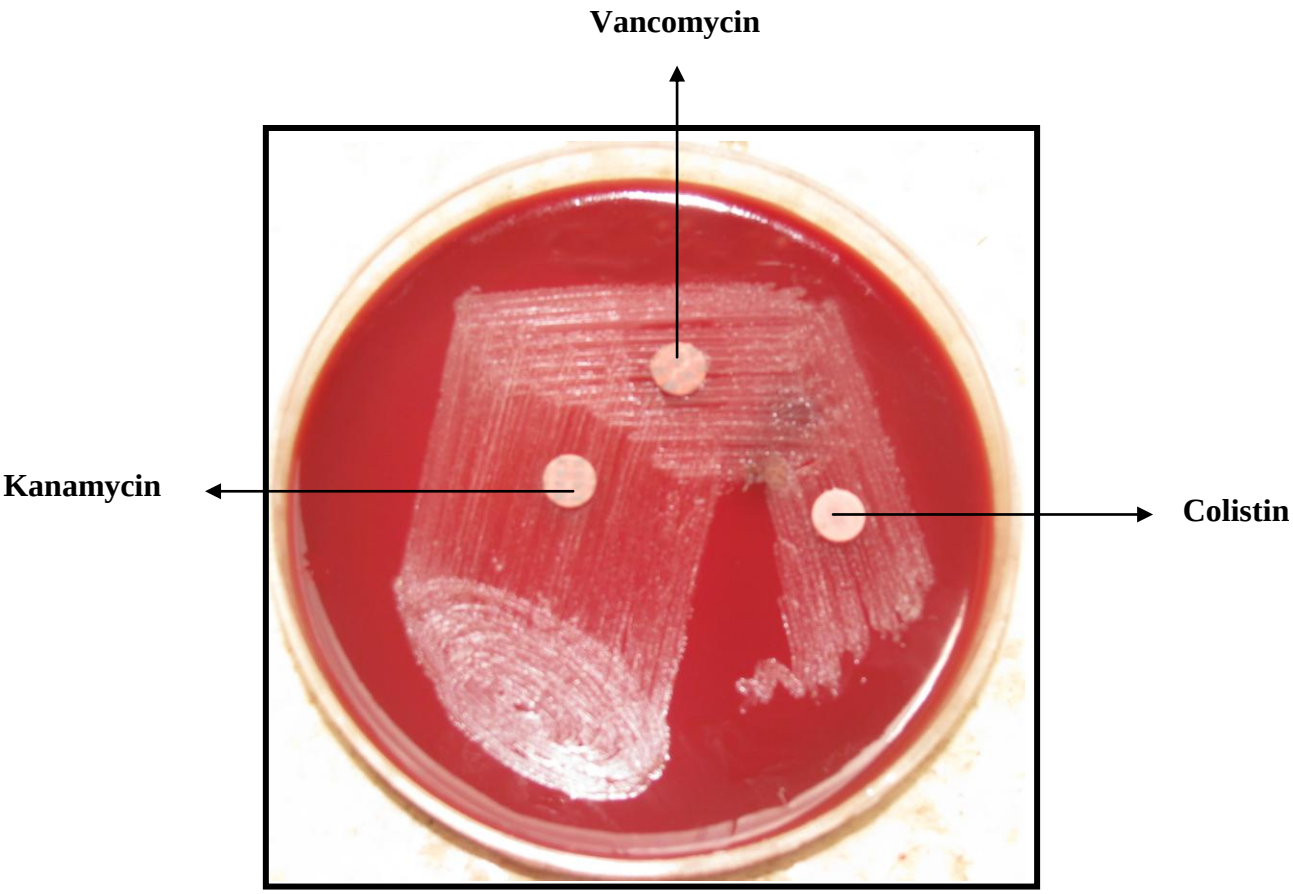


Photograph No. 6 : Prevotella spp. showing sensitivity to Colistin disc

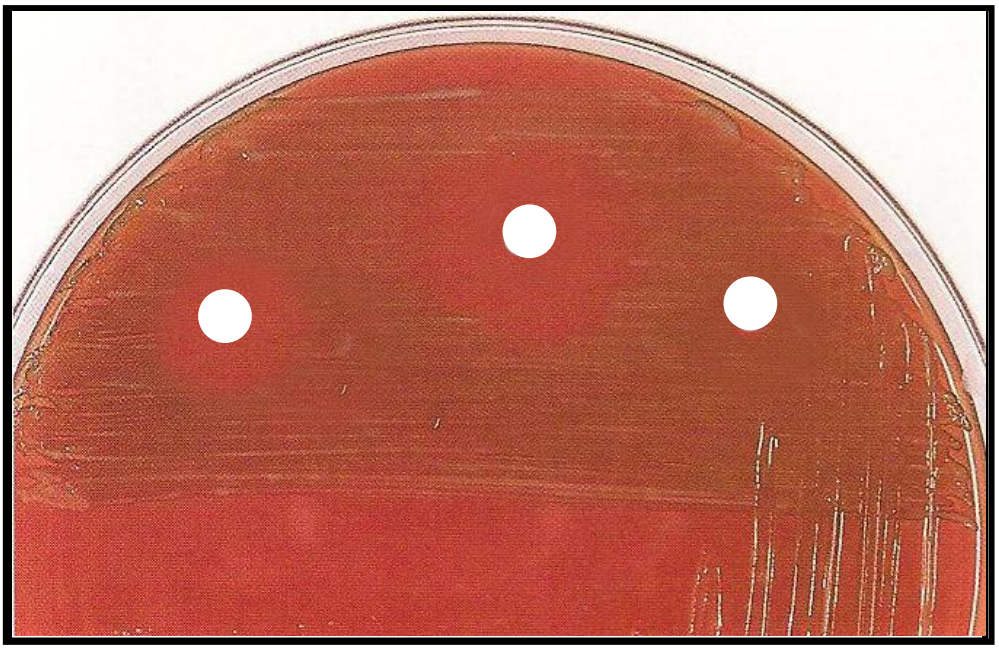
Photograph No. 7 : Peptostreptococcus anaerobius showing sensitivity to SPS disc on blood agar



