# MICROBIOLOGY OF VAGINAL DISCHARGE

## WITH EMPHASIS ON

## GARDNERELLA VAGINALIS

AYESHA BIBI MAHOMED KHARSANY

Submitted in partial fulfilment of the requirements for the degree of:

MASTER OF MEDICAL SCIENCE

In the
Department of Medical Microbiology
University of Natal
DURBAN

1990

ABSTRACT

The microbiological aetiology of vaginal discharge was studied in 208 women attending various outpatient clinics at King Edward VIII Hospital.

Specimens from the lower genital tract were collected for microscopy and culture. Vaginal wet smear examination, amine liberation test and vaginal pH estimation were performed and assessed for their reliability for the rapid diagnosis of vaginal infections.

Vaginal and endo-cervical infections were present in 163 (78,4%) women. G. vaginalis (65,4%), T. vaginalis (37,9%), genital yeasts (37,0%), M. hominis (59,6%), U. urealyticum (48,1%), anaerobic bacteria (32,6%), N. gonorrhoeae (11,1%), C. trachomatis (22,1%) and Herpes simplex virus (0,9%) were detected. Of the 104 women in whom vaginal infections were detected, bacterial vaginosis was present as the sole infection in 32 (22,2%), T. vaginalis in 35 (24,3%) and C. albicans in 23 (15,9%). Bacterial vaginosis occurred concurrently with T. vaginalis and C. albicans in 24 (16,5%) and 11 (7,5%) women respectively; whilst T. vaginalis and C. albicans occurred concurrently in 14 (9,7%) women. In 6 (4,1%) women all three infections were present. No vaginal or endo-cervical pathogens were detected in 45 (21,6%) women.

Women with bacterial vaginosis were found to be significantly colonised with G. vaginalis, M, hominis, anaerobic bacteria and curved Gram-negative bacilli ($p < 0,05$).

Vaginal wet smear microscopy detected T. vaginalis in 29% and "clue" cells in 41,3% of smears. The presence of "clue" cells (91,8%) and a positive amine test (76,7%) was significantly associated with bacterial vaginosis. Although a raised vaginal pH was also significantly associated with bacterial vaginosis, this test was less specific (65,2%) than "clue" cells (85,9%) and the amine test (95,5%). The vaginal Gram stain, as performed in this study, was found to be unreliable for the detection of "clue" cells.

G. vaginalis biotypes 1 and 5 were significantly associated with bacterial vaginosis, however the serotyping scheme did not distinguish between strains isolated from women with and without bacterial vaginosis. The antimicrobial susceptibility pattern of 93 strains of G. vaginalis was not typical of either Gram-positive or Gram-negative bacteria.

Serological tests revealed reactive syphilis serology in 47 (22,6%) and the presence of hepatitis B surface antigen in 16 (7,7%) women. Antibody to human immunodeficiency virus was detected in 4 (1,9%) women attending the colposcopy clinic.

This study clearly demonstrates the high prevalence of vaginal and/or endo-cervical infections in women locally, the majority of whom were asymptomatic. The high frequency of concurrent infections is of concern and there is a need for the recognition, and appropriate management of such infections.

DEDICATION :

To my beloved

Father - Mahomed Ebrahim Haffejee, for his love, guidance and encouragement;

Husband - Suleman for his love, patience and tolerance

Children - Irshaad and Zahira for the patience which they had to endure at their tender age

# CURRICULUM VITAE

1 Fellowship of the South African Society of Medical Laboratory Technologists (Sexually Transmitted Diseases) October 1986.

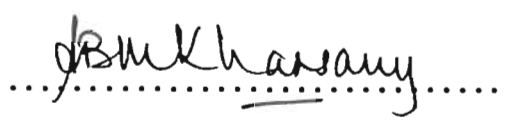
2 Pre-requisite examination in Medical Microbiology, University of Natal, November 1989.

3 Presently employed as Senior Medical Technologist in the Department of Medical Microbiology, University of Natal, Durban.

# PREFACE

This study represents original work by the author and has not been submitted in any form to another University. Where use was made of the work of others it has been duly acknowledged in the text.

The research described in this dissertation, was carried out in the Department of Medical Microbiology, Faculty of Medicine, University of Natal, under the supervision of Professors J van den Ende and J Moodley.



AYESHA BIBI MAHOMED KHARSANY

1990

Papers from this dissertation have been presented at the following congress:

1 Antimicrobial susceptibility of Gardnerella vaginalis

A B M Kharsany, J van den Ende. Anniversary Congress, Infectious Diseases and Sexually Transmitted Diseases Societies of Southern Africa, Johannesburg, October 15-20 1989. Abstract No STD 10

2 Sexually transmitted pathogens in patients with cervical intraepithelial neoplasia

A B M Kharsany, A A Hoosen, J Bagrathi, J Moodley, J van den Ende. Anniversary Congress, Infectious Diseases and Sexually Transmitted Diseases Societies of Southern Africa, Johannesburg, October 15-20 1989. Abstract No 027.

# ACKNOWLEDGEMENTS

I would like to extend my sincere thanks and gratitude to:

My supervisors, Professor Jan van den Ende, Head of the Department of Medical Microbiology, University of Natal, for his expert advice, constructive criticism and especially for his constant encouragement, guidance and teaching during the preparation of this dissertation; Professor Jack Moodley from the Department of Obstetrics and Gynaecology for his expert advice, constructive criticism and access to patients under his care.

Dr Anwar Hoosen from the Department of Medical Microbiology for his assistance with clinical assessment of all women examined and particularly for his time, constant encouragement, valuable advice and support.

Dr Piet Becker and Miss Eleanor Gouws of the Institute of Biostatistics of the Medical Research Council for assistance with statistical analyses.

Dr Cathy Ison and Mr David Harvey of St Mary's Hospital, Paddington, London, United Kingdom for supply of reagents and methodology for serotyping of *Gardnerella vaginalis* strains.

Mr Nick Kimberley of Rhône-Poulenc, United Kingdom for the supply of hydroxymetabolite of metronidazole powder for determining minimum inhibitory concentrations for G. vaginalis.

Mr Logan Pillay from the Department of Medical Microbiology for his assistance with media and reagents preparation.

The staff of the Medical Library, Medical Microbiology and Regional Laboratory Services for their kind assistance.

Mrs Moyra Kemp for the proofreading of this dissertation.

Mrs Kay George for the typing of this dissertation.

My husband Suleman, and children Irshaad and Zahira, for their understanding, patience and tolerance; my family and friends for their encouragement and support; God Almighty for the inspiration.

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# LIST OF ABBREVIATIONS

| | |
|---|---|
| ANC | Ante-natal clinic |
| BA | Horse blood agar |
| CC | Colposcopy clinic |
| CLED | Cystine lactose electrolyte deficient agar |
| $CO_2$ | Carbon dioxide |
| Ct | *Chlamydia trachomatis* |
| ELISA | Enzyme linked immunosorbent assay |
| *et al* | and others |
| FPC | Family planning clinic |
| FTA-Abs | Fluorescent treponemal antibody-absorption |
| GC | *Neisseria gonorrhoeae* |
| GV | *Gardnerella vaginalis* |
| HB | Human blood agar |
| HBsAg | Hepatitis B surface antigen |
| HIV | Human immunodeficiency virus |
| HSV | Herpes simplex virus |
| kDa | kilo dalton |
| MIC | Minimum inhibitory concentration |
| MIC50 | MIC at which 50% of isolates are inhibited |
| MIC90 | MIC at which 90% of isolates are inhibited |
| mg | milligram/s |
| mg/L | milligram/s per litre |
| mℓ | millilitre/s |
| MNYC | Modified New York City medium |
| nm | nanometer |

| | |
|---|---|
| RPR | Rapid plasma reagin |
| SHB | Selective human blood agar |
| STD | Sexually transmitted diseases clinic |
| Syph | Syphilis |
| TPHA | Treponema pallidum haemagglutination |
| ug/mℓ | microgram/s per millilitre |
| v/v | volume per volume |
| WC | Wilkens chalgren agar |
| w/v | weight per volume |

# 1.0 INTRODUCTION

Vaginal infections in adult women are amongst the commonest problems encountered in clinical practice.

In premenopausal women, a vaginal discharge of infectious origin may be either due to a cervicitis, vaginitis or a combination of both conditions. The major causes of cervicitis are sexually transmitted pathogens namely Neisseria gonorrhoeae, Chlamydia trachomatis and Herpes simplex virus. A cervical infection represents a reservoir for sexual or perinatal transmission and can lead to complications such as irritation and promotion of cervical neoplasia, salpingitis and endometritis in non-pregnant women, and chorio-amnionitis, premature rupture of membranes, and ascending infections in pregnancy and the puerperium.

In women presenting with a vaginal discharge, where a uterine cause and vaginal lesions have been excluded, the two established and recognised causative agents are Candida albicans and Trichomonas vaginalis. A further syndrome in women who present with an abnormal discharge, but in which these agents have not been implicated, is recognised. This has been variously termed - non-specific vaginitis (NSV), Gardnerella-associated vaginitis (GAV) and bacterial vaginosis (BV). These vaginal conditions are generally devoid of serious complications, however, they are common and do account for significant morbidity.

The diagnosis, aetiology, pathogenesis, epidemiology and management of bacterial vaginosis has not been fully elucidated.

Gardnerella vaginalis, a Gram-variable bacillus, first described by Leopold in 1953 (Leopold, 1953), has been implicated as the predominant aetiological agent of bacterial vaginosis (Gardner and Dukes, 1955; Vontver and Eschenbach, 1981). Its aetiological role has been described as one of synergism with a number of micro-organisms found in vaginal secretions. Recent studies have shown an association with Mycoplasma hominis (Holmes, 1984), non-sporeforming anaerobes (Pheifer et al, 1978; Spiegel et al, 1980; Taylor et al, 1982) and anaerobic curved Gram-negative rods (Hjelm et al, 1981; Skarin and Mardh, 1982) which have been named Mobiluncus species (Spiegel and Roberts, 1984).

This association has been elucidated by gas liquid chromatography (GLC) of vaginal secretions from women with bacterial vaginosis. Characteristically the lactate is reduced whilst succinate is increased (Spiegel et al, 1980). The concentration of amines such as putrescine and cadaverine are also increased (Chen et al, 1979).

Studies performed in Europe and North America have shown prevalence figures of 40% to 50% for bacterial vaginosis, 20,5% for candidiasis and 9,8% for trichomoniasis (Fleury, 1981). This compares with prevalence figures of 31% for candidiasis and 47% for trichomoniasis reported in local studies (Hoosen et al, 1981). Numerous studies on the aetiology of vaginal discharges have been performed in South Africa. Fehler et al (1984) reported on the prevalence of G.

vaginalis, but did not assess its association with bacterial vaginosis. Pochee et al (1986) have reported a 61% prevalence of bacterial vaginosis; however they made no attempt to culture the presumed aetiological agents in their study.

The aim of this study was to:

a Determine the prevalence of G. vaginalis, T. vaginalis and C. albicans in pregnant and non-pregnant women presenting at King Edward VIII Hospital, Durban.

b Attempt to confirm the role of G. vaginalis and anaerobic bacteria in bacterial vaginosis.

c Evaluate rapid bedside techniques such as wet smear microscopy, amine test and pH determination for the diagnosis of bacterial vaginosis.

d Evaluate culture media - both transport and isolation media in order to determine the efficacy of transport media for the recovery and isolation of G. vaginalis as compared to direct plating method.

e Characterise G. vaginalis isolates by various techniques including biotyping.

f Evaluate the in vitro activity of various antimicrobial agents against G. vaginalis.

# 2.0 LITERATURE REVIEW

## 2.1 COMMENSAL VAGINAL FLORA

The normal flora are micro-organisms which coexist with humans and do not cause disease under normal circumstances. Typically these flora colonise the skin, mucous membranes or those sites in contact with the environment. Some of these micro-organisms are usually fairly permanent residents while others may commonly but not invariably be present transiently. Terms used synonomously with "normal flora" include "commensal flora" and "endogenous flora". The normal flora are usually well adapted to survive under the physiological and nutritional conditions found in specific body sites; however the normal flora of the vagina are influenced by many factors and are usually altered because of hormonal cyclical changes.

Benefits provided to the host by the normal flora include the provision of competition and prevention of colonisation by pathogenic bacteria. The recognition of this has given rise to the widely accepted concept of "colonisation resistance".

Although human normal flora are regarded primarily as being of benefit to the host, they are not entirely without pathogenic potential as they may, under certain circumstances, be associated with localised infections and may occasionally spread to other body sites, particularly those which are normally sterile, to cause significant infections (Hill et al, 1984). In the case of the normal vaginal flora,

these play a significant role in defence against infection by genital pathogens (Cohen et al, 1984a). In the late 1800's, Doderlein described the prominence of facultative Gram-positive rods as part of the normal vaginal flora (Doderlein, 1894 - cited by Paavonen, 1983). These were called Doderlein bacilli, which comprised a heterogenous species of acidophilic Gram-positive bacilli, synonomous with lactobacilli.

Traditionally, the vaginal flora were assessed by vaginal smears based on the presence of Doderlein bacilli and/or their replacement by mixed predominantly coccal type flora (Leppaluoto, 1971). The association of lactobacilli with a normal clinical examination in pre-menopausal women led to the concept that only lactobacilli were normal in endogenous flora and the presence of other organisms represented an aberration.

With the advent of improved bacteriologic culture techniques for isolation and differentiation of bacteria, particularly obligate anaerobic bacteria and with the application of such sensitive techniques, numerous species have been detected either in frequent or small numbers. Apart from the lactobacilli which were the species most commonly isolated from the healthly vagina (Bartlett et al, 1977; Masfari et al, 1986; Levison et al, 1979), the vagina was also shown to be colonised by anaerobic bacteria (Bartlett et al, 1977; Larsen, 1980). Anaerobic Gram-positive cocci and Bacteroides species are the main anaerobic organisms in the vagina and cervix. These may also be present in high concentrations (Bartlett and Polk, 1984). The most prevalent bacteria of the vaginal flora of healthy women are shown in Table I.

TABLE I : CLASSIFICATION OF BACTERIA FOUND IN HUMAN VAGINA
(Huggins and Preti, 1981)

| | Aerobic | Anaerobic |
|---|---|---|
| Gram-positive cocci | Streptococcus<br>Staphylococcus<br>Micrococcus | Streptococcus<br>Peptostreptococcus<br>Peptococcus |
| Gram-positive bacilli | Lactobacillus<br>Corynebacterium<br>Listeria<br>Mycobacterium | Lactobacillus<br>Propionibacterium<br>Clostridium<br>Eubacterium<br>Bifidobacterium<br>Actinomyces |
| Gram-negative cocci | Neisseria | Veillonella<br>Acidaminococcus |
| Gram-negative bacilli | Escherichia<br>Proteus<br>Klebsiella<br>Serratia<br>Citrobacter<br>Pseudomonas<br>Acinetobacter<br>Mycoplasma<br>Ureaplasma<br>Florabacterium<br>Alcaligenes | Bacteroides<br>Fusobacterium |

Lactobacilli are generally the predominant aerobic organisms isolated followed by diptheroids and Staphylococcus epidermidis. Organisms such as Staphylococcus aureus, Lancefield Group B streptococci, Escherichia coli and Bacteroides are not frequently isolated from the vagina despite their association with upper genital tract infection (Paavonen, 1983).

Of relevance to this study is the occurrence of G. vaginalis. Some workers, McCormack et al (1977), Levison et al (1978); Sautter and Brown (1980) and Masfari et al (1986), reported a high frequency of G. vaginalis varying from 5% to 50%. Their findings differed from the results of the study of Bartlett et al (1977) in that none of the latter's subjects harboured this organism (Table II). These differences may reflect the sensitivity of isolation procedures used by various researchers. Bacteroides spp, Peptococci and Peptostreptococci are the predominant anaerobes isolated (Larsen and Galask, 1980). The presence of all other organisms isolated in small numbers reflects the increasing sensitivity of isolation procedures.

The vaginal internal environment shows considerable variation depending inter alia on age, hormonal status and sexual activity. Throughout the menstrual cycle the total number of anaerobic bacteria appears to be fairly constant. However, there is generally a 100-fold decrease in the concentration of aerobic bacteria shortly prior to menstruation. There is a great variation of specific bacteria when multiple samples are obtained from the same individual. This has been shown by Bartlett et

TABLE II : MOST PREVALENT BACTERIA OF THE VAGINAL FLORA OF HEALTHY NON-PREGNANT PRE-MENOPAUSAL WOMEN

| Bartlett et al 1977 n=22 | Levison et al 1978 n=7 |
|---|---|
| **Facultative anaerobic bacteria** | |
| Lactobacillus | Lactobacillus |
| S. epidermidis | Non enterococcal streptococci |
| Corynebacterium sp | C. vaginalis |
| | Unidentified $O_2$ catalase neg bacilli |
| **Anaerobic bacteria** | |
| Peptococci | Peptococci |
| Bacteroides spp | Peptostreptococci |
| Peptostreptococci | Bacteroides spp |

| Sautter and Brown 1980 n=7 | Masfari et al 1986 n=22 |
|---|---|
| **Facultative anaerobic bacteria** | |
| Lactobacillus | Lactobacillus |
| S. epidermidis | Coagulase negative staphylococci |
| Corynebacterium sp | C. vaginalis |
| Ureaplasma | Haemolytic strep |
| Mycoplasma | S. faecalis |
| C. vaginalis | Corynebacterium sp |
| C. albicans | |
| **Anaerobic bacteria** | |
| Bacteroides spp | Bacteroides spp |
| Peptostreptococci | Peptostreptococci |

al (1977), whose analysis of specimens obtained sequentially throughout the menstrual cycle showed considerable cyclical variation in terms of the variety and numbers of organisms present. These cyclical hormonal changes regulating the menstrual cycle influence the composition of the vaginal flora. Bartlett and Polk (1984) have suggested that the vaginal flora be regarded as a dynamic ecosystem.

## 2.2 NORMAL VAGINAL SECRETIONS

The human vagina is lined by stratified squamous epithelium with no glands. The vaginal secretions normally are a mixture of secretions originating predominantly from the cervical mucus and transudates through the vaginal epithelium, with contribution from secretions of the Bartholins and Skenes' glands, endometrial and tubal fluids; leucocytes and exfoliating epithelial cells. These cells may be superficial, intermediate or parabasal cells in origin, depending on the level and phase of stimulation by ovarian hormones. The mean amount of vaginal secretions produced by menarchal women is between 1 to 3 grams per 24 hours (Paavonen, 1983). These are usually white, of curdy, floccular consistency and importantly the odour is not disagreeable.

The characteristics of normal vaginal secretions are listed in Table III. The characteristics and the amount of vaginal secretions are influenced by oestrogen concentration and sexual stimulation and may change during the normal menstrual cycle. Such increases are regarded as physiological and may also occur during pathology.

TABLE III : CHARACTERISTICS OF NORMAL VAGINAL SECRETIONS

(Paavonen, 1983)

| | |
|---|---|
| Amount | Minimal or variable |
| Colour | Whitish, mucoid |
| Consistency | Floccular, curdy |
| Presence during examination | Pooled |
| pH | Approximately 4,5 |
| Wet mount observations | Rare leucocytes, variable number of mononuclear cells, large Gram-positive rods, vaginal epithelial cells with distinct borders |
| Organic acid content | Predominantly lactate |
| Diamines* | Absent |

* Putrescine and cadaverine

## 2.3 HOST DEFENCE MECHANISMS

Mechanisms which control the microflora of the vagina are poorly understood, however a large number of factors regulate the composition and dynamics of this microbial ecosystem.

The vagina harbours a complex microflora, but only a few micro-organisms are considered to be able to produce vaginal infections. Majority of these infections are exogenous infections and predisposing factors include: trauma, sexual activity and/or iatrogenic disruption of the micro-environment (Cohen et al, 1984a), most of them being sexually transmitted.

Generally in the body, the skin and mucous membrane form the first line of defence against invasive pathogens and the vaginal mucosa is no exception in this regard. Intact vaginal mucosa of women of reproductive age comprises a significant barrier to infection, unless breached.

Microbial interaction, regulated by hormonal influence of oestrogen and non-specific factors such as mucosal secretions containing mucus (Elstein, 1978), lysozyme (Root and Cohen, 1981), lactoferrin (Arnold et al, 1980), zinc and fibronectin (Cohen et al, 1984a) provide non-specific protection while specifically secretory IgA concentrations increase in response to specific pathogens to afford protection (Fubara and Freter, 1973).

### 2.3.1 Non-specific mechanisms

#### 2.3.1.1 Microbial interaction

The predominant species found in the vaginal flora of the majority of

women of reproductive age are lactobacilli. The detailed knowledge of the factors influencing their presence and numbers is not available, but there is good evidence that they are influenced by the level of hormones particularly oestrogens. Furthermore lactobacilli and/or their products are believed to regulate the growth and survival of other vaginal organisms.

The glycogen content of vaginal epithelial cells is under hormonal influence (Cruickshank and Sharman, 1934). Lactobacilli in the vagina break down glycogen to glucose and lactic acid resulting in a reduction of vaginal pH and thus promote an acid local environment (Hill et al, 1985). Other micro-organisms are inhibited at the low pH usually observed and this is regarded as the main reason for the beneficial antibacterial effect which lactobacilli exert in the vagina. Thus the numbers of lactobacilli and the resultant acid formation are largely dependent on the amount of glycogen in vaginal epithelial cells, thereby explaining the significant effect of oestrogens on the microbial ecology.

The presence and amount of glycogen and therefore the lactobacilli population vary at different stages of life, affecting the pH at these different stages (Table IV). Other antimicrobial factors have been attributed to lactocins produced by lactobacilli and compounds such as hydrogen peroxide ($H_2O_2$) and lactic acid are also responsible for the antibacterial activity against other organisms in the vagina (Mardh and Soltész, 1983).

