"BACTERIOLOGICAL STUDY OF LEUCORRHEA IN REPRODUCTIVE AGE GROUP WOMEN, WITH SPECIAL REFERENCE TO ANAEROBES- A ONE YEAR CROSS SECTIONAL STUDY."

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LIST OF ABBREVIATIONS

BV	Bacterial Vaginosis
BVAB	Bacterial Vaginosis Associated Bacteria
DMSO	Dimethyl sulphoxide
GNB	Gram Negative Bacilli
GPC	Gram Positive Cocci
HIV	Human Immunodeficiency Virus
IUCDs	Intra Uterine Contraceptive Devices
MIC	Minimum Inhibitory Concentration
MTZ	Metronidazole
NACO	National AIDS Control Programme
nim	Nitro imidazole resistant genes
OCPs	Oral Contraceptive Pills
PCR	Polymerase Chain Reaction
PID	Pelvic Inflammatory Disease
PROM	Premature Rupture of Membranes
RTIs	Reproductive Tract Infections
Spp.	Species
STDs	Sexually Transmitted Diseases
UTI	Urinary Tract infection
WHO	World Health Organization

ABSTRACT

INTRODUCTION:

Leucorrhea is a watery/thick, white/yellow purulent vaginal discharge, which is not blood stained, and may or may not be associated with obvious local pathology. Leucorrhea is one of the most common complaints in women of reproductive age. It may be physiological or pathological. In 90% of the women, pathological leucorrhea is mainly caused by Bacterial Vaginosis (BV), Candidal vulvovaginitis and *Trichomonas vaginalis* infection. When left untreated, it may lead to complications such as chronic pelvic inflammatory disease, chronic pelvic pain, infertility, ectopic pregnancy, PROM or chorioamnionitis.

OBJECTIVES:

1. To isolate and identify the bacteria (aerobes and anaerobes) causing leucorrhea.
2. To perform antibiotic susceptibility testing of the isolated bacteria.

MATERIALS AND METHODS:

The study was carried out from January 2016 to December 2016 in the Department of Microbiology, Jawaharlal Nehru Medical College, Belagavi.

250 Women attending the Out Patient Department of Obstetrics and Gynaecology (OBG OPD) at KLE'S Dr. Prabhakar Kore Hospital and Medical Research Centre, Belagavi with complaints of excessive watery/thick, white/yellow vaginal discharge with or without associated vaginal pruritus, burning micturition, backache or abdominal pain were included in the study.

Three high vaginal swabs were collected from the posterior fornix of these women and transported in Thioglycollate broth.

BV was diagnosed in these women by using the Amsel's clinical composite criteria according to which three of the following four criteria must be positive: a) thin, homogenous vaginal discharge, b) presence of clue cells, c) positive Whiff test and d) pH of vaginal discharge > 4.5.

Isolation and identification of aerobic as well as anaerobic bacterial isolates was done by using standard operating procedure. Antibiotic susceptibility of aerobic bacteria was done by Kirby-Bauer disk diffusion method and for anaerobic bacterial isolates, agar dilution method was used.

RESULTS:

Among 250 participants, 30% belonged to the age group of 18-25 years, 30.4% had completed only primary education, and 54.4% belonged to the middle class. 88% of them presented with thin, grey, homogenous, adherent vaginal discharge where as 12% of them had thick, white, non-fowl smelling discharge. 123 (49.2%) women were diagnosed to have BV by Amsel's clinical composite criteria and 93 (37.2%) of these women showed positive bacterial culture. In 29 women, Candida species were isolated. None of the women in the study were infected with *T. vaginalis*.

Amongst the 93 bacterial positive cultures, 86 (92.5%) were aerobes and 42 (45.2%) were anaerobes. Amongst the aerobic bacteria, *Escherichia coli* (34.9%) was the most common isolate followed by Klebsiella spp. (23.3%) and Enterococcus spp. (18.6%). Among the anaerobic bacteria, Porphyromonas spp. (55%) was isolated

frequently, followed by Peptostreptococcus spp. (17%). 65.1% of the aerobic bacteria were resistant to Ampicillin. Gentamicin and Ceftazidime were found to act well against the aerobic bacteria. Among the anaerobic bacteria, 11.9% were found to be resistant to Metronidazole. They belonged to the genus Peptostreptococcus and Bacteroides.

CONCLUSION:

Bacterial Vaginosis, which was the main cause of leucorrhea in this study, is a state of microbial dysbiosis. The normally present Lactobacilli are replaced by pathogenic facultative anaerobes. Amsel's clinical composite criteria is an easy and sensitive method to diagnose BV. However, microbiological diagnostic approach is necessary for etiological diagnosis.

Anaerobic bacteria are a major cause of BV. Their identification and antibiotic susceptibility testing are important. The emergence of resistance pattern amongst anaerobes as reported by many studies is also reflected in this study. Hence, knowing the susceptibility pattern of these pathogenic bacteria will help in appropriate and effective treatment and thereby prevents complications.

Key words: Leucorrhea, Bacterial vaginosis, Aerobes, Anaerobes, Amsel's criteria

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INTRODUCTION

Reproductive tract infections in women are infections affecting the reproductive tract and can be endogenous caused by the vaginal commensals, iatrogenic infections (resulting from abortions, insertion of Intra Uterine Contraceptive Devices, child birth) and infections transmitted sexually. RTIs are a major public health problem globally, most often seen in the reproductive age group of 20-49 years. [1]

RTIs have huge repercussions on health in the form of Pelvic Inflammatory Disease, infertility, ectopic pregnancy and adverse pregnancy outcomes like miscarriage, stillbirth, preterm birth and congenital infections. They are also known to increase the risk of HIV transmission. [2]

The National Family Survey-2 reported that 39.2% of women in India have one or more RTIs.[3]The most frequently reported symptoms by women with RTIs are abdominal pain, back pain, vaginal discharge, burning micturition, itching in genitalia and dyspareunia. About 60% of them complain of vaginal discharge, otherwise known as leucorrhea. [2]

Leucorrhea is a watery/ thick, white/ yellow/green, purulent vaginal discharge, which is not blood stained, and may or may not be associated with obvious local pathology. [3, 4]It is a condition of persistent and excessive vaginal discharge which represents desquamation of vaginal epithelial cells because of the effects of estrogen on the vaginal mucosa. [4]

Although leucorrhea is one of the most common symptoms of RTIs in women, it remains untreated in majority of them. Apart from being a source of constant

distress to the women, when left untreated, it may lead to the above mentioned complications such as chronic PID, infertility, ectopic pregnancy, etc. [5]

Leucorrhea may be physiological or pathological. In physiological vaginal discharge, there is proliferation of vaginal epithelial cells and subsequent increase in the glycogen content due to the action of estrogen. Also, estrogen decreases the viscosity of mucus which lines the vaginal epithelia resulting in watery discharge. The Lactobacilli which are normally present maintain an acidic pH between 3.8 to 4.4 which provides defense against infections.[4] This is usually seen at puberty, at the time of ovulation, during pregnancy, during the pre -menstrual phase, in women using Oral Contraceptive Pills and during anxiety.[3] Leucorrhea due to pathological causes may be due to specific vaginitis (Vaginal candidiasis, Trichomonas vaginitis) or non-specific vaginitis (Bacterial Vaginosis), vulvovaginitis, cervicitis, cervical erosions or PID.[6]

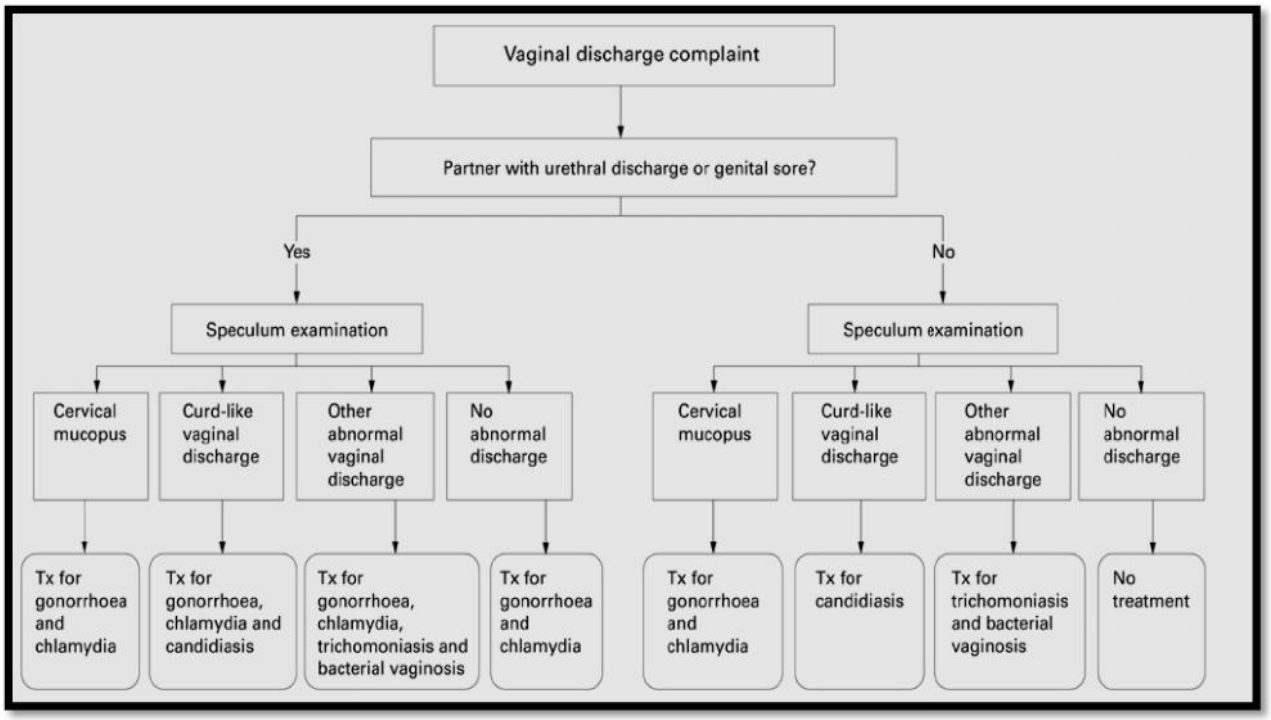
The four most frequent causes of vaginal discharge which cover almost 95% cases are Bacterial Vaginosis, Candidal vulvovaginitis, Trichomoniasis and normal physiological discharge.[7]Vaginal candidiasis is mostly due to *Candida albicans*. Asymptomatic carriage of Candida in women is 10%. Symptoms are vulval itch and soreness, with thick white non offensive discharge.[6]It is associated with diabetes, prolong use of antibiotics and pregnancy. [7]

Trichomoniasis is a Sexually Transmitted Disease. WHO estimates its prevalence to be 170 million worldwide. Thin copious greenish vaginal discharge is seen in patients with Trichomonas infection. On colposcopic examination, strawberry cervix is observed due to punctate hemorrhage. [7]

BV is the most common type of vaginal infection amongst women of reproductive age and accounts for at least 1/3 [rd] of all vulvovaginal infections. [7] Bacterial Vaginosis is termed vaginosis rather than vaginitis, because it is associated with alteration in normal flora rather than due to any specific inflammation.[3] Women with BV mainly present with thin, grey, homogenous and adherent discharge. It is usually a polymicrobial syndrome, caused by both aerobic and anaerobic bacteria.

Amongst the aerobic bacteria causing BV, Staphylococcus species, alpha hemolytic Streptococcus, *Escherichia coli*, Enterobacter, *Neisseria gonorrheae, Chlamydia trachomatis,* Mycoplasma are commonly encountered. *Gardnerella vaginalis,* Peptostreptococcus spp., Bacteroides spp., Veillonella spp., Fusobacterium spp. and Ureaplasma spp. are the frequently isolated anaerobic bacteria from women with BV. [8]

The National AIDS Control Organization recommends an algorithm for the management of vaginal discharge. This algorithm is based on socio-demographic, behavioral risk and clinical assessment to identify cervical infections and to start a syndrome management. This approach is however found to have low sensitivity, low Positive Predictive Value, leading to over treatment. [9]



NACO algorithm for management of vaginal discharge

According to the Royal College of Obstetricians and Gynecologists, the outpatient antibiotic treatment for leucorrhea should be based on one of the following regimens [10]:

- oral ofloxacin 400 mg twice daily plus oral metronidazole 400 mg twice daily for 14 days
- Intramuscular ceftriaxone 250 mg single dose followed by oral doxycycline 100 mg twice daily plus metronidazole 400 mg twice daily for 14 days.

Broad-spectrum antibiotic therapy is generally prescribed to cover *N. gonorrhoeae, C. trachomatis* and anaerobic infection, without the evidence of a laboratory report. This might lead to overtreatment and an increase in resistance of the bacteria to the commonly used antibiotics in the syndromic approach like Metronidazole.

In view of the increasing frequency ($1/3^{rd}$ of women in reproductive age)[11]and complications of pathological causes of leucorrhea, the fact that it is caused by both aerobic and anaerobic bacterial pathogens and also due to the emergence of antibiotic resistant bacteria, this study was carried out to isolate and identify the causative bacteria and their antibiogram was determined. This will help in making a rational choice of both empirical and definitive antibiotic therapy for vaginal discharge.

OBJECTIVES OF THE STUDY

1. To isolate and identify the bacteria (aerobes and anaerobes) causing leucorrhea.
2. To perform antibiotic susceptibility testing of the isolated bacteria.

REVIEW OF LITERATURE

The female reproductive tract provides a satisfactory environment for many pathogenic microorganisms and therefore multiple infections are common.[12]It is colonized by a variety of species of commensal bacteria causing no harm except under abnormal conditions.[13] RTIs are responsible for major ill-health worldwide. About 490 million cases of curable RTIs occur throughout the world, of which 79 million cases occur in India annually. [14] RTIs include both sexually transmitted infections (STIs) and non-sexually transmitted infections (non-STIs) of the reproductive tract. [15]

Vaginitis is a significant RTI with a prevalence of about 47% in developing countries.[16] Of these, Bacterial Vaginosis, Candidal vulvovaginitis and Trichomonas vaginitis are responsible for the majority of infections. Vaginitis resulting from these infections is associated with excessive vaginal discharge (leucorrhea), pruritus, vulvovaginal irritation, dyspareunia or dysuria.[17]Before going into the details of the pathophysiology of vaginal infections, it is necessary to understand the normal anatomical and physiological aspects of the vagina.

Anatomy and physiology of vagina

Vagina is a part of lower genital tract in females. It is an elastic lumen, approximately 7.5 cm long. It lies between the urethra and rectum. Vagina acts as a connector between the external or internal organs of reproduction. It is internally lined with stratified squamous epithelium and it is lubricated by secretions from the Bartholin's glands. The normal vaginal pH in women of reproductive age group is 3.5-4.5, which is maintained by lactic acid produced by the Lactobacilli.[18]

At birth, vagina of a newborn is colonized by anaerobic and aerobic bacteria acquired during passage through the birth canal. The vaginal epithelium at this time will be rich in glycogen because of the influence of placental and maternal estrogens. This results in a low pH (3.7-6.3). Several weeks after birth the epithelium becomes thin, atrophic and largely devoid of glycogen, when the pH rises to 6-8 and remains so until puberty. The predominant vaginal flora during those years comprise of Gram-positive cocci and bacilli.[19]At puberty, as the estrogen secretion increases, proliferation of the vaginal epithelial cells also increase and glycogen gets deposited in them. Subsequently, Lactobacilli proliferate and cause enzymatic breakdown of cellular glycogen, resulting in lactic acid and H_2O_2 which will lower the pH to 3.5-4.5. Lactic acid is a potent anti-microbial and prevents the proliferation of pathogenic microorganisms.[20]After menopause, Lactobacilli again diminish in numbers and mixed flora returns. [21]

The vagina, ectocervix and endocervix are all susceptible to various pathogens depending on the type of epithelium present. Squamous epithelium of vagina and ectocervix are susceptible to infection with Candida species and *T.vaginalis* whereas the columnar epithelium of endocervix is susceptible to infection with *N.gonorrheae* and *C.trachomatis.*

Microflora of vagina:

Vagina is a dynamic ecosystem with diversified species of various microbes. It is not constant and varies with changes in the hormonal levels during the female reproductive cycle. Lactobacilli are the predominant bacteria found in vagina. Among the Lactobacillus species, *Lactobacillus acidophilus* is considered to be the dominant

microbe. The other species like *Lactobacillus crispatus, L. gasseri, L. iners and L. jenseni* may also be present in the vaginal ecosystem. [18]

The vaginal flora is mostly anaerobic. Peptococcus spp., Bacteroides spp., Peptostreptococcus spp., Eubacterium spp., *Ureaplasma urealyticum* are the anaerobic bacteria found in the vaginal microbiota. Some aerobic or facultative anaerobic bacteria are also present. The lower vagina is inhabited by a mixture of these bacteria like Staphylococcus species, *Streptococcus viridans*, Group B Streptococci, Enterococcus spp., Corynebacterium spp., *E.coli,* etc. The microaerophilic *G. vaginalis* is also normally present in the vaginal microbiota.[18]

Reproductive Tract Infections and leucorrhea:

RTIs are a common public health problem in developing countries. National Family Health Survey (NFHS-3) reported that 11% women in the reproductive age group had STI/RTIs related symptom in 2014.[14] Majority of these women complain of leucorrhea. Prevalence of vaginal discharge in India is estimated to be 30%.[22]

According to Jeffcoate, leucorrhea means 'a running of which substance' and the term should be restricted to mean an excessive amount of normal discharge which dries to leave a brownish yellow stain on clothing. Literally leucorrhoea means white discharge which includes all excessive discharge which is purulent, yellow or watery but not blood stained. According to David A. Eschenbach, 1-2ml of vaginal discharge is normal and more than 3ml with malodor is copious. [23]

Vaginal discharge may be physiological or pathological. Physiological increase in vaginal discharge is seen in puberty, at the time of ovulation, during pregnancy and menstruation phase. Pathological leucorrhea is seen in conditions like

Bacterial Vaginosis or infections like vulvovaginitis caused by Candida species, *T.vaginalis* or gonocervicitis.[5]

Vaginitis which causes leucorrhoea is a clinical condition with pathological invasion of the vaginal epithelium by the microorganisms. Women in reproductive age group are at an increased risk of vaginitis. Vulvovaginal candidiasis is characterized by pruritus and cotton cheese like discharge caused by Candida species. Vaginal Trichomoniasis is associated with a copious, yellow or green and sometimes frothy discharge caused by *T. vaginalis*.[24]Amongst all the causes of vaginal infections, BV is the most common cause in non- pregnant women.

History of Bacterial Vaginosis:

Bacterial Vaginosis represents a unique upheaval of the complex vaginal bacterial flora with disappearance of Lactobacilli and overgrowth of *G.vaginalis* and other anaerobic vaginal bacteria. [25]

The composition of vaginal microflora was first described by Doderlein during the year 1892. He reported that Lactobacilli were the predominant microflora of the vaginal system. They occupy an ecological niche and play an important role in bacterial interference thereby preventing the pathogenic microorganisms to proliferate. [18] It is evident from many studies that there is a decrease in Lactobacilli in BV.

In 1914, Curtis associated anaerobic cocci and Mobiluncus with abnormal vaginal discharge. In 1921, Schroder categorized vaginal flora using gram stain into least pathogenic (consisting of Lactobacilli), intermediate stage and most pathogenic

stage that is now identified as BV. In 1950, Weaver et al. associated Bacteroides with BV and also confirmed the absence of Lactobacillus in the syndrome.[23]

In 1955, Gardner and Duke described a new microorganism *Haemophilus vaginalis*, which was later called *Gardnerella vaginalis*. They believed this agent to cause vaginitis. They also described the clinical features of this syndrome: grey malodorous homogenous vaginal discharge with pH between 5.5-6.0, absence of Lactobacillus and the presence of vaginal epithelial clue cells. This formed the basis of diagnosing BV.[23]

Advancements in anaerobic bacteriology in the 1970s led to the realization that several other anaerobic bacteria like Peptostreptococci, Prevotella, etc. could cause BV.

Bacterial Vaginosis and its microbiology:

Bacterial Vaginosis is defined as an abnormal vaginal microflora characterized by a significant reduction of the dominant bacteria Lactobacilli to extremely low levels, fewer than 10,000 colonies/ml of vaginal fluid and a marked increase in the anaerobic bacterial population.[26]However, the exact mechanism and sequence of the infective process are largely unknown due to the lack of a reflective animal model. The term was chosen to replace nonspecific vaginitis to indicate the absence of inflammatory reaction.

Clinically and microscopically BV is defined as follows: presence of thin grey homogenous vaginal discharge, vaginal pH > 4.5, liberation of amines when the vaginal discharge is mixed with 10% KOH and the presence of clue cells.[26]

Definition of BV by culture based microbiological criteria suggested the decrease or absence of Lactobacilli, which were replaced by *G. vaginalis, E.coli,* anaerobic Gram negative bacilli, Gram positive cocci and Mycoplasma spp. [27]

A number of potential microbial pathogens, singly and in combinations, have been implicated to cause BV. The list of possible agents continues to expand and includes members of a number of genera, including Gardnerella, Atopobium, Prevotella, Peptostreptococcus, Mobiluncus, Sneathia, Leptotrichia, Mycoplasma, and BV-associated bacterium 1 (BVAB1) to BVAB3. [27]

Individual microbial species as possible mediators of BV:

1) *Gardnerella vaginalis*

G. vaginalis was first isolated by Leopold in 1953. A year later, it was associated with nonspecific bacterial vaginitis by Gardner and Dukes. It is a non-motile, non -encapsulated, non- spore forming gram variable, pleomorphic rod measuring approximately 1-1.5 µm to 0.4 µm. It is a fastidious bacterium requiring complex medium for its growth. Biochemically, it is catalase and oxidase negative, hydrolyzes hippurate. It produces beta hemolytic colonies on human blood agar.[27]

G.vaginalis has a high sensitivity (100%) but low specificity (49%) in causing BV. It has various virulence factors like fimbriae and produces adhesin, which will help it to adhere to the vaginal epithelial cells. It produces vaginolysin which causes lysis of RBCs and vaginal epithelial cells in vitro. It also secretes sialidase, prolidase and putrescine which degrade mucin and cause exfoliation of the vaginal epithelial cells. It co exists with Atopobium by forming a biofilm. This is implicated in recurrence of BV and resilience to treatment with Metronidazole.

Peptidases produced by *G.vaginalis* can release peptides and amino acids from the protein rich vaginal environment which will stimulate growth of other pathogenic bacteria.

Fichorova et al. in his study showed that *G.vaginalis* causes upregulation of pro inflammatory cytokines like IL-6, IL-8, RANTES, and Soluble Leukocyte Protease Inhibitor (SLPI). Inflammatory activity of *G.vaginalis* gets synergistically enhanced by co-infection with Trichomonas vaginalis virus 1-4 species. Strong evidence supports the role of *G. vaginalis* in modifying host immunity and BV pathogenesis.[28]

2) <u>Prevotella and Porphyromonas species</u>

Prevotella and Porphyromonas are anaerobic, Gram negative, pleomorphic, non-motile coccobacilli. *Prevotella intermedia, Prevotella melaninogenica, Porphyromonas asaccharolyticus* and *Porphyromonas gingivalis*are most frequently isolated from clinical samples. They include both black pigmented and non-pigmented species. Pigment production requires prolonged incubation, especially for *P.melaninogenica*. Pigmented anaerobic GNB are bile sensitive. Based on antibiotic disk identification pattern, indole reaction and colony morphology they are grouped into 3 categories:

a) *Prevotella intermedia, P.nigrescens, P.corporis*produce dark brown to black pigment. They are indole positive, resistant to Vancomycin and Kanamycin, sensitive to Colistin.

b) *P. melaninogenica* group produce tan to light brown smooth colonies, they are indole negative, resistant to all 3 antibiotics- Vancomycin, Kanamycin and Colistin.

c) Porphyromonas species produce mucoid, dark brown to black colonies. They are indole positive, sensitive to Vancomycin and resistant to Kanamycin, Colistin.

These colonies fluoresce brick red under UV light.

Prevotella and Porphyromonas make up the "Bacteroides morphotypo" used to determine Nugent scores.[29,30]*Prevotella bivia* and black pigmented Prevotella species are significantly associated with BV.[29]Prevotella is associated with positive Whiff test, one of the clinical criteria for Amsel's test. Positive Whiff test is due to production of polyamines, including putrescine, cadaverine and trimethylamine which increase the vaginal pH and enhance the growth of anaerobic bacteria like Peptostreptococci. Synergy between Prevotella and *G.vaginalis* is also proved. Ammonia produced by the Prevotella species is used by *G.vaginalis* which in turn produces amino acids which are used by the Prevotella species.