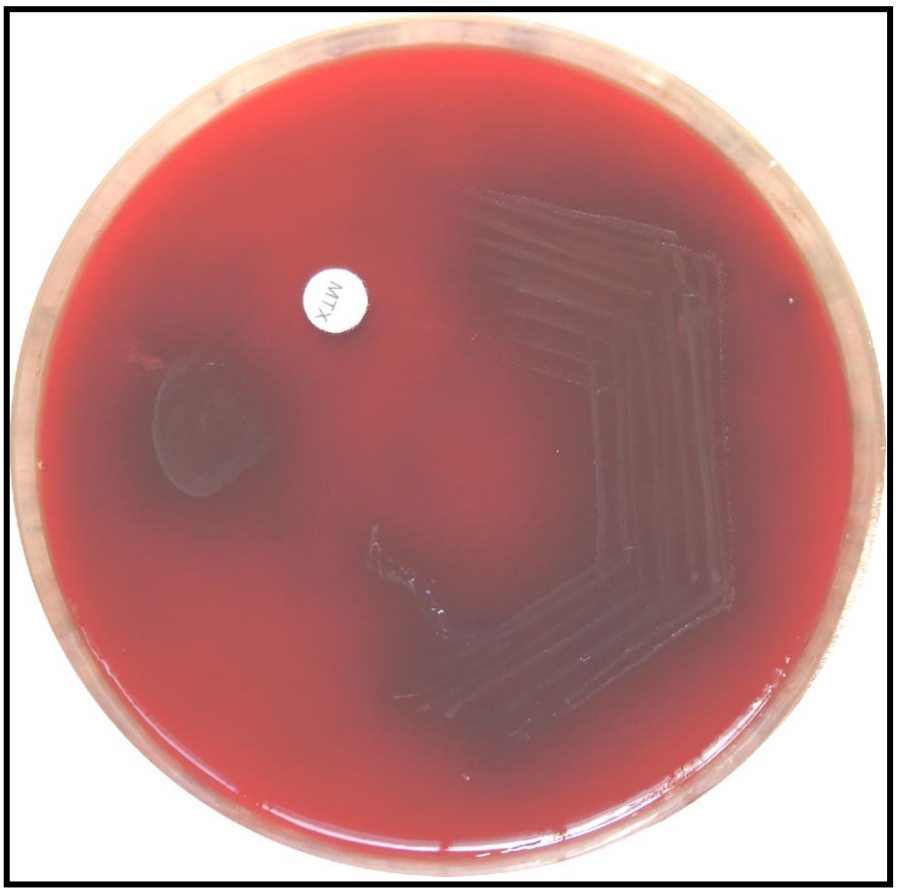
Photograph No. 8 : Bacteroides fragilis showing resistance to Kanamycin, Vancomycin & Colistin discs



Photograph 9 : Porphyromonas species showing resistance to colistin



Photograph No. 10 : Gardnerella vaginalis showing sensitivity to 50µg metronidazole disc on Columbia blood agar plate



RESULTS

Table 1 : Age wise distribution of pregnant women : **n = 120**

Age (In years)	Primigravida	Multigravida	Total (%)
18-22	33	26	59 (49.16)
23-27	23	25	48 (40)
28-32	03	10	13 (10.83)
Total	**59**	**61**	**120**

The table shows highest number of patients in the age group of 18-22 years.

Table 2 : Distribution of samples based on Clinical Composite Criteria (Amsel's Criteria)

Total	Thin white homogenous discharge (%)	pH>4.5 (%)	Amine test positive (%)	Clue cells present (%)	3 out of 4 criteria positive (%)
120	120 (100)	52 (43.33)	57 (47.5)	54 (45)	51 (42.5)

According to the Amsel's criteria BV was diagnosed in 42.5% cases clinically.

Table 3 : Scoring and Grading of vaginal smears by Nugent's method

Score	**Grade**	**Number of patients (%)**
0-3	Normal	47 (39.16)
4-6	Intermediate	31 (25.83)
7-10	BV	42 (35)

The above table shows that Frank BV was diagnosed in 35 % of the cases and 25.83% of cases belonged to intermediate grade.

Table 4 : Prevalence of BV by Non-cultural methods

	BV (%)	**NBV (%)**	**Total**
Amsel's criteria	51 (42.5)	69 (57.5)	120
Nugent's criteria	42 (35)	47 (39.16)	120*

* According to Nugent's criteria 31 (25.83%) cases belonged to intermediate grade.

Table 5 : Sensitivity, specificity, positive predictive value and negative predictive value of Amsel's criteria when compared to standard Nugent's method

Sensitivity	78.5%
Specificity	76.9%
Positive predictive value	64.7%
Negative predictive value	86.9%

Table 6 : Comparison of vaginal fluid Gram stain (Nugent's method) and Amsel's criteria :

		Amsel's criteria		**Total**
		BV	**NBV**	
Nugent's method	**BV**	33	09	42
	NBV	18	60	78
	TOTAL	**51**	**69**	**120**

The above table shows, according to Kappa statistics there is moderate agreement between the two non-cultural methods used for the diagnosis of BV. κ value = 0.529

Table 7 : Morphological predominance of organisms

Organism (Morphotype)	**Gram stain positive group (Nugent's method) BV (n = 42)**	**NBV (n=47)**
Lactobacillus	0	44
GNB/GVB	40	0
Mobiluncus	13	0
Mixed	10	3

GNB = Gram Negative Bacilli

GVB = Gram Variable Bacilli

Table 8 : Distribution of various aerobic and anaerobic culture isolates in vaginal discharge

	Aerobic organisms		**Anaerobic organisms**		**Mixed culture (Aerobic + Anaerobic)**	**Candida species**	**Total culture positive (%)**	**No growth (%)**	**Total**
	Monomicrobial flora	**Polymicrobial flora**	**Monomicrobial flora**	**Polymicrobial flora**					
BV	02	03	10	06	19	00	40 (95.2)	02 (4.7)	42
I	01	05	06	00	03	02	17 (54.8)	14 (45.2)	31
NBV	05	02	00	00	01	05	13 (27.6)	34 (72.3)	47

Highest number of culture positivity was seen in BV cases 40 (95.2%) as compared to NBV cases.

Graph : Distribution of various aerobic and anaerobic culture isolates in vaginal discharge