TABLE IV : VAGINAL FLUID pH, GLYCOGEN CONTENT, OESTROGEN AND PRESENCE OF LACTOBACILLI AT DIFFERENT STAGES OF LIFE

| Stage of development | Vaginal pH* | Glycogen° content | Ovarian activity oestrogen° | Lactobacilli |
|---|---|---|---|---|
| Neonate | 7,5 | + | + | + |
| 1 month to childhood | 7,0 | - | - | - (generally absent) |
| Puberty | 4,5 | + | + | + |
| Menarche*≠ | 4,0 | + | + | + |
| Pregnancy | 3,8 | + | + | + |
| Menopause | 6,0 | +/- | +/- | +/- |

* Adapted from Cohen, 1969; Cohen et al, 1984

° Adapted from Cruickshank and Sharman, 1934

≠ Menstrual cycle and sexual activity may affect results

\+ Present

\- Absent

+/- Decreased amounts

Eschenbach et al (1989) emphasized the importance of $H_2O_2$-producing lactobacilli in the vagina and suggested that these organisms contribute to the control of the microflora and may prevent the development of bacterial vaginosis (to be described later) in some women. The $H_2O_2$ produced by lactobacilli species may therefore represent another non-specific defence mechanism of the normal vaginal epithelium.

2.3.1.2 Vaginal mucus

Mucus, a glycoprotein is secreted by goblet-shaped epithelial cells. It passes through intracellular channels, provides lubrication for the epithelium and serves as a protective barrier against the adjacent environment (Elstein, 1978).

Bacteria usually adhere to the mucosa by specialised cell surface structures (adhesins) which promote adherence to specific cell receptors. These adhesins are usually the fimbriae (Cohen et al, 1984b). Mucus plays an important role in interfering with bacterial adherence and subsequent colonisation, by binding to bacterial surfaces prior to potential attachment to specific cell receptors.

The chemical composition of mucus also supports the growth of lactobacilli therefore helping to maintain a pH of less than 4,5.

2.3.1.3 Antimicrobial secretions

Both lysozyme (Root and Cohen, 1981) and lactoferrin (Arnold et al, 1980) have been identified in vaginal mucus and are capable of affecting microbial survival.

Lysozyme, an enzyme is capable of hydrolysing the beta 1-4 peptidoglycan linkage of micro-organisms in which this site is available, thereby allowing osmostic lysis. Not all organisms are susceptible to lysozyme and therefore would develop some sensitivity to this enzyme (Cohen et al, 1984a).

Lactoferrin is an iron binding glycoprotein. Most bacteria require iron for growth, and the presence of lactoferrin binding the iron is capable of retarding the growth of certain micro-organisms, however iron in excess of the binding capacity of lactoferrin will result in the presence of free iron thereby neutralising the inhibiting action (Arnold et al, 1980).

The presence of zinc in the secretions may play an important role at mucosal surfaces for bacterial survival. Zinc inhibits the growth of a variety of pathogens especially that of C. trachomatis, Herpes simplex virus and T. vaginalis (Cohen et al, 1985). Fibronectin, a glycoprotein, is found in a soluble form in plasma. It plays an important role in the attachment of some pathogenic bacteria to host tissues, but may also interfere with the attachment of others (Cohen et al, 1984a). Soluble fibronectin could be found in vaginal fluid during

menses and following leakage of plasma from damaged vaginal tissues. Its presence may enhance the invasiveness of certain pathogens by facilitating adherence to specific cells.

2.3.1.4 Phagocytic cells

Polymorphonuclear neutrophils and monocytes migrate to tissues in response to microbial invasion and most probably play an important defensive role at mucosal surfaces. The phagocytic cells are usually attracted directly by bacterial products or by the generation of complement components. Once attracted the bacteria will attach to phagocytic cells through ligand receptor interactions and opsonize with immunoglobulin and complement. Once opsonized the killing of the pathogen is enhanced by the neutrophil oxidative metabolism, depending on the redox state of the organism (Root and Cohen, 1981).

2.3.2 Specific immune mechanisms

The role of specific immune mechanism against pathogens in the vagina is still unclear.

2.3.2.1 Antibodies

Antibodies arise from stimulation by specific organisms and are produced locally by plasma cells in the cervical submucosal tissue. IgA antibodies are produced preferentially to IgG and IgM antibodies (McNabb and Tomasi, 1981). Secretory IgA appears to be more important

than IgG and IgM in its role of preventing adherence and of promoting killing (Fubara and Freter, 1973). The antibodies found on mucosal surfaces may interfere directly with the attachment of bacteria or neutralise viruses which could activate the complement cascade resulting in the killing of the susceptible pathogen, or may enhance opsonization and killing (intracellular lysis) of the pathogen by phagocytic cells (Root and Cohen, 1981).

#### 2.3.2.2 Cell mediated immunity

The role of lymphocyte - monocyte interaction at the vaginal mucosal surface is generally non-protective especially against recurrent infections (Cohen et al, 1984b).

## 2.4 ALTERATION OF VAGINAL FLORA

As already mentioned the vaginal ecosystem appears complex. Factors such as normal vaginal flora with the presence of lactobacilli regulated by hormones and the host's defence mechanisms all regulate the composition and dynamics of the microbial ecosystem to produce and maintain a healthy vagina.

Multiple factors influence the composition of the vaginal microflora. Some of these are the bacterial flora themselves, especially the presence of lactobacilli, hormonal and host defence mechanisms. A multiplicity of other largely host factors have also been shown to be capable of influencing the microbial habitat, these include sexual

activity, trauma, antibiotic therapy, hormonal status and iatrogenic factors. Any alteration of vaginal flora may resolve spontaneously and therefore be transient or may result in symptoms. The latter may require therapy if symptoms persist and if the aetiological agent is a micro-organism.

## 2.5 CLINICAL PRESENTATIONS

A variety of conditions which can cause vaginal discharge in normal healthy women may be due to an excessive sloughing off of epithelial cells or excessive mucoid secretions from healthy endocervical glands or due to atrophic vaginitis, desquamative inflammatory vaginitis, vaginitis emphysematosa, vaginal fistulae and vaginal ulcers. Although often diagnosed these conditions are much less common than infections.

The patients may complain of a mild to profuse grey-white discharge with the absence of irritation and odour. These conditions are usually managed with topical oestrogen or hydrocortisone cream depending on the condition and primarily of reassurance to the patient (Fleury, 1981).

Any condition that increases the amount of cervical secretion or desquamation of vaginal epithelium may give rise to a primary vaginal discharge. This may be physiological or pathological. If pathological, the causative organism is able to penetrate through epithelial deficiences. When this happens, patients usually present with a combination of symptoms such as increased vaginal discharge, vulvo-vaginal

irritation including dyspareunia or dysuria and may be accompanied with vaginal odour.

Signs and symptoms are often non-specific to permit an accurate clinical diagnosis since concurrent infections of the cervix and vagina may occur at the same time.

N. gonorrhoeae and C. trachomatis colonize the columnar epithelium, causing a purulent cervical discharge, however they are not the major causes of vaginitis of reproductive age. T. vaginalis and C. albicans are the major causes of vaginitis with vaginal irritation or inflammation respectively. In addition, another cause of vaginal discharge is a milder, unpleasant syndrome termed bacterial vaginosis.

### 2.5.1 Candidiasis

Candida species may be a common commensal of healthy people and can be recovered from the mouth and intestinal tract. In women with predisposing factors such as pregnancy, diabetes, long term antibiotic usages, malignancies and chemotherapy there may be excessive glycogen or glucose in vaginal tissues. This may give the yeast an advantage over normal vaginal bacteria, resulting in vulvovaginal candidiasis. In males Candida species has been responsible for balanitis, thus the organism could be sexually transmitted which may also result in recurrent disease.

Candidiasis is the term given to candida vaginitis and is caused by candida species. Wilkinson in 1849 reported the first case of vaginal discharge in a patient with associated yeast-like organisms (Wilkinson, 1849). In an analysis of data for 1980 and 1981, Young has shown that C. albicans was the most commonly found yeast in cases of vaginal discharge and infection, followed by Torulopsis glabrata and the other species occurring rarely (Young, 1982). Candida species has been isolated from healthy vaginas ranging from 5% to 15% in non-pregnant women to 31% in asymptomatic pregnant females (Young, 1982).

Patients presenting with candidiasis usually complain of pruritis due to intense irritation. The discharge present is characterised as milky-white with white pseudomembranes on the vaginal mucosa. There may be associated dysuria, dyspareunia and the lesions may extend to the labia and perineum causing severe excoriation. There is strong evidence that the organism is sexually transmitted (Miles et al, 1977) but a persistent reservoir in the oral cavity and gastrointestinal tract is important for recurrences (Sobel, 1984).

Vaginal candidiasis can be diagnosed clinically by its cottage cheese appearance with reddened vaginal walls or the discharge examined microscopically in wet smear preparations either in saline or potassium hydroxide or by Gram stain for the presence of yeast and pseudohyphae. The secretions can be cultured onto media for the isolation and identification of the yeast.

## 2.5.2 Trichomoniasis

Donne, in 1836 visualised motile micro-organisms in the purulent frothy leucorrhea of women presenting with vaginal discharge and vaginitis (cited by Ross, 1982).

Initially the organism T. vaginalis received little attention but is now firmly established as being sexually transmitted (McLellan et al, 1981). This is due to the fact that only a trophozoite form is present and no cystic form (Rein and Muller, 1984) thus being sensitive to light, drying and changes in temperature.

T. vaginalis is highly site specific (Rein, 1985) limiting its site of infection to the vagina in the female, therefore no reservoir such as the mouth or rectum can be found (Robinson et al, 1963). The incidence among females in developed communities ranges from 1,4% among single women (Willcox, 1960) to 44,2% in women attending a sexually transmitted diseases clinic. It is generally considered to be more common in pregnant women ranging from 4% detected in developed communities (Willcox, 1960) to 49% detected in developing (Hoosen et al, 1981).

Clinically a patient infected with T. vaginalis may vary from an asymptomatic carrier to one presenting with symptoms which may range from being mild to severe. The symptomatic patient may present with an excessive vaginal discharge which is due to an acute inflammation. The discharge is often offensive, being pale green and frothy and may also be accompanied with additional symptoms such as vulval irritation,

dysuria and frequency of micturition and occasionally dyspareunia. In severe forms the vaginal walls may be diffusely reddened throughout with punctate pink spots - the so-called "strawberry vagina" (Ross, 1982). These features are clinically diagnostic of T. vaginalis and may not require confirmation. In milder conditions and concurrent infections a diagnosis is made by the demonstration of the organism from vaginal secretions in normal saline wet smear preparations. Microscopically the organisms are slightly larger than polymorphonuclear neutrophils and characterised by their jerking motility. The organism can be detected by phase contrast microscopy, and by stained smears such as Papanicolaou, Giemsa and acridine orange, as well as by culture in various media, including modified Diamond's medium. The sensitivity of culture is far superior to staining techniques and the latter have no added advantage over the normal wet smear preparations.

Newer techniques for identifying trichomonads are the immunofluorescent antibody test using monoclonal antibodies (Krieger et al, 1988) and ELISA test and also detecting serum antibodies by these techniques. These are still experimental and seem unlikely that they may be used routinely.

### 2.5.3 Bacterial vaginosis

In 1954 and 1955 Gardner and Dukes first described the clinical entity of a mild, but generally unpleasant vaginal syndrome with a lack of an inflammatory response, naming this clinical condition as non-specific vaginitis.

Subsequently, there has been some controversy regarding the definition of this clinical condition (van der Meijden, 1984). It has been described as a vaginosis, because of the absence of an inflammatory response. Furthermore, it has been microbiologically associated with G. vaginalis (Gardner and Dukes, 1955) and non-sporeforming anaerobes (Pheifer et al, 1978; Taylor et al, 1982) which led to the condition being described as Haemophilus vaginalis vaginitis, Gardnerella (vaginalis)-associated vaginosis, non-specific vaginosis, non-specific discharge, anaerobic vaginosis and bacterial vaginosis. Thus the original description of non-specific vaginitis is no longer acceptable, since this condition is associated with the presence of specific micro-organisms and since an inflammatory response cannot be identified, therefore it should not be termed a "vaginitis".

Over the years bacterial vaginosis has been the most widely accepted definition, since it describes the bacterial association together with the lack of evidence of an inflammatory response. It also denotes "a replacement of lactobacilli of the vagina by characteristic groups of bacteria accompanied by changed properties of the vaginal fluid" (Westrom et al, 1984).

The clinical description of bacterial vaginosis as described by Gardner and Dukes (1955) has remained the most accurate and has been confirmed by many workers (Ray and Maughan, 1956; Brewer et al, 1957; Pheifer et al, 1978; Spiegel et al, 1980; Eschenbach et al, 1988). Typically patients present with a moderate increase in vaginal discharge which is often offensive, greyish, homogeneous with decreased acidity. The

discharge contains few inflammatory cells and any other recognised vaginal pathogens, such as T. vaginalis and Candida species, may be absent.

Of relevance to this syndrome is the increased offensive discharge. The offensive odour is more prominent during menstruation and after sexual intercourse (Vontver and Eschenbach, 1981). The assessment of normal vaginal secretions can be subjective and also depends on the ability of a patient to tolerate an increased discharge, which may be variable. If this condition were to be left untreated it could be distressing to the patient. Vaginal or vulval irritation could occur and dysuria and dyspareunia could be associated.

Normal vaginal secretions are usually curdy, often odourless, whitish and without any frothiness. However, unlike normal secretions, the vaginal discharge in bacterial vaginosis can be scanty to profuse, but usually moderate, homogeneous in nature and appears to adhere to the vaginal wall in a thin film. The colour is often greyish. The discharge in bacterial vaginosis has been reported to be frothy by some authors (Gardner and Dukes, 1955), a criterion often considered to be characteristic of trichomonal vaginitis.

The smell of the discharge is characteristic in that it is often unpleasant with a prominent "fishy" odour. This odour has been attributed to the presence of volatile amines such as putrescine, cadaverine (Chen et al, 1979) and trimethylamine (Brand and Galask, 1986).

The pH of normal vaginal secretions is acidic. However, in bacterial vaginosis the pH is greater than 4,5 and often 5,0 to 5,5. This shift in vaginal pH is often accompanied by a reduction or replacement of vaginal lactobacilli.

Microscopic examination of vaginal discharge from women with signs and symptoms of bacterial vaginosis characteristically reveals a striking absence of polymorphs and lactobacilli and a predominance of vaginal epithelial cells which have a granular appearance. The latter appearance is the result of bacteria adhering to the cells. These cells are characteristic and often provide the most valuable clue to the presence of this condition. These have been designated "clue" cells to emphasize their diagnostic significance (Gardner and Dukes, 1955). Microscopic examination of the Gram stained smears confirms the presence of epithelial cells with large numbers of adherent pleomorphic Gram- negative or variable bacilli.

This clinical condition of bacterial vaginosis is distinct from candidal vaginitis, but shares some similarities with trichomonal vaginitis, particularly the presence of offensive and frothy discharge.

## 2.6 DIAGNOSIS OF BACTERIAL VAGINOSIS

### 2.6.1 Signs and symptoms

The diagnosis of bacterial vaginosis was often based only on the

symptoms (Frampton and Lee, 1964) or on the observation of an abnormal vaginal discharge (Akerlund and Mardh, 1974). The ability of the patient or clinician to assess the presence and amount of discharge is subjective. This depends on the personal hygiene of the patient and the ability to tolerate an increased discharge. A study by Gardner (1980) had shown that a minority of women with infection complained of vaginal discharge and unpleasant odour. However, on specific enquiry, the majority admitted to symptoms. These patients were unaware of the significance of their symptoms and accepted these as normal, however, after appropriate therapy, improvement was noted.

The assessment of the appearance and amount of discharge by the examining clinician has also been subjective. The ability of two clinicians to assess the same patients has shown marked differences (Taylor et al, 1982) even in the assessment of the odour (Clay, 1982).

### 2.6.2 Clinical diagnosis of bacterial vaginosis

The existence of bacterial vaginosis as a clinical entity appears beyond doubt. However, there are no adequate clinical definitions of the syndrome and in addition, there have been no definite criteria for the diagnosis. McCormack et al (1977) suggested that well defined criteria were needed to be established for the uniform and reliable diagnosis of bacterial vaginosis.

Spiegel et al (1980) used specific criteria to define their patients with bacterial vaginosis. These were based firstly on the absence of

recognised pathogens such as T. vaginalis and/or yeasts on wet smear examination, and secondly on the presence of any two of the following: a) homogeneous vaginal secretions; b) pH of the discharge being greater than 4,5; c) presence of "clue" cells on wet smear examination; d) release of 'fishy' odour on addition of 10% potassium hydroxide (KOH). G.vaginalis was isolated from 100% of patients fulfilling these criteria.

Amsel et al (1983) confirmed that symptoms alone were not a reliable indication of the presence of bacterial vaginosis. They found that 50% of their patients with bacterial vaginosis were asymptomatic and 12% were aware of symptoms but did not complain about these. In view of this, they also strongly recommended the use of such specific diagnostic criteria. By combining and selecting the criteria advocated by Spiegel et al (1980) they showed improved diagnostic specificity (Amsel et al, 1983).

These criteria would be useful for the rapid and reliable diagnosis of bacterial vaginosis, in clinic settings, however, assistance from the laboratory may be necessary.

### 2.6.3 Direct microscopy

Direct microscopic examination is often used in the diagnosis of bacterial infections. Using this, Gardner and Dukes (1955) utilized wet smear preparations and described the characteristic "clue" cells (Plate 1). These cells were also seen on Gram stained smears together

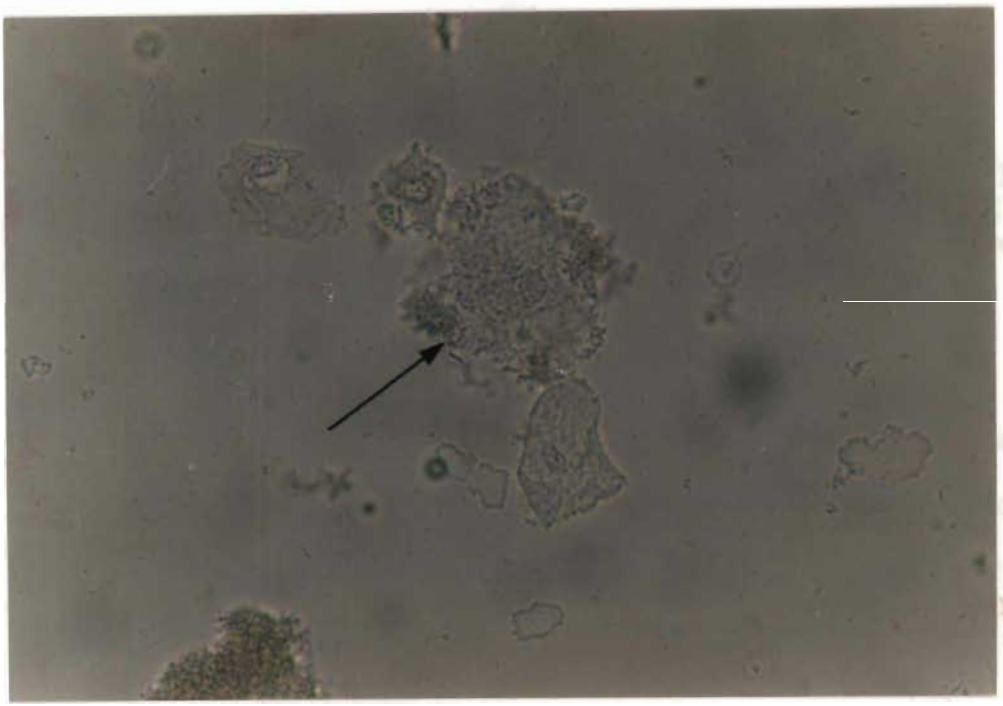

PLATE 1 :

"Clue" cells as seen on vaginal wet smear microscopic preparation (x40 objective)

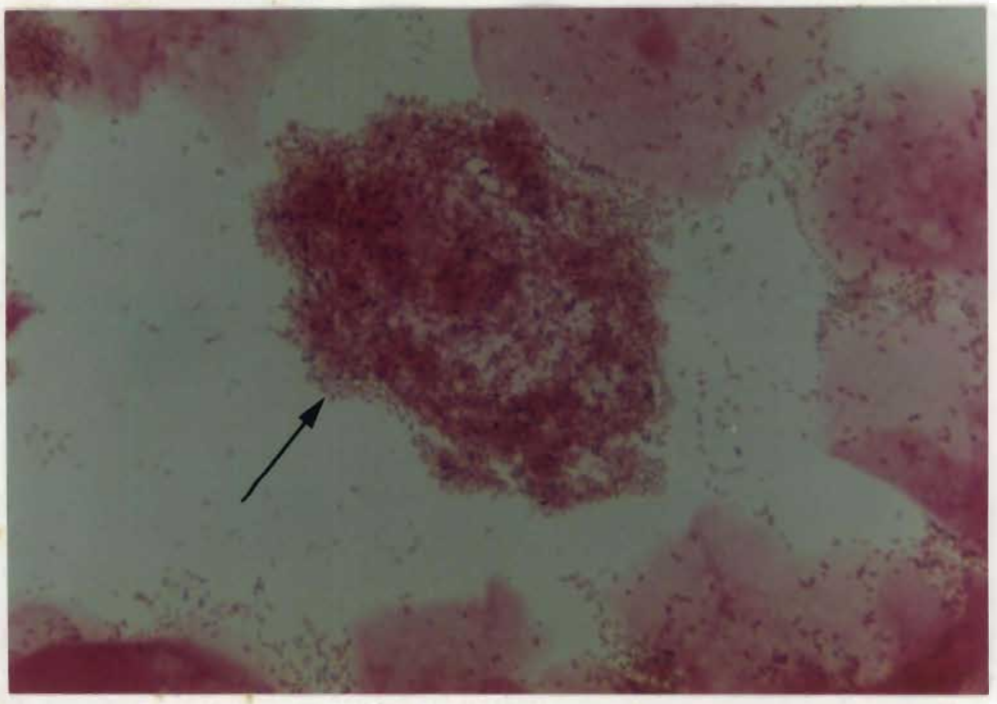

PLATE 2 :