Prevotella bivia and *P.disiens* produce collagenase and fibrolysins which degrade the mucosal surface and promote the detachment of vaginal epithelial cells. Sialidase and prolidase secreted by these species cause vaginal sloughing.[27]

Fichorova et al. and Doerflinger et al. showed that *P.bivia* increases the levels of pro inflammatory cytokines like MIP-3α, Il-1β and IL-8.[28,31]

3) <u>Anaerobic cocci</u>

Various Gram positive and Gram negative anaerobic cocci form the normal flora of the female genital tract. Peptostreptococcus,Finegoldia, Parvimonas, Gallicola, Peptoniphilus, and Anaerococcus are the anaerobic GPC.The most common anaerobic GPC isolated belong to the genus Peptostreptococcus. They are

Peptostreptococcus magnus, P.asaccharolyticus, P.prevotii, P.micros and P. anaerobius. They are large coccobacilli, arranged in chains. *P.anaerobius* produces isocaproic acid and is sensitive to Sodium Polyanethol Sulphonate (SPS). This property is used for its rapid presumptive identification.

They produce many virulence factors like lipopolysaccharide, hyaluronidase, collagenase and few are encapsulated. Delaney and Onderdonk et al. reported that the bacterial concentration of Peptostreptococci increase as the total Nugent score increases. Peptostreptococci do not adhere to vaginal epithelial cells where another anaerobic GPC, Peptoniphilus adheres to vaginal epithelial cells and is associated with persistent cases of BV. These GPCs show variable resistance to Penicillin, Clindamycin and Metronidazole.[32]

Veilonella and Megasphaera 1and 2 are Gram negative cocci which are implicated in BV. They are identified based on their morphological arrangement, nitrate reduction, catalase and the fatty acids produced. [33]

4) <u>Mobiluncus</u>

Mobiluncus species are motile, slow growing, fastidious Gram negative curved rods. They require enriched medium containing rabbit/ horse serum or fermentable carbohydrates like maltose/glycogen for growth.

The genus Mobiluncus has two species- *M.curtisii* (short curved GNB) and *M.mulieris* (long, straight or slightly curved GNB). They are sensitive to Penicillin, Clindamycin as well as Vancomycin and resistant to Colistin, Nalidixic acid. They produce malic acid and trimethylamine which are responsible for vaginal irritation and malodor. A recent study using PCR to detect Mobiluncus in vaginal specimens

reported that Mobiluncus was detected in 38% women without BV and in 84.5% women with BV.[27]

M.curtisii is resistant to Metronidazole. Predominance of *M.curtisii* and its persistence is associated with treatment failure. [34,35]*M.mulieris* is found to increase vaginal epithelial cell production of IL-1α, IL-1β , TNFα , IL-8.[36]

5) Mycoplasma and Ureaplasma

Mycoplasma and Ureaplasma are facultative anaerobes with no cell wall. Therefore, they are difficult to be identified on a Gram stained smear. Mycoplasmas are the smallest free living microbes. They require complex media for isolation. *Mycoplasma hominis* and *M.genitalium* are the two species isolated from the female genital tract. *M.hominis* is associated with BV.[37]

Kaene et al. in his study showed 53% carriage rate of *M.hominis* in women with BV and none in normal women. He also reported that women with BV had higher *Ureaplasma urealyticum* carriage rate of 65%. [38]

U.urealyticum produces agarase enzyme which causes pitting of the agar. It also expresses hemolytic activity, secretes enzymes like elastase and IgA protease which decrease mucosal immunity. It also produces phospholipase C and urease which hydrolyze urea to ammonia, which in turn precipitates pathogenesis and symptoms of BV.[39]

M.hominis and *U.urealyticum* are responsible for the pathogenesis of BV associated immune dysregulation.

6) Bacteroides species

Bacteroides species are non- motile, non- spore forming, pale staining GNB. They are usually isolated from infections below diaphragm. Most common species to be isolated from clinical samples are *B.fragilis*, followed by *B.thetaiotaomicron*. They produce small, smooth, white to grey, non- hemolytic colonies on blood agar. They are bile resistant, indole negative and produce catalase. They are resistant to all the three antibiotic identification disks: Kanamycin, Colistin and Vancomycin.

Bacteroides produce various virulence factors like catalase, proteinase and capsule. *B.fragilis* may produce enterotoxin. Polanco N has reported that the effect of enterotoxin on E-cadherin of vaginal epithelium could facilitate invasion and its possible pathogenic role in the vagina. [40]

Bacteroides spp. are the most resistant of the anaerobic bacteria. They produce β lactamase. 5-15% are resistant to Tetracycline and 30% are resistant to Clindamycin.[41]Metronidazole resistant Bacteroides have already emerged. Intermediate or high level resistances to MTZ have been reported by Alauzet et al.[42] This resistance is probably mediated by *nim* genes which are present in both chromosome and plasmid.

7) Fusobacterium

Fusobacterium species are non-motile, straight or slightly curved GNB. *F.nucleatum* is the most common species to be isolated from clinical samples followed by *F. necrophorum*, which is the most virulent species as it produces leukocidin, phospholipase A and hemolyzes RBCs.

Fusobacterium produce slightly hemolytic colonies on blood agar. The agar turns green around the colonies due to production of H_2O_2 on exposure to air. Colonies of *F.nucleatum* resemble bread crumbs and produce internal speckling.

They are resistant to Vancomycin, sensitive to Kanamycin and Colistin. Species can be identified by testing for indole, lipase, bile resistance, esculin hydrolysis. 50% of the strains produce β lactamase.[41]

8) <u>BVAB1 to BVAB3</u>

In 2005, Fredricks et al.[43]discovered 3 novel bacteria in the vaginal fluid of women with BV by PCR and subsequently confirmed by Fluorescent In situ Hybridization (FISH). They belong to the Phylum Clostridium. The 3 novel bacteria are Bacterial Vaginosis Associated Bacteria 1(BVAB1), BVAB2 and BVAB3. BVAB1 are thin curved rods, morphologically similar to Mobiluncus. BVAB2 are shorter and fatter rods, BVAB3 are long, lancet shaped rods.

BVAB1 and BVAB2 are not cultured yet. However, BVAB3 was recently cultured and renamed *Mageeibacillus indolicus*. It is an obligate anaerobe, slow growing, non-motile, non- spore forming Gram positive bacilli. It is asaccharolytic, indole positive. It does not produce urease or hydrolyze gelatin. [27]

Molecular based studies using species specific PCR, pyrosequencing and FISH showed that BVAB1 and not Mobiluncus was the dominant curved rod seen in gram stained smears with Nugent scores of 9 to 10.[44,45]

BVAB1 is associated with positive Whiff test which indicates its ability to produce amines. BVAB1 is codependent with BVAB3 and Prevotella. [27]

9) Aerobic bacteria commonly implicated in BV include *S.aureus*, Group B β hemolytic Streptococci and *E.coli*.[46]These bacteria have been noted in less than 25% of the women with BV.

In a study conducted by Khammes SSet al.[47],*S.aureus* (28%) was the most common aerobic bacteria isolated followed by *E.coli* and Klebsiella spp. 13.8% each, Enterobacter spp. (11.7%), Pseudomonas spp. (9.57%), Streptococci (2.8%) and Citrobacter spp. (1%). Afroze N et al.[3] in 2013 reported 22% *S.aureus*, 4% *E.coli* and 3.2% Streptococci in her study.

In a similar study by Duagmani et al.[48], Staphylococcus species were 55% followed by *E.coli* 8.9%, Enterobacter 6.2% and Streptococci 1.2%. The anaerobic bacterial isolates were 50.7% Peptococcus spp., 34.7% Peptostreptococcus spp., 21.8% Bacteroides spp., 10.1% Veilonella and 1.4% Fusobacterium. Sumati A et al.[49] in her study isolated 30.13% Bacteroides spp., 13% Peptococcus spp., 8.9% Peptostreptococcus spp., 8.21% Veilonella spp.,7.53% each of Prevotella and Fusobacterium spp.

Pathogenesis of BV:

BV is related to:

a) Altered vaginal flora and dysbiosis**:**A decrease in the colonization of vaginal Lactobacilli and increase in the pathogenic anaerobic bacteria.
b) Production of amines
c) Biofilm formation
d) An increased level of endotoxins stimulating pro inflammatory cytokines
e) Decreased leukocyte recruitment.

Lactobacilli are the predominant bacteria found in the normal vagina. These bacteria form a critical line of defense against potential pathogens. The symbiotic relationship between vaginal Lactobacilli and their human host is modulated by estrogen, which stimulates the vaginal epithelia to produce glycogen. The Lactobacilli metabolize glycogen present in the vaginal epithelial cells to form lactic acid which is mainly responsible for the vaginal pH to be acidic (3.5 - 4.5). The acidic environment is not permissive for growth of many potential pathogens. Also, H_2O_2 and bacitracin produced by the Lactobacilli are bactericidal. [50]

With the loss of Lactobacilli in BV, pH of the vagina increases and there is massive overgrowth of vaginal anaerobic bacteria. The difference in vaginal flora between women with and without BV was illustrated in a study that used broad range DNA probes to determine the vaginal flora of 27 women with BV and 46 controls.[43]Overall, 35 bacterial phylotypes were identified in women with BV, including 16 which were newly recognized. Women with BV had a mean of 12.6 phylotypes (range 9 to 17) per sample compared to 3.3 phylotypes (range 1 to 6) per sample in women without BV.

The proliferating anaerobes produce proteolytic carboxylase enzymes, which break down vaginal peptides into a variety of amines that are volatile and malodorous. An increase in vaginal transudation and squamous epithelial cell exfoliation is also seen which results in typical features of BV like grey, watery, adherent, fowl smelling vaginal discharge.

G.vaginalis is the key player in the pathogenesis of BV and the development of a biofilm may be an essential component of this process. *G.vaginalis* adheres to the vaginal epithelium and then becomes the scaffolding to which other species adhere.[51]This hypothesis is supported by a study of microbiota on the epithelial

surfaces of vaginal biopsy specimens from women with BV that showed 90% *G. vaginalis* to be adherent to the vaginal epithelial cells by producing biofilm, while *Atopobium vaginae* accounted for most of the remainder.[52]Subsequent desquamation of these epithelial cells would result in the classic clue cells diagnostic of the disorder.[25]

Many studies have reported increased levels of IL-1α and IL-8 which are pro inflammatory cytokines in women with BV. But they are insufficient to recruit leukocytes and also decrease their half- life by an unknown mechanism. Thus, BV is a non-inflammatory state.[27]

However, IL-1α and IL-8 enhance the risk of HIV transmission by directly stimulating HIV replication in the latent viral reservoirs and by facilitating the trafficking and stimulation of CD4+ host cells. There is also a decline in antiviral factor SLPI which increases the risk of HIV.[27]

Risk factors for BV:

Risk factors for acquisition of BV include low socio economic status, cigarette smoking, douching, recent antibiotic therapy for another condition, young age of coitarche and multiple sexual partners.[50]

a) **Low socio economic status-**Women belonging to lower socio economic status are unaware of the disease, have a poor menstrual hygiene which predisposes to BV.

b) **Sexual activity** – Epidemiological studies have found that early sexual activity, multiple sexual partners and women with a prior STD are at higher risk of BV.[53]While the majority of data support the hypothesis that BV is a sexually transmitted infection, it is not yet classified as such because of lack of a single causative agent and absence of a clear disease counterpart in males.[54] However,

epidemiologic studies are strongly supportive of sexual transmission of BV pathogens. In a systematic review and meta-analysis of 43 observational studies, sexual contact with new and multiple male and female partners was associated with an increased risk of BV, while condom use was associated with a decreased risk.[55] BV is highly prevalent (25 to 50 %) in women who have sex with women and is associated with increasing numbers of female sexual partners, a female partner with symptomatic BV, and various sexual practices, suggesting sexual transmission is an important factor.[56]

c) **Sexually transmitted infections (STIs)** – The presence of STIs is associated with an increased prevalence of BV. In a systematic review and meta-analysis of studies evaluating the association between BV infection and herpes simplex virus (HSV)-2 infection, women infected with HSV-2 had a 55% higher risk of BV infection compared with women who were HSV-2 uninfected.[57]Similarly, a five-year prospective cohort study reported that BV was both more prevalent and more persistent among HIV-infected women compared with those without HIV.[58]

d) Others- Few behaviors like using diaphragms and douching act as potential risk factors for BV. Vaginal douching may change the vaginal flora, reduce the number of Lactobacilli and encourage the anaerobic pathogens to proliferate. Although some degree of genetic susceptibility to BV is likely, no association between a gene polymorphism and BV has been established.[59]One questionnaire study reported an association between high fat diets and BV as well as an inverse relationship for BV with the intakes of folate, vitamin E, and calcium.[60]BV is not associated with chronic medical conditions (e.g. diabetes) or immunosuppressive states.

It is still unknown whether these described risk factors are causally related to BV or serve only as surrogate markers for other contributory factors.

Clinical features of BV:

Approximately, 50-75% of women with BV are asymptomatic. In symptomatic women increased greyish white vaginal discharge is frequently noticed, which may have an offensive odor that intensifies after sexual intercourse and during menstruation**.**

Other symptoms are pruritus, vulvovaginal irritation, dyspareunia, dysuria and lower abdominal pain.[17]But BV alone does not cause these symptoms. The presence of these symptoms suggests mixed vaginitis.[25]

Although BV does not involve the cervix, the disorder may be associated with acute cervicitis (endocervical mucopurulent discharge or easily induced cervical bleeding).[61]

Complications of BV:

Untreated BV will lead to complications like chronic PID, chronic pelvic pain, infertility or ectopic pregnancy. The bacterial flora that characterizes BV has been recovered from the endometria and salpinges of women having PID.

BV is a cause of endometrial bacterial colonization, plasma-cell endometritis, postpartum fever, post hysterectomy vaginal cuff cellulitis, post abortal infection.[62,63]

BV is a risk factor for HIV acquisition and transmission. BV is also a risk factor for HSV-2, gonorrhea and chlamydial infections.[64]

There are reports suggesting women suffering from BV to be at a higher risk of acquiring urinary tract infection when compared to others. Harmani et al.[65]found that 15 out of 67 (22.4%) women had both BV and UTI whereas 9.7% had UTI without BV.

In pregnancy, BV is likely to be associated with preterm deliveries due to chorioamnionitis, leading to premature rupture of membranes, which in turn leads to increased perinatal mortality. PROM is associated with 30-40% of pre term deliveries and prematurity is the cause of 85% of neonatal morbidity and mortality.

BV may be a factor in development of precancerous cervical lesions. In a systematic review and meta-analysis of cross-sectional studies, the risk of cervical intraepithelial neoplasia (CIN) or squamous intraepithelial lesions (SIL) was increased in women with BV. However, there was considerable heterogeneity among these studies. [66]

Diagnosis of BV:

1) AMSEL'S CLINICAL COMPOSITE CRITERIA:

The diagnosis of BV is usually based on Amsel's criteria, which are simple and useful in practice where microscopy is available. The first three findings are sometimes also present in patients with trichomoniasis

Amsel's criteria for diagnosis of BV (at least three criteria must be present) [67]

- Homogeneous, thin, grayish-white discharge that smoothly coats the vaginal walls.
- Vaginal pH > 4.5.

- Positive Whiff-amine test, defined as the presence of a fishy odor when a drop of 10 % potassium hydroxide is added to a sample of vaginal discharge.

- Clue cells on saline wet mount

For a positive result, at least 20 % of the epithelial cells on wet mount should be clue cells. The presence of clue cells is the single most reliable predictor of BV.

The sensitivity of Amsel's criteria for diagnosis of BV is over 90%and specificity is 77 %.

2) NUGENT'S SCORING OF THE GRAM STAINED SMEAR OF VAGINAL DISCHARGE:

Use of Nugent score to evaluate a Gram-stained smear of vaginal discharge is the diagnostic standard in research studies, but requires more time, resources and expertise.[67]

Nugent et al. reported that the three bacterial morphotypes that were recognized with the highest degree of reproducibility were Lactobacillus (large GPB), Gardnerella and Bacteroides (pleomorphic GNB) and Mobiluncus (curved GNB). These three bacterial morphotypes were used to develop a 0-10 point scoring system for the diagnosis of BV.A score of 0-3 is normal, 4-6 is intermediate and 7-10 is diagnostic of BV. [68]

3) CULTURE METHODS:

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

Mobiluncus species, another group of anaerobic bacteria is also associated with BV. But it is difficult to recover by culture methods. [68]

In a study by Rao PS et al.[69], prevalence of BV detected by culture methods was 17.42%. *G.vaginalis* was the most common isolate followed by Prevotella and Porphyromonas spp.

4) **PAPANICOLAOU SMEARS:**

Pap smear is routinely used as a cytological screening method for precancerous lesions. It has also been evaluated for BV. If there is a shift in flora from predominantly Lactobacilli to predominantly coccobacilli with or without clue cells, BV is suspected.

The Papanicolaou smear is not very reliable for the diagnosis of BV. Its sensitivity is 49 % and specificity is 93%.[70]

5) COMMERCIAL TESTS :

Commercial tests for diagnosis of BV are not widely used, given the excellent performance of Amsel's criteria, but can be useful when microscopy is not available.

- The **Affirm VP III** test is an automated DNA probe assay for detecting *G. vaginalis* when present at a high concentration. It takes less than one hour to perform and is the best option when findings on physical examination suggest BV .In one study, the combination of a positive DNA probe (concentration of *G. vaginalis* ≥2 times 10^7 CFU/mL) and vaginal pH >4.5 had a sensitivity and specificity of 95% and 99 %, respectively, for diagnosis of BV when clinical criteria were used as the diagnostic standard.[71]However, over-diagnosis is possible.

- The **OSOM BVBlue** system is a chromogenic diagnostic test based on the presence of elevated sialidase enzyme activity in vaginal fluid samples. This enzyme is produced by bacterial pathogens associated with BV including Gardnerella, Bacteroides, Prevotellaand Mobiluncus. The test can be

performed at the point of care and results are available in 10 minutes. Sensitivity ranging from 88 -94% and specificity ranging from 91 -98% have been reported when compared with Amsel's and Nugent criteria.[72]

6) GAS LIQUID CHROMATOGRAPHY:

When Lactobacilli are the predominant members in the vaginal flora, lactic acid is the main acid in the vaginal discharge. Among women with BV, succinate and acetate, metabolic products of anaerobic bacteria are detected more frequently. Spiegel et al. reported that a succinate/lactate ratio of > 0.4 based on peak heights on gas liquid chromatography analysis of vaginal discharge was correlated with clinical diagnosis of BV.[68]This method is not adaptable in routine as the laboratory equipment needed are not widely available.

7) MOLECULAR METHODS:

Quantitative PCR based assays are based upon molecular quantification of *G.vaginalis* and *Atopobium vaginae,* and other bacteria like most Megasphaera and BVAB 1 and 2. Although these tests have good sensitivity and specificity compared with standard clinical tests, they are expensive and of questionable advantage. [73]

A molecular test that assays the vaginal microbiome for evidence of Bacterial Vaginosis, vaginal candidiasis, and trichomonas is promising. [74]

A urine test that uses FISH to identify the BV biofilm on desquamated vaginal epithelial cells in urine sediment is under investigation. [75]

Treatment

The NACO recommends an algorithm for the management of vaginal discharge. This algorithm is based on socio-demographic, behavioral risk and clinical assessment to identify cervical infections and to start a syndromic management. This approach is found to cause over treatment.[9]

- Metronidazole 500 mg thrice daily for 10 days,
- Doxycycline twice daily for 10 days and
- Clotrimazole vaginal pessary

are the commonly prescribed drugs under the blanket therapy. Women should be advised to avoid consuming alcohol during treatment with MTZ as it may cause Disulfuram like reaction.

Management of sexual partners:

The results of various clinical trials indicate that a woman's response to therapy and the likelihood of relapse or recurrence are not affected by the treatment of her sex partner (s). Therefore, routine treatment of sex partners is not advisable. [64] However, this remains controversial in view of many studies showing sexual transmission may play a role in BV.[55,56]

Role of probiotics:

Probiotics containing strains of Lactobacilli like *L.acidophilus* have been shown to restore the acidic pH of vagina and inhibit growth and adhesion of pathogenic microflora. It is a cost effective management. However, its effect is variable and its reaction time against the infection is slow. Hence, using probiotics in conjunction with the anti microbials is a better option.[18]

Emergence of Metronidazole resistant bacterial strains:

Metronidazole is the most common drug prescribed in the blanket therapy used for treating anaerobic infections and *T.vaginalis*.It enters the cell as a prodrug by passive diffusion and is activated in either the cytoplasm of the bacteria or specific organelles (hydrogenosome) in the protozoa, whereas drug-resistant cells are deficient in drug activation. The metronidazole molecule is converted to a short-lived nitroso free radical by intracellular reduction. This form of the drug is cytotoxic and can bind nonspecifically to bacterial DNA, inactivating the organism's DNA and enzymes leadingto a high level of DNA breakage. Aerobic cells lack electron-transport proteins with sufficient negative redox potential; therefore, the drug is active against only bacteria with anaerobic metabolisms. In anaerobic bacteria, flavodoxin and ferredoxin act as electron acceptors.

Proposed mechanisms of resistance to Metronidazole in anaerobic bacteria are:

a) Decreased drug uptake or increased efflux
b) Decreased drug activation/change in the biological target
c) Increased oxygen scavenging capabilities (SOD/catalase/peroxidase)
d) Enhanced activity of DNA repair enzymes

Anaerobic bacteria resistant to Metronidazole have been identified since 1978.Their prevalence was 1.5% in 1995, which increased to 3.8% in 1997 and further to 7.5% in 1998.[76]Metronidazole resistant (MTZ-R) *Bacteroides fragilis*was first reported in a patient with Crohn's disease after long-term therapy with MTZ. Metronidazole resistance among Bacteroidesspp. is of concern, as these species can also be resistant to a wide variety of antimicrobial agents including β-lactams, tetracycline, clindamycin, cefoxitin and imipenem.

The genetic determinants of Metronidazole resistance are found to be the specific nitroimidazole-resistant genes (*nim*), presumably encoding a nitroimidazole reductase that converts nitroimidazole to aminoimidazole, thereby avoiding the formation of toxic nitroso radicals that are essential for antimicrobial activity. So far seven *nim* genes (*nim* A, B,C,D,E,F,G) have been described. Gal and Brazieret al.[77] studied 50 resistant isolates and found the *nim*A gene was the most common, followed by *nim*B and *nim*E. However, there is no one specific gene for MTZ resistance and multiple possible pathways for resistance exist.[78]

Resistance among anaerobic pathogens is still generally low; however, the susceptibility patterns of anaerobic bacteria are undergoing changes, and decrease in in vitro susceptibility to various antimicrobials have been reported in recent years. The practice in many laboratories of identifying obligate anaerobes by susceptibility to MTZ is a factor that contributes to probable underestimation of true resistance rates. Growths around disks are presumed to be facultative anaerobes with naturally reduced susceptibility, and these strains have not been investigated further.[76] Consequently, the treatment of anaerobic infections is generally empirical and is based on published reports of susceptibility rates. This emphasizes the need for the laboratories to periodically check for antibiotic resistance amongst anaerobes.

METHODOLOGY

Study Centre: The present study was conducted at the Department of Microbiology, Jawaharlal Nehru Medical College, Belagavi.

Source of Data: Women attending Out Patient Department of Obstetrics and Gynaecology (OBG OPD) at Karnatak Lingayat Education Society's Dr. Prabhakar Kore Charitable Hospital and Medical Research Centre, Belagavi with complaints of excessive watery/thick, white/yellow vaginal discharge with or without associated vaginal pruritus, burning micturition, backache or abdominal pain were included in this study.