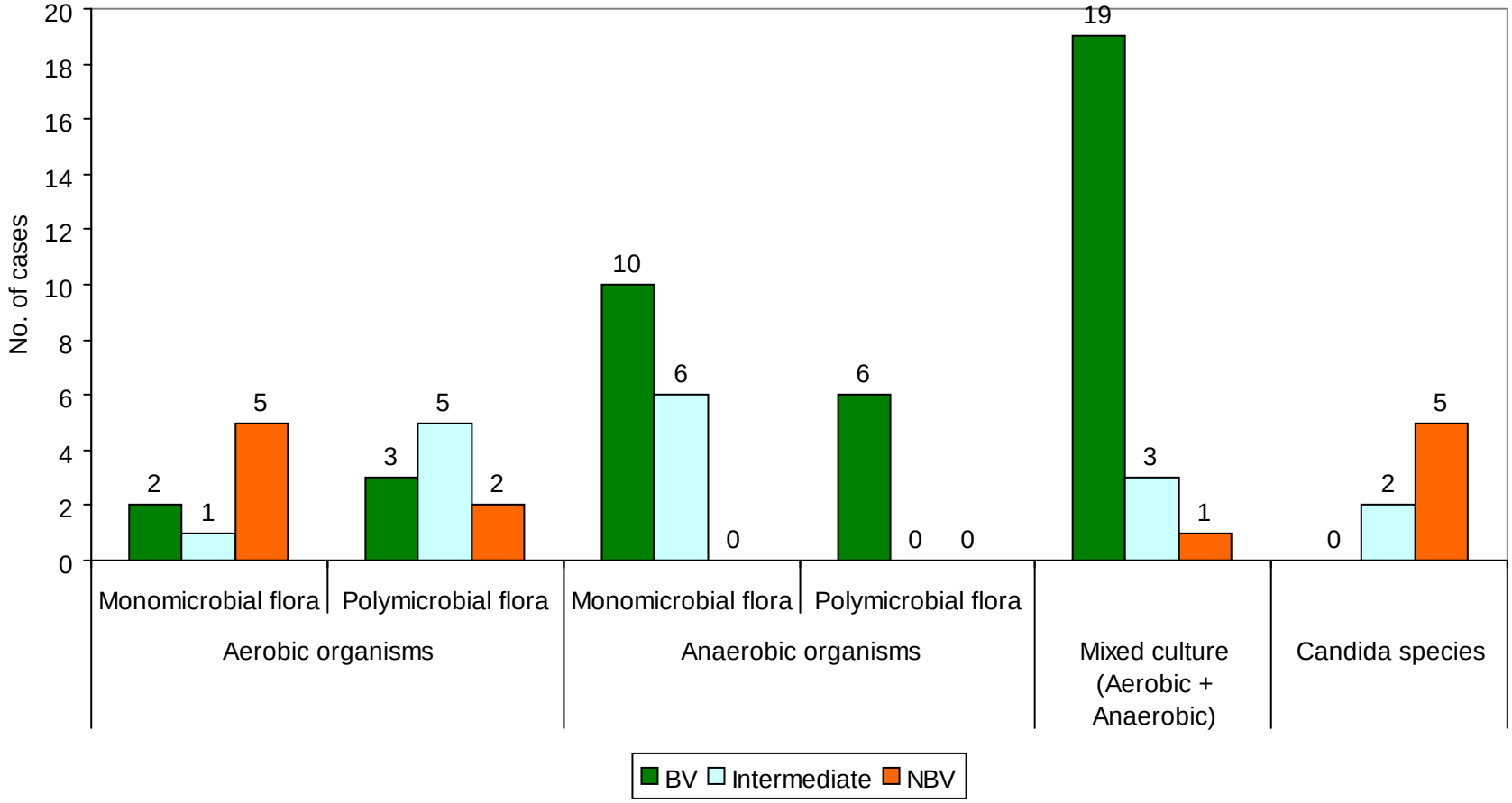


Table 9 : Anaerobic bacteria isolated from vaginal discharge

Organisms isolated	**BV n = 42 (%)**	**Intermediate n = 31 (%)**	**NBV n = 47 (%)**	**Total n = 120 (%)**	**p value**
B.fragilis	13 (30.9)	02 (6.4)	00 (00)	15 (12.5)	0.000
P.melaninogenica	11 (26.1)	01 (3.2)	00 (00)	12 (10)	0.000
P.anaerobius	08 (19)	02 (6.4)	01 (2.12)	11 (9.1)	0.006
P.intermedia	05 (11.9)	03 (9.6)	00 (00)	08 (6.6)	0.091*
Porphyromonas spp.	05 (11.9)	02 (6.4)	00 (00)	07 (5.8)	0.037

p value : Significant <0.05

The above table shows that *B.fragilis* and *P.melaninogenica* were the most common anaerobic organisms isolated.

* p value not significant.

Table 10 : Aerobic bacteria isolated from vaginal discharge

Organisms isolated	**BV n = 42 (%)**	**Intermediate n = 31 (%)**	**NBV n = 47 (%)**	**Total n = 120 (%)**	**p value**
G.vaginalis	15 (37.7)	06 (19.3)	00 (00)	21 (17.5)	0.000
Staphylococcus aureus	06 (14.2)	04 (12.9)	03 (6.3)	13 (10.8)	0.373*
CONS	05 (11.9)	01 (3.2)	04 (8.5)	10 (8.3)	0.298*
E.coli	01 (2.3)	02 (6.4)	02 (4.3)	05 (4.1)	0.459*
α hemolytic streptococci	00 (00)	02 (6.4)	01 (2.1)	03 (2.5)	0.201*
K.pneumoniae	01 (2.3)	01 (3.2)	00 (00)	02 (1.6)	0.674*

p value : Significant < 0.05

G.vaginalis was the most common microaerophilic organism isolated from BV cases.

* p value not significant.

Table 11 : Comparison of culture positives with Non-cultural methods for bacterial vaginosis

Vaginal discharge culture	**Amsel's criteria**		**Nugent's method***	
	BV n=51 (%)	**NBV n=69 (%)**	**BV n=42 (%)**	**NBV n=47 (%)**
Positive	43 (84.31)	27 (39.13)	40 (95.20)	13 (27.60)
Negative	08 (15.68)	42 (60.86)	2 (4.7)	34 (72.30)

*Among intermediate grade culture positivity was seen in 17 (54.80%) cases.

Table 12 : Antibiotic sensitivity pattern of aerobic isolates

	Ampicillin (10«g)	**Ceftazidime (30«g)**	**Amoxyclav (30«g)**	**Ciprofloxacin (5«g)**	**Oxacillin (1«g)**
***S.aureus* (n=13)**	9	9	8	10	10
***E.coli* (n=5)**	3	4	3	3	-
***K.pneumoniae* (n=2)**	2	1	0	2	-

DISCUSSION

A total of 120 pregnant women were enrolled for the study. Samples were collected from pregnant women who complained of vaginal discharge and whose period of gestation was less than 24 weeks.

The most common presenting complaints were foul smelling discharge, pruritis, pain abdomen and burning micturition. Maximum number of cases 59 (49.16%) belonged to 18-22 years of age followed by 23-27 years age group, suggesting higher prevalence of vaginal discharge in younger age group which was in accordance to a study[1].

Out of 120 cases, BV was diagnosed in 51 (42.50%) cases by Amsel's clinical composite criteria. Thin homogenous white discharge was present in all cases. In 52 (43.33%) cases pH was >4.5. Amine test (Whiff test) was positive in 57 (47.5%) cases and clue cells were present in 54 (45%) cases.

The other non cultural method that was used for the diagnosis of BV was Nugent's method of Scoring and Grading of Gram stained vaginal smears. Out of 120 cases, 42 (35%) cases were graded as having frank BV and 31 (25.83%) cases as intermediate. Intermediate grade is a transitional phase which may go in for frank BV, if left untreated.

By using Kappa statistics, it was found that there is a moderate agreement (0.529) between the two noncultural methods. Compared to Nugent's method, the positive predictive value (PPV) and negative predictive value (NPV) of Amsel's criteria was 64.7% and 86.9% respectively.

Comparison of sensitivity and specificity of Amsel's criteria when compared to standard Nugent's method of Scoring and Grading of gram stained vaginal smears in various studies

Study (Ref)	Sensitivity (%)	Specificity (%)
Present study	78.5%	76.9%
Dadhwal V et al [33].	51.2%	98%
Gutman RE et al [34].	69%	93%
Schwebke JR et al [35].	70%	94%
Gratacos E et al [36].	35%	99%
Goyal R et al [37].	60.7%	97.8%

The various studies mentioned above show different sensitivities and specificities for Amsel's criteria. The sensitivity of Amsel's criteria in the present study is in accordance to a study by Shwebke JR et al [35]. The variations when compared to other studies could be because of the subjective evaluation of the various criteria.

In a study [1], BV was diagnosed among 38.5% of symptomatic pregnant women by Nugent's method and in 40% cases by Amsel's criteria. The results of the present study were in accordance to the above study for diagnosing BV by Nugent's method.

Another study [38], conducted under National AIDS Control Organization (NACO) reported a prevalence of 32.8% BV by Gram staining method which is similar to the present study.

The intercenter variability in the interpretation of Gram stained smears from pregnant women was examined in a study [39]. This study indicated that criteria for the diagnosis of BV by using Gram's stain can be reproduced reliably between different centers and microbiologists. Clinical signs are very difficult to standardize between the clinicians and may be impossible to interpret during certain pregnancy situations such as labour.

The inverse relation between the presence and concentration of Gardnerella and Lactobacillus morphotypes in the Gram stained smears observed in the present study is in agreement with other studies [40].