"Clue" cells as seen on Gram stained smear with the presence of gardnerella morphotype predominant in bacterial vaginosis (x50 objective)

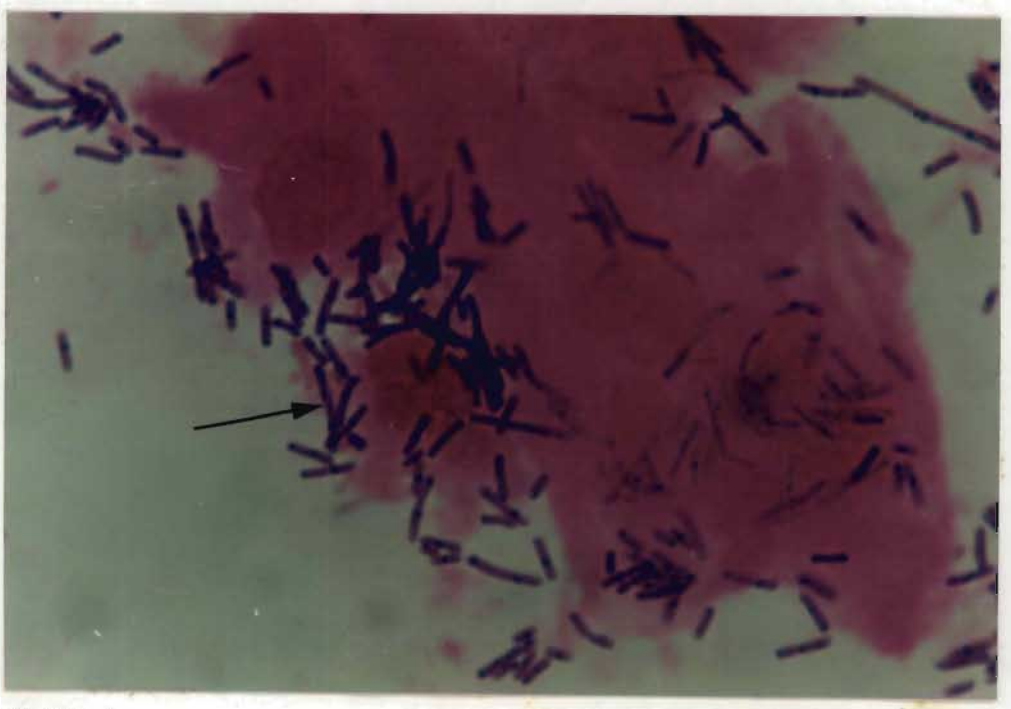

PLATE 3 :

Gram stained smear showing the presence of lactobacillus morphotype predominant in normal vaginal secretions (x100 objective)

Therefore, this study showed that any microscopic changes present in the vaginal flora could result in the isolation of small numbers of G. vaginalis. These would not necessarily produce any significant changes and could be classified as normal. The technique used by Spiegel et al (1983) gave a 90% correlation with a clinical diagnosis of bacterial vaginosis using the specific criteria as described by Amsel et al (1983). Krohn et al (1989) reported that the predictive value for a diagnosis of bacterial vaginosis was as high as 76% for the Gram stain.

Direct immunofluorescence has been used to detect adherent Gram-negative bacteria, G. vaginalis in vaginal specimens (Hansen et al, 1989). This technique was positive for G. vaginalis in 21% of specimens compared to only 12,5% of cultures (Hansen et al, 1987).

### 2.6.4 pH determination

The pH of the discharge in bacterial vaginosis is greater than 4,5, usually between 5,0 and 5,5. Gardner and Dukes (1955) found that 91% of patients with bacterial vaginosis had a pH greater than 5,0 compared to 92% of women with normal discharge who had a pH between 4,0 and 4,7. Elevated pH of vaginal fluid was found to be the most sensitive but least specific of the clinical criteria which can be used for diagnosis (Eschenbach et al, 1988). This could usually be due to presence of concurrent infections with T. vaginalis.

### 2.6.5 Amine test

The offensive "fishy" odour in bacterial vaginosis is due to the presence of cadaverine, putrescine (Chen et al, 1979) and/or trimethylamine (Brand and Galask, 1986). The addition of 10% potassium hydroxide to fresh vaginal discharges enhances the smell. The potassium hydroxide converts the non-volatile salts into highly volatile and unpleasant smelling bases. This happens rapidly, therefore the test must be "read" immediately (Pheifer et al, 1978). False positives may occur in the presence of semen and menstrual fluid.

Pheifer et al (1978) reported that 36(67%) of 54 women with an abnormal discharge and none of the 43 women with a normal discharge had positive amine test with 10% KOH. Amsel et al (1983) found 76% of women with bacterial vaginosis to have positive tests.

### 2.6.6 Culture of organisms

The isolation of G. vaginalis from vaginal fluid has been considered as the cornerstone of diagnosis. The use of improved culture techniques and selective media (Goldberg, 1976; Totten et al, 1982; Ison et al, 1982) has increased the sensitivity with which G. vaginalis may be isolated.

Spiegel et al (1980) have shown that quantitative estimation of the numbers of G. vaginalis present in the vagina had shown differences between women with and without bacterial vaginosis. Thus the presence

of small numbers of G. vaginalis in women without characteristic signs could be encountered (Amsel et al, 1983) and care should be taken in interpreting a positive culture result. Other organisms such as non-sporeforming anaerobes (Taylor et al, 1982), anaerobic curved rods (Hjelm et al, 1981) and mycoplasmas (Pheifer et al, 1978) have all been associated with bacterial vaginosis.

The isolation and identification of these organisms is difficult and usually time-consuming, and the fact that normal women may also harbour these organisms makes screening for them unrealistic.

### 2.6.7 Gas liquid chromatography

The problems encountered in the diagnosis of bacterial vaginosis resulted in the search for alternative reliable procedures.

The biochemical changes occurring in bacterial vaginosis were first noted by Spiegel et al (1980) who utilized gas liquid chromatography to analyse the short chain fatty acids present in vaginal fluids. In normal vaginal fluid lactobacilli are predominant and produce large amounts of the non-volatile lactate, whereas in patients with bacterial vaginosis the predominant organisms are G. vaginalis and anaerobes which are acetate and succinate producers. This results in a reduced lactate and increased succinate peak (Fig 1). A succinate to lactate ratio of $\geqslant$ 0,4 has a predictive value of 90% for a positive G. vaginalis culture (Spiegel et al, 1980; Ison et al, 1982).

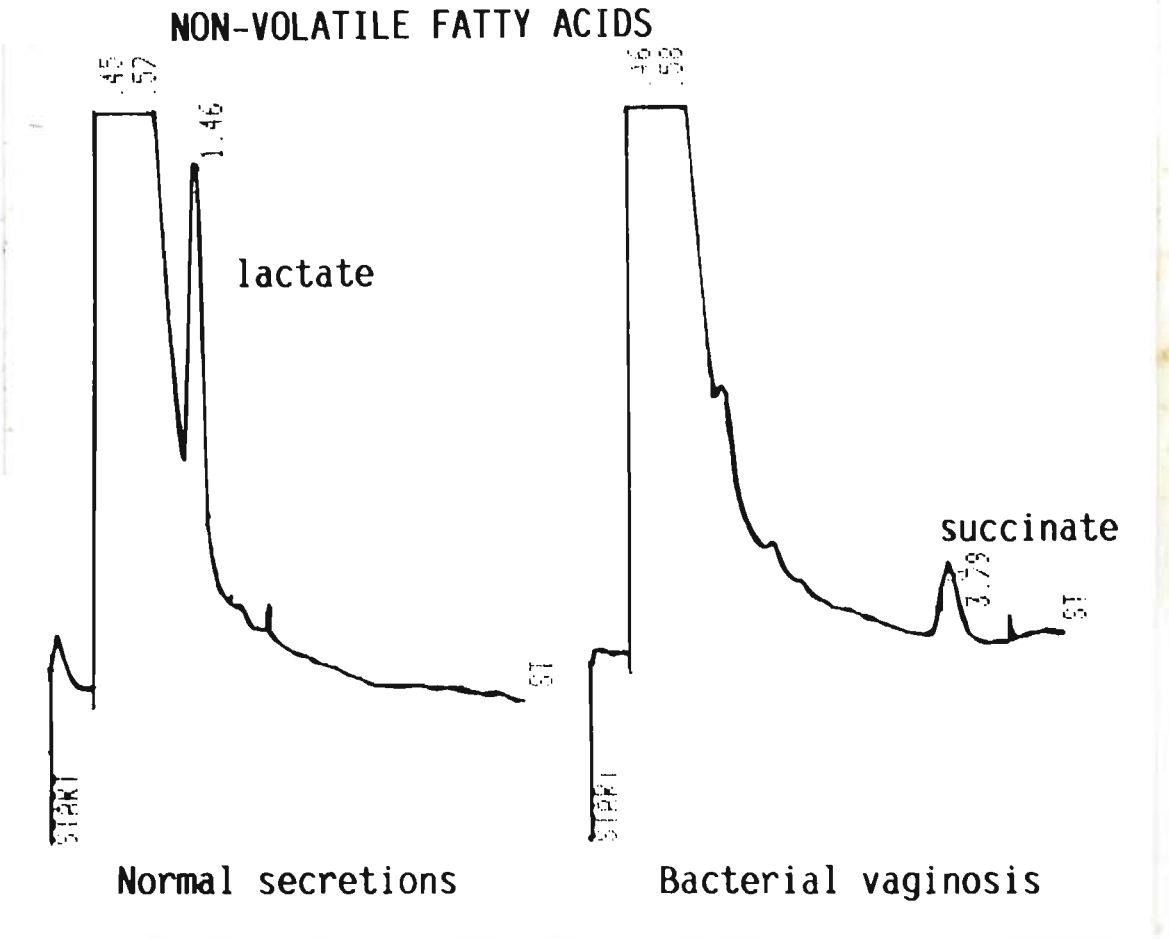


FIGURE 1 :

Gas liquid chromatographic analysis of vaginal secretions:

a Normal secretions showing a lactate peak;

b Bacterial vaginosis showing a reduced lactate peak and increased succinate peak

If volatile fatty acids such as acetate, butyrate and propionate are also detected this too may be a useful indicator of bacterial vaginosis and would slightly increase the sensitivity of the test (Jokipii et al, 1986).

This test has been useful, however false positives do occur in trichomonas vaginitis. Significantly little data are available on concurrent or mixed infections. Other disadvantages are the need for specialised equipment and the requirement of a large sample of vaginal fluid.

### 2.6.8 High-voltage electrophoresis

Chen et al (1979) have analysed vaginal washings using high-voltage electrophoresis to detect the presence of the diamines, putrescine and cadaverine in women with bacterial vaginosis.

### 2.6.9 Thin layer chromatography

A more sensitive technique, requiring a smaller sample was also advocated by Chen et al (1982). The thin-layer chromatography of dansylated vaginal washings detects the diamines. The subsequent fluorescence of the dansyldiamines is compared with that of dansyl alanine. A positive test detecting the presence of diamines was obtained in 88% of women with bacterial vaginosis and a negative test in 90% of women without bacterial vaginosis.

The high-voltage electrophoresis and thin layer chromatography are methods which require expertise and are time-consuming, and may have limited value in a diagnostic laboratory.

## 2.7 AETIOLOGY OF BACTERIAL VAGINOSIS

T. vaginalis and C. albicans are readily recognised aetiological agents of vaginitis and vaginal discharge, however, the aetiology of bacterial vaginosis is still poorly understood.

### 2.7.1 Role of Gardnerella vaginalis

Bacterial vaginosis is the term used to reflect a complex alteration of vaginal bacterial flora with the presence of increased discharge, without an apparent inflammatory response. G. vaginalis has been closely associated with bacterial vaginosis, but whether this represents a true infection is still unclear. Gardner and Dukes (1955) originally associated bacterial vaginosis with H. vaginalis (G. vaginalis) although Curtis (1914) initially described a similar organism from leucorrhoeal vaginal discharges and Leopold (1953) isolated a similar organism from urines and urethra of men and uterine cervix of women.

As the potential role of G. vaginalis increased many workers reported the isolation of G. vaginalis from the vagina of women both with and without any vaginal symptoms or discharge. The rate of isolation varied

from 13% to 92% for symptomatic patients and from 0% to 70,5% for asymptomatic patients (Table V).

There was general agreement among workers that the organism most prevalent in bacterial vaginosis was G. vaginalis. Some however disagreed. Heltai (1959), Frampton and Lee (1962) and Levison (1979), were able to routinely isolate G. vaginalis from patients without any specific signs and symptoms. In addition, McCormack et al (1977) found 29% of asymptomatic sexually inexperienced females to harbour the organism. These authors considered the organism to be part of the endogenous vaginal flora. It was soon recognised that differences in isolation rates between different studies were at least in part due to the lack of well-defined diagnostic criteria. Spiegel et al (1980) and Amsel et al (1983) suggested the diagnosis of bacterial vaginosis should be standardised. Specific guidelines and diagnostic criteria for standard clinical use were proposed. The use of more specific criteria greatly reduced the degree of variation between studies (Table VI).

Subsequent studies applying these criteria, confirmed the highly significant association of G. vaginalis with bacterial vaginosis with 8/12 studies reporting 80% or more patients with G. vaginalis and 4/12 studies reporting between 30% to 80%. However Amsel et al (1983) supported the conclusions of McCormack et al (1977) that G. vaginalis was a common component of the vaginal flora since 40% of women without bacterial vaginosis diagnosed by their criteria were also colonised as compared to 29% of sexually inexperienced women reported in the study of McCormack et al (1977).

### 2.7.2 Role of anaerobic bacteria

Until 1978, G. vaginalis was the only agent considered in the aetiology of bacterial vaginosis. Pheifer et al (1978) described the isolation of a variety of non-sporeforming anaerobes from patients with bacterial vaginosis. The total number of anaerobic bacteria was significantly higher in patients with bacterial vaginosis than in healthy controls. Their findings were subsequently confirmed by other workers (Spiegel et al, 1980; Taylor et al, 1982; Blackwell et al, 1983). The anaerobic organisms most frequently isolated were B. melaninogenicus, B. bivius, B. corrodens (ureolyticus), B. asaccharolyticus, B. disiens and anaerobic cocci (Pheifer et al, 1978; Spiegel et al, 1980; Taylor et al, 1982; Blackwell et al, 1983). The anaerobic bacterial count numbers usually equalled those of G. vaginalis. It was therefore suggested that the syndrome of bacterial vaginosis reflected a mixed infection by G. vaginalis plus anaerobic bacteria which are present in a symbiotic relationship.

More recently anaerobic bacteria, namely the so-called anaerobic curved rods (ACR) (Plate 4) have been associated with bacterial vaginosis by some workers (Hjelm et al, 1981; Sprott et al, 1982; Phillips and Taylor, 1982; Skarin and Mardh, 1982). The presence of ACR was first noted by Curtis in 1913 and 1914, who suggested that they may play a role in leucorrhoeal discharges. However, on most occasions he obtained very poor growth or failed to grow these organisms to allow proper identification. He nevertheless concluded that "the anaerobic flora,

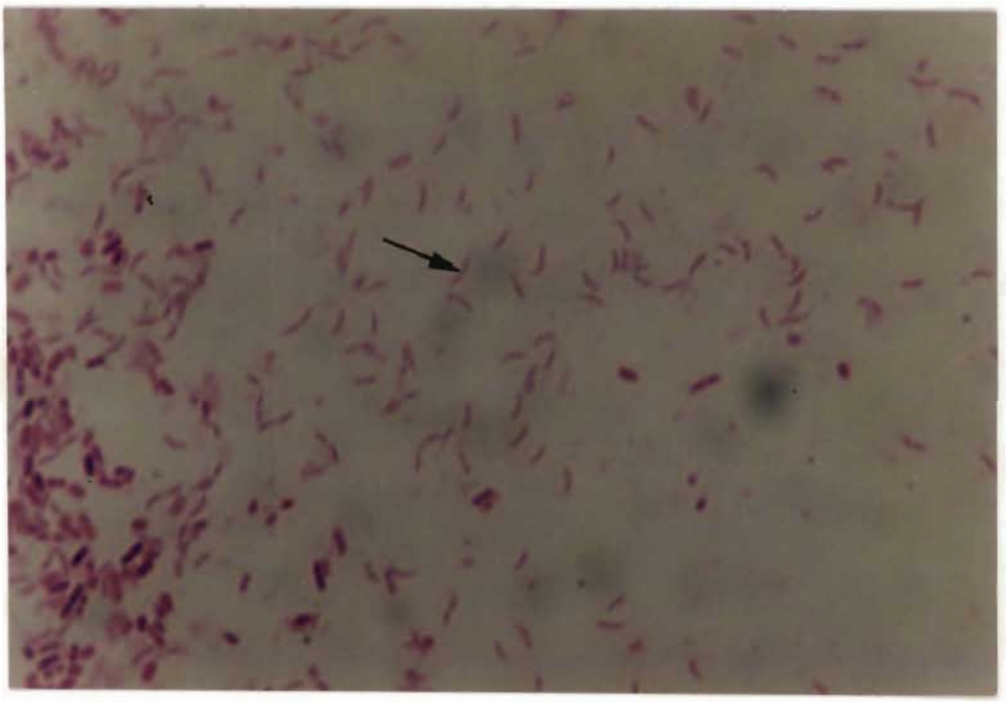

PLATE 4 :

Gram stained smear showing the presence of small Gram-negative curved rods associated with bacterial vaginosis (x100 objective)

notably its bacilli were of paramount importance and that it could play a role in leucorrhoeal discharges" (Curtis, 1913, 1914).

Since Hjelm *et al*'s (1981) rediscovery of the possible role of ACR's several other workers have performed studies supporting the association of ACR's with bacterial vaginosis. Isolation rates ranged from 8,6% to 100% in these studies (Table VII). It is however noteworthy that some workers failed to isolate ACR's or found only a low positivity rate in patients with clinical bacterial vaginosis.

ACR have been characterised and two distinct forms have been identified (Table VIII). They have been found to be genetically and biochemically distinct from any other ACR's and therefore the genus *Mobiluncus* has been proposed (Spiegel and Roberts, 1984).

### 2.7.3 Role of *Mycoplasma hominis*

The role of *M. hominis* in bacterial vaginosis was investigated by Pheifer *et al*, (1978). Although *M. hominis* was isolated from 10 of the 18 patients with bacterial vaginosis and from only one of 18 controls (Pheifer *et al*, 1978), *M. hominis* persisted in the vaginas of patients who responded to therapy for bacterial vaginosis. However, the isolation rate of *M. hominis* was not affected in patients who responded to therapy for bacterial vaginosis. It was therefore concluded that *M. hominis* could not play a significant role in the aetiology of bacterial vaginosis.

TABLE VII : PREVALENCE OF ANAEROBIC GRAM-NEGATIVE CURVED RODS IN BACTERIAL VAGINOSIS

| Reference | Symptomatic | Asymptomatic |
|---|---|---|
| | % | % |
| Hjelm et al, 1981 | 30,0 | 0 |
| Skarin, 1981 | 8,6 | - |
| Phillips and Taylor, 1982 | 11,0 | - |
| Sprott et al, 1983 | 22,5 | 5,1 |
| Thomason et al, 1984 | 100 | - |
| Pattman, 1985 | 21,6 | - |
| Hållen et al, 1987 | 50,0 | - |

TABLE VIII : ≠DIFFERENTIAL CHARACTERISTICS OF ANAEROBIC GRAM-NEGATIVE CURVED RODS (MOBILUNCUS SPECIES)

| Characteristics | M. curtisii sub sp curtisii | M. curtisii sub sp holmesii | M. mulieris |
|---|---|---|---|
| Length of cells (um) | 1,7 | 1,7 | 2,9 |
| Flagella | Multiple subterminal | Multiple subterminal | Multiple subterminal |
| Gram reaction | variable | variable | negative |
| Growth stimulated by arginine | + | + | - |
| Hippurate hydrolysed | + | + | - |
| Acid from glycogen | - | - | + |
| Acid from melibiose | +(-) | -(+) | - |
| Acid from trehalose | - | - | variable |
| Nitrate reduced | - | + | -(+) |
| CAMP reaction | weak | weak | strong |
| G + C content | 51-52% | | 40-50% |
| Type strain | ATCC 35421 | ATCC 35242 | |
| *Lipopolysaccharide | negative | negative | negative |

≠ adapted from Spiegel and Roberts, 1984

* adapted from Carlone et al, 1986

+ = positive

- = negative

+(-) usually positive

-(+) usually negative

The aetiology of bacterial vaginosis remains complex in that anaerobic flora may co-exist with G. vaginalis to cause the vaginosis. Whether they represent distinct clinical entities is still uncertain.

## 2.8 PATHOGENESIS OF BACTERIAL VAGINOSIS

There seems little doubt that G. vaginalis is present in most cases of bacterial vaginosis. Non-sporeforming anaerobes and Mobiluncus species have some role in the pathogenesis. The presence of a single species aetiology is doubtful and two types of organisms may be present and act synergistically to produce signs and symptoms. In bacterial vaginosis there is no true inflammation, no outpouring of pus. Since G. vaginalis is a surface parasite, it rarely provokes signs and symptoms on its own, and the histology of the vaginal biopsy shows no penetration of the epithelium (Gardner and Dukes, 1955).