Method of collection of data

- **Study design:** Cross-sectional study.
- **Study period**: Period of one year from January 2016 to December 2016
- **Sample size calculation:**

  $n= 4pq/ d^2$

  n= sample size

  p= 30 (isolation rate)

  q= 70 (100-p)

  d= absolute error = 20% of p = 6

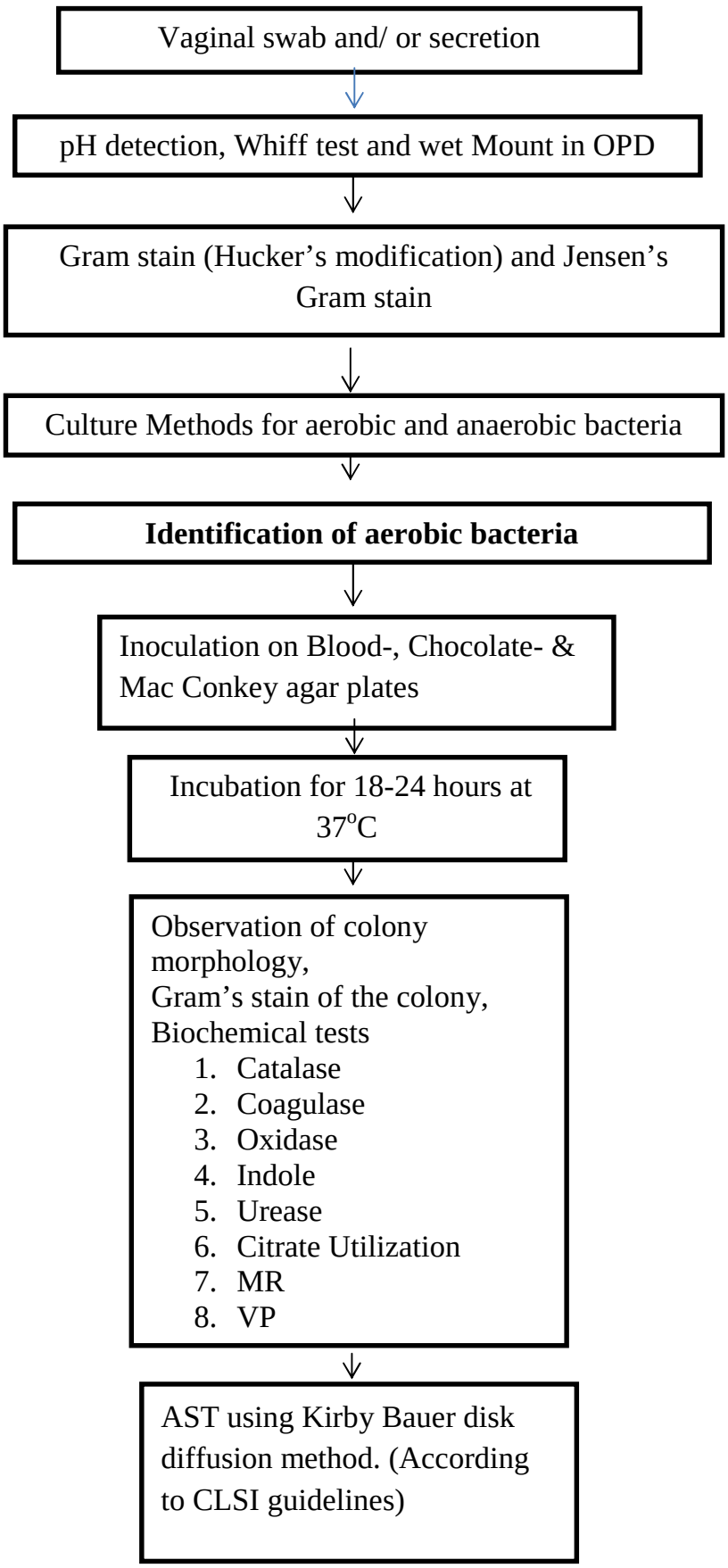
  $n=4 x30 x 70/ (6)^2$

  =233~ 240

- **Sample size:** 240 (250 samples were collected in the study)
- **Sampling Procedure:** Universal sampling method

- **Inclusion Criteria**: Married women in reproductive age group (18-45 years) with excessive vaginal discharge attending OBG OPD and who gave written informed consent for participation were included in the study.
- **Exclusion Criteria:** Vaginal bleeding at the time of sample collection, pregnant women, unmarried women, post hysterectomy women , women on hormonal therapy, women on OCPs, those with IUCDs, women on current antibiotic treatment or who had taken antibiotics within the last 2 weeks, women with genital prolapse, post-menopausal women and those with carcinoma cervix were excluded.
- **Collection of sample**
    - ✓ After obtaining informed written consent from the women selected for the study, a detailed obstetric and gynecological history was taken. Per speculum examination of the genitalia was done without using antiseptics under aseptic precautions.
    - ✓ A sterile Sim's speculum was inserted into the vagina to visualize the vagina and cervix. Any pathology of vagina and cervix like vaginitis, discharge, cervicitis, cervical erosions and tumors were looked for.
    - ✓ Three high vaginal swabs/ vaginal secretion were then collected from the posterior fornix and transported to Microbiology laboratory at JNMC within 2 hours.
    - ✓ First swab was used for making smear for Gram staining. Second swab was used for culture of aerobic bacteria. Third swab was transported in Thioglycollate medium and was used for staining and culture of anaerobic bacteria.

Flow chart showing the methodology of sample collection and processing:



Amsel's Clinical Composite criteria [79]:

- ✓ While taking the high vaginal swab, color, amount, consistency and odor of the vaginal discharge was noted.
- ✓ Vaginal pH was recorded using commercial pH strip (with in a range of pH 3.5-9.0) held in the vaginal discharge on the speculum.
- ✓ Whiff test was done by adding two drops of 10% KOH to the vaginal fluid collected on the speculum. Presence of enhanced fishy odor on sniffing was suggestive of BV.
- ✓ Wet mount of the vaginal secretion was done by adding a drop of normal saline to the vaginal discharge on a grease free clean slide. It was mounted with a coverslip and examined under the microscope for trophozoites of *T.vaginalis* and clue cells. Clue cells are vaginal epithelial cells with granular surface and blurred margins as they are studded with bacteria.
- ✓ Bacterial Vaginosis was diagnosed clinically using Amsel's criteria. Three of the following four criteria must be positive for the diagnosis of BV:
    1. Presence of thin, grey, homogenous discharge adherent to the vaginal wall
    2. Vaginal pH of > 4.5
    3. Positive Whiff test
    4. Presence of clue cells on wet mount

- **Sample processing:** Direct smear on a grease free clean glass slide was made using the first swab. The smear was stained by Gram method (Hucker's modification) [80] for an immediate presumptive diagnosis of the number and type of microorganisms present in the sample. Morphology of the organism, presence of clue cells, yeast cells or pseudohyphae in the Gram stained smear was recorded**.**

1. **Culture:**

Culture was done for both aerobic and anaerobic bacteria.

a) Aerobic culture:

The second swab was inoculated onto-

1) 5% sheep blood agar
2) Chocolate agar
3) Mac Conkey agar

- The inoculated culture plates were incubated at 37^{o}C for 24 hours aerobically.
- The isolates were identified and characterized biochemically by standard operative procedures.[81]

b) Antibiogram of aerobic bacteria:

The antibiotic susceptibility testing was done for aerobic bacterial isolates by disk diffusion method as described by Kirby-Bauer, on Mueller Hinton agar (MHA) plates. The following are the antibiotics which were tested:

Antibiotics	**Concentration per disc (µg)**
1) Ampicillin	10
2) Amoxiclav	30
3) Ceftazidime	30
4) Chloramphenicol	30
5) Ciprofloxacin	5
6) Clindamycin	2
7) Cotrimoxazole	25
8) Erythromycin	15
9) Gentamicin	10
10) Piperacillin	100
11) Vancomycin	30

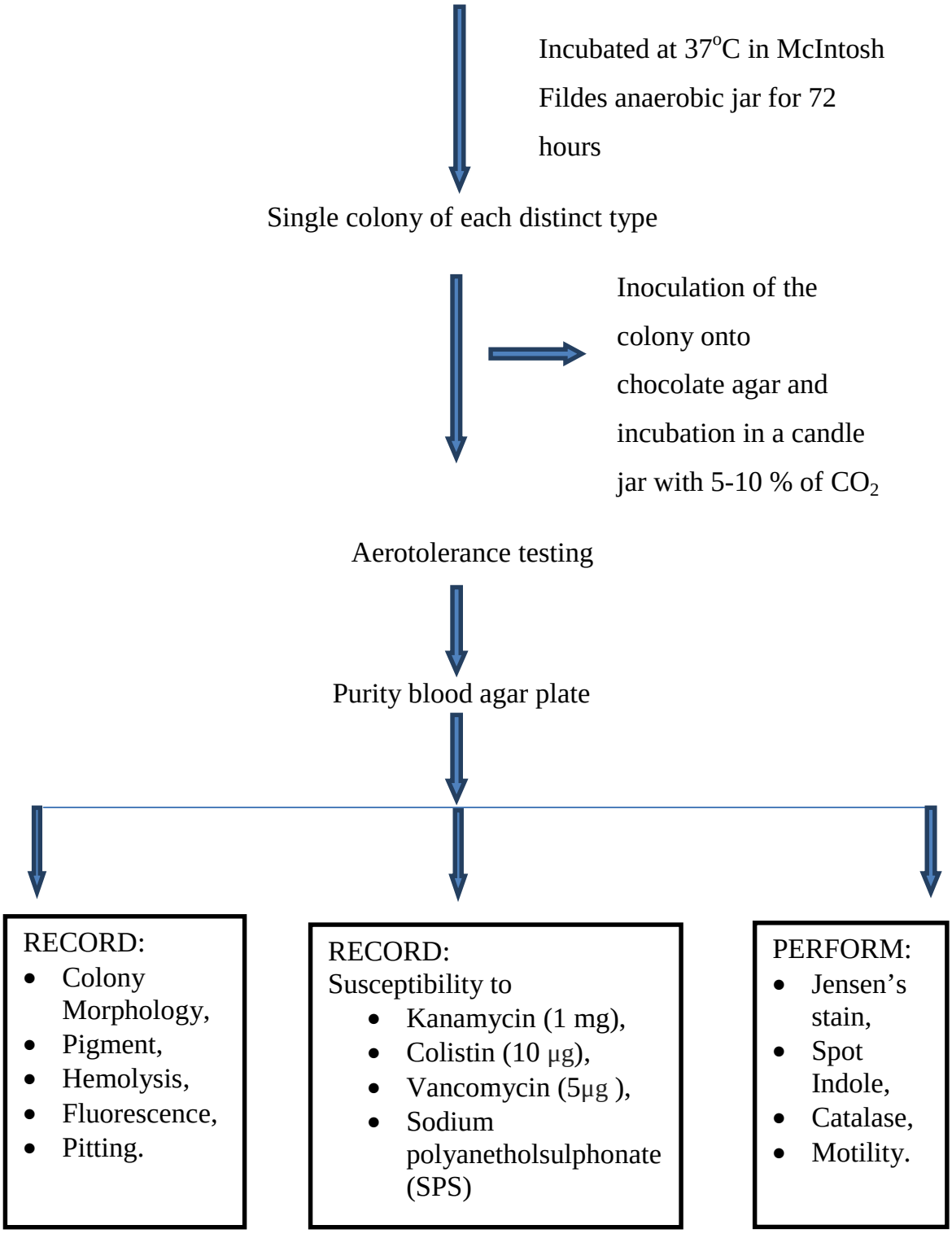
Method: For antibiotic susceptibility testing, a single colony was inoculated in peptone water and incubated at 37^{o}C for 4-6 hours. Then its turbidity was adjusted to Mc Farland's 0.5 standard and using a sterile cotton swab, lawn culture of the inoculum was done on to MHA plate and antibiotic disks were placed. This plate was incubated over night at 37^{o}C. Zone of inhibition was measured on the next day. Interpretation was recorded according to the Kirby-Bauer chart.[82]

Control strain used was *Staphylococcus aureus* ATCC 25923.

c) Culture of anaerobic bacteria: [83]

The third swab which was transported in Thioglycollate medium was inoculated onto:

Blood agar supplemented with Hemin (5 µg/ml) and Vitamin K (10 µg/ml)



Method used to obtain anaerobiosis: in the jar was "Internal gas generating system" described by Laxminarayana and Vaidhyalingam.[84]

After 72 hours of incubation at 37°C the anaerobic jar was opened. The plates were examined for the presence of bacterial colonies. Each predominant distinct colony was sub cultured onto purity blood agar plate (BAP). From a pure culture on a BAP, following were recorded.

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

Single colony of each distinct type was plated on to brucella blood agar plate with antibiotic identification disks.

- The 3 antibiotic identification disks Kanamycin 1 mg, Colistin 10 μg and Vancomycin 5 μg were placed on the first quadrant of the purity BAP, which aided in preliminary grouping of anaerobes and served to verify Gram stain. Zone of inhibition more than 10 mm was considered to be sensitive.
- A nitrate disk was placed on the 2nd quadrant for detection of nitrate reduction.
- SPS disk was used for rapid presumptive identification of Peptostreptococcus species. This bacteria shows a zone of inhibition greater than 12 mm around the disk.

Chocolate agar plate was inoculated for incubation in candle jar at 37^{o}C 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.

Catalase test and spot indole test were done from the colonies on the purity plate.

Anaerobic isolates were stored in Robertson cooked meat medium.

d) Antibiotic susceptibility testing for anaerobic bacteria:

Antibiotic susceptibility testing for anaerobic bacterial isolates was performed using agar dilution method for Metronidazole according to CLSI guidelines M11-A6. [85]

Background information:

- Antibiotic tested: Metronidazole [86]
- Potency: 94 µg/mg
- Solvent: DMSO
- Diluent: distilled water
- Number of petri plates required per dilution is: 1
- Volume of culture medium per plate: 20 ml
- (40 ml medium was prepared for each dilution)
- MIC range tested: 0.125 to 32 µg/ ml

i) Quantity of Antimicrobial powder required-

Weight (mg) = volume (ml) x concentration (µg/ ml)

Assay potency (µg/ mg)

Example: to prepare 10 ml of a stock solution of metronidazole containing 1,000 µg/ ml with potency 94 µg/ mg,

Quantity of the drug required is = 10 ml x 1000 µg/ ml = 106.38 mg

94µg/ mg

ii) Preparation of stock solution[87]**-**

Preparation of 1,000 µg/ ml stock solution:

Required volume is 0.6 ml but a stock solution of 1,000 µg/ ml can be prepared in any one of the following volumes:

Volume (ml)	Weight (mg)
1 ml	10.64
2 ml	21.28
3 ml	31.91
4 ml	42.55
5 ml	53.19
10 ml	106.38

Preparation of 100 µg/ ml stock solution:

Required volume is 0.35 ml but a stock solution of 100 µg/ ml can be prepared in any one of the following volumes:

Volume (ml)	Weight (mg)
1 ml	1.06
2 ml	2.13
3 ml	3.19
4 ml	4.26
5 ml	5.32
10 ml	10.64

The required amount of antibiotic was weighed and dissolved in DMSO. Final volume was made by adding the diluent (Distilled water).

iii) **Preparation of antibiotic dilution plates-**

40 ml of Brucella blood agar with vitamin K and hemin was prepared and allowed to cool in a water bath between 45^{o}C and 50^{o}C. Antibiotic from the stock solution was added using micropipette with sterile tips.

Dilution µg /ml	Volume taken (ml)	Stock solution
32	1.28	1,000 µg /ml
16	0.64	1,000 µg /ml
8	0.32	1,000 µg /ml
4	0.16	1,000 µg /ml
2	0.08	1,000 µg /ml
1	0.04	1,000 µg /ml
0.5	0.2	100 µg /ml
0.25	0.1	100 µg /ml
0.125	0.05	100 µg /ml

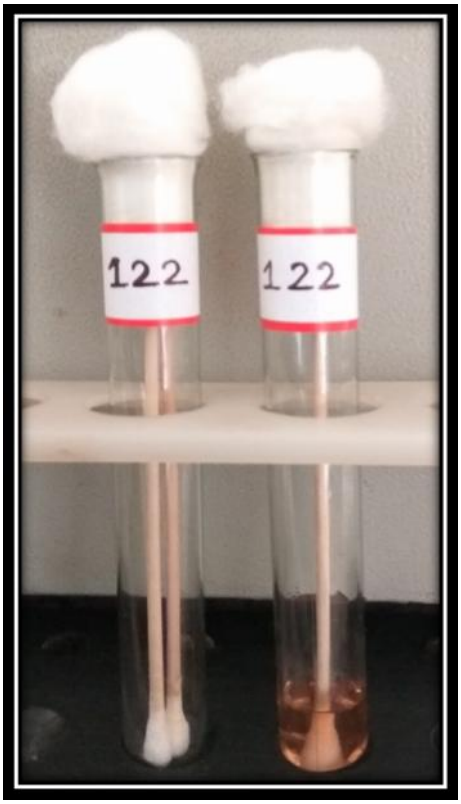
- ✓ The flask was swirled to thoroughly mix the preparation which was subsequently poured into the petri plates on a level surface to a depth of 3-4 mm. The plates were allowed to solidify at room temperature and their antibiotic concentration was labelled on them.

iv) **Procedure for MIC determination by agar dilution [85]-**

All the plates were brought to room temperature and allowed to dry. A plain medium without antibiotic was used as control.

- A suspension of test organism was prepared in Thioglycollate broth with vitamin K and hemin without indicator and its turbidity adjusted was to 0.5 McFarland standards.
- This suspension was diluted 10 times (1 in 10 dilution) using sterile saline. This suspension was tested within 15 minutes.
- The plates were arranged in increasing concentrations and the test tubes were kept as per the grid markings.
- 1-2 µl of this inoculum was transferred onto the agar plate in such a way that it formed a spot of 5-8 mm. The final inoculum on plate was 10^4 cfu/ml.
- The spots on the plate were allowed to dry (10 minutes). They were then inverted and incubated in McIntosh Fildes jar for 48 – 72 hrs.
- Following incubation, the aerobic control plate was checked for the absence of growth on all the spots. The control organism growth was recorded in each plate.
- The end point, the first negative, was read at the point where a marked change in growth appeared as compared with growth on control plate.
- The concentration of antibiotic that had completely inhibited bacterial growth was taken as MIC.
- *Clostridium difficile* ATCC 700057 strain was used as control strain.
- Susceptibility MIC range of this ATCC strain for metronidazole was 0.125-0.5 µg/ml.

PHOTOGRAPHS

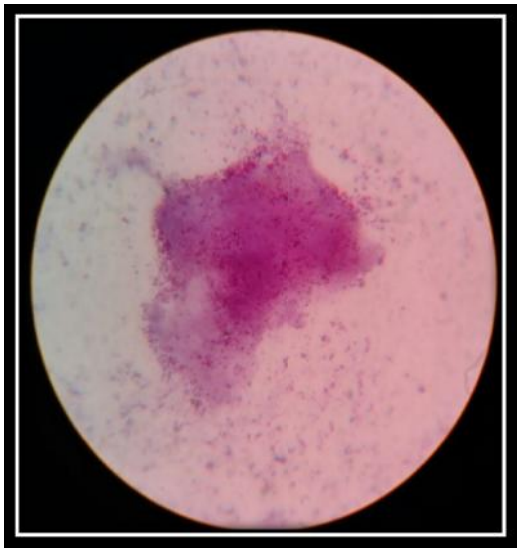


1: Swabs for aerobic and anaerobic cultures

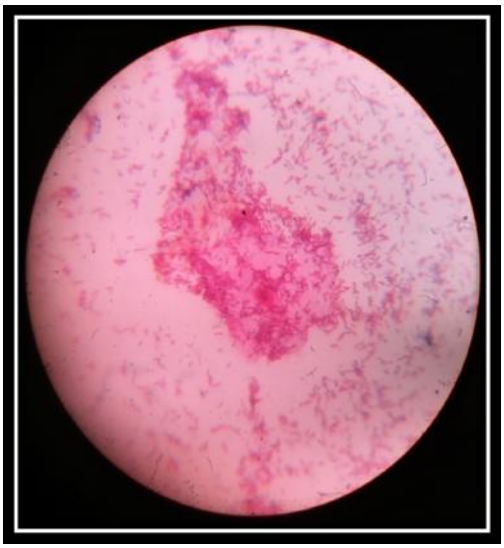
2: pH of vaginal discharge > 4.5 as seen in BV

3:Armamentarium for Whiff test

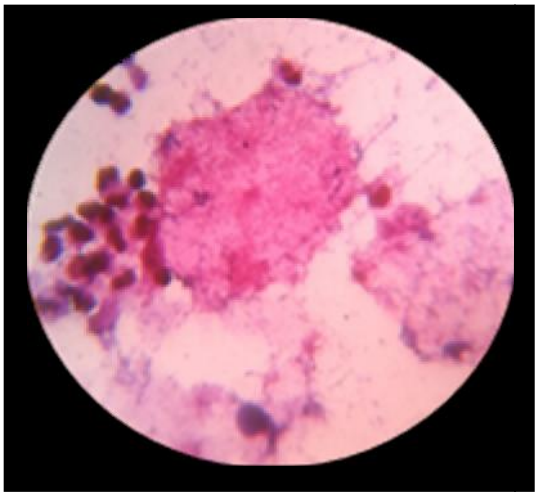
4: Clue cells on wet mount of vaginal discharge



5: Gram stained smear showing clue cells

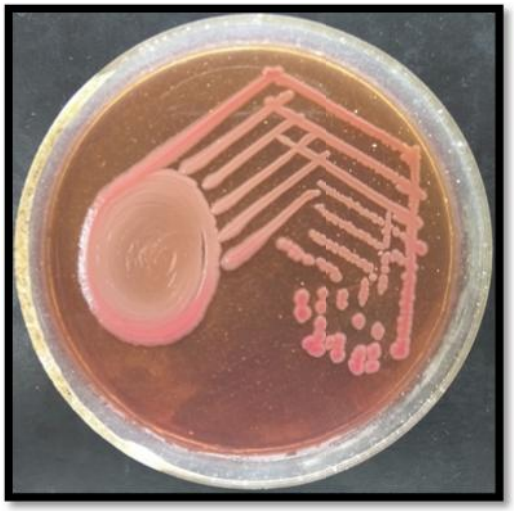


6: Gram stained smear showing clue cells and GNB

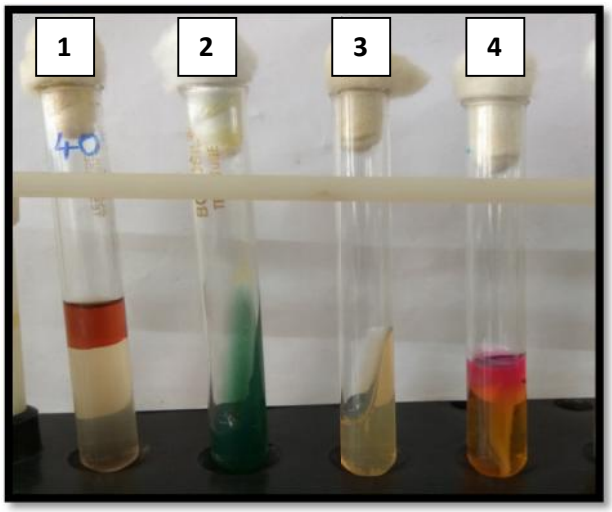


7:Gram stained smear of vaginal discharge showing budding yeast cells

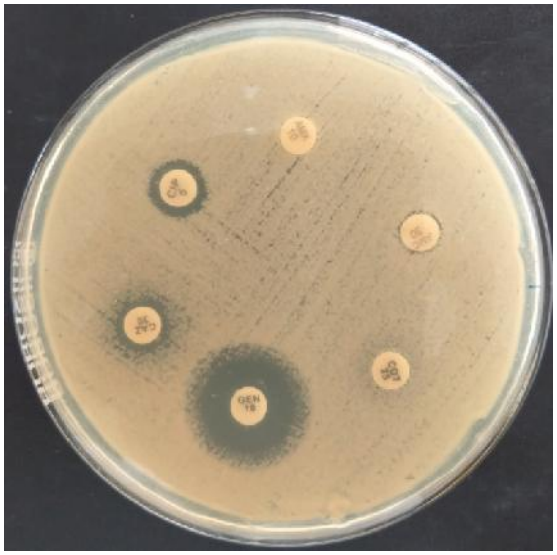
Identification of aerobic bacteria



8: Lactose fermenting colonies of *Escherichia coli* on Mac Conkey agar



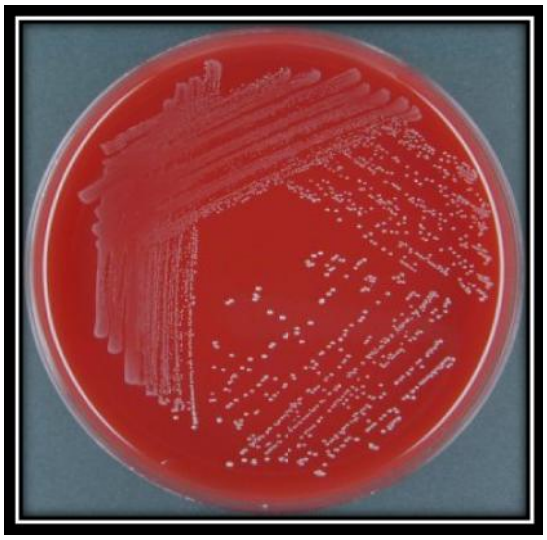
9: Biochemical reactions of *E.coli*
1=*Indole produced*, 2 = *Citrate not utilized*, 3 = *Urea not hydrolyzed*, 4=*Mannitol fermented*