Gram staining of vaginal smears are least expensive, easy to transport and requires less time to perform compared to Amsel's Composite Criteria and also this method is unaffected by physiological conditions like menstruation and recent intercourse. One more advantage of Gram stained smears is that they can be preserved for future reference.

In the present study curved Gram negative rods which represent Mobiluncus species were seen in 13 (30.95%) cases of BV and not present in cases of NBV. Mobiluncus is a good predictor of BV. The prevalence of Mobiluncus was more in the present study when compared to studies which showed 17.24% and 20% prevalence respectively [1, 41].

Culture was positive in total of 70 (58.33%) cases out of 120. Frank BV was diagnosed in 42 cases by Nugent's method. Out of these, culture was positive in 40 (95.2%) cases and in 17 (54.8%) cases from intermediate grade. Among

these 40 cases, pure anaerobes were seen in 16 cases and highest number of mixed culture was obtained in 19 cases. The culture positivity among NBV cases was 13 (27.6%) and anaerobes were not isolated.

Culture positivity was seen in 43 (84.3%) cases of BV diagnosed by Amsel's criteria and anaerobes were seen in 17 cases and mixed culture was present in 19 cases. Among NBV cases, culture was positive in 27 (39.13%) cases and anaerobes were isolated in 5 cases. Aerobic isolates were more in number in NBV as compared to BV cases.

The most common anaerobe isolated was *B.fragilis* followed by *P.melaninogenica.* The other anaerobes that were isolated were *P.anaerobius, P.intermedia* and Porphyromonas spp. The p value was significant in all the isolates except *P.intermedia.* Among aerobes, most common organism isolated was *G.vaginalis* followed by *S.aureus*. The other aerobes isolated were CONS, *E.coli*, α hemolytic streptococci and *K.pneumoniae.* p value was significant only for *G.vaginalis* among aerobes. Though few aerobes were isolated from vaginal discharge, maximum of them were from mixed isolates. Therefore, antibiotic sensitivity testing was performed by using Kirby Bauer disc diffusion method for commonly used antibiotics. Most of the aerobic isolates showed sensitivity to Ciprofloxacin, Ampicillin and Ceftazidime.

A total of 7 Candida species were isolated, 2 from intermediate grade and 5 from NBV cases and none from BV cases. This suggests that even in thin homogenous white vaginal discharge, Candida can be present and hence needs to be screened.

Evidence of association of anaerobic bacteria with BV is mounting. Different workers have isolated different types of anaerobes in vaginal discharge in different groups of patients.

Culture positivity among cases of BV was 36.4% and the most common organism isolated was Peptostreptococci in a study [2].

BV was diagnosed in 17.42% women by semi-quantitative culture. Various bacterial species isolated were Gardnerella, Candida and non sporing anaerobes in 26.05%, 38.02% and 49.99% cases respectively [9].

Prevalence of BV by culture was 63.21% and Peptostreptococci was the most common anaerobe isolate in a study conducted at Solapur [42].

The usefulness of vaginal cultures for the diagnosis of BV has been investigated less thoroughly. However, Krohn et al.[43], evaluated the sensitivity and specificity of vaginal cultures for anaerobic bacteria and *M.hominis*. She found that the presence of these organisms was a more specific indicator of BV than *G.vaginalis.*

In the present study, *G.vaginalis* was isolated from 21 cases. Out of which 15 (35.7%) were from BV cases and 6 (19.3%) were from intermediate grade. p value was significant only for *G.vaginalis.*

In a study [37] , which was done to detect BV in women in labour *G.vaginalis* was isolated in 30.8% cases. It has been demonstrated that *G.vaginalis* is a predominant organism in vaginal fluid from most women with nonspecific vaginitis. Many studies have reported the association of *G.vaginalis* with BV.

But *G.vaginalis* is also recovered from 36-55% of women without clinical signs of BV. The incidental finding of *G.vaginalis* from a routine vaginal culture should not be used to diagnose BV unless clinical signs and objective Gram's stain criteria are also present [20].

The variations in the type and rate of isolations of anaerobes reflects the difference in the population under study as well as the different methods of investigations used.

CONCLUSION

The present study showed moderate agreement between the two non-cultural methods (Amsel's criteria and Nugent's method). It was found that Gram's staining is an easier, accurate and rapid method for the diagnosis of BV and also the Gram stained smears can be stored for future reference. It also helps in the detection of intermediate grade which is a transitional phase and if not treated may go for frank BV.

It was noted that culture positivity was more in cases of BV and polymicrobial mixed isolates were significantly raised in BV as compared to NBV cases. Current microscopic wet mount analysis is useful to assess the level of infection, while qualitative cultures may not be sufficient to adequately guide effective treatment.

Hence, quantitative cultures of vaginal fluid are necessary to know the exact increase in the concentration of these organisms in the causation of BV. Large multicentered interventional and follow up studies are further needed to evaluate the effects of BV on the outcome of pregnancy.

SUMMARY

1. High vaginal swabs from the posterior fornix of the vagina, were collected from 120 pregnant women complaining of vaginal discharge, attending the OBG Out Patient Department of KLE'S Dr. Prabhakar Kore Hospital and MRC., Belgaum. The samples were processed in the Department of Microbiology, J. N. M. C., Belgaum.
2. The age of pregnant women varied from 18-32 years, with the highest number of cases between 18-22 years of age (49.16%). Out of 120, 59 women were primigravida and 61 were multigravida.
3. The most common presenting symptom was foul smelling offensive vaginal discharge.
4. BV was diagnosed in 51 (42.5%) cases by Amsel's criteria and in 42 (35%) cases by Nugent's method (Non cultural methods).
5. By using Kappa statistics, we found that there is a moderate agreement (0.529) between the two non-cultural methods.
6. Sensitivity of Amsel's criteria was 78.5%, specificity was 76.9%, positive predictive value was 64.7%, and negative predictive value was 86.9% when compared to Nugent's method.
7. Out of 120 cases of vaginal discharge, total culture positivity was seen in 70 (58.33%) cases. The commonest anaerobic isolates were *B.fragilis, P.melaninogenica* and *P.anaerobius*. Among aerobes, *G.vaginalis, S.aureus* and Coagulase negative. Staphylococcus were the predominant organisms isolated.

8. Among BV cases diagnosed by Nugent's method, culture positivity was seen in 40 (95.2%) cases. Culture was positive in 43 (84.31%) out of 51 cases of BV and 27 (39.13%), out of 69 NBV cases as diagnosed by Amsel's criteria.
9. Most of the aerobic isolates were sensitive to Ciprofloxacin, Ampicillin and Ceftazidime.