Transmission of infection to volunteers was demonstrated by Gardner and Dukes (1955) who inoculated 15 healthy women vaginally with vaginal secretions of patients with bacterial vaginosis. Eleven of the 15 women developed clinical signs and symptoms within 10 days and G. vaginalis was isolated from all 11. A further 13 women were inoculated with pure cultures of G. vaginalis. Only 3 of the 13 patients became colonised by the organism and only one developed clinical signs and symptoms of bacterial vaginosis.

On the basis of these findings Gardner and Dukes believed that they had satisfactorily fulfilled Koch's postulates which require the organism to be observed in disease, isolated and grown in pure culture, give

rise to disease when inoculated into a susceptible host and be recovered from the diseased host. Koch's postulates were not entirely fulfilled since vaginal secretions were used and Koch's postulates are aimed at a single pathogen. Since G. vaginalis was the only organism isolated from the secretions, it may reflect the isolation media used were not sensitive enough to detect other vaginal flora which are usually present in vaginal secretions. Thus, the authors may have transmitted a mixed infection with more than one pathogen, which may also be part of the normal flora and also exist in a symbiotic relationship. Therefore it would be difficult to fulfil Koch's postulates.

Criswell et al (1969) showed the capacity of G. vaginalis to induce infection in volunteer subjects. Five of the 9 patients given approximately $2x10^{10}$ colony forming units of a 12 hour culture became colonised with G. vaginalis and had clinical evidence of bacterial vaginosis and only 2 of the 20 women given a 24 hour culture of the same inoculum produced the same symptoms. The differences in these results may be due to the age of the inoculum when grown in a biphasic medium. This appeared to be an important factor, since optimal viability was obtained in 12 hours when the organisms were in a logarithmic phase of growth. Viability decreased after 24 hours when the organisms were in a stationary phase (Criswell et al, 1969). Although the minimum infecting dose of G. vaginalis is yet unknown it seems that a high infective dose was necessary to produce infection. It is not clear whether reservoirs providing such high infective doses exist in nature. At present available evidence thus suggests that G. vaginalis infection is transmissible, but this is not yet conclusive.

In bacterial vaginosis, the odour of vaginal discharge is offensive, with a characteristic smell; this is considered to be due to the presence of diamines such as putrescine and cadaverine, (Chen et al, 1979). Since G. vaginalis is known to produce acetic acid (Moss and Dunkelberg, 1969) but not produce these amines, other vaginal anaerobic bacteria can. It would therefore seem that mixed infections may be synergistic in rendering the environment alkaline, whilst presence of putrescine may be a growth factor for the survival of G. vaginalis (Chen et al, 1979).

In bacterial vaginosis the presence of so-called "clue" cells is often characteristic. "Clue" cells are vaginal squamous epithelial cells studded with organisms seen during wet smear microscopy. G. vaginalis does not have adhesins but may be coated with protein and carbohydrate which act as adhesins (Peeters and Piot, 1985) and in vitro it has the ability to adhere to epithelial cells more so than lactobacilli (Mardh and Westrom, 1976). Regarding pathogenesis, it is unclear whether G. vaginalis adheres to epithelial cells and causes shedding or whether adherence occurs on exfoliated cells. Chen et al (1979) have suggested that amines such as cadaverine and phenethylamine present in vaginal washings are skin irritants and these may contribute to the exfoliation of epithelial cells. With regard to pathogenesis, the ability of G. vaginalis to adhere to epithelial cells is unclear.

A possible relationship between G. vaginalis and anaerobic bacteria exists in the pathogenesis of bacterial vaginosis resulting in an

increased production of amines, raising vaginal pH at a level ideal for the growth of G. vaginalis (Piot and Vanderheyden, 1982).

To further elucidate the pathogenesis of bacterial vaginosis, the grivet monkey, whose genital tract shows close morphological and physiological similarities to that of the human vagina, was used as an experimental model for vaginosis (Mardh et al, 1984). The vaginas were inoculated with ACR's alone or in combination with G. vaginalis. The results of the monkey challenged with G. vaginalis alone were invalid due to a technical error, however, the monkey challenged with G. vaginalis and a long variant of anaerobic curved rod produced within 6 days distinct signs and symptoms of bacterial vaginosis. The vaginal discharge was thin, grey, translucent and did not show an increase in leucocytes. In addition, the vaginal mucosa appeared normal without any macroscopic signs of inflammation (Mardh et al, 1984).

The vaginas of three different primate species were challenged with G. vaginalis (Johnson et al, 1984). Ten pig-tailed macaques became colonised between 11 to 39 days whereas four tamarins and three chimpanzees failed to become colonised. However, specific signs associated with bacterial vaginosis, that is, "clue" cells, raised vaginal pH, increased levels of volatile fatty acids were not detected. The failure to produce disease with specific signs and symptoms may have been due to the different physiology and microbial flora of the primate genital tract and/or the need for the presence of other anaerobic organisms to act synergistically to produce disease.

It remains unclear what initially causes the imbalance of normal vaginal flora, resulting in an overgrowth of organisms in bacterial vaginosis, thereby allowing them to act as pathogens. It may be a transient change in the physiological state of the patient or due to the male sexual partner, providing a passive reservoir for reinfection (Ison and Easmon, 1985).

## 2.9 EPIDEMIOLOGY OF BACTERIAL VAGINOSIS

Bacterial vaginosis has been considered to be a sexually associated rather than a sexually transmitted condition (Easmon, 1986). Sufficient data have been provided for this. The acquisition of the organism G. vaginalis after the onset of sexual intercourse has been shown by Gardner and Dukes (1955, 1959) who described 2 and 6 of their patients who acquired G. vaginalis after marriage. Lee and Schmale (1973) recovered G. vaginalis from 21 of 154 young women who acquired the organism after release from a correctional school for the weekend.

G. vaginalis has further been associated with sexual activity since it has been found more frequently in women with a history of marital instability (Josey et al, 1976), promiscuity (Gardner et al, 1957), pregnancy (McCormack et al, 1977), abortion (Amsel et al, 1983), in those with multiple sexual partners, those receiving oral contraceptives (McCormack et al, 1977) and in patients using intrauterine contraceptive devices (Amsel, 1983).

G. vaginalis has rarely been found to be present in the vagina before puberty (Kummel, 1963, cited by Taylor-Robinson, 1985). Its absence in such females provides additional evidence that its presence may be associated with sexual activity. However, this has been disputed by McCormack (1977) who found 29% of asymptomatic sexually inexperienced women to be colonised. Likewise Amsel et al (1983) found 4/18 sexually inexperienced women to be colonised with G. vaginalis; none of these subjects fulfilled their diagnostic criteria for bacterial vaginosis.

There is overwhelming evidence that G. vaginalis is often present in association with recognised sexually transmitted pathogens. In particular it has been found together with T. vaginalis (Ray and Maughan, 1956; Brewer et al, 1957; Josey et al, 1976; McCormack et al, 1977; Taylor et al, 1982; Blackwell and Barlow, 1982; Amsel et al, 1983) and N. gonorrhoeae (Levison et al, 1977; Blackwell and Barlow, 1982) and occasionally with Candida species (Ray and Maughan, 1956; Josey et al, 1976). All these studies provide significant evidence associating G. vaginalis with sexual activity, however, if bacterial vaginosis is to be considered a sexually transmitted infection, the presence of a male reservoir needs to be established.

Leopold (1953) was the first to show that G. vaginalis could be isolated more frequently from the urine of men whose wives had vaginal cultures positive for G. vaginalis than from randomly selected men.

Gardner and Dukes (1955) showed a prevalence of 96% in the urethras of male sexual partners of women harbouring G. vaginalis. High prevalences of colonisation in males have been confirmed (Table IX).

In addition Ison et al (1985) found G. vaginalis in 38% of semen samples from unselected men attending an infertility clinic. Although the minimum infecting dose of G. vaginalis is not yet known, it has been suggested that semen could act as a vehicle for the sexual transmission of G. vaginalis (Easmon, 1986).

Colonisation of the male urethra by G. vaginalis usually results in an asymptomatic carrier state (Gardner, 1980; Taylor-Robinson, 1985) and the existence of this established male reservoir could explain the recurrent infections experienced by some female patients. Successful therapy with sulphonamide creams (Pheifer et al, 1978 and Blackwell et al, 1983) and metronidazole has prevented recurrences of colonisation in women with G. vaginalis infections. Recurrence after successful therapy further supports the concept of reinfection from the male partner. Pheifer et al (1978) found that reinfection rates in women after successful therapy were lower when their sexual partner used barrier contraceptives or when they refrained from sexual intercourse. The lower incidence associated with barrier methods was confirmed by Bramley (1981). These findings all suggest that effective therapy of both female patients and their male partners should diminish the frequency of recurrences.

TABLE IX : OCCURRENCE OF *GARDNERELLA VAGINALIS* IN MALES

| Reference | Specimen | Sexual contacts of *G. vaginalis* +ve women | | Unselected men | |
|---|---|---|---|---|---|
| | | Total tested | No +ve (%) | Total tested | No +ve (%) |
| Leopold, 1953 | Urine | 9 | 4(44,5) | 965 | 54(5,5) |
| Josephson and Thomason, 1986 | Urine | NT | NT | 14,178 | 322(3,2) |
| Gardner and Dukes, 1955 | Urethra | 47 | 45(96) | 2 | 0(0) |
| Gardner and Dukes, 1959 | Urethra | 101 | 91(90) | 38 | 1(3) |
| Pheifer *et al*, 1978 | Urethra | 34 | 27(79) | 3 | 0(0) |
| Blackwell *et al*, 1982 | Urethra | NA | NA(30) | NT | NT |
| Kinghorn *et al*, 1982 | Urethra | NT | NT | 194 | 14(7,2) |
| Dawson *et al*, 1982 | Urethra | NT | NT | 430 | 49(11,4) |
| Holst *et al*, 1985 | Urethra | 19 | 0(0) | 309 | 14(4,2) |
| Ison and Easmon, 1985 | Semen | NT | NT | 58 | 22(38) |
| Abdennader *et al*, 1990 | Urethra | 40 | 14(35) | 44 | 5(11) |

NT = Not tested

NA = Not variable

G. vaginalis has also been reported by some workers to be more prevalent in certain population groups. In particular it has been isolated more frequently in women of the non-white population (Lewis and O'Brien, 1969; Gardner et al, 1957; Dunkelberg, 1962; McCormack et al, 1977).

Other potential factors have been studied. Variables such as age, marital status, menstrual history and personal hygiene have not been shown to have a significant influence on the prevalence of vaginal colonisation by G. vaginalis.

Numerous studies have been done to determine the prevalence of G. vaginalis in women of different population groups and female patients attending various clinics. Not unexpectedly considerable variations in the prevalence of G. vaginalis culture positivity have been found. This wide variation is not surprising since these include patients with bacterial vaginosis or vaginal discharge due to other causes or those with no apparent or abnormal vaginal disease. These studies are summarised in Table X and it is clear that G. vaginalis may be commonly found in a significant proportion of women.

## 2.10 GARDNERELLA VAGINALIS

### 2.10.1 Taxonomy

Since it was first recognised, the taxonomy of G. vaginalis has

TABLE X : SUMMARY OF STUDIES ON THE PREVALENCE OF GARDNERELLA VAGINALIS IN VARIOUS CLINIC POPULATIONS

| Reference | Clinic | Type of patient | Race | No. of subjects investigated | No. (%) with vaginal culture positive for G. vaginalis | |
|---|---|---|---|---|---|---|
| | | | | | No | % |
| Gardner and Dukes, 1955 | Private | O, G | White | 1181 | 141 | 12,0 |
| Ray and Maughan, 1956 | Clinic | NK | NK | 447 | 68 | 15,2 |
| Gardner et al, 1957 | Private | NK | White | 2251 | 274 | 12,2 |
| Delaha et al, 1964 | Private | G, P, PP | NK | 121 | 27 | 22,3 |
| Lewis and O'Brien, 1969 | Clinic | NK | NK | 475 | 137 | 28,8 |
| Dunkelberg, 1970 | Clinic | STD | White/Negro | 200 | 62 | 31,0 |
| Bramley et al, 1981 | Clinic | FP | NK | 522 | 27 | 5,0 |
| Osborne et al, 1982 | Clinic | G | NK | 383 | 67 | 17,5 |
| Ratnam and Fitzgerald, 1983 | Clinic | G, FP, STD | NK | 1585 | 417 | 26,3 |
| Mirza et al, 1983 | Clinic | STD | NK | 100 | 75 | 75,0 |
| Kelsey et al, 1987 | Private | FP | NK | 173 | 13 | 7,7 |
| Lefèvre et al, 1988 | Clinic | NK | NK | 392 | 147 | 37,5 |
| *Fehler et al, 1984 | Clinic<br>Clinic | STD<br>FPC | Urban Black<br>Urban Black | 205<br>101 | 96<br>56 | 47,0<br>55,0 |
| *Petersen et al, 1986 | Clinic<br>Clinic | STD<br>Non G | Rural Black<br>Rural Black | 50<br>50 | 30<br>27 | 60,0<br>54.0 |

* = Southern African studies
O = Obstetric
G = Gynaecology
P = Pregnant
PP = Post-partum
STD = Sexually transmitted disease
FP = Family planning
NK = Not known

presented problems. In part this has been due to its variable staining by Grams' method. In addition, as described in section 2.10.4 (Identification), workers initially did not apply uniform criteria for its identification. Leopold (1953) first reported the isolation of small Gram-negative rod-shaped bacteria from patients with prostatitis and cervicitis, however, no attempt was made to classify the organism. Two years later, in 1955, Gardner and Dukes (Gardner and Dukes, 1955) described a similar fastidious organism from vaginal secretions which was implicated in bacterial vaginosis. They proposed the name Haemophilus vaginalis based on colonial morphology, growth in thioglycollate broth and characteristic biochemical profile (Table XI). This organism was initially isolated on blood containing media, which led to its inclusion into the genus Haemophilus.

Although Edmunds (1960a) described the organism as a small Gram-negative non-sporing bacillus resembling H. influenzae, he suggested that microscopically "...it has a tendency to grouping in parallel rows and to show some angling, with two bacilli meeting at an acute angle at their ends, rather like diptheroid bacilli and there is associated with this a tendency to Gram positivity in occasional bacilli. These tendencies are much increased by growth in fluid media or in media containing fermentable carbohydrates". This description would be appropriate for corynebacteria, however he justified the use of the generic name Haemophilus since the organism appeared to require X factor (haemin) although it did not require V factor (nicotinamide adenine nucleotide) (Edmunds, 1960b).

TABLE XI : GARDNERELLA VAGINALIS AS DESCRIBED BY GARDNER AND DUKES IN 1955.

- Gram-negative, pleomorphic rod
- Non-motile
- Growth on sheep blood agar after 24-48 hours at 37°C; forming minute pinpoint colourless, transparent colonies 0,05-0,2mm in diameter.
- Growth in thioglycollate broth microaerophilic with small puff-ball appearance
- Oxidase-negative
- Acid produced from: glucose, maltose, arabinose, rhamnose, laevulose, xylose
- No action on: lactose, sucrose, mannitol, trehalose, glycerol, raffinose, salacin or dulcitol
- Sensitive to bacitracin, tetracycline
- Resistant to penicillin, polymyxin and streptomycin

Lapage (1961) was the first to demonstrate that neither X factor nor V factor were essential for its growth, invalidating its placement in the genus Haemophilus, since no organism can be placed in the genus Haemophilus unless it requires X and/or V factors or any other definable coenzyme-like substance (Zinneman, 1967). However, these factors were stimulatory (Dukes and Gardner, 1961). This bacterium, he concluded did not belong to the genus Haemophilus (Lapage, 1961).

Zinneman and Turner (1963) proposed the name Corynebacterium vaginale on morphological grounds since it was a Gram-positive rod, showing club formation, polar granules and characteristic chinese letter arrangement resembling that seen with corynebacteria. Dunkelberg et al (1969), endorsed its placement in the genus Corynebacterium on these grounds. However, unlike other corynebacteria the organism was catalase negative. Further, doubts on its classification as Corynebacterium were the conflicting reports on its cell wall structure.

Certain features associated with Gram-positive organisms were demonstrated : electron microscopy showed it to form a cell wall with septa (Reyn et al, 1966) and Jones and Weitzman (1971) showed it to contain citrate synthase. The cell wall of H. vaginalis 594 (G. vaginalis NCTC 10287) contained 6-deoxytalose but not arabinose which is normally present in corynebacteria (Vickerstaff and Cole, 1969). In comparative studies performed with Escherichia coli, this strain 594 was found to possess a multi-layered cell wall containing 11 to 14 amino acids, a low mucopeptide content and no techoic acid - all features considered to be consistent with Gram-negative organisms,

whilst diaminopimelic acid was not demonstrated (Criswell et al, 1971). Although an outer membrane was not readily apparent (Costerton et al, 1974) these results indicated that this organism had properties closer to those of Gram-negative organisms. Further support was by Greenwood and Pickett (1980) who showed the presence of a "lipopolysaccharide-like" fraction associated with the cell wall of G. vaginalis.

In contrast, the presence of simple amino acid profiles including lysine was reported by Harper and Davis (1982), who also did not find diaminopimelic acid (Criswell et al, 1971; Harper and Davis, 1982) which is usually found in Gram-negative cell walls and in a few Gram-positive species. Subsequent electron micrscopy studies confirmed the presence of a very thin cell wall, absence of an outer membrane or any other lamellae structure (Sadhu et al, 1989). These results were in agreement with those of Reyn et al (1966).

In contrast to Greenwood and Pickett's (1980) work, Sadhu et al (1989) failed to demonstrate 2-keto-3 deoxy-D-mannose-2-octonoic acid, heptose and hydroxy fatty acids which are specific for the lipopolysaccharide (LPS) of Gram-negative baccili. Thus the results confirm G. vaginalis cell walls to be that of Gram-positive organisms, however, they are unusually thin in most cells.

Overall these results favour G. vaginalis to have cell walls more closely to resemble that of Gram-positive bacteria, however the walls appear to be unusually thin. Clearly this organism does not belong to

the genus Haemophilus or Corynebacterium. Attempts were made to place it in genera such as Lactobacillus, Butyribacterium and Propionibacterium, however placement in the latter three did not apply as G. vaginalis produces acetic acid as the principal end product (Moss and Dunkelberg, 1969) rather than lactic, butyric or propionic acid produced by these three genera respectively. Further, the guanine plus cytosine content of the DNA of G. vaginalis (42 mol %) distinguishes it from true Corynebacterium spp (51 mol %) and Propionibacterium spp (53 mol %).

Numerical taxonomic analysis performed on numerous clinical isolates of G. vaginalis showed that all strains form one tight cluster with a 95% similarity. These could not be split into species or biovars (Greenwood and Pickett, 1980; Piot et al, 1980). The same researchers performed the DNA homology studies which showed little homology between G. vaginalis strains and related species. On the basis of these findings Greenwood and Pickett (1979, 1980) recommended the creation of a new genus to accommodate this clearly different bacterium. They proposed the name Gardnerella in honour of Doctor H L Gardner. Only one species has been identified so far and the genus cannot be assigned to any family because of its unusual cell wall characteristics. The accepted name is now Gardnerella vaginalis and its features are detailed in Table XII.

TABLE XII : CHARACTERISTICS OF GARDNERELLA VAGINALIS STRAIN : 594 OF GARDNER AND DUKES =ATCC 14018 = NCTC 10287 (Greenwood and Pickett, 1980)

- Pleomorphic, Gram-negative to Gram-variable rod shape
- Non-encapsulated, non-motile
- Facultatively anaerobic
- Temperature requirements : optimum 35°C to 37°C; growth occurs within range of 25°C to 42°C
- pH requirements: optimum 6,0 to 6,5; No growth at pH 4,0
- Haemolysis
  - Beta-haemolysis on human and rabbit blood agar
  - No haemolysis on sheep blood agar
- Fastidious in its growth requirements, does not require X or V factor
- Fermentative; acetic acid is major end product
- Antigenic structure : seven serological groups
- Pathogenicity:
  Major role in "non-specific" bacterial vaginitis
- Source : isolated from human genital/urinary tract. Worldwide distribtuion
- DNA base composition : 43+/- 1 mol % G+C

## 2.10.2 Antigenic structure/determinants

Although the morphological, cultural and biochemical characteristics of G. vaginalis has been studied in considerable detail, reports on its antigenic structure are somewhat scarce. Various workers have investigated the antigenic structure of G. vaginalis. One object of such studies is the recognition of specific antigenic determinants so that there could be a possibility of investigating serovars based on antigenic differences.

Edmunds (1962) utilized whole cell suspensions as antigen to produce a panel of 13 antisera raised in rabbits. These were used to develop a serogrouping scheme based on serogroup antigens. Since G. vaginalis organisms do not often produce smooth suspensions they are unsatisfactory for use in agglutination tests. By utilizing the precipitation test, these antisera grouped 36 of the 50 strains of G. vaginalis studied, placing them in one of 7 main serological groups. Three of the 50 strains were non-groupable with these 13 antisera and 11 strains gave indefinite reactions. Edmunds was unable to show significant correlation between different antigenic groups and the clinical origin or biochemical properties of the strains. The antigens used were not further characterised.

Vice and Smaron (1973) and Svarva and Maeland (1982) also raised antisera in rabbits using whole cell suspensions. These were used exclusively in an indirect fluorescent antibody method for the identification of G. vaginalis. Vice and Smaron (1973), used 6

reference strains to prepare antisera. The strain ATCC 14018 (G. vaginalis NCTC 10287) grown diphasically produced the most satisfactory antisera. After absorption with L. acidophilus the antisera reacted with 6 reference strains and 10 clinical strains of G. vaginalis, and no cross-reactions were demonstrated with strains of C. diphtheria, C. xerosis and L. acidophilus.