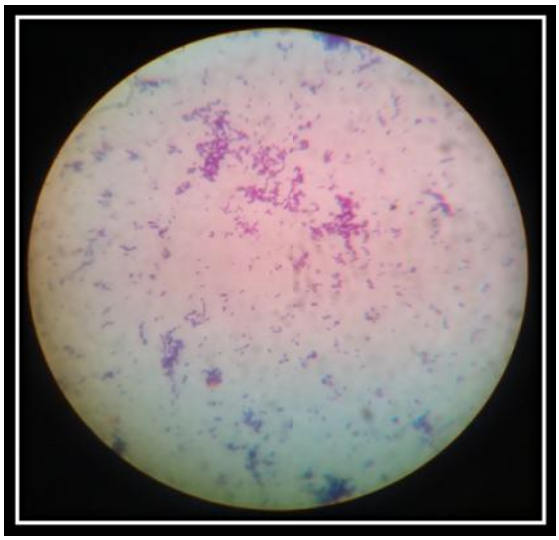
10: Antibiogram of aerobic bacterial isolate showing sensitivity only to Gentamicin

Identification of anaerobic bacteria

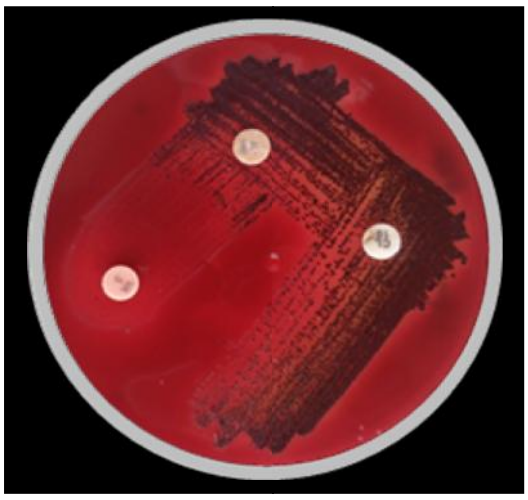
11: McIntosh Filde's anaerobic jar



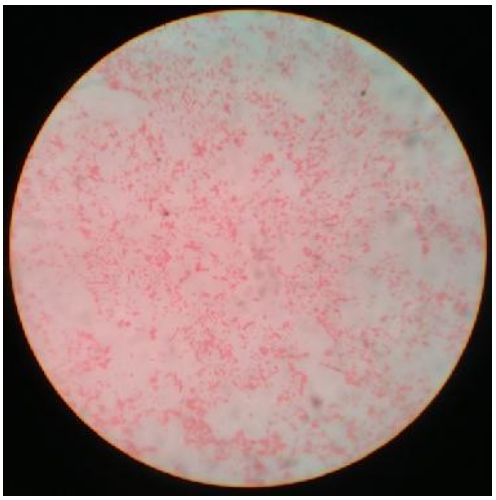
12: Minute grey opaque colonies of Peptostreptococuus spp. on Brucella Blood Agar



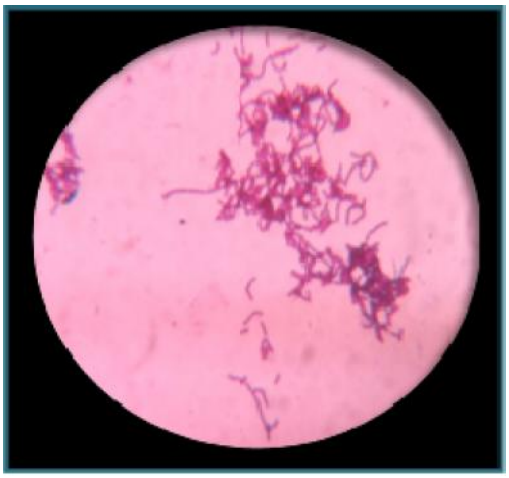
13: Gram stained smear of Peptostreptococuus spp. showing GPC in chains



14: Black opaque colonies of Porphyromonas spp. on brucella blood agar showing sensitivity to Vancomycin and resistance to Colistin and Kanamycin



15: Gram stained smear of Porphyromonas spp. showing pleomorphic GNB

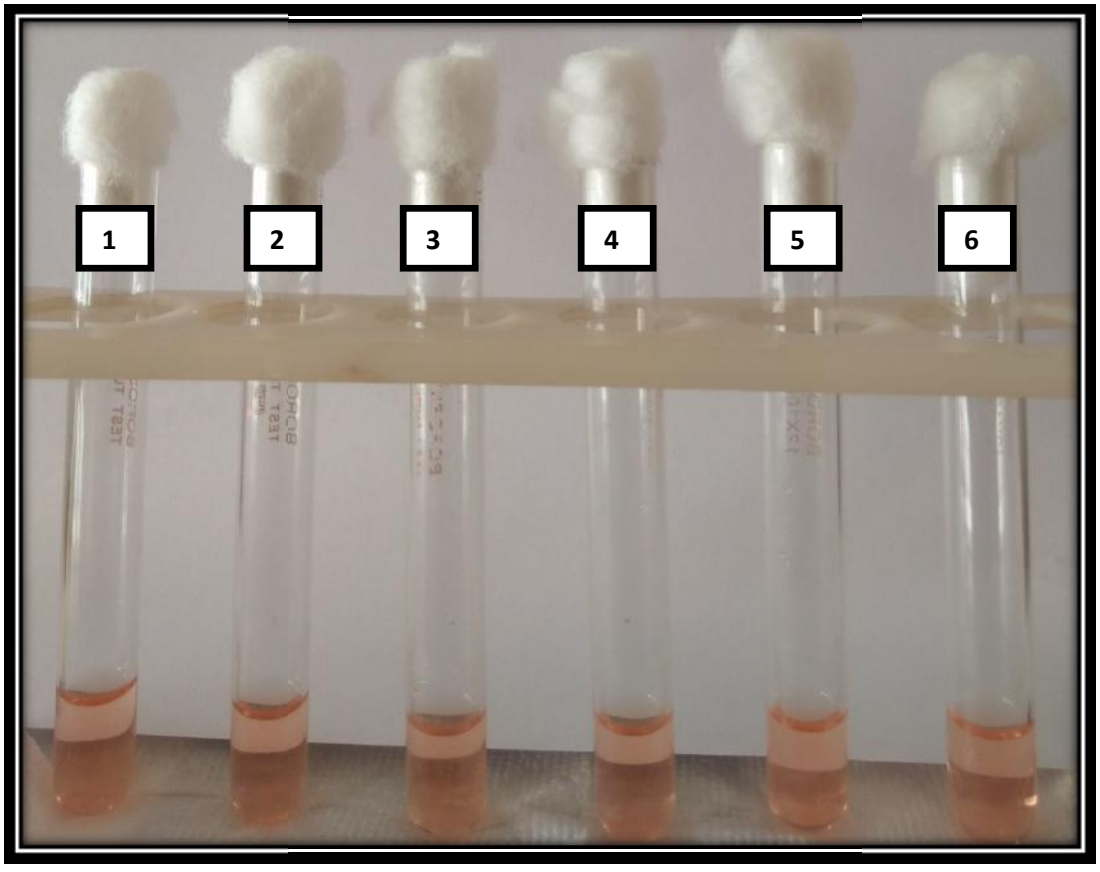


16: Gram stained smear of Fusobacterium showing curved GNB

17: Robertson cooked meat medium

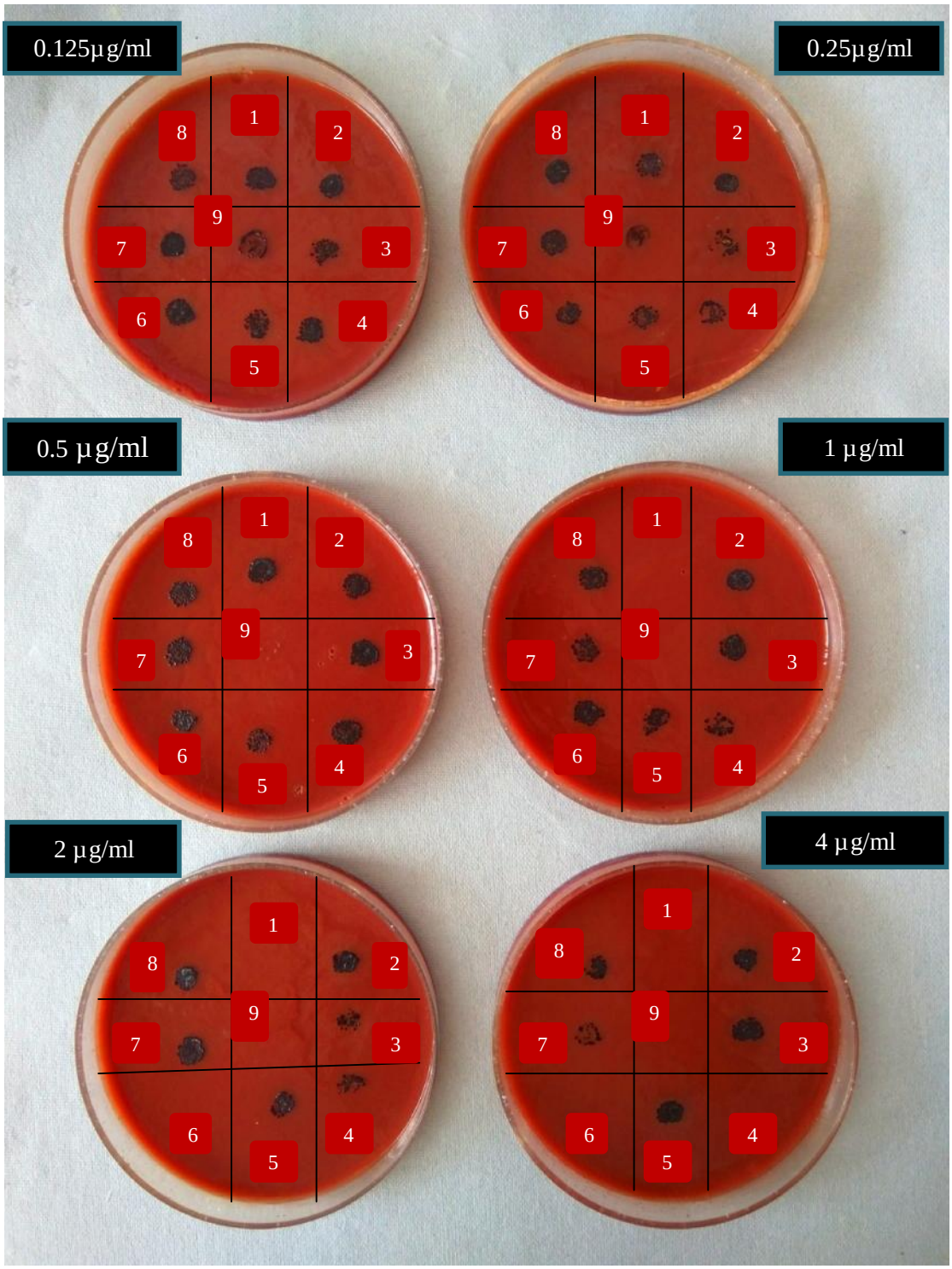
Antibiotic susceptibility testing of the anaerobic bacterial isolates

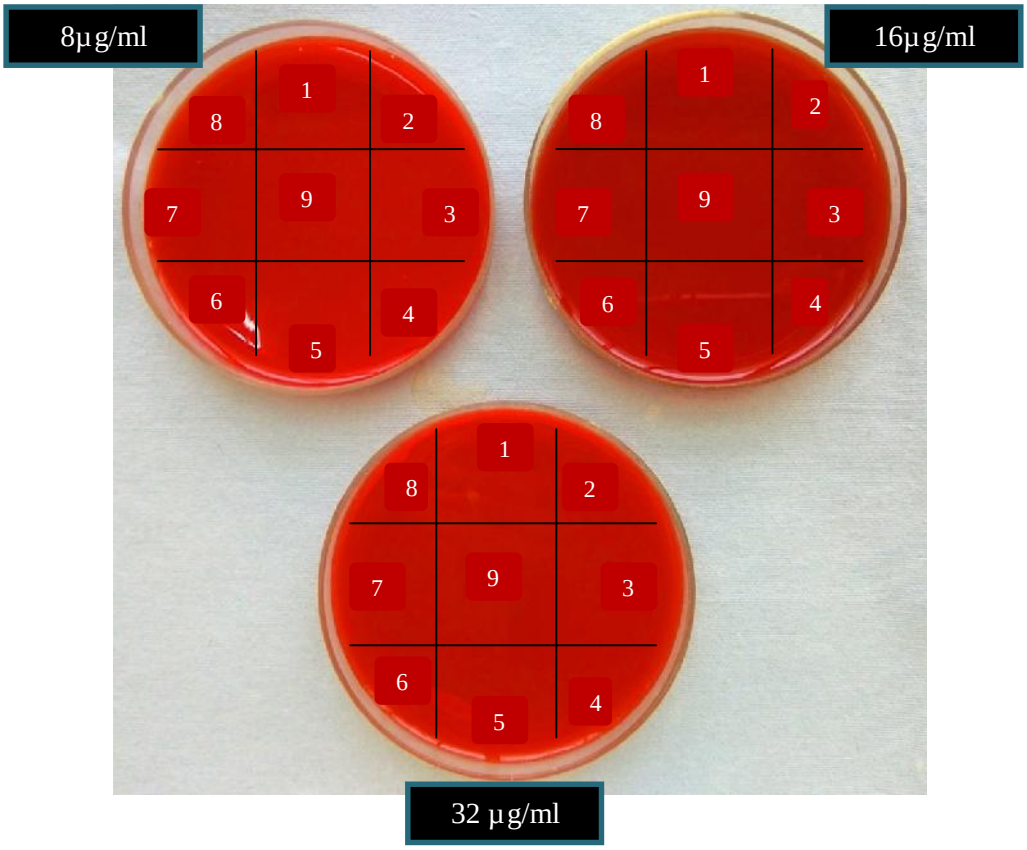
18: Stock solutions of Metronidazole



19: Suspension of anaerobic bacterial isolates (*1 to 6*) in Thioglycollate broth

20:Agar dilution method used to test susceptibility of Porphyromonas spp. to Metronidazole (n=9)





MIC of isolate 9= 0.5µg/ml, MIC of isolate 1= 1 µg/ml

MIC of isolate 6 = 2 µg/ml, MIC of isolate 4=4 µg/ml

MIC of isolates 2,3,5,7 and 8= 8 µg/ml

All the isolates are sensitive to Metronidazole

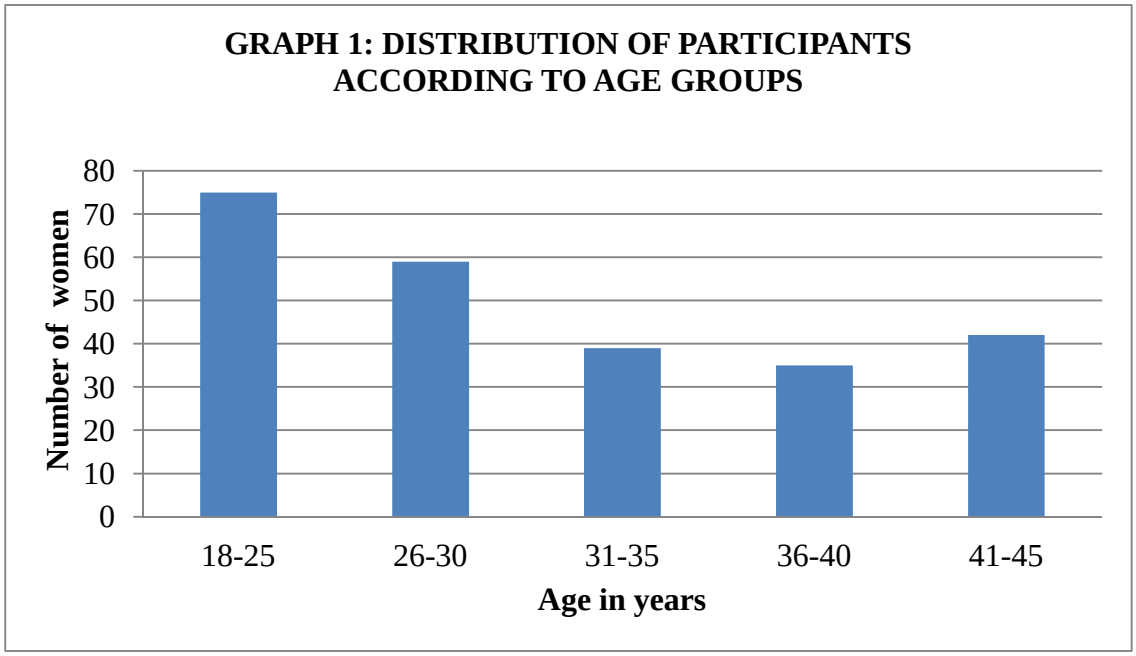
RESULTS

A total of 250 samples were collected from married women in their reproductive age group presenting with complaints of leucorrhea to the OBG OPD at Dr. Prabhakar Kore Charitable Hospital and Research Centre from January 2016 to December 2016.

The samples were processed for isolation, identification and antibiotic sensitivity of both aerobic and anaerobic bacteria.

TABLE1: DISTRIBUTION OF PARTICIPANTS ACCORDING TO AGE GROUPS

Age (in years)	Number of women (N=250)	Percentage (%)
18-25	**75**	**30**
26-30	**59**	**23.6**
31-35	**39**	**15.6**
36-40	**35**	**14**
41-45	**42**	**16.8**



In the present study,

- The participants belonged to reproductive age of 18-45 years.
- Maximum number of women (30%) belonged to the age group of 18-25 years.
- 14% of them were in the age group 36-40 years.

TABLE 2: EDUCATION STATUS OF THE PARTICIPANTS

Education status	Number of participants	Percentage (%)
Illiterates	**15**	**6**
Primary education	**76**	**30.4**
Middle school	**42**	**16.8**
Secondary education	**51**	**20.4**
Graduates	**66**	**26.4**
Total	**250**	

In the present study,

-most of the participants (30.4%) had completed only primary education.

-6% were illiterate.

TABLE 3: SOCIO ECONOMIC STATUS OF THE PARTICIPANTS ACCORDING TO MODIFIED BG PRASAD CLASSIFICATION

Socio economic status	Monthly per capita income in Rupees	Number of participants	Percentage (%)
Upper class	≥5357	17	6.8
Upper middle class	2652-5356	77	30.8
Middle class	1570-2651	136	54.4
Lower middle class	812-1569	20	8
Lower class	<811	0	0
Total		250	

In the present study,

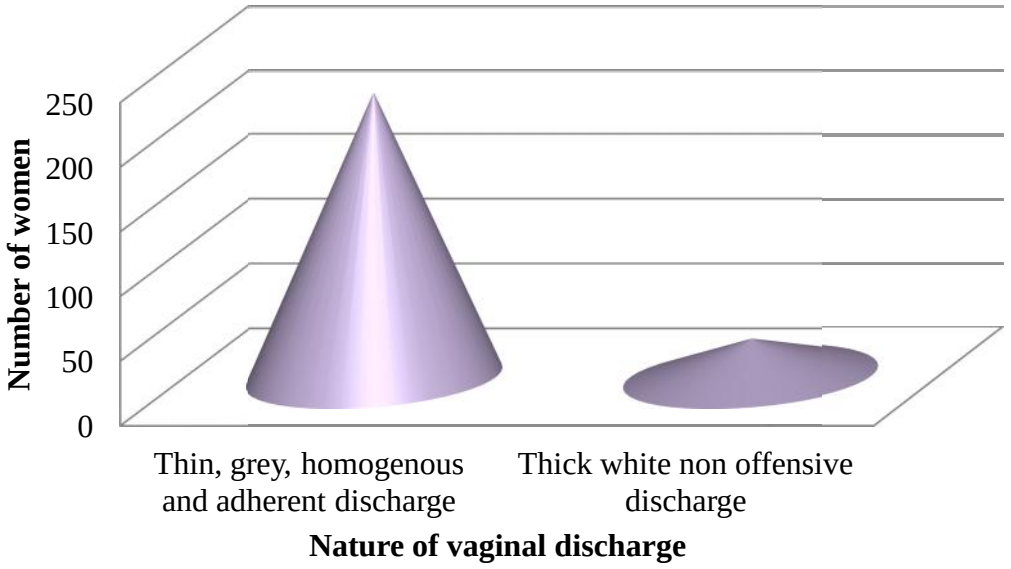
-most of the participants (30.8%) belonged to middle class.

-only 6.8% were from upper class

TABLE 4: NATURE OF VAGINAL DISCHARGE IN THE PARTICIPANTS

Nature of vaginal discharge	Number of women	Percentage (%)
Thin, grey, homogenous, adherent discharge	**220**	**88**
Thick, white, non-offensive discharge	**30**	**12**
Total	**250**	**100**

GRAPH 2: NATURE OF VAGINAL DISCHARGE IN THE PARTICIPANTS



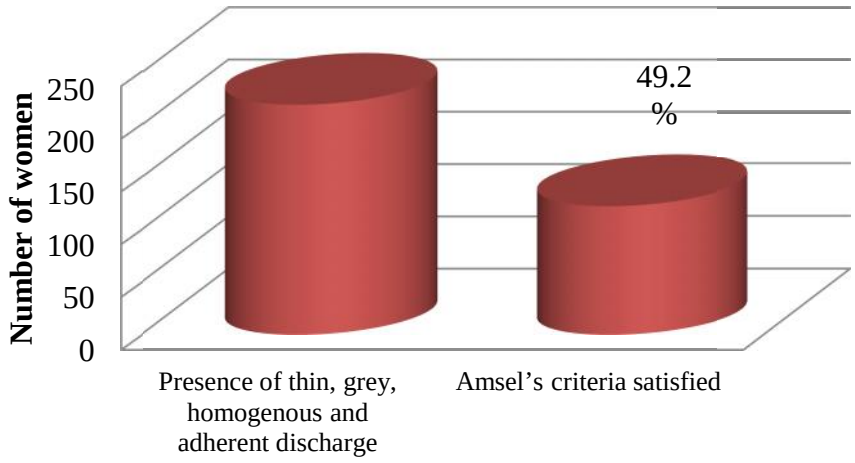
The participants presented with two types of vaginal discharges:

- 88% of them had thin, grey, homogenous adherent discharge
- 30 (12%) of them had curdy, thick, white, non- offensive discharge.

TABLE 5: PREVALENCE OF BACTERIAL VAGINOSIS BY AMSEL'S CRITERIA

Presence of thin, grey, homogenous and adherent discharge	Vaginal discharge of pH> 4.5	Positive Whiff test	Presence of clue cells	Number of women satisfying Amsel's criteria
220	141	41	145	123

GRAPH 3: PREVALENCE OF BV BY AMSEL'S CRITERIA

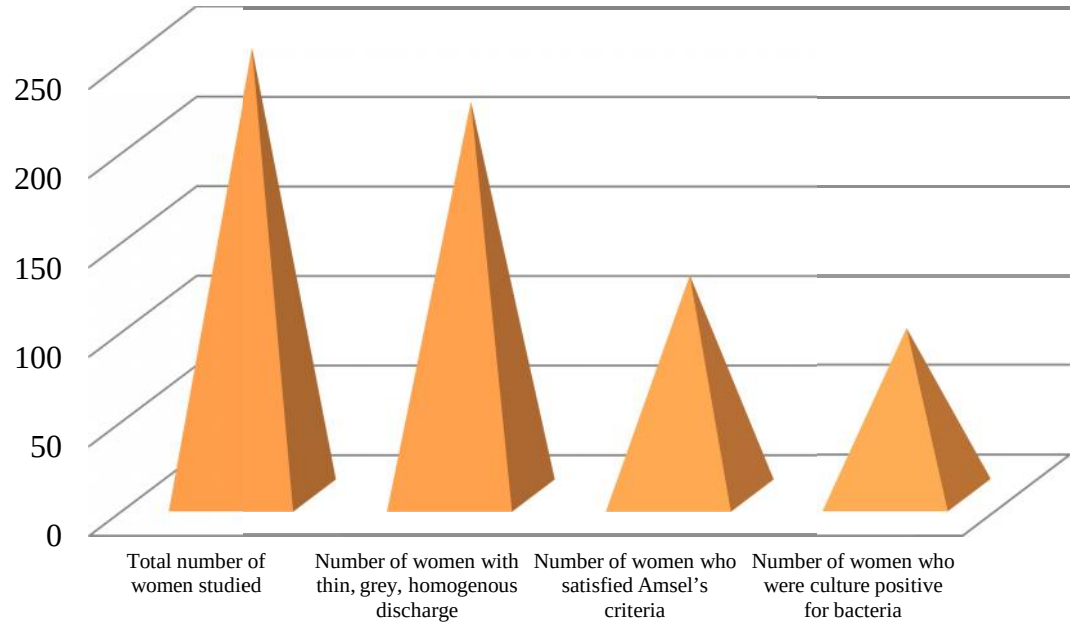


In this study, 220 (88%) women presented with thin, grey, homogenous discharge. Out of them, 123 (49.2%) satisfied Amsel's criteria and were diagnosed to have Bacterial Vaginosis.