BIBLOGRAPHY

1. Mathew R, Kalyani J, Bibi R, Mallika M. Prevalence of bacterial vaginosis in antenatal women. Indian J Pathol Microbial. 2001 : 44(2) : 113-6.
2. Aggarwal A, Devi P, Jain R. Anaerobes in bacterial vaginosis. Indian J Med Microbiol. 2003 ; 21 (2) : 124-6.
3. Laboratory considerations. Forbes BA, Sahm DF, Weissfeld AS. Bailey and Scott's Diagnostic Microbiology. 12th ed. Elsevier. St. Louis. Missouri. 2007.
4. Howard AJ, Ison CA. Haemophilus, Gardnerella and other bacilli. Collee JG, Fraser AG, Marmion BP, Simmons A. Mackie and MacCartney Practical Medical Microbiology. 14th ed. Elsevier. New Delhi. 2006.
5. Eschenbach DA. History and review of bacterial vaginosis. Am J Obstet Gynecol. 1993 ; 169 (2) : 441-5.
6. Faro S, Martens M, Maccato M, Hammil H, Pearlman M. Vaginal flora and pelvic inflammatory disease. Am J Obstet Gynecol. 1993 ; 169 (2) : 470-4.
7. Brian I, Duerden, Drasar BS. Anaerobes in human disease. 1st ed. Edward Anord. London, Melbourne, Auckland. 1991.
8. Hill GB. The microbiology of bacterial vaginosis. Am J Obstet Gynecol. 1993; 169 (2) : 450-4.
9. Rao PS, Devi S, Shriyan A, Rajaram M, Jagadishchandra K. Diagnosis of bacterial vaginosis in a rural set up : Comparison of clinical algorithm, smear scoring and culture by semi quantitative technique. Indian J Med Microbiol. 2004 ; 22 (1) : 47-50.

10. Sobel JD. Bacterial vaginosis. Annu Rev Med 2000 ; 51 : 349-56.
11. Nelson DB, Macones G. Bacterial vaginosis in pregnancy : Current Findings and Future Directions. Epidemiologic Reviews. 2002 ; 24 : 102-8.
12. McGregor JA, French IJ, Richter R, Franco-Buff A, Johnson A. Antenatal microbiologic and maternal risk factors associated with prematurity. Am J Obstet Gynecol. 1990 ; 163 : 465-73.
13. Taha TE, Hoover DR, Dallabetta GA. Bacterial vaginosis and disturbances of vaginal flora : Association with increased acquisition of HIV AIDS. 1998 ; 12 : 1699-706.
14. http://www.cdc.gov/std/treatment /206/vaginal -discharge.htm. Assessed on 10/10/2009.
15. Harmanli OH, Cheng GY, Nyirjesy P, Chatwani A, Gaughan JP. Urinary tract infections in women with bacterial vaginosis. Obstet Gynecol. 2000 ; 95 : 710-2.
16. Hillebrand L, Harmanli OH, Whiteman V. Urinary tract infections in pregnant women with bacterial vaginosis. Am J Obstet Gynecol. 2002 ; 186 : 916-7.
17. McGregor JA, French JI, Seo K. Premature rupture of membranes and bacterial vaginosis. Am J Obstet Gynecol. 1993 ; 169 (2) 463-6.
18. Purwar M, Ughade S, Bhagat B. Bacterial vaginosis in early pregnancy and adverse pregnancy outcome. J Obstet Gynecol Res 2001 ; 27 : 175-81.
19. Gibbs RS. Chorioamnionitis and bacterial vaginosis. Am J Obstet Gynecol. 1993 ; 169 (2) : 460-2.
20. Hillier SL.. Diagnostic microbiology of bacterial vaginosis. Am J Obstet Gynecol. 1993 ; 169 (2) : 455-9.

21. Tokyo C, Aktepe OC, Cevrioglu AS, Altndi M, Dilek HF. Bacterial vaginosis : Comparison of Pap smear and microbiological test results. Modpathol. 2004 ; 17 : 857-60.
22. Thomason JL, Gelbart SM, Wilcoski LM, Peterson AK, Jilly BJ, Hamilton PR. Proline Aminopeptidase Activity as a Rapid Diagnostic Test to Confirm Bacterial Vaginosis. Obstet Gynecol 1988 ; 71 (4) : 607-11.
23. Fredricks DN, Fiedler TL, Thomas KK, Oakley BB, Marrazzo JM. J Clin Microbiol. 2007 ; 45 (10) : 3270-6.
24. Thies F, Konig W, Konig B. Rapid diagnosis of BV by terminal restriction fragment length polymorphism profiling. Proceedings of the 16th European Congress of Clinical Microbiology and Infectious Diseases. 2006 April 1-4 ; NICE , France.
25. Menard SP, Fenollar F, Henry M, Bretelle F, Raoult D. Molecular Quantification of Gardnerella vaginalis and Atopobium vaginae loads to predict Bacterial Vaginosis. CID. 2008 ; 47 : 33-43.
26. Devillard E, Burton JP, Reid G. Complexity of vaginal microflora as analysed by PCR denaturing gradient gel electrophoresis in a patient with recurrent bacterial vaginosis. Infect Dis Obstet Gynecol. 2005 ; 13(1) : 25-30.
27. Morency AM, Bujold E. The effect of Second-Trimester Antibiotic Therapy on the Rate of Preterm birth. J Obstet Gynaecol Can. 2007 ; 29 (1) : 35-44.
28. Parent D, Bossens M, Bayot D, Kirkpatrick C, Graf F, Wilkinson FE et al. Therapy of bacterial vaginosis using exogenously applied Lactobacillus acidophilus and a low dose of estriol a placebo-controlled multicentric clinical trial. Arzneimittelforchung. 1996 ; 46 : 68-73.

29. Chimura T, Funayama T, Murayama K, Numazaki M. Ecological treatment of bacterial vaginosis. Jpn. J. Antibiot. 1995 ; 48 : 432-6.
30. Patil K, Swapna S. Vaginal Clindamycin and clotrimozole to reduce the incidence of preterm delivery. A hospital based longitudinal study (dissertation). Rajiv Gandhi University. 2006.
31. Vaidhyalingam PK, Lakshminarayana CS, Doraswami MAR. Internal Gas Generator – A system suitable for creating anaerobic atmosphere. Ind Journ Surg. 1980 ; 42 : 154-9.
32. Sutter VL, Citron DM, Edelstein MAC, Finegold SM, Wadsworth. Anaerobic Bacteriology Manual. 4th Ed. Star Publishing Company, California. 1985.
33. Dadhwal V, Hariprasad R, Mittal S, Kapil A. Prevalence of bacterial vaginosis in pregnant women and predictive value of clinical diagnosis. Arch Gynecol Obstet. 2009.
34. Gutman RE, Peipert JF, Weitzen S, Blume J. Evaluation of Clinical methods for diagnosing bacterial vaginosis. Obstet Gynecol. 2002 ; 105 (3) : 551-6.
35. Schwebke JR, Hillier SL, Sobel JD, McGregor JA, Sweet RL. Validity of vaginal gram stain for the diagnosis of bacterial vaginosis. Obstet Gynecol. 1996 ; 88 : 573-6.
36. Gratacos E, Figueras F, Barranco M, Ros R, Andreu A, Alonso PL et al. Prevalence of bacterial vaginosis and correlation of clinical to Gram stain diagnostic criteria in low risk pregnant women. Eur J Epidemiol. 1999 ; 15 (10) : 913-6.
37. Goyal R, Sharma P, Kour I, Aggarwal N, Talwar V. Diagnosis of bacterial vaginosis in women in labour. JKScience. 2005 ; 7 (1) : 1-4.