The findings of Svarva and Maeland (1982) were similar to those of Vice and Smaron (1973), but they observed reduced reactivity with one of the 206 clinical isolates of G. vaginalis studied and confirmed lack of cross-reactivity of the anti-Gardnerella sera with bacteria of other species.

Using an Ouchterlony immunodiffusion test Smaron and Vice (1974) studied the antigenic relationship between isolates of G. vaginalis. The antiserum was raised in rabbits against strain 594 of G. vaginalis. On the basis of the precipitin bands obtained, they showed that all strains of G. vaginalis examined, had a common antigenic determinant. However, the pattern of precipitin bands varied between strains and when strains were under different culture conditions. A distinct band of reaction (identity) was obtained with 12 of the 15 strains tested and this appeared to reflect one antigen of high molecular weight. There was no antigenic relationship between G. vaginalis and C. diphtheria, C. cervicis, C. xerosis and L. acidophilus.

Broth cultures of G. vaginalis have been found to contain three extracellular soluble antigens, which have been designated (i), (o) and

(m). These antigens are heat stable, resist protease but are susceptible to periodate treatment and thus may be polysaccharide or glycoprotein in nature (Smaron and Vice, 1977). Absorption studies suggested that these antigens may be cell wall antigens situated at or near the surface of the organism.

To identify the antigenic components of G. vaginalis, sensitive techniques such as sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting have been used (Boustouller et al, 1986). Although inter-strain variation in immunogenic polypeptide profiles was demonstrated, a common antigen of molecular mass 41 kilo daltons (kDa) was found in all 23 strains tested. Both proteins and lipopolysaccharides can be separated by SDS-PAGE, and to determine the nature of the species-specific antigen, the G. vaginalis extract was treated with proteinase K prior to electrophoresis. Since bands developed only from the untreated material, it was concluded that the antigen was protein in nature. However, the authors were unable to determine whether the antigens were situated on the bacterial cell surface or not, since homogenised suspensions were used.

Identification of this large molecular weight protein antigen, which appears to be common to all G. vaginalis strains could open the way for raising species specific monoclonal antibodies for diagnostic application.

Other workers have attempted to develop a serotyping system for G. vaginalis based on other antigens. Ison et al (1987) raised antisera which were directed against different immunodominant proteins ranging from 40 kDa to 90 kDa in size. These antisera were used to develop a serotyping scheme for G. vaginalis.

### 2.10.3 Isolation

G. vaginalis is fastidious in its growth requirements and therefore cannot be easily cultivated in vitro. Leopold (1953) first isolated the organism on medium containing rabbits blood (Casman's) after 48 hours incubation. The colonies were generally minute with definite haemolysis. Thereafter, Gardner and Dukes (1955) also isolated the organism on medium containing sheep blood.

Dunkelberg and McVeigh (1969) demonstrated the growth requirements of G. vaginalis to include five of the B-vitamins (riboflavin, thiamine, niacin, folic acid and biotin), purines, pyrimidines, inorganic salts, trace metals and some amino acids. These substances were usually present together with a fermentable carbohydrate in the dextrose starch agar which they used as an isolation medium.

The growth of the organism is enhanced when cultures are incubated in an atmosphere with increased carbon dioxide and also when incubated anaerobically.

In view of the fastidious nature of the organism, a variety of culture media have been developed and tested. Blood containing media (Leopold, 1953; Gardner and Dukes, 1955; Edmunds 1960a), chocolate agar (Pheifer et al, 1978), dextrose starch agar (Dunkelberg and McVeigh, 1969), peptone starch dextrose agar (PSD) (Dunkelberg et al, 1970) and human blood agar (Edmunds, 1960a; Greenwood et al, 1977; Totten et al, 1982; Ison et al, 1982) will all support the growth of G. vaginalis. The cultural characteristics of G. vaginalis on these are detailed in Table XIII.

These various media all have some advantages and disadvantages. On PSD agar, G. vaginalis produces characteristic colonies but the use of a dissecting microscope is necessary for their detection. Chocolate agar supports its growth, however, the colonies may be difficult to differentiate from those of other vaginal flora.

The acidity of the medium is also important, G. vaginalis grows at an optimal pH of 6 to 6,5 (Edmunds, 1960a) and the choice of peptone in providing the vitamins, purines and pyrimidines is critical. Since the organism utilises these to produce acetic acid (Moss and Dunkelberg, 1969) which may lower the pH to less than 4,5 which is inimical to G. vaginalis, subculture is necessary every 48 hours. The organism does not grow on MacConkey, Thayer-Martin and tellurite containing agar (Taylor-Robinson, 1985).

Since G. vaginalis grows poorly and is usually difficult to isolate in the presence of other vaginal flora, attempts have been made to produce

media with both selective and indicator properties. A modification of the PSD agar was made by replacing soluble starch with corn starch and bromocresol purple as a pH indicator (Smith, 1975). Only organisms such as G. vaginalis which utilise starch will grow well on this medium and produce a yellow colour. A modification of this medium took advantage of the ability of G. vaginalis to hydrolyse starch resulting in a zone of clearing around each colony (Mickelson et al, 1977).

The original success of Casman's agar (Casman, 1947), and media containing sheep blood prompted the investigation of other blood containing media for the isolation of G. vaginalis. Greenwood et al (1977) developed their so-called vaginalis agar which contained 1% proteose peptone and 5% whole human blood. This medium supported the growth of G. vaginalis, colonies of which produced unique beta-haemolysis and could thus easily be recognised. Furthermore, the use of a dissecting microscope was not necessary.

The inclusion of selective agents such as colistin, nalidixic acid (Goldberg and Washington, 1976) and amphotericin B (Spiegel et al, 1980) to suppress the growth of other vaginal organisms was introduced almost at the same time as the re-introduction of media containing blood (Goldberg and Washington, 1976). Since human blood was found to be more sensitive, Totten et al (1982) modified Columbia colistin-nalidixic acid (CNA) agar by overlaying this base with agar containing 5% human blood agar plus amphotericin B to create bilayer plates. The advantage of this medium is that the beta-haemolysis is more distinct and the high degree of selectivity enables small numbers of G. vaginalis to be detected.

Ison et al (1982) investigated the selective ability of human blood agar containing amphotericin B and nalidixic acid with either gentamicin or with colistin. The medium containing gentamicin was more effective than colistin in suppressing other vaginal organisms whilst the production of beta-haemolysis was not affected. In addition they confirmed the differential beta-haemolysis phenomenon, namely that G. vaginalis produces diffuse beta-haemolysis on human blood agar (Plate 5) and not on horse blood agar (Plate 6). This differentiation is a useful aid in the rapid identification of the organism.

As most sexually transmitted pathogens are fastidious in nature and do not survive delay in transport prior to culture, specimens are often cultured directly onto appropriate media immediately after collection or otherwise sent to the laboratory in suitable transport media.

The ideal transport medium should maintain viability of the organism(s) in transit and allow the recovery of the maximum number of organisms, even if they are present in small numbers. Difco proteose peptone, 1,5% in distilled water (Dunkelberg et al, 1970), Stuart transport media (Akerlund and Mardh, 1974; Jolly, 1983), heart infusion broth supplemented with 1% proteose peptone No 3 (Bailey et al, 1979) and Amies' medium (Josey et al, 1976; Ison et al, 1982), have proved satisfactory as transport media for specimens for the recovery of G. vaginalis. In a subsequent study by Bailey et al (1979) it was found

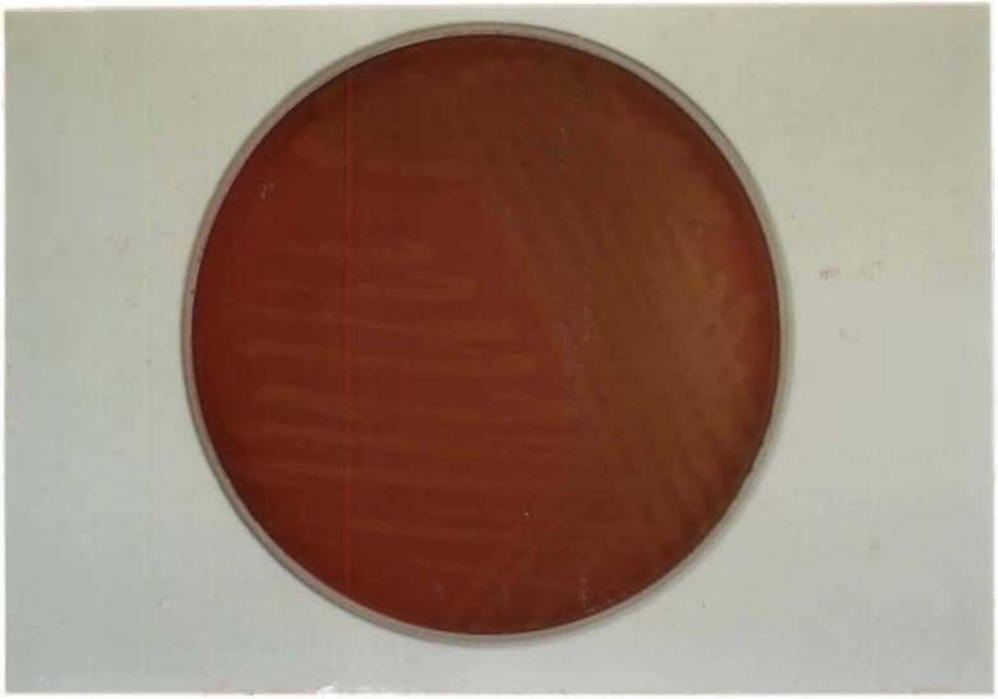

PLATE 5 :

Production of diffuse beta-haemolysis on human blood agar by Gardnerella vaginalis

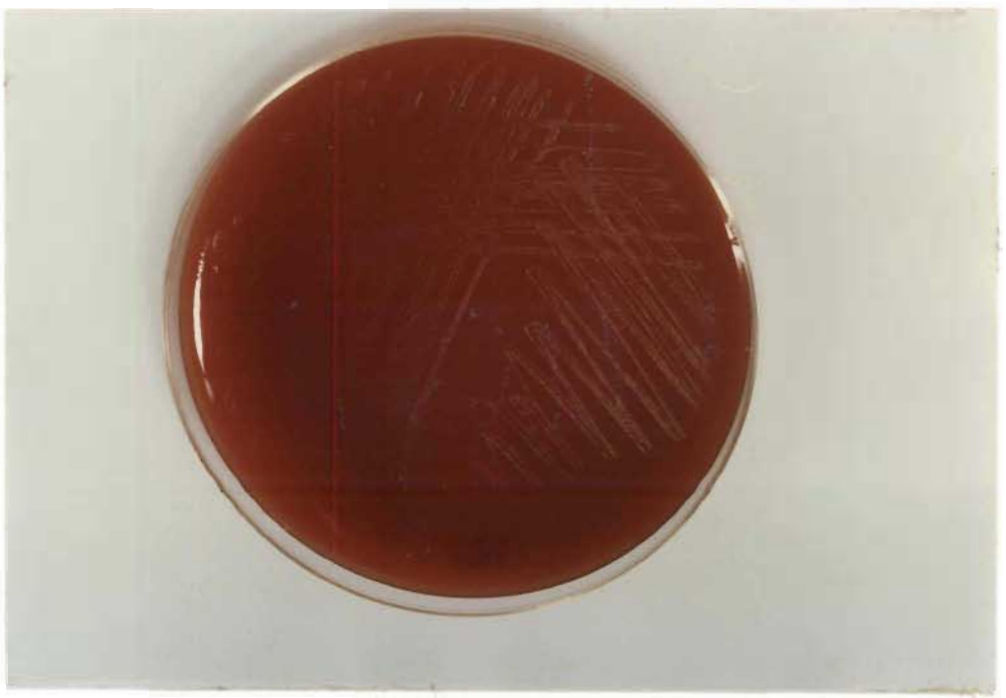

PLATE 6 :

Absence of beta-haemolysis on horse blood agar by Gardnerella vaginalis

that isolation rates of G. vaginalis were not significantly affected when specimens were inoculated directly onto media or inoculated after some delay. They demonstrated a delay of up to 6 hours appeared to have little effect on the viability of the organisms. However, it is possible that longer delays may be associated with lower yields.

### 2.10.4 Identification

Ideally identification schemes are based on colonial morphology, Gram stain reaction, biochemical tests and may include susceptibility to certain drugs and dyes. Since G. vaginalis is the organism primarily implicated in bacterial vaginosis, it is necessary to differentiate and distinguish it from other catalase negative, small Gram-variable rods, which commonly occur in vaginal specimens from women with and without vaginitis.

The identification of G. vaginalis has been complicated by its previously confused taxonomy and the variety of identification schemes used in the past. Detailed schemes for identification have since aided resolution of its taxonomic position (Section 2.10.1), but these schemes are not ideally suited to routine diagnostic clinical laboratory use, because of their time-consuming nature (Greenwood and Pickett, 1979, Piot et al, 1980, 1982b; Taylor and Phillips, 1983).

The identification scheme described by Dunkelberg et al (1970b) was based on the recognition of typical colonial morphology after 48 hours incubation on peptone starch dextrose agar (PSD) using a dissecting

microscope. The organism was confirmed by a negative catalase reaction, inhibition of growth by hydrogen peroxide and fermentation of the carbohydrates glucose, maltose and starch. This method was also successfully used by Lee and Schmale (1973) and Akerlund and Mardh (1974).

Carbohydrate fermentation has been used extensively in identification protocols. However, these have been difficult to perform and require some degree of expertise. Furthermore, they are time-consuming and reproducibility has been reported to be poor (Edmunds, 1962; Dunkelberg et al, 1970; Piot et al, 1980).

The incubation time required to detect acid production is usually between 48 to 72 hours. Greenwood et al (1977) used a buffered substrate for carbohydrate fermentation; this gave rapid results in approximately 3 hours. The fermentation of sucrose, xylose and mannose (Taylor and Phillips, 1983) has been found to be extremely variable. Since the majority of strains of G. vaginalis ferment starch and maltose consistently (Dunkelberg et al, 1977; Bailey et al, 1979), it may seem ideal to include these in identification protocols. However, the value of these carbohydrates is limited as they are also fermented by other similar vaginal organisms (Piot, 1982b; Taylor and Phillips, 1983). A negative mannitol reaction has also been used as a useful negative control (Piot et al, 1982b). Overall there is a general view that carbohydrate fermentation tests are difficult to perform, poorly reproducible and are of little discriminating value. Thus their routine application in the diagnostic setting is limited.

Ideally, tests which give a rapid and reliable presumptive identification, should be selected for use in clinical diagnostic laboratories.

The Gram stain could be used but is of limited value because G. vaginalis has the structural and biochemical characteristics of a Gram-negative bacterium, yet appears as both Gram-negative and Gram-positive bacilli in young cultures (Plate 7). Thus, it could readily be mistaken for unclassified vaginal coryneform organisms.

A specific indirect fluorescent antibody test has been used for the identification of G. vaginalis (Redmond and Kotcher, 1963; Vice and Smaron, 1973; Svarva and Maeland, 1982). The antiserum raised in rabbits against reference strain ATCC 14018 gave good fluorescence with all except one strain of G. vaginalis tested. The antisera also discriminated well between G. vaginalis and other vaginal organisms including lactobacilli. The use of this test would be ideal for the rapid identification of G. vaginalis since the method used had excellent sensitivity and specificity, however the antiserum was not commercially available.

Other identification protocols which included fermentation of starch, raffinose and hippurate hydrolysis together with diffuse beta-haemolysis were proposed. Using a combination of these the majority of

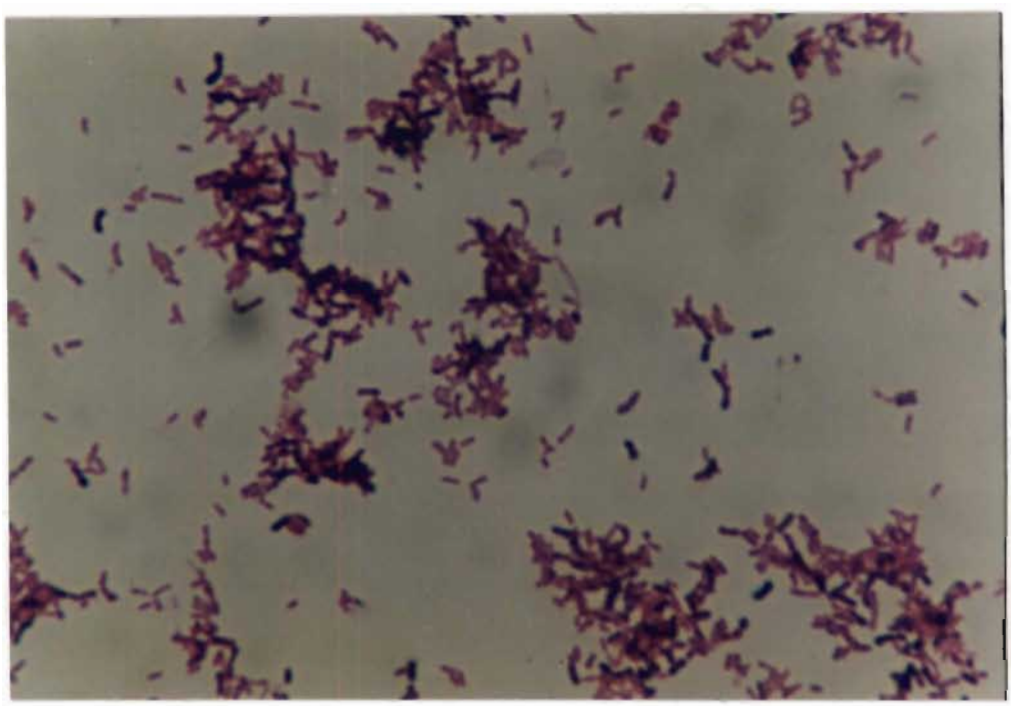

PLATE 7 :

Gram stained smear of a 24-hour culture of Gardnerella vaginalis, showing typical pleomorphic Gram-variable rods

the strains of G. vaginalis were identified within one hour (Yong and Thompson, 1982; Jolly, 1983) and were differentiated from other G. vaginalis-like organisms which gave different patterns. Based on the above tests, a standardised commercially available system has been used by Lien and Hillier (1989). This system may be useful for the identification of G. vaginalis in laboratories where human blood bilayer plates are not available.

Inhibition tests have also been used in diagnostic laboratories. Park et al (1968) relied on growth inhibition by alpha-haemolytic streptococci or pneumococci in addition to colonial morphology and Gram stain reaction. All 30 strains tested were reliably identified. Reimer and Reller (1985) found that previously identified strains of G. vaginalis were inhibited by some strains of alpha-haemolytic streptococci and by paper discs containing sodium polyanethole sulfonate (SPS). Most strains of G. vaginalis are inhibited in disc diffusion tests by high concentrations of metronidazole (50ug), trimethoprim (5mg) and bile (10%), but resistant to sulphonamides (1mg) (Piot et al, 1982b). These tests have been found to discriminate well and are simple to perform, but require at least 24 hours incubation.

Taylor and Phillips (1983) have recommended the use of seven tests which are easy to perform and interpret. These are beta-haemolysis on human blood agar, sensitivity to trimethoprim (5mg) and metronidazole (128ug), resistance to a low concentration of metronidazole (32ug), growth in the presence of 2% sodium chloride, growth on nutrient agar and the production of lactic acid from glucose.

A criterion which has been found most useful, is the production of diffuse beta-haemolysis on human blood agar on primary isolation. Thus, this feature has been proposed for the rapid presumptive identification (Smith et al, 1979; Totten et al, 1982) of G. vaginalis. However, other oganisms such as lactobacilli and streptococci also produce beta-haemolysis (Smith et al, 1979; Piot et al, 1980; Taylor and Phillips, 1983) reducing the reliability of this criterion.

It has been shown that the use of beta-haemolysis together with colonial morphology, negative catalase test and typical Gram stain reaction can correctly identify 90% to 97% of G. vaginalis strains (Piot et al, 1982b). Ison et al (1982) have used horse blood agar together with human blood agar and have shown that G. vaginalis produces diffuse beta-haemolysis on human blood agar and not on horse blood agar. Demonstration of differential haemolysis can aid in the rapid presumptive identification on primary isolation.

Research laboratories may require to perform additional tests to obtain optimal discrimination. The use of alpha and beta-glucosidase together with hippurate and starch hydrolysis have been found to give a high discriminative value of up to 99% (Piot et al, 1982b).

To date there has been no consensus as to which tests may be most appropriate for the identification of G. vaginalis. Minimal criteria for the identification of G. vaginalis from specimens of genital tract have been suggested by Taylor-Robinson et al (1984b). They are of the opinion that occurrence of beta-haemolysis, typical colonial morphology

with a Gram-negative to variable staining reaction (Plate 7) would strongly suggest that the organism was G. vaginalis. However, if the isolate was from a site other than the genital tract, a larger number of tests would need to be employed; these could include the use of a test for catalase production and inhibition by metronidazole (50ug) (Table XIV).

### 2.10.5 Biotyping

Since G. vaginalis has been isolated from vaginal fluid of women with and without bacterial vaginosis, it has necessitated the characterisation of strains. However, biochemical reactions used did not differentiate the strains, thus techniques such as biotyping were developed to enable this characterisation.

The first simple and reproducible biotyping scheme was developed by Piot et al (1984). Their scheme was based on the reactions obtained for lipase, hippurate hydrolysis and beta-galactosidase activity (ONPG). Eight biotypes were identified (Table XV) (Piot et al, 1984). The commonest biotypes were 1, 2 and 5 which included over 80% of G. vaginalis isolates tested (Piot et al, 1984).

This scheme was further developed by Benito et al (1986) with the addition of fermentation reactions of arabinose, galactose and xylose (Benito et al, 1986). This scheme identified 17 biotypes with four in Group I, five in Group II, four in Group III, and four in Group IV (Table XVI).