TABLE 6:PREVALENCE OF BACTERIAL VAGINOSIS BY CULTURE METHOD

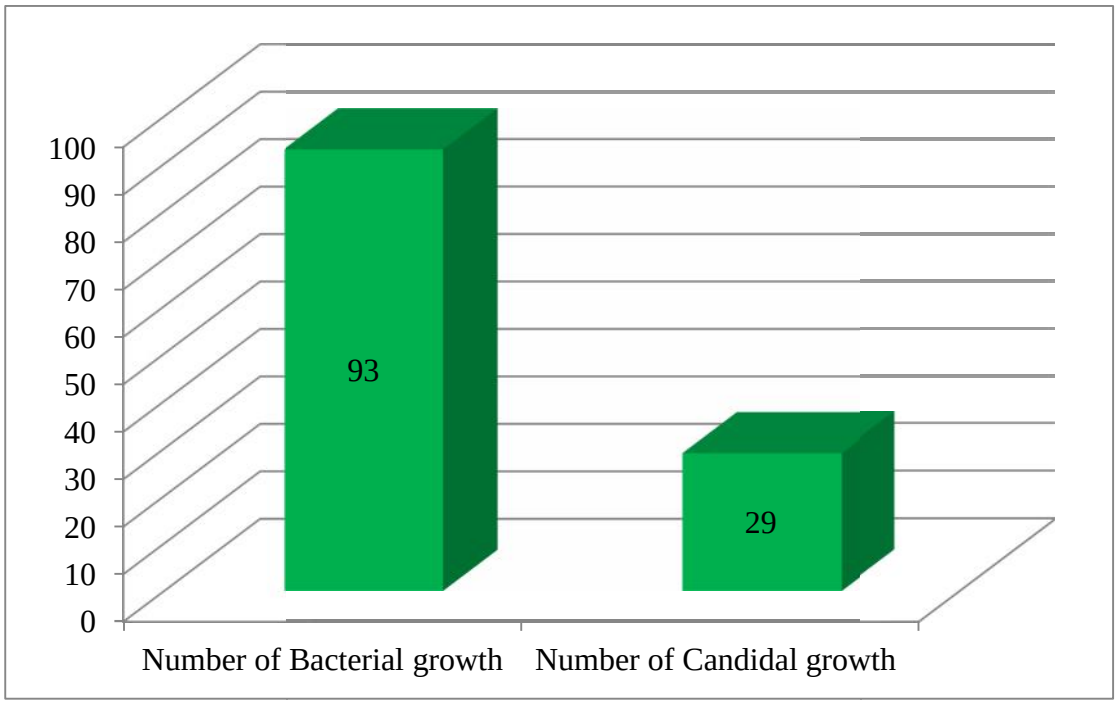
Total number of women studied	Number of women with thin, grey, homogenous discharge	Number of women satisfying Amsel's criteria	Number of women who were culture positive for bacteria
250	220	123	93

GRAPH 4: PREVALENCE OF BV BY CULTURE METHOD



As is evident from the graph, amongst the 250 participants, 93 (37.2%) were positive for bacterial culture.

GRAPH 5: DISTRIBUTION OF BACTERIAL VAGINOSIS AND VAGINAL CANDIDIASIS IN THE PARTICIPANTS BY CULTURE METHOD

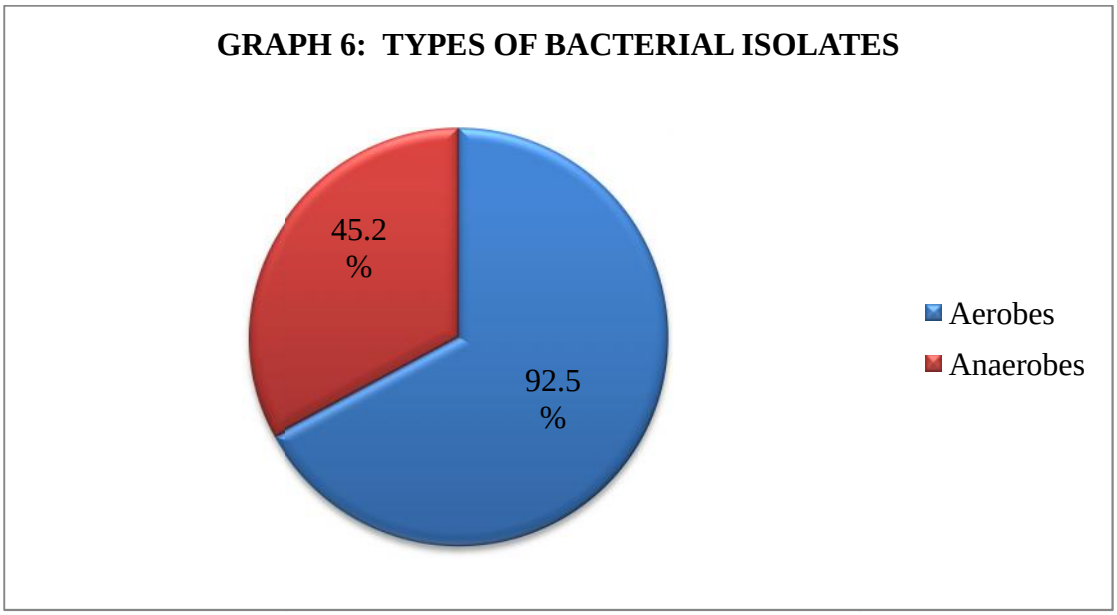


In our study, 93 participants had positive culture for bacteria and 29 were positive for culture of Candida species.

TABLE 7: TYPES OF BACTERIAL ISOLATES

Type of bacteria	Number	Percentage (%)
Aerobes	**86**	**92.5**
Anaerobes	**42**	**45.2**

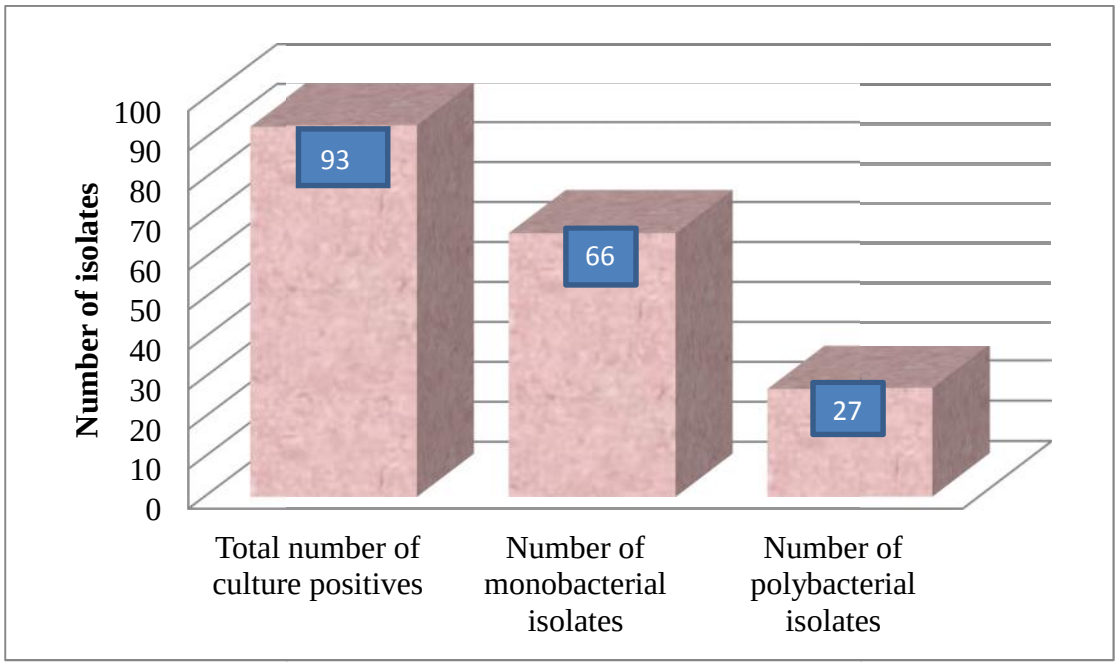
GRAPH 6: TYPES OF BACTERIAL ISOLATES



In the present study,

- Amongst 93 bacterial culture positives, 86 (92.5%) were aerobic bacteria
- 42 (45.2%) were anaerobic bacteria.

GRAPH 7: NUMBER OF MONOBACTERIAL AND POLYBACTERIAL ISOLATES

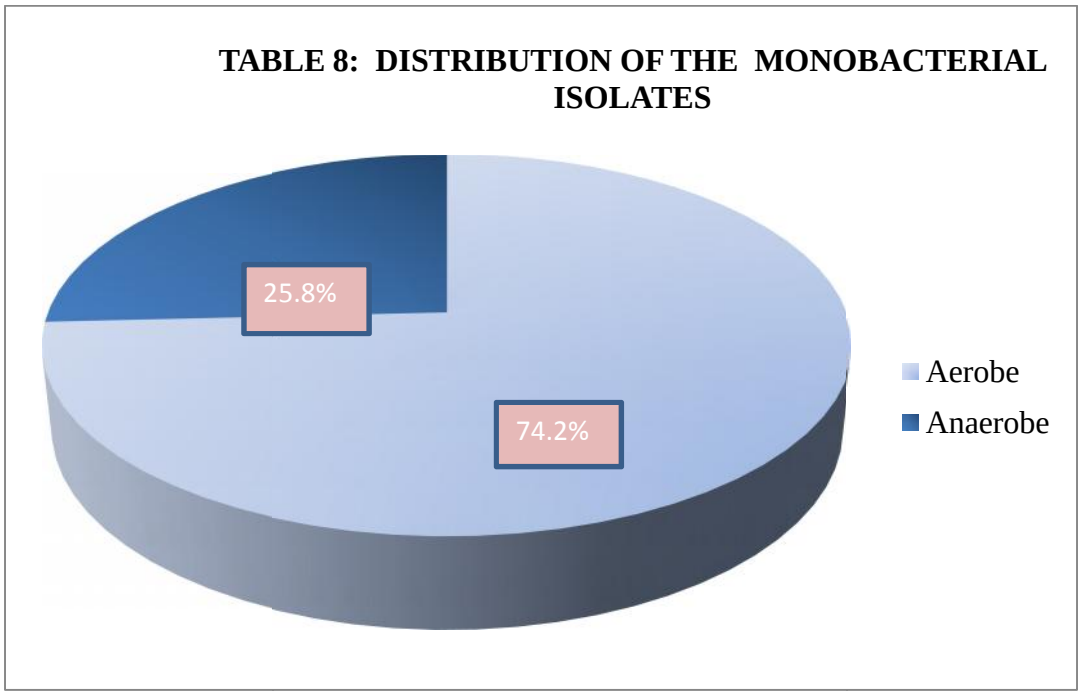


In the present study,

- Out of 93 culture positives, 66 (71%) were monobacterial isolates.
- In 27 (29%) women, polybacterial isolates were observed.

TABLE 8: DISTRIBUTION OF THE MONOBACTERIAL ISOLATES

Type of the monobacterial isolate	Number	Percentage (%)
Aerobe	**49**	**74.2**
Anaerobe	**17**	**25.8**



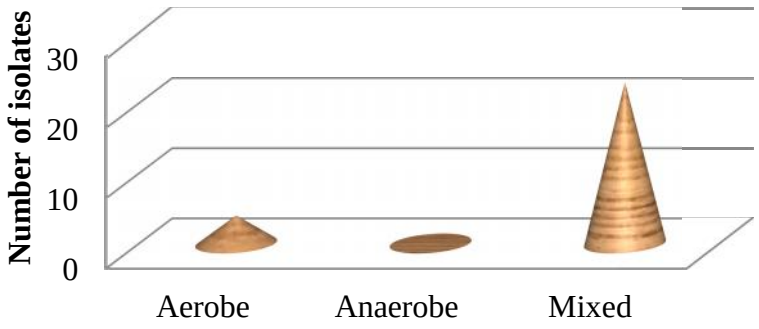
In the present study,

- Out of 66 monobacterial isolates, 49 (74.2%) were aerobes.
- In 17 (25.8%) samples, only anaerobic bacteria were observed.

TABLE 9: DISTRIBUTION OF THE POLYBACTERIAL ISOLATES

Type of the polybacterial isolate	Number	Percentage (%)
Aerobe	**4**	**14.8**
Anaerobe	**0**	**0**
Mixed	**23**	**85.2**
Total	**27**	**100**

GARPH 9: DISTRIBUTION OF THE POLYBACTERIAL ISOLATES



In this study,

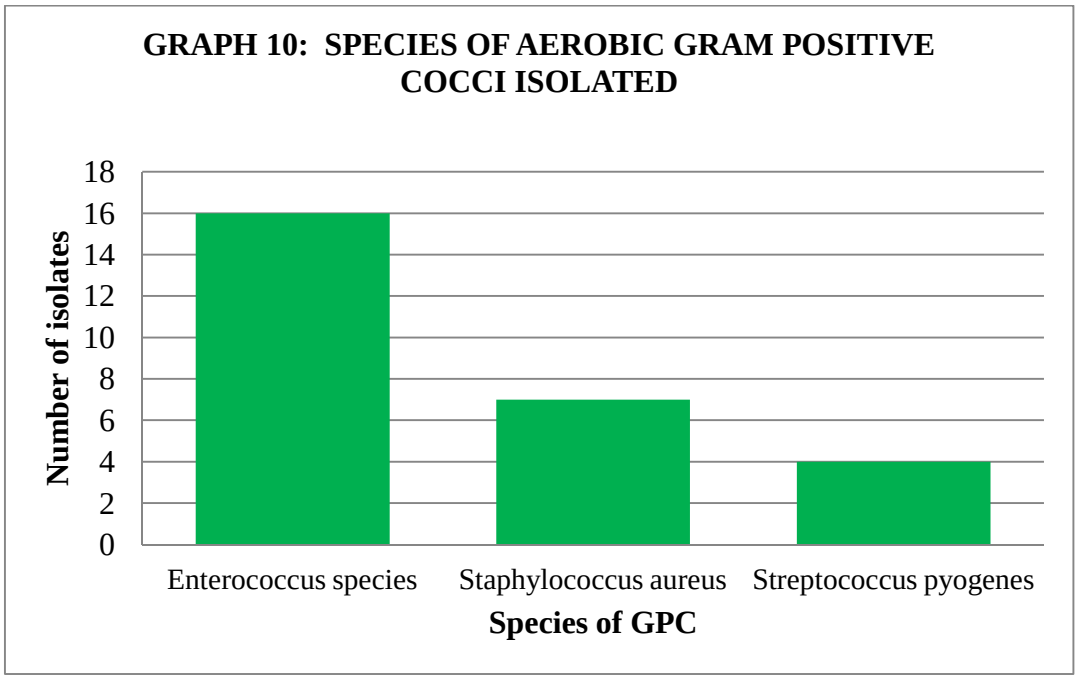
- Amongst 27 women who showed polybacterial growth, 4 were only multiple aerobes and in no woman were multiple anaerobic bacteria isolated.
- In 23 (85.2%) women, there was a mixed infection by both aerobes and anaerobes. Out of these 23 samples, 17 (73.9%) samples had two isolates and 6 (26.1 %) had three isolates.
- Synergy with aerobic bacteria was most commonly noticed with Porphyromonas spp. (11), Bacteroides (4) and Fusobacterium (4) spp.

CULTURE OF AEROBIC BACTERIA:-

Out of 250 samples, 86 aerobic bacteria were isolated. 27 (32.1%) were Gram positive cocci and 59 (68.6%) were Gram negative bacilli.

TABLE 10: SPECIES OF AEROBIC GRAM POSITIVE COCCI ISOLATED

Species of GPC isolated	Number (N= 27)	Percentage (%)
Enterococcus species	**16**	**59.3**
Staphylococcus aureus	**7**	**25.9**
Streptococcus pyogenes	**4**	**14.8**

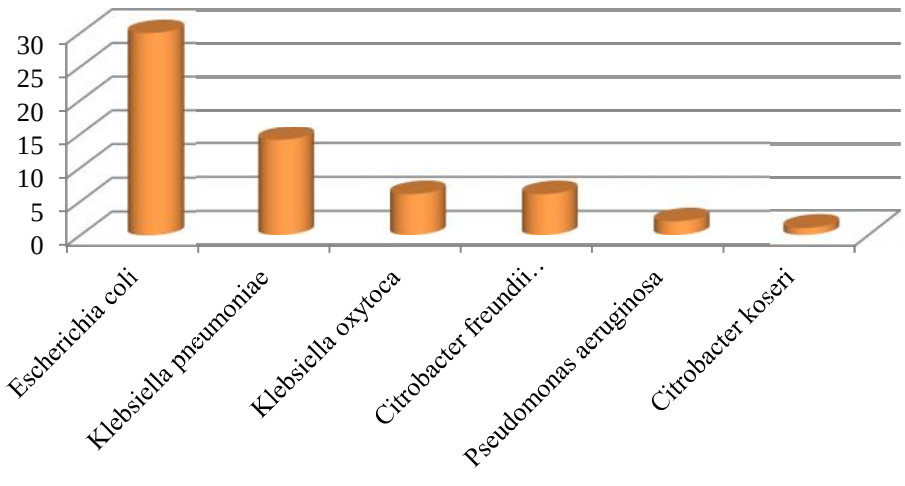


In this study, among the GPC, Enterococcus species (59.3%) was the most common bacteria isolated, followed by *Staphylococcus aureus* (25.9%) and *Streptococcus pyogenes* (14.8%).

TABLE 11: SPECIES OF AEROBIC GRAM NEGATIVE BACILLI ISOLATED

Species of GNB	Number (N= 59)	Percentage (%)
Escherichia coli	**30**	**50.8**
Klebsiella pneumoniae	**14**	**23.7**
Klebsiella oxytoca	**6**	**10.2**
Citrobacter freundii complex	**6**	**10.2**
Pseudomonas aeruginosa	**2**	**3.4**
Citrobacter koseri	**1**	**1.7**

GRAPH 11: SPECIES OF AEROBIC GRAM NEGATIVE BACILLI ISOLATED



In the present study, *Escherichia coli* (50.8%) were the most common GNB isolated followed by *Klebsiella pneumoniae* (23.7%).

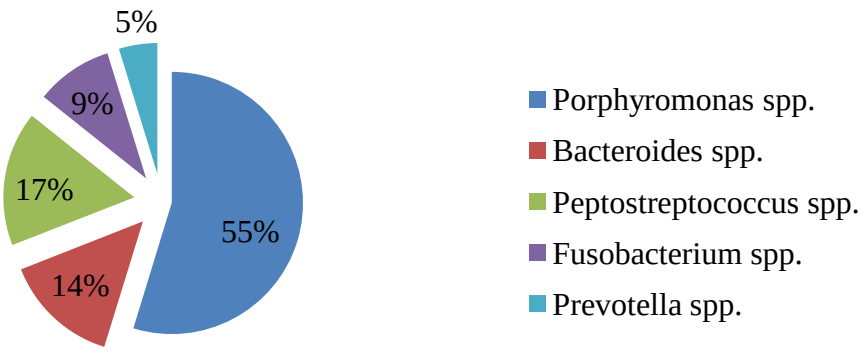
CULTURE OF ANAEROBIC BACTERIA:-

Out of 250 samples, 42 anaerobic bacteria were isolated. Out of them,7(16.7%) were Gram positive cocci and 35 (83.3%) were Gram negative bacilli.

TABLE 12: ANAEROBIC BACTERIA ISOLATED

Anaerobic bacteria isolated	Number (N=42)	Percentage (%)
Porphyromonas spp.	**23**	**54.8**
Peptostreptococcus spp.	**7**	**16.7**
Bacteroides spp.	**6**	**14.3**
Fusobacterium spp.	**4**	**9.5**
Prevotella spp.	**2**	**4.8**

GRAPH 12: ANAEROBIC BACTERIA ISOLATED



As seen in the above table and graph, more than half of the anaerobic bacteria isolated were Porphyromonas spp. (54.8%), followed by Peptostreptococcus spp. (16.7%).

ANTIBIOTIC SUSCEPTIBILITY TESTING OF AEROBIC BACTERIAL ISOLATES

Antibiotic susceptibility testing for aerobic bacterial isolates was done by Kirby Bauer disk diffusion method for the commonly used antibiotics whose results are as shown in the tables 13(a) and 13 (b).

TABLE 13 (a): ANTIBIOGRAM OF AEROBIC GRAM POSITIVE COCCI

		Antibiotic Sensitivity															
Isolates	**Number**	**Amc**		**Amp**		**C**		**Cd**		**Cip**		**Cot**		**E**		**Va**	
		S	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**	**S**	**R**
Enterococcus spp.	**16**	**7**	**9**	**8**	**8**	**10**	**6**	**-**	**-**	**5**	**11**	**-**	**-**	**6**	**10**	**16**	**0**
S.aureus	**7**	**4**	**3**	**2**	**5**	**-**	**-**	**3**	**4**	**1**	**6**	**2**	**5**	**5**	**2**	**-**	**-**
S. pyogenes	**4**	**4**	**0**	**3**	**1**	**3**	**1**	**-**	**-**	**1**	**3**	**-**	**-**	**2**	**2**	**-**	**-**

In the present study, 74.1% of theGPC were found to be resistant to Fluoroquinolones (Ciprofloxacin), 51.9% eachto Ampicillin and Macrolides (Erythromycin) each.

A combination of Ampicillin and Clavulanic acid performed better, with55.6% GPC showing sensitivity.

None of the Enterococcus spp.was resistant to Vancomycin.

TABLE 13 (b): ANTIBIOGRAM OF AEROBIC GRAM NEGATIVE BACILLI

Isolates	Number	Amc		Amp		Caz		Cip		Cot		Gen		Pi	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R
E.coli	30	12	18	10	20	19	11	14	16	13	17	22	8	-	-
Klebsiella spp.	20	6	14	7	13	15	5	14	6	8	12	11	9	-	-
Citrobacter spp.	7	0	7	0	7	5	2	5	2	3	4	6	1	-	-
P.aeruginosa	2	0	2	0	2	2	0	-	-	-	-	2	0	2	0

In the present study,

- Most (71.2 %) of the GNB were resistant to Ampicillin, followed by Amoxicillin+ clavulanic acid (69.5%).
- GNB showed higher sensitivity to Ceftazidime and Gentamicin, 69.5% each.

ANTIBIOTIC SUSCEPTIBILITY TESTING OF ANAEROBIC BACTERIAL ISOLATES

TABLE 14: SUSCEPTIBILITY PATTERN OF ANAEROBIC BACTERIAL ISOLATES TO METRONIDAZOLE

Organism	Sensitive (≤8 μg/ml)	Intermediate (16 μg/ml)	Resistant (≥32 μg/ml)
Porphyromonas spp. n= 23	**23**	**0**	**0**
Peptostreptococcus spp. n= 7	**3**	**0**	**4**
Bacteroides spp. n= 6	**4**	**1**	**1**
Fusobacterium spp. n= 4	**4**	**0**	**0**
Prevotella spp. n= 2	**2**	**0**	**0**
Total = 42	**36**	**1**	**5**

In the present study,

- 36 (85.7%) anaerobic bacterial isolates were sensitive to metronidazole.
- 1 (2.4%) isolate was with an intermediate MIC of 16 μg/ml
- 5 (11.9%) were resistant to metronidazole
- 4 (57.1%) out of 7 isolates of Peptostreptococcus spp. and 1 (16.7%) out of 6 Bacteroides spp. were resistant to metronidazole.
- 1 isolate of Bacteroides spp. was intermediate (MIC 16μg/ml) in its susceptibility to Metronidazole.
- None of the Porphyromonas spp., Fusobacterium spp. or Prevotella spp. was resistant to Metronidazole.

DISCUSSION

A total of 250 women were enrolled in the present study. Samples were collected from married women in their reproductive age group who complained of excessive vaginal discharge.