38. Bhalla.P,Chawla R,Garg S,Singh MM,Rain U, Bhalla R et al. Prevalence of bacterial vaginosis among women in Delhi, India. Indian J Med Res. 2007 ; 125 : 167-72.
39. Nugent RP, Krohn MA, Hillier SL. Reliability of diagnosing Bacterial Vaginosis is improved by a standardized method of Gram stain interpretation. J. Clin. Microbiol. 1991 ; 29 (2) : 297-301.
40. Spiegel CA, Amsel R, Eschenbach A, Schoenknecht F, Holmes KK. Anaerobic bacteria in non-specific vaginitis. N Engl J Med. 1980 ; 303 (11) : 601-7.
41. Hallen A, Pahlson C, Forsum U. Bacterial vaginosis in women attending STD clinic : diagnostic criteria and prevalence of Mobiluncus spp. Genitourin med. 1987 ; 63 : 386-9.
42. Sumati AH, Saritha NK. Bacterial vaginosis with special reference to anaerobes. Indian J Pathol Microbiol. 2009 ; 52 (1) : 56-8.
43. Krohn MA, Hillier SL, Eschenbach DA. Comparison of methods for diagnosing bacterial vaginosis among pregnant women. J Clin Microbiol. 1989 ; 27 (6) : 1266-71.

PROFORMA

Name : **Age :** **Sex :**

Hospital No : **ID :**

Ref. By :

Obstetric History :

Presenting complaints :

History of presenting illness :

Past History :

Diagnosis :

Specimen :

MICROBIOLOGICAL INVESTIGATIONS

- Macroscopic examination
- pH of the discharge
- Amine test
- Wet mount
- Gram's staining

Cultural methods :

- MacConkey agar
- Blood agar
- Columbia blood agar
- Anaerobic incubation

CONSENT FOR PARTICIPATION IN RESEARCH WORK

Mrs ……., we are requesting you to enroll yourself in the study titled "**ISOLATION AND IDENTIFICATION OF ORGANISMS ASSOCIATED WITH BACTERIAL VAGINOSIS IN PREGNANT WOMEN WITH PER VAGINAL DISCHARGE. A ONE YEAR CROSS SECTIONAL STUDY"** conducted by Dr. Gulnar Parveen Kazi, post-graduate student in Microbiology, under the guidance of Dr.(Mrs) Sumathi Hogade at J.N.M.C, Belgaum under KLE University.

You have been requested to participate in research because you are into a study group. During the study you are supposed to answer to the best of your knowledge.

Your participation in research is voluntary. Your decision whether or not to participate in the study will not affect your relationship with JNMC, Belgaum. If you decide to participate you are free to withdraw at any time.

The purpose of research is to isolate and identify the various organisms associated with bacterial vaginosis and to compare two non-cultural techniques for the diagnosis.

Procedure involved

Microbiological investigations are done to detect various organisms from the clinical sample.

Risks and benefits

There are no extra risks involved and benefits are to be evaluated.

Privacy and confidentiality

The only people to know that your are a research subject are members of the research team, no information will be disclosed to others without your written permission, except.

1. In emergency to protect your rights and welfare.
2. If required by law.

Authorization to publish results

When the results of the research are published or discussed in a conference no information will be displayed that would disclose your identity. Any information obtained in connection with this study and that can be identified with you will remain confidential.

Financial incentives for participation

You will not be paid / offered gifts for participation in the research. You will not be reimbursed for expenses.

Consent statement

I undersigned _____ have been explained in my vernacular language about the study and my participation in the study is voluntary, I have been given enough time to clear my doubts and rights as study participant.

In case you have any questions related to the study you can contact Dr. Gulnar. Parveen. Kazi, Mobile No. 9449070522 in case you have any questions about your rights as a study participant you can contact Dr. V. D. Patil, 0831-2471350.

Signature or left thumb print of participants or legally authorized representative

Participant's name : _____ Sign : _____

_____

Witness name : _____ Sign : _____

_____

Experimenter's name _____ Sign : _____

_____

Date : _____

Place : _____

MASTER CHART

S.No	Hosp. No	Age (In yrs)	AMSEL'S CRITERIA					NUGENT'S METHOD	Anaerobic bacteria isolated	G.Stain	Catalase test	Spot Indole test	Nitrate test	Susceptibility				Pigment production	Flouresence	Pitting	Aerobic & other organisms isolated
			Type of Discharge	pH	Amine Test	Clue Cells (Wet mount)	Three out of Four criteria positive	Gram's Stain						SPS	K	V	C				
1	940404	24	THW	6.0	-	Absent	-	7+	P.mel	GNB	-	+	-	-	R	R	S	Black	Brick red	-	NOGC
2	944920	24	THW	6.0	-	Absent	-	2+	NOGC												S.aureus, Candida spp.
3	887729	19	THW. FS	5.5	+	Absent	+	5+	NOGC												NOGC
4	903785	20	THW	6.0	+	Present	+	7+	P.mel	GNB	-	+	-	-	R	R	S	Black	Brick red	-	G.vaginalis
5	951143	25	THW	6.0	+	Absent	+	5+	NOGC												NOGC
6	951166	19	THW	4.0	-	Absent	-	2+	NOGC												Candida spp.
7	908982	21	THW	5.5	-	Absent	-	6+	NOGC												NOGC
8	899901	22	THW. FS	4.0	+	Absent	-	7+	NOGC	-	-	-	-	-	-	-	-	-	-	-	NOGC
9	895974	21	THW	5.5	+	Present	+	7+	P.anae	GPC in clusters & chains	-	+	-	S	-	-	-	-	-	-	G.vaginalis
10	887607	25	THW	4.0	-	Present	-	6+	NOGC												NOGC
11	960369	22	THW	4.0	-	Absent	-	2+	NOGC												Candida spp.
12	946523	23	THW	4.0	-	Absent	-	5+	NOGC												NOGC
13	960368	19	THW	4.0	-	Present	-	6+	NOGC												NOGC
14	873868	22	THW. FS	5.0	+	Present	+	6+	NOGC												NOGC
15	936855	23	THW	4.0	+	Absent	-	7+	NOGC												S.aureus
16	954256	29	THW	4.0	-	Absent	-	2+	NOGC												Candida spp.
17	983049	20	THW	3.5	+	Absent	-	5+	NOGC												Candida spp.
18	976330	25	THW	5.0	+	Present	+	7+	B.fragilis	GNB	-	-	-	-	R	R	R	-	-	-	G.vaginalis
19	970368	22	THW	5.5	-	Absent	-	6+	NOGC												NOGC
20	951207	21	THW	4.0	+	Present	+	7+	P.mel	GNB	-	+	-	-	R	R	S	Black	Brick red	-	E.coli
21	951122	22	THW	4.0	-	Present	-	2+	NOGC												NOGC
22	968799	22	THW	4.0	+	Present	+	7+	NOGC												NOGC