TABLE XIV : MINIMAL CRITERIA FOR THE IDENTIFICATION OF *GARDNERELLA VAGINALIS*

(Taylor-Robinson *et al*, 1984b)

Minimum

- Diffuse beta-haemolysis on human blood bilayer agar media with Tween 80
- Typical colonial morphology
- Gram-negative to variable reaction

Additional

- Catalase negative
- Any zone of inhibition with metronidazole (50ug)

TABLE XV : BIOTYPES OF GARDNERELLA VAGINALIS
(Piot et al, 1984)

| | BIOCHEMICAL TESTS | | |
|---|---|---|---|
| Biotype | Hippurate | Lipase | Beta-galactosidase (ONPG) |
| 1 | + | + | + |
| 2 | + | + | - |
| 3 | - | + | - |
| 4 | - | + | + |
| 5 | + | - | - |
| 6 | + | - | + |
| 7 | - | - | - |
| 8 | - | - | + |

\+ = Positive reaction

\- = Negative reaction

TABLE XVI : BIOTYPES OF *GARDNERELLA VAGINALIS*
(Benito *et al*, 1986)

| | BIOCHEMICAL TESTS | | | | | |
|---|---|---|---|---|---|---|
| Biotype | Hippurate | ONPG | Lipase | Arabinose | Galactose | Xylose |
| GROUP I | | | | | | |
| 1a | + | + | + | + | + | + |
| 1b | + | + | + | + | + | - |
| 1c | + | + | + | - | - | + |
| 1d | + | + | + | - | - | - |
| GROUP II | | | | | | |
| IIa | + | - | + | - | - | - |
| $IIa_1$ | + | - | - | - | - | - |
| IIb | + | - | + | - | - | + |
| IIc | + | - | + | + | + | + |
| IId | + | - | + | + | + | - |
| GROUP III | | | | | | |
| IIIa | - | + | + | + | + | + |
| IIIb | - | + | + | + | + | - |
| IIIc | - | + | + | - | - | - |
| IIId | - | + | + | - | - | + |
| GROUP IV | | | | | | |
| IVa | - | - | + | - | - | - |
| $IVa_1$ | - | - | - | - | - | - |
| IVb | - | - | + | + | + | - |
| IVc | - | - | + | + | + | + |

\+ = Positive reaction
\- = Negative reaction
ONPG = Beta-galactosidase

A comparison of the biotypes as defined by Benito et al (1986) showed that strains identified as Group I corresponded to the biotype I of Piot; those of Group II to biotype 2; Group IIa to biotype 5, Group III to biotype 4 and those of Group IV to biotype 3. However, Benito et al (1986) found no combinations corresponding to biotypes 6 and 8.

The results of these two studies did not show any predominance of a particular biotype amongst strains of G. vaginalis isolated from women with bacterial vaginosis or amongst those from asymptomatic women. However, the isolation of the identical biotypes from sex partners when both were sampled within 24 hours, suggested that G. vaginalis may be sexually transmitted.

Geographically the biotypes were similarly distributed, that is, those isolated from women in Seattle (USA), Nairobi (Africa) and Antwerp (Belgium) (Piot et al, 1984). In contrast, geographical differences were found using the scheme of Benito et al (1986).

Thus, to date application of these biotyping schemes has not enabled workers to distinguish any differences between G. vaginalis strains isolated from symptomatic and asymptomatic women.

### 2.10.6 Antimicrobial susceptibility

In order to identify those antimicrobial agents which may potentially be effective for the treatment of Gardnerella-associated infections,

several workers have determined the *in vitro* susceptibility of *G. vaginalis* to a variety of antimicrobial agents.

Gardner and Dukes (1955) determined the susceptibility of *G. vaginalis* by the disc diffusion method and found the organism to be sensitive to bacitracin and tetracycline, usually resistant to chloramphenicol and resistant to penicillin, polymyxin and streptomycin. In contrast, other workers have reported the organism to be sensitive to penicillin (Frampton and Lee, 1964; Akerlund and Mardh, 1974, Levison *et al*, 1979).

Frampton and Lee (1964) examined 18 clinical isolates of *G. vaginalis* using the disc diffusion method and found all strains sensitive to penicillin, erythromycin and tetracycline. They demonstrated resistance to polymyxin and colistin. Akerlund and Mardh (1974) reported 10 strains of *G. vaginalis* to be sensitive to penicillin, tetracycline, chloramphenicol, streptomycin but resistant to sulphonamides.

McCarthy *et al* (1979) determined the minimum inhibitory concentration (MIC) of several agents for strains of *G. vaginalis* including type strain 594. All strains were sensitive to ampicillin and penicillin with MICs of less than 0,06mg/ℓ and less than 0,124mg/ℓ respectively. All were resistant to sulphadiazine at a concentration of 128 mg/ℓ and also resistant to colistin and nalidixic acid. The resistance of the organism to all these agents except trimethoprim and vancomycin has been used for the development of a selective medium for the isolation of *G. vaginalis* from clinical specimens.

The metronidazole susceptibility of eight clinical isolates and two reference strains (ATCC 14018, ATCC 14019) were determined by the tube dilution method both aerobically and anaerobically by Pheifer et al, (1978). MIC for the two reference strains were 3mg/ℓ (ATCC 14018) and 6mg/ℓ (ATCC 14019) and for the eight clinical isolates, these ranged from 2 to 12mg/ℓ. An additional 12 strains were tested by the agar dilution method and these isolates showed a gradual reduction of growth at 0,5mg/ℓ and with complete inhibition by 4 to 8mg/ℓ anaerobically.

Although G. vaginalis has been shown to be only variably sensitive to metronidazole, its hydroxymetabolite, being the major metabolite in the body was investigated to determine its relative antibacterial activity (Ralph 1983).

Ralph (1983) found G. vaginalis to have a metronidazole MIC of 4mg/ℓ whilst for the hydroxymetabolite this was only 1mg/ℓ. Easmon et al (1982) reported similar results although the MICs obtained in their study were slightly higher. These results were confirmed by the studies of Jones et al (1985), however, the median MIC of metronidazole for G. vaginalis was 32mg/ℓ and of the hydroxymetabolite was 4mg/ℓ and three strains were resistant to both agents with MICs of 128mg/ℓ. Clearly the hydroxymetabolite seems more active than the parent compound on a weight to weight basis. However, it remains unclear as to why metronidazole therapy is so successful clinically in bacterial vaginosis even though G. vaginalis, the organism implicated in its aetiology usually exhibits reduced sensitivity to this agent. Other nitroimidazoles tested include tinidazole with MIC 90 (MIC at which 90%

of isolates are inhibited) of 16mg/ℓ and 32mg/ℓ, showing similar activity to metronidazole (Shanker and Munro, 1982; Piot, 1984).

The fluoroquinolones have generally demonstrated lower activity against G. vaginalis than against many other bacteria; the MIC 90 for ciprofloxacin, A56619 and A56620 against G. vaginalis being 4, 8 and 4mg/ℓ respectively. The MIC90 for the same agents against N. gonorrhoeae was 0,008, 0,03 and 0,015mg/ℓ respectively, which is considerably lower. These agents may play a role in the treatment of bacterial vaginosis just as metronidazole which is moderately active against G. vaginalis in vitro, is useful in the treatment of bacterial vaginosis (Tjiam et al, 1986).

### 2.10.7 Extra-vaginal infections caused by Gardnerella vaginalis

G. vaginalis has been found to be a common inhabitant of the normal vagina and associated with bacterial vaginosis. The potential of the organism to cause invasive disease is uncertain, although it has been isolated from sites other than the vagina.

The organism was first reported from the urine of men by Leopold (1953). It has also been found in the male urethra (Dawson et al, 1982; Kinghorn et al, 1982; Chowdury, 1986) and semen (Ison and Easmon, 1985). Although the presence of the organism in the male genital tract is primarily associated with asymptomatic carriage (Gardner, 1980; Dawson et al, 1982), it has been implicated in urinary tract infection (Abercrombie et al, 1978), non-gonoccocal urethritis (Chowdury, 1986)

and bacteraemia in a man following prostatectomy (Patrick and Garnett, 1978). It has also been associated with balanoposthitis (Kinghorn et al, 1982; Burdge et al, 1986) and bacteraemia secondary to a pulmonary abscess (Legrand et al, 1989).

G. vaginalis has been found more frequently in the urinary tract of women than men (Josephson and Thomason, 1986). The isolation of the organism from the bladder of women was first described by McFadyen and Eykyn (1968) who found an incidence of 16% - (159 of 1000) suprapubic aspirates of healthy pregnant women; similarly McDowall et al (1981) reported an incidence of 18% in mid-stream urine specimens. The isolation rate was higher (58%) in pregnant women who had underlying renal disease. The presence of the organism in patients' urine was not associated with a pyuria and in spite of not receiving treatment, they remained asymptomatic.

Fairley and Birch (1983) observed squamous epithelial cells with adherent bacteria resembling "clue" cells in the bladder aspirates of persons whose urine was culture positive for G. vaginalis. They suggested that "clue" cells from the vaginal mucosa may provide an effective vehicle of spread from the vagina to the urinary tract.

G. vaginalis has frequently been found in association with U. urealyticum in the urine of pregnant women (Savige et al, 1983) and in renal transplant patients (Birch et al, 1981) and it is possible that these two organisms may interact in the urinary tract, enabling G. vaginalis to be a potential pathogen.

Edmunds (1959) reported an association between isolation of G. vaginalis from high vaginal swabs and the presence of puerperal pyrexia and leucorrhoea. G. vaginalis has also been isolated from endometrial samples from women with post-partum or post-caesarean section infections and septic abortions (Regamy and Schoenknecht, 1973). Eschenbach et al (1984) showed that organisms associated with bacterial vaginosis, that is G. vaginalis and/or anaerobes were isolated from 61 of 100 women with post-partum endometritis, with G. vaginalis being the most common isolate from both the blood and endometrium. Bacterial vaginosis was also associated more often in women giving birth prematurely (49%) than at full term (24%).

Most patients presenting with bacteraemia with G. vaginalis have been obstetric patients (Vontver and Eschenbach, 1981). It appears that the organism may be an opportunistic pathogen spreading to the blood stream following trauma to the tissues of the genital tract (Venkataramani and Rathbun, 1976; Reimer and Reller, 1984). It is unclear whether a positive blood culture represents a true infection or a transient bacteraemia, since other organisms occurring in the lower genital tract were also isolated from the blood culture (Reimer and Reller, 1984), suggesting that bacteraemia reflects the spread of vaginal flora into areas of tissue damage with subsequent spread to the blood stream. Furthermore, the studies have also reported that despite initial presentation with high fever and abdominal pain, the patients recovered without appropriate antibiotic therapy and complications such as meningitis or endocarditis were not encountered.

G. vaginalis has been isolated from cord blood of neonates, (Platt, 1971; Monif and Baer, 1974). None of these infants appeared to be clinically infected. Platt (1971) found G. vaginalis in the blood of one child who subsequently died and from the scalp lesions of another who survived.

From reports available, it would appear that patients at greater risk of developing urinary tract infections are women, those with chronic renal disease and especially pregnant women, as the vagina, which may be colonised with G. vaginalis lies in close proximity to the urinary tract. Although G. vaginalis was found to be non-invasive (Gardner and Dukes, 1955) the organism could spread from the vagina to other body sites including the bloodstream. Invasion of the blood stream usually occurred during or after parturition, thus the infection is a result of opportunistic spread of G. vaginalis, dependent on the underlying predisposing conditions of the host.

# 3.0 PATIENTS AND METHODS

## 3.1 STUDY PERIOD

The clinical portion of this study was carried out between November 1987 and March 1988. During this period all clinical assessments were performed and specimens collected.

## 3.2 PATIENTS

A total of 208 women attending four different out-patient clinics at King Edward VIII Hospital (KEH) Durban, were investigated.

KEH is a 2 000 bed hospital situated in central Durban. The hospital has a number of out-patient clinics and serves a large, predominantly Black urban and peri-urban resident population and also serves as a referral centre for the Natal-KwaZulu region.

The study group comprised:

55 women presenting to the family planning clinic (FPC) for advice on contraception;

52 pregnant women presenting to the ante-natal clinic (ANC) for their first ante-natal consultation;

51 women presenting to the sexually transmitted diseases (STD) clinic, either as patients with vaginal symptoms such as vaginal

discharge and/or vaginal irritation or itching or as contacts of patients with known sexually transmitted diseases;

50 patients attending the colposcopy clinic (CC). These patients were referred from the hospital family planning clinic or from peripheral family planning clinics for follow-up of abnormal uterine cervical cytology, suggestive of cervical intra-epithelial neoplasia (CIN).

Patients were excluded from the study if they were menstruating or if they had taken any antimicrobial agent(s), or received any local therapy for vaginitis within the previous month, or had douched on the day of the examination.

Patients were informed of the purpose of the study and the need for non-invasive procedures including a vaginal examination. Only patients giving (verbal) consent were included in the study.

A confidential questionnaire was administered to each woman by interview. Information elicited included the following: patient's age, marital status, pregnancies, contraception used, number of sexual partners and current genital symptoms.

## 3.3 CLINICAL METHODS

Each patient was examined by the attending doctor. Examination included a general examination and a comprehensive gynaecological examination. During the latter, the patient was placed in the lithotomy position and

an unlubricated sterile speculum passed into the vagina. The amount, type and consistency of vaginal secretions were assessed and clinical specimens collected. Clinical data were recorded on a standard form.

In the case of patients attending the colposcopy clinic, a colposcopic examination was performed and cervical biopsies were performed routinely as part of a diagnostic examination. Pregnant women were subjected to routine ante-natal examination.

## 3.4 SPECIMEN COLLECTION

Specimens were collected from the vagina, endo-cervix, urethra and rectum. Venous blood was collected aseptically from a peripheral vein. Specimens were processed in the order described (Table XVII). The formulations for all media used are detailed in Appendix A.

### 3.4.1 Vaginal specimens

A sterile polypropylene disposable pipette (Sterilin, Polychem, South Africa) was used to collect vaginal discharge and secretions from the lateral and posterior fornices. The vaginal pH was measured directly from the pipette using narrow range pH paper. The remaining discharge was mixed into 1,5mℓ of sterile physiological saline and a drop was placed on a glass slide for wet smear microscopic examination. For the culture of T. vaginalis 0,5mℓ of the saline mixture was inoculated into modified Diamond's medium. The remaining saline mixture was stored at -20°C and subsequently examined by gas liquid chromatography for volatile and non-volatile organic acids.

TABLE XVII : PROCESSING OF SPECIMENS*

| Nature/site of specimen | Microscopic Examination | | | | Additional tests | | |
|---|---|---|---|---|---|---|---|
| | Wet smear | Gram stain | Pap stain | If for Ct | pH | KoH | GLC |
| Vagina | +[a] | + | + | - | + | + | + |
| Endo-cervix | - | + | + | + | - | - | - |
| Urethra | - | - | - | + | - | - | - |
| Rectum | - | - | - | - | - | - | - |
| MSU | +[b] | - | - | - | - | - | - |
| Blood | - | - | - | - | - | - | - |

| | Culture | | | | | | | | Serology | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | GC | HSV | GV | MH UU | AN | Yeast | Trich | Coli-forms | Syph | Hep B | HIV |
| Vagina | - | - | + | + | + | + | + | - | - | - | - |
| Endo-cervix | + | + | - | + | - | - | - | - | - | - | - |
| Urethra | + | - | + | - | - | - | - | - | - | - | - |
| Rectum | + | - | - | - | - | + | - | - | - | - | - |
| MSU | - | - | + | - | - | + | - | + | - | - | - |
| Blood | - | - | - | - | - | - | - | - | + | + | + |

* See text section 3.4

\+ Specimen tested

\- Specimen not tested

a Vaginal wet smear microscpy

b Direct urine microscopy

For each patient, further vaginal specimens were obtained by means of sterile cotton-tipped swabs and immediately (at the clinic) used for the following:

i the preparation of a smear for Gram staining;

ii mixing with 0,25mℓ of 10% potassium hydroxide (KOH) for the detection of the characteristic "fishy" odour;

iii inoculation onto selective human blood agar (SHBA) and horse blood agar (BA) for the isolation of G. vaginalis;

iv inoculation onto pre-reduced Wilkins-Chalgren anaerobic agar (Oxoid, UK) supplemented with horse blood for the isolation of anaerobic bacteria;

v inoculation onto Sabourauds dextrose agar (Difco, USA) for the isolation of yeasts;

vi inoculation of Shepard's A7 agar and U9 broth for isolation of genital mycoplasmas.

vii one swab specimen was placed into Stuart transport medium (Oxoid, UK) and another into modified Cary-Blair transport medium. This was to determine the efficacy of transport media for the recovery and isolation of G. vaginalis as compared to the direct plating method (iii above).

A wooden spatula was used to collect material from the vaginal wall and a smear made on a glass slide. This was immediately fixed using cytological fixative and subsequently stained with modified Papanicolaou's stain.

### 3.4.2 Endo-cervical specimens

Endo-cervical specimens were collected after wiping the ecto-cervix with sterile cotton wool swabs to clear any adherent vaginal secretions. Three sterile cotton-tipped and one sterile dacron-tipped (plastic shaft) swabs were sequentially inserted into the endo-cervical canal and rotated firmly to obtain cervical cells and exudate. Of the cotton-tipped swabs:

i one was inoculated onto modified New York City Medium (MNYC) for the isolation of N. gonorrhoeae. The same swab was also used for the preparation of a smear for Gram staining;

ii one was inoculated onto Shepard's A7 agar for genital mycoplasmas;

iii one was placed in virus transport medium (VTM) for culture of Herpes simplex virus.

The dacron-tipped swab was rolled onto an unmasked area of a MicroTrak glass slide. The material was air-dried, fixed in acetone and stored at -70°C for subsequent direct immunofluorescent (IF) staining for antigen detection for C. trachomatis (Microtrak, Syva, USA).

An Ayre's spatula was used to prepare a cervical smear for Papanicolaou's staining.

### 3.4.3 Urethral specimens

The urethral meatus was first wiped clean with a sterile cotton wool swab after which the para-urethral glands were gently massaged and then

two endo-urethral specimens were obtained using calcium alginate-tipped swabs (Calgiswab Type 1, Inolex, USA).

i one was inoculated onto MNYC medium and SHBA .

ii one was used for preparation of a smear for direct IF staining for C. trachomatis (as in dacron-tipped endo-cervical swab).

### 3.4.4 Rectal specimens

A rectal mucosal swab was obtained by inserting a cotton-tipped swab into the anus for approximately 3cms and sweeping the tip peripherally against the mucosa. This was plated out onto MNYC and Sabourauds dextrose agar for the detection of N. gonorrhoeae and yeasts respectively.

### 3.4.5 Mid-stream urine specimen

Each patient was supervised and requested to pass a mid-stream urine specimen into a sterile container. This was held on ice and transported to the laboratory within 2 hours for further processing.

### 3.4.6 Serological specimens

Serum was separated aseptically from the clotted blood specimens and kept at -20°C prior to the performance of serological tests for syphilis, and the detection of hepatitis B surface antigen and antibody to human immunodeficiency virus (HIV).

### 3.4.7 Transport of specimens

All inoculated plates were immediately placed into candle extinction jars and transported to the laboratory within 2 hours, where the plates were streaked out and then incubated appropriately (see separate sections). The VTM were held at 4°C on ice and transported to the laboratory where they were held at -70°C until they could be inoculated onto cell monolayers.

## 3.5 MICROBIOLOGICAL METHODS

Microscopic examination of the vaginal wet smears, estimation of vaginal pH and the amine liberation tests were conducted immediately after collection of specimens at the respective clinics. All further processing of specimens was done in the laboratory.

### 3.5.1 Vaginal wet smear microscopic examination (direct microscopy)

Wet smears (see section 3.4.1) were examined by light microscopy (Nikon) under low power magnification (X40 objective) within 10 minutes of specimen collection for the presence of epithelial cells, inflammatory cells, "clue cells", motile trichomonads, yeasts and bacteria. The attachment of large numbers of bacteria to vaginal epithelial cells with obscure cellular and nuclear outlines were defined as "clue cells".

### 3.5.2 pH estimation

The pH of the vaginal fluid was estimated by the application of a drop of vaginal secretion from the collecting pipette to a narrow range (4-6) pH paper (Whatman, BDH, England) and reading against the colour chart provided by the manufacturer.

### 3.5.3 Amine liberation test

A cotton-tipped swab containing vaginal secretions was added to 0,25mℓ of 10% potassium hydroxide (KOH) (w/v) solution in a test tube. The production of a fishy odour was considered to be a positive reaction, indicative of the release of aromatic amines (Pheifer et al, 1978).

### 3.5.4 Stained smears

#### 3.5.4.1 Gram stain

Vaginal smears were prepared by transferring vaginal secretions to glass slides. The slides were air-dried, heat-fixed and Gram-stained. Smears were examined under oil immersion (X100 objective) and quantitated for the presence of "clue" cells, Gram-variable bacilli, yeasts, lactobacilli, G. vaginalis-like organisms, Gram-negative curved rods (ACR) and other bacteria.

Endo-cervical smears were Gram stained and examined for the presence of inflammatory cells and intra- and/or extracellular Gram-negative

diplococci suggestive of N. gonorrhoeae. The presence of more than 10 inflammatory cells per oil immersion field was considered to be indicative of cervicitis (Paavonen et al, 1986).

3.5.4.2 Papanicolaou (Pap) stained smears

Vaginal and cervical smears were prepared as described in 3.4.1 and 3.4.2 and stained with the Pap stain. The smears were microscopically examined for the presence of "clue cells" with pleomorphic bacilli (suggestive of G. vaginalis), yeasts, T. vaginalis, koilocytes - suggestive of human papilloma virus (HPV) infection and dysplasia.