In our study, maximum number of cases 75 (30%) belonged to 18-25 years of age followed by 23.6% in 26-30 years age group, suggesting a higher prevalence of leucorrhea in younger age group. This was in accordance to a study conducted by Leela KP et al.[5] in which 30% of women belonged to the age group 15-25 years. Two other studies by Afroze N et al.[3] and Sarwat F et al.[11] reported similar findings of 44.8% and 54% respectively .This can be attributed to the probability of women being more sexually active in this age group.

In the present study, 97.2% of the women were from urban area, 30.4% had completed only primary education, 74.4% were housewives and 54.4% women belonged to class III of socio economic status according to modified BG Prasad classification. These findings were similar to the observed data by Afroze N et al.[3], Bhalla P et al.[88] and Rani U et al.[24] Substandard hygiene, ignorance, low socio economic status and traditional taboo against openness about these diseases are the usual factors responsible for the high prevalence in these women.

The most common presenting complaints of the women included in our study were foul smelling vaginal discharge, pruritus, irregular menstrual cycle, lower abdominal pain and burning micturition. Excessive vaginal discharge can be physiological or pathological. Physiological leucorrhoea is caused by congestion of the vaginal mucosal membranes due to hormonal stimulation. This may occur during pregnancy.[89] Hormonal therapy, IUCDs and OCPs increase physiological

leucorrhea.[47,90]In our study, pregnant women, women with IUCDs and on OCPs were excluded.

Women with BV are at an increased risk of acquisition of infections with HIV, *N. gonorrhoeae, C. trachomatis,* and HSV- 2and present with recurrent leucorrhea.[64] These infections are sexually transmitted. None of the women in our study gave history of any of these STDs.

Amongst 250 women, 220 (88%) of them had excessive thin, grey, homogenous malodorous discharge and 30 (12%) had thick, white, non-offensive discharge. Chaudhary V et al.[91] reported that 87.8% of the women in their study had excessive vaginal discharge. Chen et al. in his two studies conducted in 1979 and 1982 suggested that putrescine and cadaverine were the two most abundant amines in vaginal fluid of women with untreated non-specific vaginitis which were the cause for malodor of vaginal discharge.

Out of 250 women studied, BV was diagnosed in 123 (49.2%) women using Amsel's clinical composite criteria, which is considered to be the gold standard for the diagnosis of Bacterial Vaginosis.[3,92] Similar prevalence of BV was documented by Puri KJ et al.[93] (45%), and Kamara P et al.[94](44.1%).

Amsel's clinical composite criteria require three out of four of the following to be satisfied:

- Presence of thin, grey, homogenous discharge,
- Vaginal pH of > 4.5,
- Positive Whiff test and
- Presence of clue cells.

In the present study, thin, grey, homogenous discharge was present in 220 (88%) of the women studied. In 141 (56.4%) of them, vaginal pH was > 4.5. Whiff test was positive in 41 (16.4%) and clue cells were present in 145 (58%) women. Similar findings were reported in a study conducted by Rani U et al.[24]Out of 130 women, 35.38% of them had profuse vaginal discharge, 96% had a vaginal pH of > 4.5, 44% showed positive Whiff test and 42% of them had clue cells. Presence of >20% clue cells on a wet mount is the single most reliable predictor of BV.[95]

In the present study, out of 250 women, 30 of them had thick, white non offensive vaginal discharge. 29 (11.6%) of the total women studied were culture positive for Candida species. Joshi et al.[96] and Bandi et al.[97] have reported similar prevalence (10%) of vaginal candidiasis in their respective studies. Khammes S et al.[47] found 11.9% of candida vaginosis in their study. In most of the studies vaginal candidiasis has been found to be the cause of leucorrhea, next only to BV. It is more commonly seen in pregnant women compared to non- pregnant women.

Out of 250 women studied, 93 (37.2%) of them were positive for bacterial culture, 11.6% had candidal vaginosis and 3 of them had mixed infection. 131 (52.4%) samples were culture negative. According to Donger G et al.[98], in 10-58% cases etiological diagnosis cannot be found. This may be due to infection with *C.trachomatis, M.hominis* or other fastidious bacteria which are difficult to grow.

Amongst the 93 bacterial cultures, 86 (92.5%) yielded aerobic bacterial isolates and in 42 (45.2%) anaerobic bacteria were isolated. 66 (71%) isolates were monobacterial and 27 (29%) were polybacterial.

In BV, Lactobacilli are replaced with the increased population of pathogenic facultative and anaerobic bacteria. Amongst the 86 aerobic bacterial isolates, 27 (32.1%) were Gram positive cocci and 59 (68.6%) were Gram negative bacilli. This coincides with a study conducted by Khamees et al.[47]7 (16.7%) of the anaerobic bacterial isolates were Gram positive cocci and 35 (83.3%) were Gram negative bacilli. This was in accordance with the study conducted by Sumati A et al.[49] in which 74% of the anaerobic bacteria isolated were GNB.

The most common aerobic bacteria isolated was *E. coli* (34.9%) followed by Klebsiella species (23.3%). The others were Enterococcus spp., *S.aureus*, Citrobacter spp., *S. pyogenes* and *P. aeruginosa.* Mulu W et al. in an Ethiopian study isolated *E.coli*, Pseudomonas spp. and *S.aureus* frequently.[99] In a study conducted by Afzal H et al. at Faisalabad, the aerobic bacterial isolates comprised of 21.9% *S. aureus*, 16.6% *S. pyogenes* and 14.6% *E. coli.* [100] In another study conducted by Duangmani C et al. in Bangkok, 53.8% were *Staphylococcus epidermidis*, 1.2% *S.aureus,* 35.8% were *alpha hemolytic Streptococci*, 8.9%*E. coli* and 5.2% *Enterobacter* species [48].

Table showing different aerobic bacterial isolates in four different studies:

Study	Place	*E.coli*	Klebsiella spp.	Citrobacter spp.	*Pseudomonas aeruginosa*	Enterococcus spp.	*S.aureus*	*S.pyogenes*
Present	Belagavi	34.9%	23.3%	8.1%	2.3%	18.6%	8.1%	4.7%
Afzal H et al.	Faisalabad	14.6%	-	-	-	-	21.9%	16.6%
Duangmani C et al.	Bangkok	8.9%	-	-	-	-	1.2%	-
Khamees SS et al.	Lybia	14.2%	13.6%	-	-	-	21.8%	-

Amongst the anaerobic bacterial isolates, Porphyromonas spp. (54.8%) was the most common followed by Peptostreptococcus spp. (16.7%), Bacteroides spp. (14.3%), Fusobacterium spp. (9.5%) and Prevotella spp. (4.8%). In a study by Agarwal et al.[101] at Amritsar in 2003, 53.3% Peptostreptococcus spp., 16.7% Bacteroides spp., 10% Prevotella spp., 4% Fusobacterium spp. were isolated. In a similar study by Sumati A et al.[49], 30.1% Bacteroides spp., 13% Peptococcus spp., 8.9% Peptostreptococcus spp., 12% Veillonella spp. 7.5% each of Prevotella spp. and Fusobacterium spp. were isolated. The difference in the type of and rate of isolation of these bacteria reflects the difference in the study population, different time period and methods of investigations.

Table showing different anaerobic bacterial isolates in three different studies:

Study	Place	Porphy-romonas spp.	Pepto-strepto-coccus spp.	Bacteroides spp.	Fusobacterium spp.	Prevotella spp.	Peptococci spp.	Veilonella spp.
Present	Belagavi 2016	54.8%	16.7%	14.3%	9.5%	4.8%	-	-
Agarwal et al	Amritsar 2003	14.6%	53.3%	16.7%	4%	10%	21.9%	16.6%
Sumati A et al	Belagavi 2009	-	8.9%	30.1%	7.5%	7.5%	13%	12%

The WHO and NACO have developed and advocated the Syndromic management approach for the treatment of vaginal discharge. This approach is based on the identification of a relatively constant combination of symptoms and signs and on the knowledge of the most common causative organisms of these syndromes and their antimicrobial susceptibility. Antimicrobial regimens are chosen to cover the major pathogens responsible for the syndromes. Metronidazole is the drug most

commonly prescribed in the blanket therapy which targets anaerobic bacteria and *T. vaginalis*. The main disadvantage of this management is the cost of over diagnosis and over treatment when multiple antimicrobials are given to patient where infection is caused by none or only one organism. Also to be considered are the risks of adverse drug reactions, alteration of normal vaginal flora and also the potential for developing antibiotic resistance in the community.[9,102]

In the present study, susceptibility of aerobic bacterial isolates was tested for the commonly used antibiotics in daily practice. Maximum resistance (74.1%) amongst the GPC was to Ciprofloxacin. 51.9% of GPC and 65.1% of the GNB were resistant to Ampicillin. Resistance to Cotrimoxazole was found to be 43.8%. This was in accordance with a study conducted by Mulu W et al.[99] in which resistance to Ampicillin and Cotrimoxazole was found to be 62.2%-82.2%. In the same study, *S.aureus* showed resistance ranging between 67-83% to Ampicillin and Cotrimoxazole. This coincides with our study in which 71.4% *S. aureus* were resistant to Ampicillin and Cotrimoxazole.

Semisynthetic Penicillins like Amoxicillin combined with a beta lactam inhibitor like Clavulanic acid proved to be better for the GPC. Our study found that 55.6% of GPC to be susceptible to this combination.

Aminoglycosides like Gentamicin and a third generation Cephalosporin (Ceftazidime) were more efficient with 69.5% of the total aerobic isolates showing sensitivity to each of them. This coincides with the findings of Mulu W et al.[99]

Our study showed 48.1% isolates to be sensitive to Erythromycin which is comparable to 68% recorded by Afzal H et al.[100] in his study.

Susceptibility testing of anaerobic isolates was done for MTZ as it is the most common drug used against anaerobes in the syndromic management. In our study, out of 42 anaerobic bacterial isolates, 36 (85.7%) were sensitive, 1 (2.4%) was intermediate and 5 (11.9%) were resistant to Metronidazole.

Amongst the 5 Metronidazole resistant isolates, four were Peptostreptococcus spp. and one was Bacteroides spp. with a high MIC $\geq$32 μg/ ml. None of the Porphyromonas spp., Fusobacterium spp. or Prevotella spp. showed resistance to Metronidazole.

Though Metronidazole is still considered to be the drug of choice for anaerobic bacteria, an increase in their resistance to the said drug is being noticed. *Bacteroides fragilis* with a high MIC $\geq$32 μg/ ml have been isolated in various studies. [103]The genes responsible for resistance to MTZ are *nim* genes which are present on chromosomes as well as plasmids. They can be transferred by conjugation. These Metronidazole resistant *B.fragilis* isolates are a cause of concern as they are resistant to a wide variety of antimicrobial agents including beta lactams, tetracycline, clindamycin, cefoxitin and imipenem. [104]Doxycycline, which belongs to the tetracycline group of antibiotics, is also a part of the blanket therapy. Infection due to MTZ resistant strains when managed by the syndromic approach may fail therapeutically. This emphasizes the need to assess more accurately the susceptibilities of clinical anaerobic bacterial isolates.

CONCLUSION

Our study showed that leucorrhea is mainly due to BV or Candidal vaginosis. As these infections can be treated effectively with specific agents, microbiological diagnostic approach is ideal for the etiological diagnosis of symptomatic vaginal discharge.

However, in resource constrained setting, Amsel's clinical composite criteria can be used for the diagnosis of BV which is an easy and efficient method.

It was observed that the microflora of vagina is a diverse mixture of aerobic and anaerobic bacteria. Aerobic bacteria were the predominant bacterial population. GNB were more common than GPC amongst both aerobes and anaerobes. *E.coli* and Porphyromonas spp. were the most frequently isolated aerobic and anaerobic bacteria respectively.

Aerobic bacterial isolates were found to be mainly resistant to Ciprofloxacin and Ampicillin. A combination of Ampicillin and Clavulanic acid proved better against GPC where as GNB had a higher sensitivity to Gentamicin and third generation Cephalosporins. Metronidazole resistant anaerobic bacteria were found to be prevalent.

Periodic antibiogram of the bacterial strains should be carried out to be aware of the changes in their resistance pattern. This will help us in formulating a better empirical or definitive therapy for treating women with symptomatic vaginal discharge.

The role of *nim* genes in causing resistance of the anaerobic bacterial isolates to Metronidazole can be further studied and co related with the results of phenotypic methods of detection of resistance.

SUMMARY

- The present study was conducted on 250 women in their reproductive age group with complaints of excessive vaginal discharge, attending the OBG OPD at KLE'S Dr. Prabhakar Kore Charitable Hospital and Reference Centre, Belagavi between the period January to December 2016.
- Three high vaginal swabs were collected from these women and processed in the Department of Microbiology, JNMC, Belagavi.
- Most of the women (30%) were in the age group of 18-25 years, 30.4% had finished only primary education and 54.4% of them belonged to the middle class.
- The most common presenting complaint of the women was foul smelling watery vaginal discharge.
- 49.2% of the women were diagnosed to have Bacterial Vaginosis by Amsel's clinical composite criteria.
- 93 women were culture positive for bacteria and 29 were culture positive for Candida.
- Amongst the 93 bacterial culture positive women, 86 were aerobic bacterial isolates and 42 were anaerobic bacteria.
- *E.coli*, followed by Klebsiella spp. and Enterococcus spp. were the most commonly isolated aerobic bacteria.
- Amongst the anaerobic bacteria, Porphyromonas spp. was the most frequently isolated bacteria followed by Peptostreptococcus spp. and Bacteroides spp.
- Most of the aerobic isolates showed resistance to Ciprofloxacin and Ampicillin. Aerobic GPC showed better sensitivity to a combination of Amoxicillin with Clavulanic acid where as aerobic GNB were had a higher sensitivity to Gentamicin and Ceftazidime.

- Amongst the 42 anaerobic bacterial isolates, 5 of them were resistant to Metronidazole. Four of these resistant isolates belonged to Peptostreptococcus spp. and one was Bacteroides spp. One other Bacteroides spp. had intermediate MIC.

APPENDIX

1. Gram stain Procedure: Hucker's modification: [80]

Principle: After treatment with decolorizing agents, gram positive bacteria retain para- rosaniline dyes and appear violet colorwhile gram negative bacteria lose the dye and take up counter stain and appear pink in color.

Procedure:

a) A clean grease free glass slide was labelled and a thin smear was made on it using the first high vaginal swab and allowed to air dry.

b) The smear was fixed by passing the slide three to four times through the flame of a Bunsen burner.

c) Slide was then placed on the slide rack and the smear overlaid with crystal violet solution.

d) After 20 seconds, the slide was washed thoroughly with tap water.

e) Subsequently, the smear was overlaid with Gram iodine solution for 20 seconds and washed again with water.

f) The smear was held between the thumb and fore finger and the surface flooded with a few drops of acetone-alcohol decolorizer, until no color washed off.

g) The smear was washed with running water and placed back on the staining rack. Surface of the smear was overlaid with safranin (counter stain) for 10 seconds and washed with running water.

h) The slide was placed in an upright position in a rack, allowing excess water to drain off.

i) The stained smear, after being dried was examined under 100 X (oil) immersion objective lens.

Quality control: Gram positive: *Staphylococcus aureus* ATCC 25923

Gram negative: *Escherichia coli* ATCC 25922

2. Jensen's Gram stain Procedure:[81]

This method uses alcohol as decolorizer and weak neutral red as counterstain. It is recommended for examination of smears for gonococci.

a) A thin smear was made from the first swab, dried, fixed and then placed on the staining rack.
b) The smear was covered with 0.5% methyl violet for 30 seconds.
c) The slide was tilted and sufficient Lugol's (1%) iodine was used to wash away the stain. The smear was covered with fresh iodine for 30 seconds.
d) The slide was tilted and iodine was washed off with ethanol till color ceased to come out of the smear.
e) The slide was then washed with running water.
f) Neutral red (0.1%) was the counterstain used. The smear was covered with it for 2 min.
g) The slide was washed with running water and blotted to dry.
h) It was then examined under oil immersion objective.

3. Culture of anaerobic bacteria:

Brucella blood agar (BBA) supplemented with Hemin and Vitamin K was used.

Brucella agar base with hemin and vitamin k (M1039) was purchased from HIMEDIA Company. Its composition was as follows:

Ingredients	**Gms / Litre**
Casein enzymic hydrolysate	10.000
Peptic digest of animal tissue	10.000
Yeast extract	2.000
Dextrose	1.000
Sodium chloride	5.000
Sodium bisulphite	0.100
Hemin	0.010
Vitamin K1	0.010
Agar	15.000

Final pH (at 25°C) 7.0±0.2

- 5% sterile defibrinated sheep blood was added to this media aseptically at 50^{o}C.
- The medium contains casein enzymic hydrolysate, peptic digest of animal tissue and yeast extract as sources of carbon, nitrogen and essential growth nutrients including B-complex vitamins. Dextrose serves as a source of energy. Addition of blood provides nutrients and helps to differentiate hemolytic organisms. Presence of hemin and Vitamin K1 supports growth of other fastidious bacteria like Bacteroides species and gram-positive spore bearers like Clostridium species.

4. Method used for obtaining anaerobiosis : in the jar was by " internal gas generating system" described by Lakshminarayana and Vaidhyalingam.[84]

Principle of internal gas generating system:

In this system, hydrogen and carbon dioxide gas mixture required for creating anaerobiosis is obtained from the following reactions:

a) Citric acid + sodium bicarbonate ⟶ Sodium citrate + carbon dioxide

$$C_6H_8O_7 + 3NaHCO_3 \longrightarrow Na_3C_6H_5O_7 + 3H_2O + 3CO_2$$

b) Sodium borohydride + water ⟶ Sodium metaborate + Hydrogen

$$NaBH_4 + 2\,H_2O \longrightarrow NaBO_2 + 4H_2$$

Operation of the internal gas generator system:

a) 1 g of Sodium borohydride was taken in a 30 ml test tube.

b) 1 g of Sodium bicarbonate and 1 g of citric acid were taken in a 5 ml test tube, which was placed inside the 30 ml test tube.

c) The stem of a 20 ml funnel was plugged lightly with cotton to control the flow of water. The funnel was placed in 30 ml test tube in such a way that the stem of funnel dips into 5 ml test tube. Entire unit was kept inside the anaerobic jar with the indicator. 20 ml of distilled water was poured in the funnel just before closing the lid of the jar.

The water poured into the funnel drips into the 5 ml test tube liberating CO_2. Carbon di oxide being heavier stays within displacing the air. Once the 5 ml test tube is filled with water, it overflows into the 30 ml test tube liberating hydrogen, which being the lighter gas, rushes out with CO_2.

d) The palladium catalyst reduces the oxygen present within the jar to form water. Catalyst is exothermic, so warming of the lid of the jar can be felt.

Catalyst:

Cold catalyst which contained pellets of alumina coated with finely divided palladium (Baker platinum Ltd., London) was used. It was reactivated every time before use by drying at 150^{o}C- 160^{o}C for 1-2 hours.

Indicator for anaerobiosis:

- **Fildes and Mcintosh indicator**- 3 Stock solutions were prepared:

a) A solution of 6% glucose in distilled water

b) 6 ml of 0.1 N NaOH diluted to 100 ml with distilled water.

c) 3 ml of 0.5% w/v solution of methylene blue diluted to 100 ml of distilled water.

- Each time the indicator solution was required equal parts of the 3 stock solutions were mixed together in a test tube and the mixture was boiled until methylene blue was reduced.
- The tube of colorless methylene blue was immediately placed in anaerobic jar
- If anaerobic conditions were secured and maintained the indicator solution remained colorless.

- **Bacteriological indicator**- *Pseudomonas aeruginosa* ATCC 25922 was inoculated on to a nutrient agar plate and kept inside the jar along with the other plates. This bacterium is aerobic. A growth free culture plate indicates a successful anaerobiosis.

5. **Catalase test [83]:** Growth from the purity blood agar was removed onto a glass slide. A drop of 15% hydrogen peroxide was added and observed for evolution of bubbles.
6. **Spot indole test [83]:** A loopful of growth from a pure culture on the blood agar plate was removed and smearedon filter paper that was saturated with 1% paradimethylaminocinnamaldehyde in 10% (V/V) concentrated HCl.

   A positive reaction was indicated by the rapid development of blue color around the growth. Negative reaction gave no color change or a pinkish color.
7. **Nitrate test [83]:** Test was done using nitrate disk. The disk was removed from the surface of the blood agar plate and placed in a clean petridish. One drop each of Nitrate reagents A and B were added. Development of pink to red color indicated that nitrate has been reduced to nitrite.

If no color developed in few minutes, a small amount of zinc dust was added and waited for 5 minutes. Development of red color indicated that nitrate was not reduced. If no color developed, nitrate was reduced beyond nitrite (positive test).

Preparation of Nitrate reagents:

Solution A-

Sulfanilic acid- 0.5 g

Glacial acetic acid- 30 ml

Distilled water- 120 ml

Solution B-

1,6- Cleve's acid- 0.2 g

(5-amino-2-naphthalenesulfonic acid)

Glacial acetic acid-30 ml

Distilled water- 120 ml

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ANNEXURE I - ETHICAL CLEARANCE LETTER

K.L.E.UNIVERSITY'S
JAWAHARLAL NEHRU MEDICAL COLLEGE,
NEHRU NAGAR, BELAGAVI-590010 (KARNATAKA-INDIA)
(Accredited 'A' Grade by NAAC)

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Ref: MDC/DOME/ 385 **Date: 19/11/2015**

To,

Dr.
PG student in Microbiology,
J.N.Medical College,
BELAGAVI.

Sub: Institutional Ethical Clearance for the study.

With reference to the above, we wish to inform you that your proposed research project titled **"BACTERIOLOGICAL STUDY OF LEUCORRHEA IN REPRODUCTIVE AGE GROUP WOMEN, WITH SPECIAL REFERENCE TO ANAEROBES – A ONE YEAR CROSS SECTIONAL STUDY",** is ethical and justifiable. The proposed research project has been cleared by the JNMC Institutional Ethics Committee on Human Subjects Research.

(Dr. Arathi Darshan)
Member Secretary
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

(Dr. Ganga Pilli)
Chairman,
JNMC Institutional Ethics Committee
on Human Subjects Research,
J.N.Medical College, Belagavi.

ANNEXURE II - CONSENT FORM

TITLE: Bacteriological Study of Leucorrhea in Reproductive Age Group Women, With Special Reference to Anaerobes- A One Year Cross Sectional Study

Study Investigator Dr.
Post Graduate Student,
Department of Microbiology,
Jawaharlal Nehru Medical College,
KLE University, Belagavi – 590 010

Guide Dr.

Co-guide Dr.

INTRODUCTION:

Leucorrhea is a watery/thick, white/yellow purulent vaginal discharge, which is not blood stained. It is an excessive amount of normal discharge. Pathological leucorrhea is caused by various bacteria, both aerobes (like *Staphylococcus aureus, Gardnerella vaginalis, Escherichia coli, Streptococcus spp.*) and anaerobes (like *Bacteroides, Peptostreptococcus, Veillonella*, etc.)

When left untreated, it may lead to complications such as chronic pelvic inflammatory disease, chronic pelvic pain, infertility or ectopic pregnancy.

OBJECTIVE OF THE STUDY:

The purpose of research is to isolate and identify the bacteria (aerobes and anaerobes) causing leucorrhea and to determine antibiotic susceptibility of the isolated bacteria.

PROCEDURE INVOLVED:

You are requested to participate in this study which will help to provide appropriate and effective treatment. During the study you will be asked some questions and you are supposed to answer to the best of your knowledge.

If you agree to be a part of this study, you will be subjected to per speculum examination. Vaginal pH will be assessed and vaginal swabs will be collected for further processing.

RISKS AND BENEFITS:

There are no risks involved and benefit is to know the causative bacteria and to which antibiotics it is susceptible, so that appropriate treatment can be given.

ALTERNATIVES:

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

PRIVACY AND CONFIDENTIALITY:

The only people to know that you are a research subject are members of the research team. No information about you or provided by you during research will be disclosed to others without your written permission, except in emergency to protect your rights and welfare.

AUTHORIZATION TO PUBLISH RESULTS:

When the results of research are published or discussed in a conference, no information will be displaced that would disclose your identity. Any information that is 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 any gifts/incentives for participating in the study. You will not be reimbursed for expenses.

In case you have any questions related to the study, you can contact the study investigator,

In case you have any questions about your rights as a participant, you can contact Dr.Ganga S Pilli, Professor of Pathology and Chairman of Institutional Ethics Committee, JNMC (Office no. 0831-2471350 Ext: 4052)

CONSENT STATEMENT

I, the undersigned _____have been explained in my vernacular language about the study and my participation in the study is voluntary. If I want, I can withdraw at any time. Also I have been given enough time to clear my doubts and rights as study participant.

Signature or left hand thumb print of participant or legally authorized representative.