S.No	Hosp. No	Age (In yrs)	AMSEL'S CRITERIA					NUGENT'S METHOD	Anaerobic bacteria isolated	G.Stain	Catalase test	Spot Indole test	Nitrate test	Susceptibility				Pigment production	Flouresence	Pitting	Aerobic & other organisms isolated
			Type of Discharge	pH	Amine Test	Clue Cells (Wet mount)	Three out of Four criteria positive	Gram's Stain						SPS	K	V	C				
23	988940	25	THW	6.0	+	Present	+	7+	P.anae	GPC in clusters & chains	-	+	-	S	-	-	-	-	-	-	NOGC
24	311821	25	THW. FS	6.5	+	Present	+	7+	Por.	GNCB	-	+	-	-	S	S	R	Brown	-	-	NOGC
									P.inter	GNB beaded	-	+	-	-	R	R	S	Black	Brick red	-	
25	980368	28	THW	4.0	-	Present	-	1+	NOGC												NOGC
26	997880	23	THW	4.0	-	Present	-	1+	NOGC												NOGC
27	988985	31	THW. FS	4.0	-	Absent	-	2+	NOGC												NOGC
28	997951	19	THW. FS	5.5	+	Present	+	7+	NOGC												G.vaginalis
29	966265	22	THW	4.0	-	Present	-	2+	NOGC												Candida spp.
30	966109	26	THW. FS	5.0	+	Present	+	7+	B.fragilis	GNB	-	-	-	-	R	R	R	-	-	-	K.pneumonia
31	987563	24	THW	4.0	+	Absent	-	7+	P.mel	GNB	-	+	-	-	R	R	S	Black	Brick red	-	G.vaginalis
32	989761	22	THW	4.0	-	Absent	-	4+	NOGC												NOGC
33	954466	28	THW	4.0	+	Absent	-	2+	NOGC												NOGC
34	1005178	24	THW	6.0	-	Absent	-	7+	B.fragilis	GNB	-	-	-	-	R	R	R	-	-	-	S.aureus
35	1005909	21	THW	4.0	+	Absent	-	1+	NOGC												NOGC
36	971726	26	THW	4.0	-	Absent	-	4+	NOGC												NOGC
37	962520	19	THW	4.0	-	Absent	-	2+	NOGC												NOGC
38	967168	24	THW	6.0	+	Present	+	7+	B.fragilis	GNB	-	-	-	-	R	R	R	-	-	-	G.vaginalis
39	975476	25	THW. FS	4.0	-	Absent	-	2+	NOGC												NOGC
40	946079	22	THW	4.0	+	Present	+	7+	P.mel	GNB	-	+	-	-	R	R	S	Black	Brick red	-	S.aureus
41	979167	19	THW	4.0	-	Present	-	4+	Por.	GNCB	-	+	-	-	S	S	R	Brown	-	-	NOGC
42	980686	20	THW	4.0	-	Absent	-	2+													E.coli, CONS
43	1041843	24	THW	4.0	-	Absent	-	2+	NOGC												Candida spp.
44	982659	20	THW	5.0	+	Present	+	7+	P.inter	GNB (beaded)	-	+	-	-	R	R	S	Black	Brick red	-	G.vaginalis
45	1041898	26	THW	5.0	+	Present	+	4+	B.fragilis	GNB	-	-	-	-	R	R	R	-	-	-	S.aureus
									P.inter	GNB (beaded)	-	+	-	-	R	R	S	Black	Brick red	-	

S.No	Hosp. No	Age (In yrs)	AMSEL'S CRITERIA					NUGENT'S METHOD	Anaerobic bacteria isolated	G.Stain	Catalase test	Spot Indole test	Nitrate test	Susceptibility				Pigment production	Flouresence	Pitting	Aerobic & other organisms isolated
			Type of Discharge	pH	Amine Test	Clue Cells (Wet mount)	Three out of Four criteria positive	Gram's Stain						SPS	K	V	C				
46	1041922	24	THW	5.0	-	Absent	-	2+	NOGC												NOGC
47	1041713	27	THW	5.0	+	Present	+	7+	Por.	GNCB	-	+	-	-	S	S	R	Brown	-	-	CONS
48	319490	23	THW	4.0	-	Absent	-	2+	NOGC												CONS
49	1050874	28	THW	5.0	+	Present	+	4+	P.inter	GNB (beaded)	-	+	-	-	R	R	S	Black	Brick red	-	S.aureus, G.vaginalis
50	1050866	22	THW	5.0	-	Absent	-	2+	NOGC												NOGC
51	1078994	22	THW. FS	4.0	-	Absent	-	2+	NOGC												NOGC
52	1000820	23	THW	5.0	+	Present	+	7+	B.fragilis	GNB	-	-	-	-	R	R	R	-	-	-	NOGC
53	1046273	20	THW	4.0	+	Absent	-	2+	NOGC												NOGC
54	1042639	22	THW	4.0	-	Absent	-	2+	NOGC												NOGC
55	1065319	24	THW. FS	4.0	+	Present	+	7+	B.fragilis	GNB	-	-	-	-	R	R	R	-	-	-	G.vaginalis
56	1038056	26	THW	4.0	-	Absent	-	2+	NOGC												NOGC
57	943390	21	THW. FS	4.0	+	Present	+	7+	P.mel	GNB	-	+	-	-	R	R	S	Black	Brick red	-	NOGC
									P.anae	GPC in clusters	-	+	-	S	-	-	-	-	-	-	
58	1076244	22	THW	4.0	-	Absent	-	2+	NOGC												NOGC
59	1078873	28	THW	4.0	-	Absent	-	1+	NOGC												NOGC
60	315907	22	THW	4.0	+	Absent	-	4+	P.anae	GPC in clusters	-	+	-	S	-	-	-	-	-	-	NOGC
61	999219	24	THW	4.0	-	Absent	-	1+	NOGC												NOGC
62	1060290	24	THW	4.0	-	Absent	-	2+	NOGC												S.aureus
63	1068042	22	THW	5.0	+	Present	+	4+	NOGC												NOGC
64	1043092	22	THW	4.0	-	Absent	-	2+	NOGC												E.coli
65	1055415	20	THW	6.0	-	Absent	-	4+	NOGC												K.pneumonia
66	1013795	23	THW	5.0	+	Present	+	7+	B.fragilis	GNB	-	-	-	-	R	R	R	-	-	-	NOGC
									P.anae	GPC in clusters	-	+	-	S	-	-	-	-	-	-	
67	1030149	22	THW	5.0	-	Absent	-	7+	B.fragilis	GNB	-	-	-	-	R	R	R	-	-	-	G.vaginalis
68	1076833	19	THW	4.0	-	Absent	-	2+	NOGC												NOGC

S.No	Hosp. No	Age (In yrs)	AMSEL'S CRITERIA					NUGENT'S METHOD	Anaerobic bacteria isolated	G.Stain	Catalase test	Spot Indole test	Nitrate test	Susceptibility				Pigment production	Flouresence	Pitting	Aerobic & other organisms isolated
			Type of Discharge	pH	Amine Test	Clue Cells (Wet mount)	Three out of Four criteria positive	Gram's Stain						SPS	K	V	C				
69	1055384	23	THW	4.0	+	Present	+	7+	P.inter	GNB (beaded)	-	+	-	-	R	R	S	Black	Brick red	-	S.aureus
70	1068179	22	THW	4.0	-	Absent	-	2+	NOGC												NOGC
71	1029820	19	THW	4.0	-	Absent	-	4+	NOGC												CONS, E.coli, G.vaginalis
72	1045619	20	THW	4.0	-	Absent	-	2+	NOGC												NOGC
73	1041843	24	THW	4.0	+	Absent	-	2+	NOGC												NOGC
74	312003	20	THW	5.0	+	Present	+	7+	P.anae	GPC in clusters	-	+	-	S	-	-	-	-	-	-	CONS
									P.mel	GNB	-	+	-	-	R	R	S	Black	Brick red	-	
75	312205	25	THW	5.0	+	Absent	+	2+	NOGC												NOGC
76	1041744	24	THW. FS	4.0	-	Absent	-	2+	NOGC												NOGC
77	1110291	21	THW	4.0	-	Present	-	2+	NOGC												NOGC
78	840677	24	THW	4.0	-	Absent	-	2+	NOGC												NOGC
79	312302	19	THW	6.0	+	Present	+	4+	NOGC												S.aureus, G.vaginalis
80	1133997	25	THW. FS	4.0	-	Absent	-	2+	NOGC												NOGC
81	312204	23	THW	5.0	+	Present	+	7+	Por.	GNCB	-	+	-	-	S	S	R	Brown	-	-	NOGC
82	313345	25	THW	4.0	-	Absent	-	4+	NOGC												G.vaginalis, Alpha H.S
83	312664	20	THW	4.0	-	Absent	-	2+	NOGC												NOGC
84	308313	28	THW	6.0	+	Present	+	7+	B.fragilis	GNB	-	-	-	-	R	R	R	-	-	-	NOGC
									P.inter	GNB (beaded)	-	+	-	-	R	R	S	Black	Brick red	-	
85	306638	21	THW	6.0	+	Absent	+	4+	B.fragilis	GNB	-	-	-	-	R	R	R	-	-	-	G.vaginalis
86	1030865	19	THW. FS	6.0	+	Absent	+	4+	NOGC												NOGC
87	1052317	21	THW	6.0	-	Present	+	7+	NOGC												CONS, G.vaginalis
88	308540	26	THW	6.0	-	Present	+	4+	Por.	GNCB	-	+	-	-	S	S	R	Brown	-	-	NOGC
89	1052258	22	THW	4.0	+	Present	+	7+	P.mel	GNB	-	+	-	-	R	R	S	Black	Brick red	-	G.vaginalis