3.5.5 Examination of mid stream urine specimen

Using a calibrated loop, 10uℓ of unspun urine specimen was inoculated onto SHBA, HB agar and cystine lactose electrolyte deficient agar (CLED). The centrifuged deposits were examined microscopically using a light microscope (Nikon) and X40 objective for the presence of inflammatory cells, red blood cells, epithelial cells, trichomonads and casts.

3.5.6 Culture and identification of micro-organisms

The formulations and methods for the preparation of media used appear in appendix A.

### 3.5.6.1 Gardnerella vaginalis

G. vaginalis was isolated on SHBA (5% v/v) and BA (5% v/v) agar, incubated for 48 hours at 37°C in 6% $CO_2$.

The SHBA medium was used for primary isolation only. All suspicious colonies with characteristic diffuse beta-haemolysis on SHBA medium were sub-cultured onto human blood agar without antibiotics (HB). After single sub-culture all isolates were stored in sterile human group O red cells at -70°C. Isolates were subsequently further identified in batches.

Identification of G. vaginalis was made on the basis of colonial morphology, typical pleomorphic Gram-variable bacilli, negative oxidase and catalase tests and characteristic diffuse beta-haemolysis on human but not horse blood agar (Ison et al, 1982) (see section 2.10.3).

The identity of all isolates was confirmed by the presence of alpha-glucosidase and absence of beta-glucosidase activity. A zone of inhibition around a 5ug trimethoprim (Oxoid) disc and a 50ug metronidazole (Mast) disc and no zone of inhibition to a 1mg sulphathiazole (Mast) disc were additional criteria used in the identification of G. vaginalis.

### 3.5.6.2 Anaerobic bacteria

Anaerobic bacteria were isolated on pre-reduced Wilkens-Chalgren anaerobic agar (Oxoid) supplemented with 5% (v/v) horse blood.

Vaginal specimens were inoculated onto a third of the plate and a 5ug metronidazole (Mast) disc was placed on the initial inoculum. The plates were streaked out for single colonies and transferred into a gas pak jar (Baltimore Biological Laboratories-BBL) containing moistened paper and incubated at 37°C for 5 days under anaerobic conditions provided by using Anaerocult A (Merck).

Anaerobic bacteria were recognised by their inhibition by metronidazole (Phillips and Taylor, 1987) and inability to grow aerobically.

3.5.6.3 Genital yeasts

Yeasts were isolated on Sabouraud's dextrose medium which was incubated for 48 hrs at 37°C in air. All yeasts isolated were inoculated into fresh normal human serum and incubated at 37°C for 3 hours and observed for the production of pseudohyphae (germ tube test) (Cooper and Silva-Hutner, 1985). Isolates that produced pseudohyphae were identifed as C. albicans and all other isolates were identified by using the API 20C auxanogram (API Systems SA, France). The numerical profile obtained for the unknown yeast was compared with those given by the analytical profile index (API + 2021).

3.5.6.4 Genital mycoplasmas

For the isolation of genital mycoplasmas swabs were inoculated onto A7 agar plates (Shepard and Lunceford, 1976) and U9 broths (Shepard and Lunceford, 1970). The plates and broths were incubated in gas pak (BBL) jars microaerophically using BBL gas pak anaerobic systems at 37°C for

48 hours. After 48 hours incubation A7 agar plates were examined using a Nikon light microscope with X10 objective for the presence of characteristic colonies of Ureaplasma urealyticum and Mycoplasma hominis. Colonies of U. urealyticum were identified as dark golden brown colonies of variable size. The dark brown colonies were due to the manganese reaction since ureaplasmas are urease positive. Colonies of M. hominis were identified by their typical fried egg appearance, much larger than U. urealyticum and failure to produce the manganese reaction. The U9 broth cultures were observed twice daily for up to 7 days for a colour change, that is from orange to pink (due to alkalinity resulting from urea hydrolysis) with no turbidity, whereupon they were immediately sub-cultured onto A7 agar plates and incubated and examined as described above.

3.5.6.5 Trichomonas vaginalis

T. vaginalis was identified by screening the vaginal wet smear using X40 objective for motile trichomonads, exhibiting characteristic morphology and jerky movement.

Culture was performed in Diamond's medium (Diamond, 1957) modified by the addition of amikacin (100ug/mℓ) ampicillin (1mg/mℓ) and amphotericin B (5ug/mℓ). The inoculated broths were incubated at 37°C in 6% $CO_2$ for up to 7 days. Wet smears were made daily from cultures and examined microscopically for the presence of T. vaginalis as described above.

### 3.5.6.6 Neisseria gonorrhoeae

N. gonorrhoeae was isolated on MNYC which consisted of gonococcal agar base (Oxoid) with the addition of yeast autolysate supplement (Oxoid) and lincomycin, colistin, amphotericin B, trimethoprim (LCAT) antibiotic supplement (Oxoid).

Swab specimens were inoculated directly onto the medium and incubated for 48 to 72 hours at 37°C in 6% $CO_2$.

N. gonorrhoeae was identified as an oxidase positive, Gram-negative coccus, producing typical results in carbohydrate utilisation tests, namely utilisation of glucose but not maltose, sucrose and lactose. All isolates were screened for penicillinase production using chromogenic cephalosporin as substrate (Nitrocefin-Glaxo, England) (O'Callaghan 1972). Whatman's filter paper No 1 (Whatman, England) was impregnated with nitrocefin solution (0,05% w/v). Colonies were smeared onto the filter paper; a deep pink colour appearing within 2 minutes, being indicative of penicillinase production . A known positive (N. gonorrhoeae) and negative (N. gonorrhoeae) were included as control organisms.

### 3.5.6.7 Chlamydia trachomatis

C. trachomatis was identified using a direct antigen detection test (MicroTrak, Syva, USA).

Smears of urethral and endo-cervical exudate (section 3.4.2) were examined using the "MicroTrak" C. trachomatis direct fluorescent antibody reagent. The reagent contains monoclonal antibodies specific to the principle membrane protein of C. trachomatis and Evans blue counterstain in a protein stabilised buffer solution. Tests were performed according to the manufacturer's instructions. A summary of the procedure is given below:

The acetone-fixed smears were stained using 30uℓ of diluted monoclonal antibody which was spread over the smear. The slides were incubated at room temperature for 15 minutes in a moist chamber. At the end of the incubation the slides were gently rinsed in distilled water for 10 seconds and air dried. A drop of mounting fluid was added and slides mounted with a coverslip and examined with a Nikon fluorescent microscope at a wavelength of 540nm. A positive diagnosis was made on the presence of one or more elementary bodies. Elementary bodies were identified as characteristically brightly staining with a distinctive disc shape and green colour. Elementary bodies were confirmed using x100 objective. Positive and negative control slides provided by the kit manufacturer were set up in parallel with the test slides.

3.5.6.8 Culture for Herpes simplex virus

The presence of Herpes simplex virus in endo-cervical specimens was detected by culture in monkey kidney cells. Inoculated monolayers were incubated at 37°C and observed daily for up to 14 days. Herpes simplex virus was identified by its characteristic cytopathic effects and its identity confirmed by staining with fluorescein isothiocyanate labelled

monoclonal antibody (MA Bioproducts, Maryland, USA) against Herpes simplex virus (Types 1 and 2).

3.5.6.9 Culture of G. vaginalis from specimens in transport media

Vaginal secretions, collected from the posterior fornix were inoculated into Stuart and modified Cary-Blair transport media (section 3.4.1). These were allowed to stand at room temperature overnight in the laboratory; thereafter they were plated onto SHBA and HB agar for the isolation of G. vaginalis. For each set of specimens the growth obtained from swabs inoculated from the two transport media were graded and compared with one another and with the growth obtained on the directly inoculated media.

## 3.6 GAS LIQUID CHROMATOGRAPHIC ANALYSIS OF VAGINAL SECRETIONS TO DETERMINE SUCCINATE : LACTATE RATIOS

Previously preserved saline suspensions of vaginal secretions were prepared for gas liquid chromatography by the method adapted by Spiegel et al, (1980).

### 3.6.1 Extraction of non-volatile acids

The sample (0,5mℓ) was acidified with 0,1mℓ of 50% aqueous sulphuric acid (v/v), mixed with 1mℓ of methanol and incubated in a 55°C heating block for 30 minutes. Water (0,5mℓ) was added and the non-volatile acids were extracted with 0,25mℓ of chloroform. The solution was gently mixed and centrifuged for 5 minutes at 1000g to separate the chloroform

layer from the aqueous layer. The chloroform layer was carefully removed for analysis.

### 3.6.2 Extraction of volatile acids

The sample (0,5mℓ) was acidified with 0,1mℓ of 50% aqueous sulphuric acid (v/v) and 1mℓ ethyl ether added. The mixture was gently inverted repeatedly and centrifuged for 5 minutes at 1000g to separate the aqueous layer from the ethyl ether layer. The ethyl ether layer was carefully removed for analysis.

### 3.6.3 Gas liquid chromatography

Gas liquid chromatography was performed using Hewlett Packard - 5790A Series gas chromatograph. This was attached to a Hewlett Packard integrator 33908 for the recording of results. A 2 microlitre volume was injected onto a column. The following operating temperatures were employed: column temperature 170°C, detector temperature 250°C. Helium was the carrier gas at 50mℓ/minute.

The ratio of the succinate peak to that of the lactate peak was then calculated by measuring the heights of the peaks in millimeters. A non volatile fatty acid standard included pyruvic, lactic, oxalacetic, oxalic, methyl malonic, malonic, fumaric, succinic acids (Supelco) and a volatile fatty acid standard included formic, acetic, propionic, isobutyric, butyric, isovaleric, valeric, isocaproic, caproic and heptanoic acids (Supelco).

## 3.7 ANTIMICROBIAL SUSCEPTIBILITY OF GARDNERELLA VAGINALIS

Minimum inhibitory concentrations (MIC) were determined by the agar dilution method.

### 3.7.1 Bacterial strains

All previously confirmed vaginal isolates of G. vaginalis were tested for their in vitro susceptibility to antimicrobial agents. Isolates stored at -70°C were thawed and inoculated onto HB agar and incubated in air plus 6% $CO_2$ for 48 hours. Three control strains were included with every test batch. These were reference cultures of Staphylococcus aureus NCTC 6571, Escherichia coli NCTC 10418 and G. vaginalis NCTC 10915.

### 3.7.2 Antimicrobial agents

The antimicrobial agents tested were as powders of stated potencies for laboratory use supplied by pharmaceutical companies. The antibiotics were dissolved in appropriate solvents to prepare stock solutions. These were then diluted in water to provide a final range of concentration from 128mg/L to 0,001 mg/L when tested.

The antibiotics tested were:

| | Antibiotic | Source |
|---|---|---|
| 1 | Metronidazole | Rhôna-poulenc, UK |
| 2 | 2-Hydroxymetabolite of metronidazole RP20396 | Rhôna-poulenc, UK |
| 3 | Tinidazole | Sigma Chemical Company, USA |
| 4 | Penicillin G | Glaxo Pharmaceuticals, UK |
| 5 | Ampicillin | Beechams Pharmaceuticals, UK |
| 6 | Cefamandole | Eli-Lilly, USA |
| 7 | Cefoxitin | MSD, UK |
| 8 | Cefuroxime | Glaxo Pharmaceuticals, UK |
| 9 | Cefotaxime | Roussel, France |
| 10 | Ceftriaxone | Roche, Switzerland |
| 11 | Aztreonam | Squibb, USA |
| 12 | Imipenem | MSD, UK |
| 13 | Tetracycline | Upjohn Company, USA |
| 14 | Minocycline | Lederle Laboratories |
| 15 | Erythromycin | Abbott Laboratories, SA |
| 16 | Clindamycin | Upjohn Company, USA |
| 17 | Vancomycin | Eli-Lilly, USA |
| 18 | LY 146032 | ELi-Lilly, USA |
| 19 | Chloramphenicol | Parke Davis, UK |
| 20 | Amikacin | Bristol Laboratories, UK |
| 21 | Rifampicin | Ciba-Geigy, USA |
| 22 | Ciprofloxacin | Bayer Miles, USA |
| 23 | Sulphamethoxazole(S) | Wellcome, USA |
| 24 | Trimethoprim (T) | Wellcome, USA |
| 25 | Cotrimoxazole (T/S 1:19) | |

### 3.7.3 Culture media

HB agar was prepared as described in appendix A, but kept molten at 50°C. Freshly prepared, appropriately diluted antibiotic solutions in 5mℓ amounts was added to 20mℓ of molten agar. This was mixed thoroughly and dispensed into 9cm sterile petri dishes. The agar medium was allowed to solidify at ambient temperature and dried at 37°C for 30 minutes. All plates were kept at 4°C and used within 24 hours of preparation. Antibiotic free plates were used for growth control.

### 3.7.4 Inoculum preparation

Colonies of G. vaginalis from a 48 hour HB agar culture were suspended in Mueller Hinton broth (MHB) and the turbidity adjusted to match that of a 0,5 MacFarland standard. A 1 in 10 dilution was then prepared in MHB for inoculation onto agar containing antibiotics. Inoculation of media was made within 30 minutes of adjusting the inoculum.

### 3.7.5 Minimum inhibitory concentration (MIC) determination

The diluted suspensions were applied to agar plates using a Cathra replicator (Rousseau and Harbec, 1987). The final inoculum applied to the testing media was approximately $10^5$ colony forming units (CFU). Antibiotic free plates were inoculated at the beginning and end of each set of antibiotic plates to ensure viability of organisms and to check for potential contamination.

All plates were incubated at 37°C in air in 6% $CO_2$ for 48 hours except those for metronidazole, tinidazole and 2-hydroxymetabolite of metronidazole were incubated anaerobically in gas pak jars (BBL) at 37°C. After incubation plates were examined for growth. The growth on the antibiotic plates was compared to the growth on the antibiotic free plates. Very fine hazy growth or single colonies were considered to indicate no growth.

The MIC of each antibiotic was defined as the lowest concentration of an antibiotic which inhibited growth of the organism (Plate 8).

## 3.8 BIOTYPING OF GARDNERELLA VAGINALIS

G. vaginalis isolates were biotyped according to the scheme described by Piot et al (1984). The biochemical tests used were hippurate hydrolysis, lipase and beta-galactosidase activity (appendix B). The pattern of results obtained from these tests was used to determine the biotype of each isolate.

## 3.9 SEROTYPING OF GARDNERELLA VAGINALIS

Whole cell antigen of G. vaginalis was trapped on nitrocellulose membrane. This was reacted with antibody directed against immunodominant protein and visualised using anti-rabbit immunoglobulin linked to alkaline phosphatase for the polyclonal antibody, and anti-mouse immunoglobulin linked to phosphatase for the monoclonal antibody.

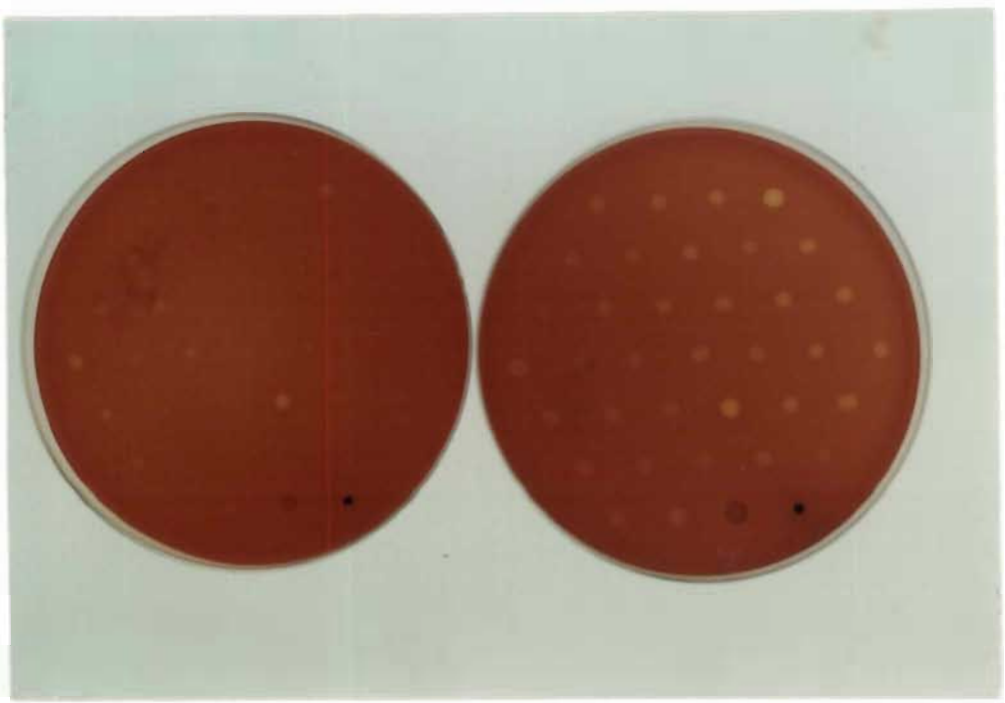

PLATE 8 :

Antibiotic containing agar plates to determine the minimum inhibitory concentration for *Gardnerella vaginalis*

The technique used was a dot blotting enzyme-linked assay which was convenient and enabled many strains to be tested against a variety of antisera.

### 3.9.1 Bacterial strains

All previously confirmed G. vaginalis isolates were serotyped.

### 3.9.2 Preparation of antigen

G. vaginalis was grown on the HB agar for 48 hours in 6% $CO_2$. Suspensions of bacteria were prepared in saline. The optical density (OD) was calculated at a wavelength of 540nm on a spectrophotometer by using a 1 in 10 dilution of the original suspension. The OD of the suspension was adjusted to 0,05.

### 3.9.3 Control organisms

Suspensions of control organisms were prepared and tested every time serotyping was performed

Controls 1 G. vaginalis 170(C)

2 G. vaginalis NCTC 10915

3 Lactobacillus acidophilus

Control strains 1 and 2 were provided by Doctor Cathy Ison (St Mary's Hospital, Paddington, London).

### 3.9.4 Antisera

The antisera used were also kindly provided by Doctor Cathy Ison. These were specially prepared for research purposes by Coralabs Research, United Kingdom. The antibodies raised to G. vaginalis were directed to proteins of differing molecular weights. One polyclonal and four monoclonal antisera were used as a panel for the serotyping scheme.

### 3.9.5 Immune Dot Blot Technique

A nitrocellulose membrane 0,45 um (Anderman and Co., Ltd) measuring 12x8cm was soaked in sterile saline for 10 minutes. The membrane was blotted between paper to remove excess saline and placed in Bio Dot blotter (Bio-Rad Laboratories).

A volume of 100uℓ of adjusted bacterial suspensions was added to each well of the 96 well Bio Dot apparatus. Duplicate dots were prepared for each test organism and controls for 5 antibodies resulting in 10 dots per organism.

The Bio Dot was allowed to stand for 20 minutes thereafter the bacteria were concentrated onto the membrane by the application of a vacuum. The membrane was carefully removed from the Bio Dot and soaked in blocking buffer on an orbital shaker for 60 minutes at 37°C. The membranes were immersed in 20mℓ of the following serotyping antisera diluted in washing buffer with 0,05% (w/v) sodium azide.

| antisera | working dilution v/v |
|---|---|
| Monoclonal 1 | 1: 10,000 |
| Monoclonal 2 | 1: 20,000 |
| Monoclonal 3 | 1: 5,000 |
| Monoclonal 5 | 1:100,000 |
| Polyclonal C | 1:200,000 |

The membranes were incubated overnight at 37°C on an orbital shaker. Thereafter they were rinsed in saline and then in washing buffer for three 15 minute washes.

20mℓ of anti-rabbit immunoglobulin linked to alkaline phosphatase (Dakopatts D306) diluted 1:5000 (v/v) in washing buffer was added to membrane reacted with antibody C.

20mℓ of anti-mouse conjugate (Dakopatts 314) diluted 1:3000 (v/v) with washing buffer was added to each of the other membranes. Membranes were incubated again for 5 hours on a shaker at 37°C.

The washing steps were repeated. The complexes of antigen and antibody were visualised by immersing each membrane in 20mℓ substrate for exactly 10 minutes while being shaken at room temperature. The reaction was halted by washing the membranes in distilled water. These were then blotted and air dried.

### 3.9.6 Reading of Membranes

The intensity of the colour obtained for the test dots were visually

compared and graded in comparison to those obtained for the immunizing strains (Plate 9). This aided to check the specificity of the antiserum and in the interpretation of results.

The reaction obtained with that of the polyclonal antisera was compared with G. vaginalis 170 (C) strain and the reaction obtained with that of the monoclonal antisera was compared with G. vaginalis NCTC 10915 strain.

Lactobacillus acidophilus was non-reactive and negative results were compared with this strain.

The membranes were read macroscopically by three observers and scored as follows

0 = no reaction (negative)

1 = weak reaction

2 = reaction more than that of 1 but less than that of the control strain

3 = strong reaction of greater than or equal to in intensity to that of the control strain.

The duplicate results of each observer were summed and thereafter the scores from all three observers were summed. This could possibly give a result of any value from 0 to 18. A cut off point of 12 and over was considered positive. The value of 12 was found to give acceptable typability and reproducibility.