Participant's Name signature

Witness Name signature

Experimenter's Name signature

Date:

Place:

ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ಸಮ್ಮತಿ

ಶೀರ್ಷಿಕೆ: ಸಂತಾನೋತ್ಪತ್ತಿ ವಯಸ್ಸಿನ ಗುಂಪಿನ ಮಹಿಳೆಯರಲ್ಲಿ ಲ್ಯುಕಾರಿಯ ಉಂಟುಮಾಡುವ ಬ್ಯಾಕ್ಟೀರಿಯಾದ ವಿಜ್ಞಾನಾಧ್ಯಯನ, ವಿಶೇಷ ಉಲ್ಲೇಖ ಅನೇರೊಬ್ಸ್ ಗಳ ಕುರಿತಾಗಿ - ಒಂದು ವರ್ಷದ ವಿಭಾಗಾಧ್ಯಯನ.

ಅಧ್ಯಯನದ ಪರೀಕ್ಷಕರು	ಡಾ ಸ್ನಾತಕೋತ್ತರ ವಿದ್ಯಾರ್ಥಿ, ಮೈಕ್ರೋಬಯಾಲಜಿ ಇಲಾಖೆ, ಜವಾಹರಲಾಲ್ ನೆಹರೂ ಮೆಡಿಕಲ್ ಕಾಲೇಜ್, ಕೆ ಎಲ್ ಇ ವಿಶ್ವವಿದ್ಯಾಲಯ, ಬೆಳಗಾವಿ - ೫೯೦೦೧೦
ಮಾರ್ಗದರ್ಶಕರು	ಡಾ
ಸಹಾಯಕ ಮಾರ್ಗದರ್ಶಕರು	ಡಾ

ಪರಿಚಯ:

ಲ್ಯುಕಾರಿಯಾ ಒಂದು ರಕ್ತದ ಕಲೆ ಹೂಂದಿರದ,ನೀರಿನಂತಹ / ದಪ್ಪ, ಬಿಳಿ / ಹಳದಿ ಪ್ಯುರುಲೆಂಟ್ ಯೋನಿ ಡಿಸ್ಚಾರ್ಜ್ ಆಗಿರುತ್ತದೆ. ಇದು ಒಂದು ವಿಪರೀತ ಪ್ರಮಾಣದ ಸಾಮಾನ್ಯ ವಿಸರ್ಜನೆ. ಪೆಥಾಲಜಿಕಲ್ ಲ್ಯುಕಾರಿಯಾ ವಿವಿಧ ಬ್ಯಾಕ್ಟೀರಿಯಾಗಳಿಂದ ಉಂಟಾಗುತ್ತದೆ, ಇದರಲ್ಲಿ ಎರೊಬ್ಸ್ ಗಳು (*ಸ್ಟ್ಯಾಫಿಲೋಕೊಕಸ್ ಔರೆಸ್, ಗಾಡ್ನೆರೆಲ್ಲಾ ವಜೈನಾಲ್ಸಿ, ಎಸ್ಚರೀಶಿಯ ಕೋಲಿ, ಸ್ಟ್ರೆಪ್ಟೊಕೊಕಸ್* ಎಸ್ಪಿಪಿ. *ನಂತಹ)* ಹಾಗು ಅನೇರೊಬ್ಸ್ ಗಳು (*ಬ್ಯಾಕ್ಟೀರಾಯ್ಡಿಸ್ , ಪೆಪ್ಟೊಸ್ಟ್ರೆಪ್ಟೊಕೊಕಸ್, ವೈಲ್ಲೋನೆಲ್ಲಾ* ಮುಂತಾದವುಗಳು) ಒಳಗೊಂಡಿರುತ್ತವೆ.ಚಿಕಿತ್ಸೆ ನೀಡದೆ ಹಾಗೆ ಬಿಟ್ಟರೆ, ಇದು ತೀವ್ರ ಶ್ರೋಣಿಯ ಉರಿಯೂತದ ಕಾಯಿಲೆ, ದೀರ್ಘಕಾಲದ ಶ್ರೋಣಿ ಕುಹರದ ನೋವು, ಬಂಜೆತನ ಅಥವಾ ಅಪಸ್ಥಾನೀಯ ಗರ್ಭಧಾರಣೆಯ ಜಟಿಲ ಕಾರಣವಾಗಬಹುದು.

ಈ ಸಂಶೋಧನೆಯ ಉದ್ದೇಶ,

ಲ್ಯುಕಾರಿಯಾ ಉಂಟುಮಾಡುತ್ತಿರುವ ಬ್ಯಾಕ್ಟೀರಿಯಾವನ್ನು (ಎರೊಬ್ಸ್ ಮತ್ತು ಅನೇರೊಬ್ಸ್) ಪ್ರತ್ಯೇಕಿಸುವುದು ಹಾಗು ಗುರುತಿಸುವುದು.

ಒಳಗೊಂಡಿರುವ ವಿಧಾನ:

ನಿಮ್ಮನ್ನು ಈ ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸಲು ಕೋರುತ್ತೇವೆ;ನೀವು ಇದರಲ್ಲಿ ಭಾಗವಹಿಸುವುದರಿಂದ ಸೂಕ್ತ ಮತ್ತು ಪರಿಣಾಮಕಾರಿಯಾದ ಚಿಕಿತ್ಸೆ ನೀಡಲು ಸಹಾಯವಾಗುತ್ತದೆ. ಅಧ್ಯಯನದ ಸಮಯದಲ್ಲಿ ನಿಮ್ಮನ್ನು ಕೆಲವು ಪ್ರಶ್ನೆಗಳನ್ನು ಕೇಳಲಾಗುತ್ತದೆ ಮತ್ತು ತಾವು ತಮಗೆ ಅರಿವಿರುವಷ್ಟನ್ನ ಉತ್ತರಿಸಬೇಕು.ನೀವು ಈ ಅಧ್ಯಯನದ ಭಾಗವಹಿಸಲು ಒಪ್ಪಿದ್ದಲ್ಲಿ, ನಿಮ್ಮನ್ನು ಪರ್ ಸೈಕುಲಂ ಪರೀಕ್ಷೆಗೆ ಒಳಪಡಿಸುತ್ತೇವೆ. ಯೋನಿ ಪಿಎಚ್ ಮೌಲ್ಯಮಾಪನ ಮಾಡಲಾಗುತ್ತದೆ ಮತ್ತು ಯೋನಿ ಸ್ವೇದದೋಧಕಗಳು ಮತ್ತಷ್ಟು ಪ್ರಕ್ರಿಯೆಗೆ ಸಂಗ್ರಹಿಸಲಾಗುವುದು.

ಆಪಾಯಗಳು ಮತ್ತು ಲಾಭಗಳೆ:

ಇದರಲ್ಲಿ ಯಾವುದೇ ಆಪಾಯಗಳೊಳಗೊಂಡಿರುವುದಿಲ್ಲ; ಇದರಿಂದ ಲಾಭ ಎಂದರೆ,ಸುಲಭವಾಗಿ ಕಾರಕ ಬ್ಯಾಕ್ಟೀರಿಯಾವನ್ನ ತಿಳಿಯಬಹುದು ಮತ್ತು ಅದು ಯಾವ ಪ್ರತಿಜೀವಕಗಳಿಂದ ಶಮನವಾಗುತ್ತದೆ ಎಂಬುದು ತಿಳಿಯುತ್ತದೆ, ಇದರಿಂದ
ಸೂಕ್ತ ಚಿಕಿತ್ಸೆ ನೀಡಲು ಸಹಾಯವಾಗುವುದು.

ಪರ್ಯಾಯ:

ಸಂಶೋಧನೆಯಲ್ಲಿ ನಿಮ್ಮ ಭಾಗವಹಿಸುವಿಕೆ ವ್ಯೆಯಕ್ತಿಕವಾಗಿದ್ದು. ಅಧ್ಯಯನದಲ್ಲಿ ಭಾಗವಹಿಸುವುದೂ ಇಲ್ಲವೋ ಎಂಬ ನಿಮ್ಮ ನಿರ್ಧಾರದಿಂದ; ಜವಾಹರಲಾಲ್ ನೆಹರೂ ವೈದ್ಯಕೀಯ ಕಾಲೇಜು ಮತ್ತು ನಿಮ್ಮ ನಡುವಿನ ಸಂಬಂಧದ ಮೇಲೆ ಯಾವುದೇ ಪರಿಣಾಮ ಬೀರುವುದಿಲ್ಲ. ನೀವು ಭಾಗವಹಿಸಲು ನಿರ್ಧರಿಸಿದರೆ ; ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ನಿಮ್ಮ ನಿರ್ಧಾರವನ್ನು ಬದಲಿಸಲು ನೀವು ಪೂರ್ಣ ಸ್ವತಂತ್ರರಾಗಿರುತ್ತೀರಿ.

ಗೌಪ್ಯತೆ:

ಸಂಶೋಧನೆಯ ತಂದದವರನ್ನ ಹೊರತುಪಡಿಸಿ ಬೇರೆಯಾರಿಗೂ ನೀವು ಸಂಶೋಧನೆಗೆ ಒಳಪಟ್ಟ ವ್ಯಕ್ತಿ ಎಂಬುದು ತಿಳಿದಿರುವುದಿಲ್ಲ.ನಿಮ್ಮ ಹಕ್ಕುಗಳು ಮತ್ತು ಒಳಿತನ್ನ ರಕ್ಷಿಸಲು ತುರ್ತು ಪರಿಸ್ಥಿತಿಯನ್ನು ಹೊರತು ಪಡಿಸಿ ನಿಮ್ಮ ಲಿಖಿತ ಅನುಮತಿಯಿಲ್ಲದೆ ನಿಮ್ಮ ಬಗ್ಗೆ ಅಥವಾ ಸಂಶೋಧನೆಯ ಸಮಯದಲ್ಲಿ ನೀವು ಒದಗಿಸಿದ ಮಾಹಿತಿ ಬಗ್ಗೆ ಇತರರಿಗೆ ಬಹಿರಂಗಪಡಿಸಲಾಗುವುದಿಲ್ಲ.

ಫಲಿತಾಂಶಗಳನ್ನು ಪ್ರಕಟಿಸುವ ಅಧಿಕಾರ:

ಸಂಶೋಧನೆಯ ಫಲಿತಾಂಶಗಳನ್ನ ಪ್ರಕಟಿಸಿದಾಗ ಅಥವಾ ಕಾನ್ಫರೆನ್ಸ್ ಗಳಲ್ಲಿ ಚರ್ಚಿಸಿದಾಗ ನಿಮ್ಮ ಗುರುತನ್ನು ಬಹಿರಂಗಪಡಿಸಬಹುದಾದ ಯಾವುದೇ ವಿಷಯದ ಪ್ರಸ್ತಾಪ ಮಾಡಲಾಗುವುದಿಲ್ಲ.ಈ ಅಧ್ಯಯನಕ್ಕೆ ಸಂಬಂಧಪಟ್ಟಂತೆ,ನಿಮ್ಮಿಂದ ಪಡೆದ ಯಾವುದೇ ಮಾಹಿತಿ ನಿಮ್ಮಜೊತೆ ಗುರುತಿಸುವಂತಿದ್ದಲ್ಲಿ ಅದನ್ನು ಕೂಡ ಗೌಪ್ಯವಾಗಿದಲಾಗುವುದು.

ಪಾಲ್ಗೊಳ್ಳುವಿಕೆಗಾಗಿನ ಪಣಕಾಸು ಪ್ರೋತ್ಸಾಹ:
ಈ ಸಂಶೋಧನೆಯಲ್ಲಿ ಭಾಗವಹಿಸಲು ನಿಮಗೆ ನಾವು ಯಾವುದೇ ಪಣಪಾವತಿ ಅಥವಾ ಉದುಗೊರೆಯನ್ನ ನೀಡುವುದಿಲ್ಲ ಮತ್ತು ನಿಮ್ಮ ಯಾವುದೇ ವೆಚ್ಚಗಳನ್ನ ನಾವು ಬರಿಸುವುದಿಲ್ಲ.

ನಿಮ್ಮ ಹಕ್ಕುಗಳ ಬಗ್ಗೆ ಯಾವುದೇ ಪ್ರಶ್ನೆಗಳನ್ನು ಹೊಂದಿದ್ದರೆ, ನೀವು ಸಂಪರ್ಕಿಸಬಹುದು
ಡಾ ಗಂಗಾ ಎಸ್ ಪಿಳ್ಳೈ, ವ್ಯಾಧ್ಯಾಪಕರು ವೆಧಾಲಜಿ ಇಲಾಖೆ ಮತ್ತು ಅಧ್ಯಕ್ಷರು ಇನ್ಸ್ಟಿಟೂಷನಲ್ ಎತಿಕ್ಸ್ ಕಮಿಟಿ, ಜೆ ಎನ್ ಎಮ್ ಸಿ
(ಕಚೇರಿ # 0831-2471350 ಎಕ್ಸ್ಟೆ : 4052)

ಅನುಮತಿ

ಈ ಕೆಳಗೆ ರುಜುಮಾಡಿರುವ _____ನಾನಗೆ ನನ್ನ ದೇಶೀಯ ಭಾಷೆಯಲ್ಲಿ ಈ ಅಧ್ಯಯನದ ಬಗ್ಗೆ ವಿವರಿಸಲಾಗಿದೆ ಮತ್ತು ಅಧ್ಯಯನದಲ್ಲಿ ನನ್ನ ಭಾಗವಹಿಸುವಿಕೆ ವೈಯಕ್ತಿಕವಾಗಿದ್ದು. ನಾನು ಬಯಸಿದರೆ, ಯಾವುದೇ ಸಮಯದಲ್ಲಿ ಹಿಂದಕ್ಕೆ ಸರಿಯಬಹುದು. ಹಾಗೆಯೇ ನಾನು ಅಧ್ಯಯನಭಾಗಿಯಾಗಿ ನನ್ನ ಅನುಮಾನಗಳನ್ನ ಪರಿಹರಿಸಿಕೊಳ್ಳಲು ಮತ್ತು ಹಕ್ಕುಗಳ ಬಗ್ಗೆ ತಿಳಿದುಕೊಳ್ಳಲು ಸಾಕಷ್ಟು ಸಮಯ ನೀಡಲಾಗಿದೆ.

ನ್ಯಾಧೀಯ ಅಥವಾ ಕಾನೂನು ಬದ್ಧ ಪ್ರತಿನಿಧಿಯ ಸಹಿ ಅಥವಾ ಎಡಗೈ ಹೆಬ್ಬೆರಳು ಮುದ್ರಣ.

ಭಾಗವಹಿಸುವವರ ಹೆಸರು _____ ಸಹಿ _____

ಸಾಕ್ಷಿದಾರರ ಹೆಸರು _____ ಸಹಿ _____

ಪ್ರಯೋಗಿಕರ ಹೆಸರು _____ ಸಹಿ _____

ದಿನಾಂಕ: ಸ್ಥಳ:

अनुसंधान में भागीदारी के लिए सहमति

शीर्षक: प्रजनन आयु समूह महिलाओं में ल्यूकोरिया के जीवाणु, विशेष संदर्भ के साथ एनरौबस् के , एक वर्ष क्रॉस अनुभागीय अध्ययन

अध्ययन अन्वेषक: डॉ

स्नातकोत्तर छात्र,
माइक्रोबायोलॉजी विभाग,
जवाहर लाल नेहरू मेडिकल कॉलेज,
के ए लई विश्वविद्यालय,
बेलगावी - ५९००१०

गाइड: डॉ

सह-गाइड: डॉ

परिचय:

ल्यूकोरिया एक पानी का / मोटी, सफेद / पीले पीप योनि स्राव होता है, जो खून से सना हुआ नहीं है| ये सामान्य मुक्ति का एक अत्यधिक राशि है। रोग ल्यूकोरिया विभिन्न बैक्टीरिया के कारण होता है, एरौबस् *(जैसे स्टाफीलोकोकस ऑरीअस, गर्द्नेरेल्ला वेजिनेलिस, एस्चेरिचिया कोलाई, स्ट्रैपटोकोकस एसपीपी)* और एनरौबस् (जैसे *बैक्टेरॉइड्स, वीलोनेल्ला,* आदि) ।
इलाज के बिना, जीर्ण श्रोणि सूजन रोग, जीर्ण पैल्विक दर्द, बांझपन या अस्थानिक गर्भावस्था के रूप में जटिलताएं पैदा हो सकती है।

अध्ययन का उद्देश्य:

अध्ययन का उद्देश्य ल्यूकोरिया के जीवाणु की पहचान करना और पृथक जीवाणुओं की एंटीबायोटिक संवेदनशीलता का निर्धारण करना है।

कार्यविधि:

आपको इस अध्ययन में भाग लेने के लिए अनुरोध कर रहे हैं ताकी उचित और प्रभावी उपचार उपलब्ध कराने में मदद मिले। अध्ययन के दौरान आपसे कुछ सवाल पूछे जाऐंगे और आप अपने ज्ञान के अनुसार जवाब दें।

अगर आप इस अध्ययन का एक हिस्सा होने के लिए सहमत हैं, तो आपका योनि पीएच मूल्यांकन किया जाएगा और योनि के स्त्राव आगे की प्रसंकरण के लिए एकत्र किया जाएगा।

विपत्ति और लाभ:

आपको इस अध्ययन से कोई विपत्ति नहीं हैं और लाभ ये है कि ल्यूकोरिया के बैक्टीरिया और एंटीबायोटिक दवाओं का पता कर सकते है और उचित चिकित्सा दिया जा सकता है ।

विकल्प:

अनुसंधान के क्षेत्र में आपकी भागीदारी स्वैच्छिक है। अध्ययन में भाग लेने या न लेने कि आपका निर्णय जवाहर लाल नेहरू मेडिकल कॉलेज के साथ अपने रिश्ते को प्रभावित नहीं करेगा। आप भाग लेने का फैसला करते हैं तो आप किसी भी समय वापस लेने के लिए स्वतंत्र हैं।

गोपनीयता :

केवल शोध टीम लोगों को पता होगा कि आप शोध विषय के सदस्य हैं। शोध के दौरान आपके द्वारा प्रदान किये कोई सूचना, आपके लिखित अनुमति के बिना, आपात स्थिति में छोड़कर (आपके कल्याण की रक्षा करने के लिए), उन्मुक्त नहीं किया जाएगा।

प्राधिकार परिणामों को प्रकाशन:

शोध के परिणामों को प्रकाशित या एक सम्मेलन में चर्चा कर रहे हैं, तो आप के कोई जानकारी का खुलासा नहीं होगा। इस अध्ययन के संबंध में प्राप्त की आपकी जानकारी गोपनीय रहेगा।

भागीदारी के लिए वित्तीय प्रोत्साहन:

आपको अध्ययन में भाग लेने के लिए किसी भी उपहार / प्रोत्साहन की पेशकश नहीं किया जाएगा। आपकी खर्च के लिए प्रतिपूर्ति नहीं की जाएगी।

इस अध्ययन में आपको एक भागीदार के रूप में अपने अधिकारों के बारे में किसी भी सवाल है, तो **डॉ. गंगा एस पिल्लै**, पैथोलॉजी के प्रोफेसर और संस्थागत आचार समिति के अध्यक्ष, जवाहर लाल नेहरू मेडिकल कॉलेज (कार्यालय 0831-2471350, एक्सटेंशन: 4052) को संपर्क कर सकते हैं ।

सहमति वक्तव्य

मुझे, अधोहस्ताक्षरी _____ को इस अध्ययन और मेरी भागीदारी के बारे में, मेरे स्थानीय भाषा में, विस्तार से बताया गया है। मैं चाहता हूँ तो, मैं किसी भी समय इस अध्ययन से वापस ले सकता हूँ । इसके अलावा, मुझे अध्ययन भागीदार के रूप में अपने संदेह और अधिकार स्पष्ट करने के लिए पर्याप्त समय दिया गया है।

भागीदार या कानूनी रूप से अधिकृत प्रतिनिधि के हस्ताक्षर या बाएं हाथ अंगुली की छाप

प्रतिभागी का नाम हस्ताक्षर

गवाह का नाम हस्ताक्षर

प्रयोगकर्ता का नाम हस्ताक्षर

तारीख:
जगह:

ANNEXURE III - PROFORMA

Name: **Occupation:**

Age: **Education:**

OPD number: **Marital status:**

Address: **Monthly income:**

Phone number: **Referred by:**

Presenting complaints:

H/o. similar complaints in the past:

and treatment taken, if any

Current antibiotic therapy, if any:

Hormonal therapy, if any:

OCP/IUCD:

H/o. STD:

LMP:

Surgical history:

<u>Per speculum Examination</u>:

Quantity and type of discharge, color, odor:

Prolapse:

Signs of malignancy:

Laboratory investigation:

- Gram stain

Culture for aerobes:

Blood agar:

(Chocolate agar)

Mac conkey agar:

- Biochemical reactions:

Catalase	Coagulase	Oxidase	Indole	Urease	Citrate	Mannitol	Motility	TSI

- Antibiogram using disc diffusion method

Culture of Anaerobes

- Growth on Brucella Blood Agar with vitamin K and Hemin
- Colony morphology
- Pigment
- Haemolysis
- Fluorescence
- Pitting
- Antibiotic identification disc- Kanamycin- Vancomycin-
  Colistin- SPS-
- Jensen Grams stain of individual colony
- Catalase test
- Spot Indole test
- Antibiogram using agar dilution method

ANNEXURE - IV– LETTER FOR PROVISION OF *C.difficile* ATCC 700057 STRAIN

KLE University's
Jawaharlal Nehru Medical College, Belgaum-10
Department of Microbiology

Dr. Sumati Hogade MD. Professor of Microbiology
College Office: 0831-2471350, Depart: 0831-2473777 Extn: 4068 Fax No 0831-2470759
Email: principal@jnmc.edu. Web Site:www.jnmc.edu.

Ref : MDC/Micro/ Date: 27.01.2017

To,
Dr. Renu Bharadwaj,
Professor and Head of Department,
Department of Microbiology,
B. J Medical College,
Pune- 411001.

Through proper channel

Subject:- Requirement of *Bacteroides fragilis* ATCC 25285 and *Clostridium difficile* ATCC 700057

Respected Madam ,

I have spoken to your esteemed self , regarding the above mentioned subject. I require *B. fragilis* ATCC-25285 strain and *C. difficile* ATCC 700057 strain for my dissertation purpose in our department. In this regard I request you to kindly give the strain in a suitable medium.
I will send you an e-mail regarding the same.

Thanking you

C.difficile Standard strain given Received from CMC, Vellore 20/3/2017

Yours Sincerely,

Dr. Sumati Hogade
Prof. & Head of Microbiology,
J.N.Medical College, KLE University,
Belagavi.

Post graduate student,
J.N.Medical College,
KLE University
Belagavi.