S.No	Hosp. No	Age (In yrs)	AMSEL'S CRITERIA					NUGENT'S METHOD	Anaerobic bacteria isolated	G.Stain	Catalase test	Spot Indole test	Nitrate test	Susceptibility				Pigment production	Flouresence	Pitting	Aerobic & other organisms isolated
			Type of Discharge	pH	Amine Test	Clue Cells (Wet mount)	Three out of Four criteria positive	Gram's Stain						SPS	K	V	C				
90	303634	28	THW. FS	4.0	+	Present	+	7+	P.mel	GNB	-	+	-	-	R	R	S	Black	Brick red	-	NOGC
									P.anae	GPC in clusters	-	+	-	S	-	-	-	-	-	-	
91	1041922	20	THW	6.0	+	Present	+	2+	P.anae	GPC in clusters	-	+	-	S	-	-	-	-	-	-	NOGC
92	305776	24	THW	5.0	+	Present	+	7+	P.mel	GNB	-	+	-	-	R	R	S	Black	Brick red	-	S.aureus
93	305399	22	THW	5.0	+	Absent	+	4+	NOGC												NOGC
94	1104375	21	THW	5.0	+	Absent	+	2+	NOGC												CONS
95	305797	22	THW	6.0	+	Present	+	7+	P.inter	GNB (beaded)	-	+	-	-	R	R	S	Black	Brick red	-	NOGC
96	307587	21	THW	4.0	-	Absent	-	2+	NOGC												NOGC
97	305987	25	THW	4.0	-	Present	-	2+	NOGC												NOGC
98	304044	25	THW	5.0	-	Present	+	7+	NOGC												G.vaginalis, CONS
99	3067109	26	THW	6.0	+	Present	+	7+	P.anae	GPC in clusters	-	+	-	S	-	-	-	-	-	-	NOGC
									B.fragilis	GNB	-	-	-	-	R	R	R	-	-	-	
100	328028	20	THW	5.0	+	Absent	+	4+	NOGC												Alpha H.S, S. aureus
101	307890	21	THW. FS	5.0	+	Present	+	7+	B.fragilis	GNB	-	-	-	-	R	R	R	-	-	-	G.vaginalis
102	325840	28	THW	5.0	-	Present	+	4+	P.mel	GNB	-	+	-	-	R	R	S	Black	Brick red	-	NOGC
103	323960	23	THW	5.0	+	Present	+	7+	Por.	GNCB	-	+	-	-	S	S	R	Brown	-	-	NOGC
104	328004	26	THW	5.0	+	Absent	+	4+	NOGC												E.coli, G.Vaginalis
105	1133973	26	THW	4.0	-	Present	-	4+	P.anae	GPC in clusters	-	+	-	S	-	-	-	-	-	-	NOGC
106	1087300	30	THW	4.0	-	Present	-	7+	Por.	GNCB	-	+	-	-	S	S	R	Brown	-	-	CONS
107	1110319	20	THW. FS	4.0	-	Absent	-	2+	NOGC												NOGC
108	1110202	25	THW	4.0	-	Absent	-	4+	NOGC												NOGC
109	1129474	22	THW	4.0	-	Absent	-	7+	B.fragilis	GNB	-	-	-	-	R	R	R	-	-	-	NOGC
110	1096718	31	THW	5.0	+	Present	-	4+	P.inter	GNB (beaded)	-	+	-	-	R	R	S	Black	Brick red	-	NOGC

S.No	Hosp. No	Age (In yrs)	AMSEL'S CRITERIA					NUGENT'S METHOD	Anaerobic bacteria isolated	G.Stain	Catalase test	Spot Indole test	Nitrate test	Susceptibility				Pigment production	Flouresence	Pitting	Aerobic & other organisms isolated
			Type of Discharge	pH	Amine Test	Clue Cells (Wet mount)	Three out of Four criteria positive	Gram's Stain						SPS	K	V	C				
111	1087489	22	THW	4.0	-	Present	+	7+	NOGC												S.aureus, G.vaginalis
112	1114917	21	THW	4.0	-	Absent	-	2+	NOGC												NOGC
113	1144738	21	THW	4.0	+	Absent	-	2+	NOGC												CONS, Alpha H.S
114	1147409	23	THW	5.0	+	Present	+	7+	P.anae	GPC in clusters	-	+	-	S	-	-	-	-	-	-	NOGC
115	1052315	22	THW	4.0	+	Absent	-	2+	NOGC												NOGC
116	1043498	28	THW	5.0	-	Absent	-	4+	NOGC												NOGC
117	1092071	25	THW	5.0	-	Present	+	7+	B.fragilis	GNB	-	-	-	-	R	R	R	-	-	-	G.vaginalis
118	1169701	21	THW	4.0	-	Absent	-	2+	NOGC												NOGC
119	1142627	18	THW	6.0	+	Present	+	7+	P.mel	GNB	-	+	-	-	R	R	S	Black	Brick red	-	NOGC
120	1115161	24	THW	4.0	-	Absent	-	2+	NOGC												S.aureus

KEY TO MASTER CHART

αHS	:	α Hemolytic Streptococcus
B.fra	:	Bacteroides fragilis
CONS	:	Coagulase Negative Staphylococcus
E.coli	:	Escherichia coli
FS	:	Foul smelling
G.vaginalis	:	Gardnerella vaginalis
GNB	:	Gram Negative Bacilli
GNCB	:	Gram Negative Coccobacilli
K.pneumoniae	:	Klebsiella pneumoniae
NOGC	:	No organisms grown in culture
P.anae	:	Peptostreptococcus anaerobius
P.inter	:	Prevotella intermedia
P.mel	:	Prevotella melaninogenica
Por	:	Porphyromonas spp.
R	:	Resistant
S	:	Sensitive
S.aureus	:	Staphylococcus aureus
THW	:	Thin homogenous white