Contact No: 9448866944, Email ID:sumatihogade@yahoo.co.in

ANNEXURE – VI KEY TO MASTER CHART

Education:	I=Illiterate
	P=Primary education
	M=Middle school
	S=Secondary education
	G=Graduate
Type of discharge:	TG=Thin, grey
	TW=Thick, white
Whiff test	P=Positive
	N=negative
Clue cells:	P=Present
	A=Absent
Amsel's criteria	S=Satisfied
	N=Not satisfied
Aerobes	VC=Vaginal Commensals
Anaerobes	NOGC=No organisms grown on culture
Candida	P=Present
	A=Absent
AST for aerobes	Amc=Amoxicillin + Clavulanic acid
	Amp= Ampicillin
	C=Chloramphenicol
	Caz=Ceftazidime
	Cip=Ciprofloxacin
	Cd=Clindamycin
	Cot=Cotrimoxazole

	E=Erythromycin
	Gen=Gentamicin
	Pi=Piperacillin
	Va=Vancomycin
AST for anaerobes	MTZ=Metronidazole
AST results	S=Sensitive
	R=Resistant

Sl. No.	OPD number	Age in years	Education	SE status	Nature of discharge	pH	Whiff test	Clue cells	Amsel's criteria	Aerobic culture	Anaerobic culture	Candida species	Amp	Amc	Cip	Cot	Caz	Gen	E	C	Cd	Pi	Va	MTZ
1	3712405	23	S	II	TG	6.5	N	A	N	VC	NOGC	N												
2	3741832	45	I	IV	TG	6.5	N	A	N	VC	VC	N												
3	3742021	30	I	III	TG	6.5	N	A	N	VC	VC	N												
4	3725880	43	M	III	TG	6.5	N	A	N	VC	VC	N												
5	3606562	44	G	I	TG	7	N	P	S	VC	Peptostreptococcus spp.	N												S
6	3743178	30	P	III	TG	7.5	N	P	S	VC	Peptostreptococcus spp.	N												R
7	3755309	22	S	III	TG	5.5	N	A	N	VC	VC	N												
8	3545024	32	S	III	TG	7	N	P	S	VC	VC	N												
9	3234222	28	M	II	TG	8	N	A	N	VC	VC	N												
10	3721061	20	P	IV	TG	7.5	N	P	S	VC	NOGC	N												
11	3636432	24	I	III	TG	7	N	P	S	VC	NOGC	N												
12	1231030	38	P	III	TG	6.5	N	A	N	VC	VC	P												
13	3409969	32	S	III	TG	7	N	P	S	*E.coli*	VC	N	S	S	S	R	S	S						
14	3769170	30	M	III	TG	4.5	N	A	N	VC	VC	N												
15	3724036	37	I	III	TG	7	N	P	S	*K. pneumoniae*	NOGC	N	R	R	S	R	R	S						
16	3770829	32	P	III	TG	6	N	P	S	VC	VC	P												
17	3771777	40	M	III	TG	7	N	P	S	*S. pyogenes*	Prevotella , Peptostrptococcus spp.	N	S	S	S				S	S				S
18	3775300	38	S	III	TG	7	N	P	S	*S. aureus*	VC	N	S	S	S	R			S		R			
19	3775762	33	P	III	TG	7	N	P	S	VC	VC	N												
20	3775843	29	P	III	TW	4.5	N	P	S	VC	VC	N												
21	3775889	35	P	IV	TG	4	N	A	N	VC	VC	N												
22	978457	45	M	III	TG	6.5	N	A	N	VC	VC	N												
23	3778858	32	P	III	TG	7.5	N	P	S	VC	VC	N												
24	3778984	22	M	III	TW	6	N	P	S	VC	VC	P												
25	344926	24	P	IV	TG	7	N	P	S	*S. pyogenes*	VC	N	S	S	R				R	S				
26	3751403	27	G	II	TG	7	N	P	S	*K. pneumoniae, C. freundii*	Porphyromonas spp.	N	R	R	S	R	S	S						S
27	3715756	27	S	III	TG	7.5	N	P	S	*S. aureus*	Porphyromonas spp.	N	R	R	R	S			S		R			S
28	1491001	32	P	III	TG	7.5	N	P	S	*C.koseri*	Fusobacterium spp.	N	R	R	R	R	R	S						S
29	3795380	45	M	II	TG	7	N	P	S	VC	VC	N												
30	3795551	30	P	III	TG	7	N	P	S	*S. aureus*	Porphyromonas spp.	N	R	R	R	S			S		R			S
31	3795557	43	I	III	TG	6.5	N	P	S	VC	VC	N												
32	2860528	39	P	IV	TG	6	N	P	S	VC	Bacteroides spp.	P												S
33	3790460	35	G	III	TG	6	N	P	S	VC	VC	N												
34	3988218	21	P	III	TG	3.5	N	A	N	VC	VC	N												
35	3792410	22	G	II	TG	6	N	A	N	VC	VC	N												
36	3829673	45	M	II	TG	5.5	N	A	N	VC	VC	N												
37	3320274	28	M	III	TG	6	N	P	S	VC	VC	N												
38	3840024	45	P	III	TG	7	N	P	S	*K. pneumoniae*	Porphyromonas spp.	N	R	R	S	R	S	S						S
39	3840420	37	P	IV	TG	5.5	P	P	S	*C.freundii*	VC	N	R	R	S	R	S	S						
40	1385762	34	M	II	TG	6	N	P	S	*E.coli*	VC	N	R	R	S	R	S	S						
41	3438301	30	G	III	TG	4.5	N	A	N	VC	VC	N												
42	3874366	26	S	II	TG	5.5	N	A	N	VC	NOGC	N												
43	3419794	29	G	III	TG	6.5	N	P	S	VC	Porphyromonas spp.	N												S
44	3858241	30	I	IV	TG	5	N	A	N	VC	VC	N												
45	3419795	28	S	III	TG	6	N	P	S	*E.coli*	VC	N	R	R	R	R	R	R						
46	3419802	29	P	III	TG	4	N	P	N	VC	VC	P												

Sl. No.	OPD number	Age in years	Education	SE status	Nature of discharge	pH	Whiff test	Clue cells	Amsel's criteria	Aerobic culture	Anaerobic culture	Candida species	Amp	Amc	Cip	Cot	Caz	Gen	E	C	Cd	Pi	Va	MTZ
47	3869536	40	P	III	TG	4	N	A	N	VC	VC	N												
48	3874670	25	S	II	TG	6	N	P	S	Enterococcus spp.	VC	N	S	R	R				S	R			S	
49	3874365	25	P	IV	TG	5	N	A	N	VC	VC	N												
50	3867860	36	G	III	TG	6.5	N	P	S	Enterococcus spp.	Peptostreptococcus spp.	N	S	R	R				R	R			S	R
51	3822296	28	P	III	TG	5	N	A	N	VC	VC	N												
52	3881663	40	P	II	TG	6	N	P	S	VC	VC	N												
53	3895441	34	S	II	TG	6	N	P	S	VC	Peptostreptococcus spp.	N												S
54	3895108	44	S	II	TG	4.5	N	A	N	VC	VC	N												
55	3905347	27	P	III	TG	6	N	P	S	*E.coli*	Bacteroides spp.	N	R	R	S	R	S	S						I
56	3905142	36	M	III	TG	3.5	N	P	N	VC	VC	P												
57	3905548	26	M	III	TG	3.5	N	P	N	VC	VC	N												
58	3905551	34	M	III	TG	6	P	P	S	VC	Bacteroides spp.	N												S
59	3174284	40	M	III	TG	5.5	N	P	S	VC	Porphyromonas spp.	N												S
60	3893704	45	P	II	TG	6.5	P	A	S	*C.freundii*	VC	N	R	R	R	R	R	S						
61	3923704	22	S	III	TW	5.5	N	P	S	VC	Porphyromonas spp.	P												S
62	3923959	34	P	IV	TG	3.5	N	A	N	VC	VC	N												
63	3922541	43	S	II	TG	6.5	N	P	S	VC	VC	N												
64	3935184	18	G	II	TG	6.5	P	P	S	*S. aureus*	VC	N	S	S	R	R			S		S			
65	3845140	45	S	III	TG	3	N	A	N	VC	VC	N												
66	3908169	45	I	III	TW	7	P	P	S	*E.coli*	Porphyromonas spp.	N	R	R	R	R	R	R						S
67	3938623	43	I	IV	TG	6	P	P	S	*S. pyogenes, S. aureus*	Porphyromonas spp.	N	R	S	R	R			S	R	S			S
68	3938741	24	P	III	TW	6.5	P	P	S	Enterococcus spp.	VC	N	R	R	R				R	S			S	
69	3929047	42	P	III	TG	4.5	N	P	S	Enterococcus spp.	VC	N	R	S	S				S	S			S	
70	3560823	22	M	III	TG	6	N	P	S	Enterococcus spp.	VC	N	R	S	R				R	S			S	
71	3947603	40	S	III	TG	6.5	N	P	S	VC	Porphyromonas spp.	N												S
72	3163416	34	M	II	TW	6.5	P	P	S	VC	Porphyromonas spp.	N												S
73	3025897	43	P	II	TG	5	N	A	N	VC	VC	N												
74	3799069	25	M	III	TG	6	N	P	S	VC	Porphyromonas spp.	N												S
75	3455328	40	I	IV	TG	6	N	P	S	VC	VC	N												
76	3952722	29	P	III	TG	4.5	N	A	N	VC	VC	N												
77	3952582	40	I	IV	TG	5	N	P	S	VC	VC	N												
78	3896370	38	I	III	TG	3.5	N	A	N	VC	VC	N												
79	3952758	40	I	IV	TG	6.5	N	P	S	Enterococcus spp.	VC	N	S	S	R				S	R			S	
80	3876188	42	P	III	TG	5.5	N	P	S	VC	VC	N												
81	3743298	30	M	III	TG	6.5	P	P	S	*S. pyogenes*	VC	N	S	S	R				R	S				
82	3051455	42	P	III	TG	5	N	P	S	*K. pneumoniae*	VC	N	S	S	S	S	S	S						
83	3961265	30	P	III	TG	5.5	P	P	S	*E.coli*	VC	N	S	S	S	S	S	R						
84	3823521	25	P	II	TG	7	N	P	S	*E.coli*	VC	N	R	R	S	R	R	R						
85	3962748	40	P	IV	TG	6.5	P	P	S	*E.coli,C.freundii*	Peptostreptococcus spp.	N	R	R	S	S	S	S						R
86	3962733	24	P	II	TG	6	P	A	S	Enterococcus spp.	VC	N	S	S	S			R	S	S			S	
87	3309129	27	I	III	TG	6	N	P	S	Enterococcus spp.	VC	N	S	S	S		S	S	R	S			S	
88	3962473	45	P	IV	TG	3.5	N	A	N	VC	VC	N												
89	3970995	43	P	III	TG	3.5	N	A	N	VC	VC	N												
90	3962792	43	P	III	TG	6.5	P	P	S	*E.coli*	Porphyromonas spp.	N	R	R	R	R	S	S						S
91	3700838	25	P	III	TG	6	N	P	S	VC	VC	N												
92	3978913	31	I	III	TG	7	P	P	S	*E.coli*	VC	N	S	S	R	S	S	S						
93	3915637	30	P	III	TG	3.5	N	A	N	VC	VC	N												

Sl. No.	OPD number	Age in years	Education	SE status	Nature of discharge	pH	Whiff test	Clue cells	Amsel's criteria	Aerobic culture	Anaerobic culture	Candida species	Amp	Amc	Cip	Cot	Caz	Gen	E	C	Cd	Pi	Va	MTZ
94	3774358	25	P	III	TG	6	N	P	S	*E.coli*	VC	N	S	S	R	S	S	S						
95	3820376	22	P	III	TG	3.5	N	A	N	VC	VC	N												
96	3897297	28	P	II	TG	3	N	A	N	VC	VC	P												
97	3798391	37	M	III	TW	3.5	N	P	N	VC	VC	P												
98	3999677	42	P	III	TG	4	N	A	N	VC	VC	N												
99	3904150	21	S	II	TG	3	N	A	N	VC	VC	N												
100	4018411	22	G	III	TG	3	N	A	N	VC	VC	N												
101	3983653	25	G	III	TG	5	N	A	N	VC	VC	N												
102	3980518	30	P	III	TG	6	N	A	N	VC	VC	N												
103	3040976	21	M	II	TG	5	N	P	S	VC	VC	N												
104	2222106	33	P	II	TW	4	N	P	N	VC	VC	P												
105	3996689	30	P	IV	TG	3.5	N	A	N	VC	VC	N												
106	4024943	34	P	II	TG	3.5	N	A	N	VC	VC	N												
107	4027891	40	M	III	TG	4	N	A	N	VC	VC	N												
108	3906654	34	M	II	TG	6	N	P	S	VC	Porphyromonas spp.	N												S
109	3845746	44	P	II	TG	4.5	N	P	N	VC	VC	N												
110	2963546	35	P	III	TG	3.5	N	P	N	VC	VC	P												
111	4069392	44	M	III	TG	4	N	A	N	VC	VC	N												
112	942031	29	S	II	TG	4	N	A	N	VC	NOGC	N												
113	4041906	23	S	II	TG	6	N	P	S	VC	Porphyromonas spp.	N												S
114	2154173	19	S	II	TG	3.5	N	P	N	VC	VC	N												
115	4059454	43	P	II	TW	5.5	N	P	S	VC	Porphyromonas spp.	N												S
116	4069933	20	S	II	TG	5	N	P	S	VC	Porphyromonas spp.	N												S
117	917468	35	P	II	TG	6	P	P	S	VC	Porphyromonas spp.	N												S
118	4069638	22	G	II	TG	3	N	A	N	VC	VC	N												
119	3934796	25	G	III	TG	6.5	P	P	S	*E.coli, K.pneumoniae*	VC	N	R/R	S/S	S/S	S/S	S/S	R/S						
120	3488655	20	G	III	TG	3	N	A	N	VC	VC	N												
121	4045662	22	S	II	TG	3.5	N	P	N	VC	VC	N												
122	4069512	35	M	II	TG	3.5	N	A	N	VC	VC	N												
123	3889941	24	G	II	TG	5	N	P	S	VC	VC	N												
124	3761238	40	M	II	TG	4.5	N	A	N	VC	VC	N												
125	4069976	43	P	III	TG	4	N	A	N	VC	VC	N												
126	4031172	35	P	III	TG	7	P	P	S	*E. coli, K.oxytoca*	VC	N	R/R	R	R/R	R/R	R/R	S/S						
127	4060250	38	P	III	TG	6	N	P	S	*E. coli, K.oxytoca*	VC	N	R/R	R	R/R	R/R	R/R	S/R						
128	1995509	25	S	III	TG	4	N	A	N	VC	VC	N												
129	4050511	23	M	II	TG	4	N	A	N	VC	NOGC	N												
130	1324570	25	G	I	TG	4	N	P	N	VC	VC	N												
131	1320168	40	P	III	TG	5.5	N	P	S	VC	VC	N												
132	3609207	24	G	IV	TW	6	N	P	S	*K. pneumoniae*	NOGC	N	S	R	S	S	S	R						
133	4042759	26	S	III	TW	6	P	P	S	*K. oxytoca*	VC	N	S	R	S	S	S	R						
134	3999289	44	P	III	TG	3	N	A	N	VC	VC	N												
135	2886978	35	S	III	TG	5	N	A	N	VC	VC	N												
136	4072765	40	P	IV	TG	5.5	P	P	S	*E.coli*	VC	N	R	R	R	R	S	R						
137	3645590	43	P	IV	TG	6	N	P	S	*S. aureus*	VC	N	R	R	R	R		R	R		S			
138	3850166	26	P	II	TG	4	N	P	N	VC	VC	N												
139	2246732	45	M	II	TG	3	N	A	N	VC	VC	N												
140	3694104	25	P	III	TW	4	N	A	N	VC	VC	P												
141	3749648	25	G	I	TG	4	N	A	N	VC	VC	N												
142	4072699	37	M	III	TW	3	N	A	N	VC	VC	P												
143	3838505	33	S	III	TG	4	N	A	N	VC	VC	N												

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144	4081895	31	G	II	TG	4	N	A	N	VC	VC	N												
145	4068399	34	P	III	TG	5.5	N	P	S	VC	NOGC	N												
146	3681189	41	P	III	TG	6	P	P	S	*Enterococcus spp., K.pneumoniae*	VC	N	S/R	R/R	R/S	/S	/S	R	R/	S/			S	
147	1612271	38	M	III	TG	6	N	P	S	*K. oxytoca*	VC	N	S	S	R	R	S	R						
148	4004217	29	G	III	TG	5.5	P	P	S	*K. pneumoniae*	VC	N	S	S	R	R	S	R						
149	4073869	26	M	III	TG	3	N	A	N	VC	NOGC	N												
150	4110864	35	P	III	TG	6.5	N	P	S	VC	VC	N												
151	4048073	35	M	II	TG	4.5	P	A	S	*K. pneumoniae*	VC	N	R	R	S	R	S	R						
152	4082393	27	G	II	TG	6	P	P	S	*K. pneumoniae*	VC	N	R	R	S	R	S	R						
153	4111342	33	M	II	TG	6	N	A	N	VC	VC	N												
154	4027740	27	G	II	TG	6.5	N	A	N	VC	VC	N												
155	3990511	38	S	II	TG	5	N	P	S	*E.coli*	Bacteroides spp.	N	R	R	R	S	R	R						S
156	3876143	27	G	II	TG	4	P	P	S	*E.coli*	Bacteroides spp.	N	R	R	R	S	R	S						R
157	4016949	33	P	II	TG	5	N	P	S	*P.aeruginosa*	VC	N	R	R			S	S	S			S		
158	4045441	26	G	I	TG	3.5	N	A	N	VC	VC	N												
159	4106315	35	P	III	TG	3.5	N	A	N	VC	VC	N												
160	3659475	23	G	III	TG	4	N	A	N	VC	VC	N												
161	4097957	43	M	II	TG	4	N	A	N	VC	VC	N												
162	4098235	32	S	III	TG	4	N	P	N	VC	VC	N												
163	4098671	42	P	III	TG	5.5	N	P	S	Enterococcus spp.	VC	N	R	R	S				S	S		S	S	
164	1738996	40	S	III	TG	7	P	P	S	*K. oxytoca*	VC	N	S	S	S	S	S	S						
165	4098723	31	G	II	TG	3.5	N	A	N	VC	NOGC	N												
166	4082425	32	S	II	TW	6	N	P	S	VC	VC	P	R	R	R	R	R	R						
167	4133019	20	G	II	TG	4	N	A	N	VC	VC	N												
168	4133025	23	G	III	TG	6	P	P	S	*E.coli*	Fusobacterium spp.	N	R	R	R	R	R	S						S
169	4133017	27	G	I	TG	6.5	P	A	S	*E.coli*	Fusobacterium spp.	N	S	S	S	S	S	S						S
170	3840302	28	G	II	TG	7.5	N	P	S	*E.coli*	Fusobacterium spp.	N	R	R	R	R	S	S						S
171	2910723	21	G	I	TG	3.5	N	A	N	VC	VC	N												
172	1091177	30	S	II	TG	4	N	P	N	VC	VC	N												
173	4121630	21	S	II	TG	4	N	A	N	VC	VC	N												
174	4098517	29	G	III	TG	4	N	A	N	VC	VC	N												
175	3848517	22	M	II	TG	3.5	N	P	N	VC	VC	N												
176	4014676	23	G	II	TG	4.5	N	P	N	VC	Porphyromonas spp.	N												S
177	2773627	29	S	II	TW	3.5	N	A	N	VC	VC	P												
178	2343903	26	G	II	TG	5	P	A	S	Enterococcus spp.	VC	N	R	R	R				R	R			S	
179	2657961	21	G	II	TG	4	N	A	N	VC	NOGC	N												
180	2905979	18	G	II	TG	6	N	P	S	Enterococcus spp.	VC	N	R	R	R				R	R			S	
181	4129338	41	P	III	TG	3.5	N	A	N	VC	VC	N												
182	1567742	30	M	II	TG	6	P	P	S	*E.coli, K.pneumoniae*	Bacteroides spp.	N	R	R	R	R	R	S						S
183	4129463	20	G	III	TG	3.5	N	A	N	VC	NOGC	N												
184	4104749	44	S	II	TG	4	N	P	N	VC	VC	N												
185	2392787	28	P	III	TG	4	N	P	N	VC	NOGC	N												
186	4129733	25	G	III	TG	3.5	N	P	N	VC	VC	N												
187	2992591	20	G	III	TG	3.5	N	P	N	VC	VC	N												
188	4093052	26	S	III	TG	4	N	A	N	VC	VC	N												
189	4129696	20	G	III	TW	4.5	N	A	N	VC	VC	P												
190	4122087	21	S	II	TW	4	N	A	N	VC	VC	P												
191	4122148	27	S	II	TG	4	N	A	N	VC	NOGC	N												
192	4094676	45	P	II	TG	5.5	P	P	S	*E.coli*	Porphyromonas spp.	N	S	S	S	S	S	S	S					S

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193	4144541	45	P	III	TW	3.5	N	A	N	VC	VC	P												
194	4102396	19	G	III	TW	4	N	P	N	VC	VC	P												
195	4106016	20	G	I	TG	4	N	A	N	VC	NOGC	N												
196	4115879	35	P	II	TG	4	N	P	N	VC	VC	N												
197	4021418	41	S	III	TG	3.5	N	A	N	VC	VC	N												
198	4110462	25	G	I	TG	4	N	P	N	VC	VC	N												
199	4110105	35	P	II	TW	3.5	N	A	N	VC	VC	P												
200	4004054	26	S	I	TG	4	N	P	N	VC	VC	N												
201	3008886	28	G	III	TG	4	N	P	N	VC	VC	N												
202	4132736	28	G	III	TG	6.5	P	A	S	*K. pneumoniae*	VC	N	R	R	S	S	S	S						
203	4144707	41	P	II	TG	4	N	P	N	VC	VC	N												
204	4144245	22	G	III	TG	5	N	P	S	VC	VC	N												
205	1676982	31	G	I	TW	3.5	N	P	N	VC	VC	P												
206	4144248	23	G	III	TW	3	N	P	N	VC	VC	P												
207	4144265	38	M	III	TG	5	N	A	N	VC	VC	N												
208	1766657	40	M	III	TG	5	N	P	S	VC	Peptostreptococcus spp.	N												R
209	4126042	27	G	III	TG	4	N	A	N	VC	VC	N												
210	4123733	26	P	III	TG	4	N	A	N	VC	VC	N												
211	4126133	23	G	II	TG	3.5	N	P	N	VC	VC	N												
212	3925632	25	G	III	TG	6	P	P	S	Enterococcus spp.	VC	N	S	S	S				S	S			S	
213	4144844	24	G	I	TG	4	N	A	N	VC	VC	N												
214	4058594	25	S	III	TG	6.5	P	P	S	*E.coli*	VC	N	R	R	R	R	S	S						
215	3741835	41	I	III	TG	6	N	P	S	*P.aeruginosa*	VC	N	R	R			S	S	S			S		
216	33342072	20	G	I	TG	6	N	P	S	*E.coli*	VC	N	S	S	S	S	S	S	S					
217	4125985	24	G	III	TG	5.5	N	P	S	*S. aureus*	VC	N	R	S	R	R			R		R			
218	3893836	26	G	II	TG	6	N	P	S	*E.coli*	VC	N	R	R	S	R	R	R						
219	2335811	32	S	II	TG	5	N	A	N	VC	VC	N												
220	4068170	26	S	III	TG	6	N	P	S	VC	VC	N												
221	3328060	36	G	I	TW	3.5	N	A	N	VC	VC	P												
222	3723473	36	M	II	TW	4	N	A	N	VC	VC	P												
223	3923352	20	G	III	TW	3.5	N	A	N	VC	VC	P												
224	4123745	22	S	III	TW	3.5	N	A	N	VC	VC	P												
225	3925307	24	G	III	TG	6	P	P	S	*E.coli*	VC	N	S	S	S	S	S	S						
226	4123603	21	G	III	TG	7.5	N	P	S	*E.coli*	VC	N	S	S	S	S	S	S						
227	3700896	23	S	III	TG	5.5	N	P	S	VC	VC	N												
228	4013910	30	G	II	TG	6	N	P	S	*K. pneumoniae*	VC	N	R	R	S	R	S	S						
229	4142072	30	S	II	TG	6	P	A	S	VC	VC	N												
230	4153773	26	G	III	TG	3.5	N	A	N	VC	VC	N												
231	4102973	38	S	III	TG	4	N	A	N	VC	VC	N												
232	4090320	43	M	III	TG	4.5	N	A	N	VC	VC	N												
233	4168867	22	P	IV	TG	6.5	P	A	S	*E.coli*	VC	N	S	S	R	R	S	S						
234	4037192	40	S	III	TG	5.5	N	P	S	Enterococcus spp.	VC	N	S	S	R				R	S			S	
235	3853751	20	G	III	TG	3.5	N	P	N	VC	VC	N												
236	4126076	44	M	III	TG	6	P	P	S	*E.coli*	Porphyromonas spp.	N	R	R	R	R	R	S						S
237	4091106	43	P	II	TW	3	N	A	N	VC	VC	P												
238	3980230	21	S	I	TW	3.5	N	A	N	VC	VC	P												
239	4105850	30	G	I	TW	3	N	A	N	VC	VC	P												
240	3978842	21	G	I	TG	5.5	N	P	S	VC	VC	N												
241	3949352	38	S	III	TG	4	N	P	N	VC	VC	N												
242	4015345	26	S	II	TG	6	N	P	S	Enterococcus spp.	VC	N	R	R	R				R	S			S	

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243	4087059	40	M	III	TG	5	P	A	S	VC	VC	N												
244	4137373	26	G	III	TG	6.5	N	P	S	*C.freundii*	Porphyromonas spp.	N	R	R	S	S	S	S						S
245	4123371	19	G	III	TG	4.5	N	P	N	VC	VC	N												
246	4060094	34	P	III	TG	6	P	P	S	*E.coli, K.pneumoniae*	Prevotella spp.	N	R/S	S/S	S/S	S/S	S/S	S/S						S
247	4137450	25	S	III	TG	4.5	N	A	N	VC	VC	N												
248	3743815	26	G	I	TG	5.5	P	P	S	*C.freundii*	Porphyromonas spp.	N	R	R	S	S	S	R						S
249	4086925	35	S	III	TG	4.5	N	A	N	VC	NOGC	N												
250	3991393	19	G	III	TG	6	P	P	S	Enterococcus spp.	VC	N	R	R	R				R	R			S	