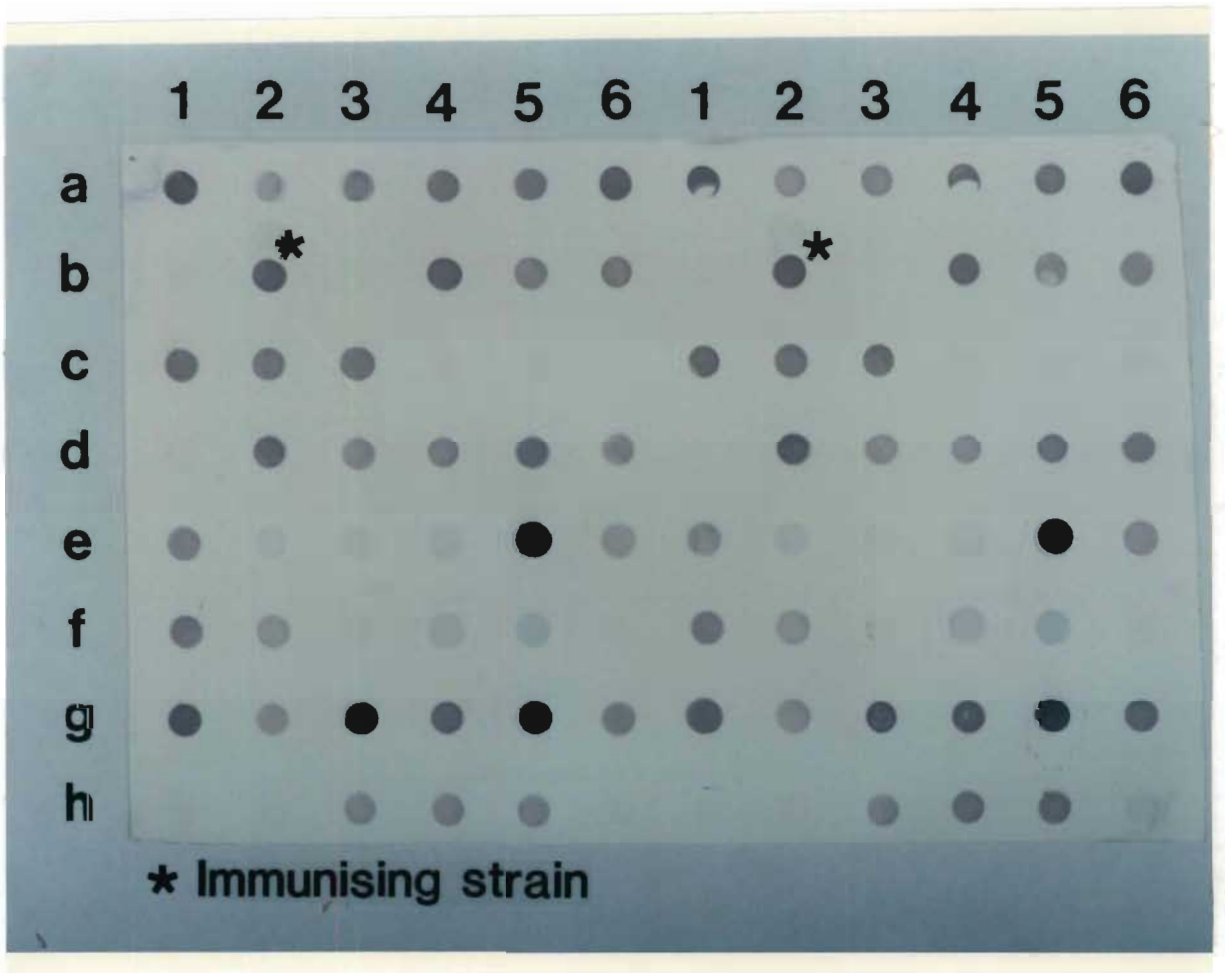


PLATE 9 :

Nitrocellulose membrane showing varying intensities of colour which was compared to the immunizing strain

## 3.10 SEROLOGICAL METHODS

### 3.10.1 Syphilis

The tests carried out for syphilis were the non-specific Rapid Plasma Reagin, (RPR) (Brewer Diagnositc Kits, BBL Microbiology Systems, Maryland, USA) both qualitative and quantitative.

The specific tests were Treponema pallidum haemagglutination (TPHA) test (Fujirebio, Tokyo, Japan) and the indirect fluorescent treponemal antibody absorption (FTA-Abs) test using treponemal antigen, sorbent and fluorescein labelled anti-human immunoglobulin conjugate (Bio Merieux, France). Specific fluorescent treponemal antibodies for the IgG and IgM classes were also tested for.

### 3.10.2 Hepatitis B surface antigen

A radio immunoassay (Ausria-Abbott Laboratories) was used to detect hepatitis B surface antigen (HBsAg) in serum specimens.

### 3.10.3 Human immunodeficiency virus (HIV)

Sera were tested for antibodies to HIV-1 using Abbott recombinant HIV1/HIV-2 ELISA test (Abbott Laboratories). All positive sera were confirmed by Western blotting (Diagnostic Biotechnology, Singapore).

## 3.11 ANALYSIS OF DATA

Where required patient data were analysed using the Chi-square test with Yates' correction. The level of significance was set at 95% and recorded as $p \leqslant 0,05$.

The sensitivity, specificity, predictive value of positive and negative tests were calculated as follows:

$$\text{Sensitivity} = \frac{\text{No. of true positive tests}}{\text{Total no. of women with bacterial vaginosis tested}} \times 100$$

$$\text{Specificity} = \frac{\text{No. of true negative tests}}{\text{Total no. of women without bacterial vaginosis tested}} \times 100$$

$$\text{Predictive value of positive test} = \frac{\text{No. of true positive tests}}{\text{Total no. of positive tests}} \times 100$$

$$\text{Predictive value of negative test} = \frac{\text{No. of true negative tests}}{\text{Total no. of negative tests}} \times 100$$

# 4.0 RESULTS

The subjects admitted to this study were recruited consecutively at the four clinics. Inclusion was made irrespective of the clinical diagnosis in individual patients.

## 4.1 PATIENT DATA

The patient data are presented in Table XVIII. All patients were Black between the ages of 16 to 52 years with a mean age of 26,1 years. There were no significant differences in the mean ages of women attending the four different clinics, however, the mean age of the women attending the colposcopy clinic (CC) was 31,2 years.

A high proportion, 130 (62,5%) women were unmarried whilst the remaining were married or paying lobola. Paying lobola was considered to be tantamount to marriage, because it traditionally signifies a commitment to one partner with a view to marriage and during this time sexual intercourse continues. There were more unmarried women in the sexually transmitted diseases (STD) clinic (68,8%) and colposcopy clinic (CC) (72,0%) groups compared to the ante-natal clinic (ANC) (51,9%) and family planning clinic (FPC) (58,2%) groups. The mean parity of the women was 2,1 with a range from 0 to 7. There were no significant differences in parity between the groups.

The majority of the women in the STD group (66,7%) did not use any form of contraception compared to 24% of women from the CC group. Parenteral

TABLE XVIII : DATA OF PATIENTS ATTENDING VARIOUS CLINICS

| | STD patients n=51 | CC patients n=50 | ANC patients n=52 | FPC patients n=55 | Total patients n=208 |
|---|---|---|---|---|---|
| Age in years | | | | | |
| mean | 26,5 | 31,2 | 26,4 | 25,1 | 26,1 |
| range | 18-44 | 18-52 | 16-37 | 16-38 | 16-52 |
| Marital status | | | | | |
| single | 35 (68,8) | 36 (72,0) | 27 (51,9) | 32 (58,2) | 130 (62,5) |
| paying lobola*/ married | 16 (31,2) | 14 (28,0) | 25 (48,1) | 23 (41,8) | 78 (37,5) |
| Parity | | | | | |
| mean | 1,8 | 2,4 | 2,1 | 2,2 | 2,1 |
| range | 0-7 | 0-7 | 0-6 | 0-7 | 0-7 |
| Contraception | | | | | |
| no contraception | 34 (66,7) | 12 (24,0) | NA | 0 (0) | |
| parenteral progesterone | 15 (29,4) | 22 (44,0) | | 40 (72,7) | |
| oral contraceptive | 2 (3,9) | 14 (28,0) | | 11 (20,0) | |
| IUD | - | - | | 4 (7,3) | |
| condom | - | 1 (2,0) | - | - | |
| tubal ligation | - | 1 (2,0) | - | - | |
| No. of sexual partners (in the past year) | | | | | |
| mean | 1,1 | 1,1 | 1,0 | 1,1 | 1,1 |
| range | 1-2 | 1-2 | 1-2 | 1-2 | 1-2 |
| (total to date) | | | | | |
| mean | 2,5 | 2,4 | 2,1 | 2,1 | 2,3 |
| range | 1-10 | 1-10 | 1-7 | 1-10 | 1-10 |

Figures in parenthesis denote percentages

* Signifies a commitment to marriage - see text
NA Not applicable
IUD Intra-uterine contraceptive device

progesterone was by far the most popular method of contraception. Significantly only one woman admitted to the use of condom (barrier method) by the sexual partner.

There were no differences in the mean number of sexual partners which was 1,1 for the past year and 2,3 overall. However, the total number of sexual partners per individual woman ranged from 1 to 10.

## 4.2 CLINICAL CHARACTERISTICS

### 4.2.1 Urinary symptoms on enquiry

Data relating to urinary symptoms are provided in Table XIX. A total of 24,0% of women complained of burning on micturition. This was sometimes associated with frequency or urgency.

### 4.2.2 Vaginal symptoms on enquiry

Women attending the CC, ANC, FPC and approximately half of those attending the STD clinics did not present with a complaint of vaginal discharge, however, on specific questioning, 54,8% (Table XX) of women admitted to having vaginal discharge which ranged from mild (59,6%) to profuse (14,0%). The colour of the discharge varied from white/grey to blood stained. In women who had vaginal discharge an offensive odour and pruritis vulvae were associated in only 37,5% and 30,3% respectively.

TABLE XIX : URINARY SYMPTOMS ON ENQUIRY

| | STD patients n=51 | | CC patients n=50 | | ANC patients n=52 | | FPC patients n=55 | | Total patients n=208 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % | No | % |
| Burning on micturition | 20 | 39,2 | 9 | 18,0 | 9 | 17,3 | 12 | 21,8 | 50 | 24,0 |
| Frequency of micturition | 11 | 21,6 | 5 | 10,0 | 8 | 15,4 | 9 | 16,4 | 33 | 15,9 |
| Urgency | 5 | 9,8 | 1 | 2,0 | 3 | 5,8 | 4 | 7,3 | 13 | 6,3 |

TABLE XX : VAGINAL SYMPTOMS ON ENQUIRY

| | STD patients n=51 | | CC patients n=50 | | ANC patients n=52 | | FPC patients n=55 | | Total patients n=208 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % | No | % |
| Vaginal discharge present | 34 | 66,7 | 28 | 56,0 | 24 | 46,2 | 28 | 50,9 | 114 | 54,8 |
| Quantity of discharge* when present | | | | | | | | | | |
| mild | 12 | 35,3 | 19 | 67,8 | 20 | 83,4 | 17 | 60,7 | 68 | 59,6 |
| moderate | 14 | 41,2 | 8 | 28,6 | 2 | 8,3 | 6 | 21,4 | 30 | 26,4 |
| profuse | 8 | 23,5 | 1 | 3,6 | 2 | 8,3 | 5 | 17,9 | 16 | 14,0 |
| Colour of discharge* | | | | | | | | | | |
| white/grey | 20 | 58,8 | 15 | 53,6 | 16 | 66,7 | 10 | 35,7 | 61 | 53,5 |
| yellow | 9 | 26,5 | 11 | 39,3 | 5 | 20,8 | 16 | 57,1 | 41 | 36,0 |
| green | 3 | 8,8 | 0 | 0 | 2 | 8,3 | 2 | 7,2 | 7 | 6,1 |
| blood-stained | 2 | 5,9 | 2 | 7,1 | 1 | 4,2 | 0 | 0 | 5 | 4,4 |
| Odour | 28 | 54,9 | 17 | 34,0 | 14 | 26,9 | 19 | 34,5 | 78 | 37,5 |
| Pruritis | 28 | 54,9 | 15 | 30,0 | 9 | 17,3 | 11 | 20,0 | 63 | 30,3 |

* Percentages quoted when discharge was present

### 4.2.3 Clinical signs

The presence of a vaginal discharge was ascertained by the examining clinician who reported whether it was present around the introitus with pooling in the vaginal fornices. Using these criteria a discharge was diagnosed in 95,1% of all women (Table XXI). The graded quantity of the discharge ranged from mild in 61,6% of women to profuse in 10,1% of women.

## 4.3 PREVALENCE OF MICRO-ORGANISMS

### 4.3.1 Vaginal micro-organisms

On the basis of positive cultures from vaginal specimens, G. vaginalis was isolated from 136/208 (65,4%) women (Table XXII). There were no significant differences in isolation among the four groups. The overall prevalence of T. vaginalis was 37,9%; the highest being ANC group (51,9%) followed by the STD group (47,0%), CC group (28,0%) and FPC group (25,5%). The prevalence of yeasts (37,0%) in the vagina was significantly lower than G. vaginalis, with the highest prevalence in the ANC group (55,8%). M. hominis and U. urealyticum were isolated more often from women attending the STD clinic (70,6% and 64,7% respectively) and CC clinic (68,0% and 60,0% respectively) than the other two groups. Anaerobic bacteria were present in 32,6% of women overall.

TABLE XXI : CLINICAL SIGNS OF ALL WOMEN STUDIED

| | STD patients n=51 | | CC patients n=50 | | ANC patients n=52 | | FPC patients n=55 | | Total patients n=208 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % | No | % |
| Presence of vaginal discharge on examination | 51 | 100 | 47 | 94,0 | 49 | 94,2 | 51 | 92,7 | 198 | 95,1 |
| Quantity of discharge on examination | | | | | | | | | | |
| mild | 19 | 37,3 | 34 | 72,3 | 28 | 57,1 | 41 | 80,4 | 122 | 61,6 |
| moderate | 25 | 49,0 | 10 | 21,3 | 12 | 24,5 | 9 | 17,6 | 56 | 28,3 |
| profuse | 7 | 13,7 | 3 | 6,4 | 9 | 18,4 | 1 | 2,0 | 20 | 10,1 |

TABLE XXII : MICRO-ORGANISMS DETECTED* IN VAGINAL SPECIMENS

| | STD patients n=51 | | CC patients n=50 | | ANC patients n=52 | | FPC patients n=55 | | Total patients n=208 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % | No | % |
| G. vaginalis | 34 | 66,6 | 34 | 68,0 | 28 | 53,8 | 40 | 72,6 | 136 | 65,4 |
| T. vaginalis | 24 | 47,0 | 14 | 28,0 | 27 | 51,9 | 14 | 25,5 | 79 | 37,9 |
| Yeasts | 13 | 25,5 | 20 | 40,0 | 29 | 55,8 | 15 | 27,2 | 77 | 37,0 |
| M. hominis | 36 | 70,6 | 34 | 68,0 | 23 | 44,2 | 31 | 56,4 | 124 | 59,6 |
| U. urealyticum | 33 | 64,7 | 30 | 60,0 | 40 | 77,0 | 33 | 60,0 | 100 | 48,1 |
| Anaerobic bacteria | 21 | 41,2 | 16 | 32,0 | 15 | 28,0 | 16 | 29,0 | 68 | 32,6 |

* By microscopy and/or culture

### 4.3.2 Endo-cervical micro-organisms

The endo-cervical micro-organisms detected were N. gonorrhoeae, C. trachomatis, Herpes simplex virus, M. hominis and U. urealyticum (Table XXIII). N. gonorrhoeae was isolated from 31,4% patients in the STD group followed by ANC (5,8%), FPC (5,5%) and CC (2,0%) groups. Overall the prevalence of C. trachomatis was higher than that of N. gonorrhoeae, being present in 37,3% of women in the STD group followed by CC (20,0%), ANC (19,2%) and FPC (12,7%) groups. Herpes simplex virus was isolated only from 2 (3,9%) women in the STD group. These women had no vesicular or ulcerative lesions in the genital area on clinical examination. Overall M. hominis and U. urealyticum were detected in the endocervices of 47,1% and 43,3% women respectively. Cervical Papanicolaou stained smears revealed human papilloma virus (HPV) infections predominatly in CC patients (26/50; 52%), with only one patient each from the STD and ANC groups being infected.

## 4.4 PREVALENCE OF BACTERIAL VAGINOSIS

The women studied were classified into those with and without bacterial vaginosis. This classification was based on the presence of any three of the criteria proposed by Amsel et al (1983) namely: i) an abnormal vaginal discharge, ii) a raised vaginal pH, iii) the presence of amines and iv) the presence of "clue" cells on vaginal wet smear microscopy. Using these criteria 73 (35,1%) of the women were diagnosed as having clinical bacterial vaginosis. The other 135 (64,9%) did not fulfil these diagnostic criteria for bacterial vaginosis (Table XXIV).

TABLE XXIII : MICRO-ORGANISMS DETECTED* IN ENDO-CERVICAL SPECIMENS

| | STD patients n=51 | | CC patients n=50 | | ANC patients n=52 | | FPC patients n=55 | | Total patients n=208 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % | No | % |
| N. gonorrhoeae | 16 | 31,4 | 1 | 2,0 | 3 | 5,8 | 3 | 5,5 | 23 | 11,1 |
| C. trachomatis | 19 | 37,3 | 10 | 20,0 | 10 | 19,2 | 7 | 12,7 | 46 | 22,1 |
| Herpes simplex virus | 2 | 3,9 | 0 | 0 | 0 | 0 | 0 | 2 | 2 | 0,9 |
| M. hominis | 29 | 56,9 | 27 | 54,0 | 20 | 38,5 | 22 | 40,0 | 98 | 47,1 |
| U. urealyticum | 16 | 31,4 | 21 | 42,0 | 34 | 65,4 | 19 | 34,5 | 90 | 43,3 |
| Human papilloma virus | 1 | 1,9 | 26 | 52,0 | 1 | 1,9 | 0 | 0 | 28 | 13,5 |

* By microscopy and/or culture

TABLE XXIV : PREVALENCE OF BACTERIAL VAGINOSIS

| | Bacterial vaginosis present | | Bacterial vaginosis not present | |
|---|---|---|---|---|
| | No | % | No | % |
| Sexually transmitted diseases clinic (STD) n=51 | 22 | 43,1 | 29 | 56,9 |
| Colposcopy clinic (CC) n=50 | 18 | 36,0 | 32 | 64,0 |
| Ante-natal clinic (ANC) n=52 | 13 | 25,0 | 39 | 75,0 |
| Family planning clinic (FPC) n=55 | 20 | 36,4 | 35 | 63,6 |
| Total n=208 | 73 | 35,1 | 135 | 64,9 |

## 4.5 ASSOCIATION OF RECOGNISED GENITAL PATHOGENS WITH BACTERIAL VAGINOSIS

Of the 208 women studied, vaginal infections alone were present in 104 (50,0%), endo-cervical infections alone in 18 (8,7%) and coexisting vaginal and endo-cervical infections in 41 (19,7%). No vaginal or endo-cervical infections were detected in 45 (21,6%) women (Fig 2).

Figure 3 represents all vaginal infections which occurred in a total of 145 (69,7%) women. In 6 (4,2%) patients all three infections occurred (bacterial vaginosis, T. vaginalis and C. albicans) concurrently. Forty nine patients had dual infections comprising either bacterial vaginosis with T. vaginalis (16,6%); bacterial vaginosis with C. albicans (7,6%) and T. vaginalis with C. albicans (9,7%).

The micro-organisms found in association with and without bacterial vaginosis in the four clinic groups are detailed in Tables XXV (STD), XXVI (CC), XXVII (ANC) and XXVIII (FPC).

### 4.5.1 Vaginal infections

In women with bacterial vaginosis (n=73), T. vaginalis was present in association with bacterial vaginosis in 24 (33,9%). C. albicans was associated less frequently occurring alone in 11 (15,1%) women and T. vaginalis together with C. albicans was present in 6 (8,2%) women. Of all the infections, T. vaginalis was found most frequently in association with bacterial vaginosis in women from the

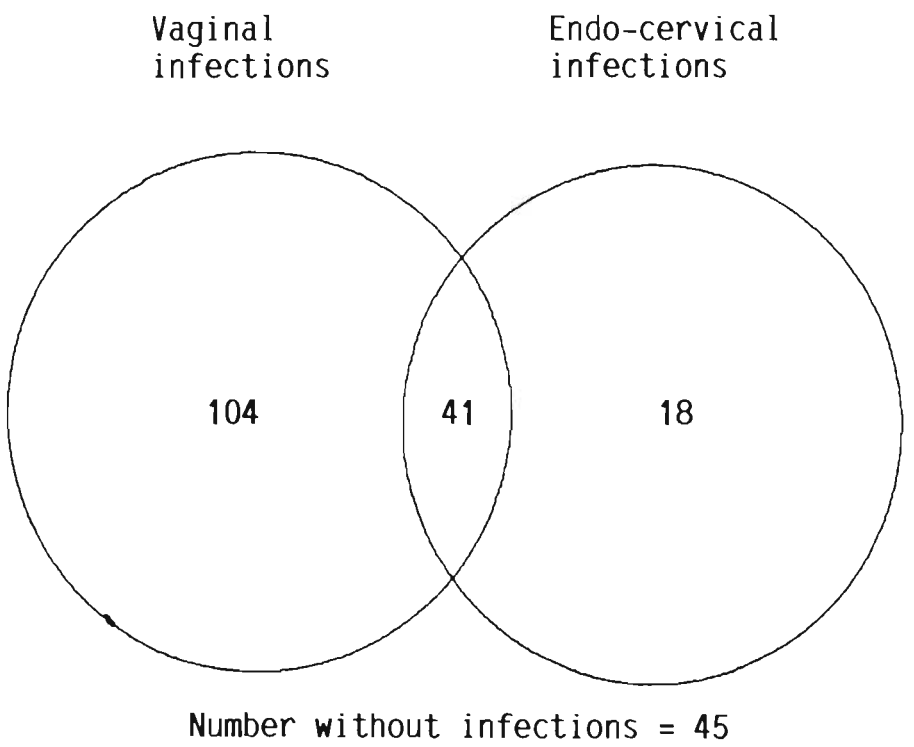


FIGURE 2 :

The prevalence of vaginal and endo-cervical infections

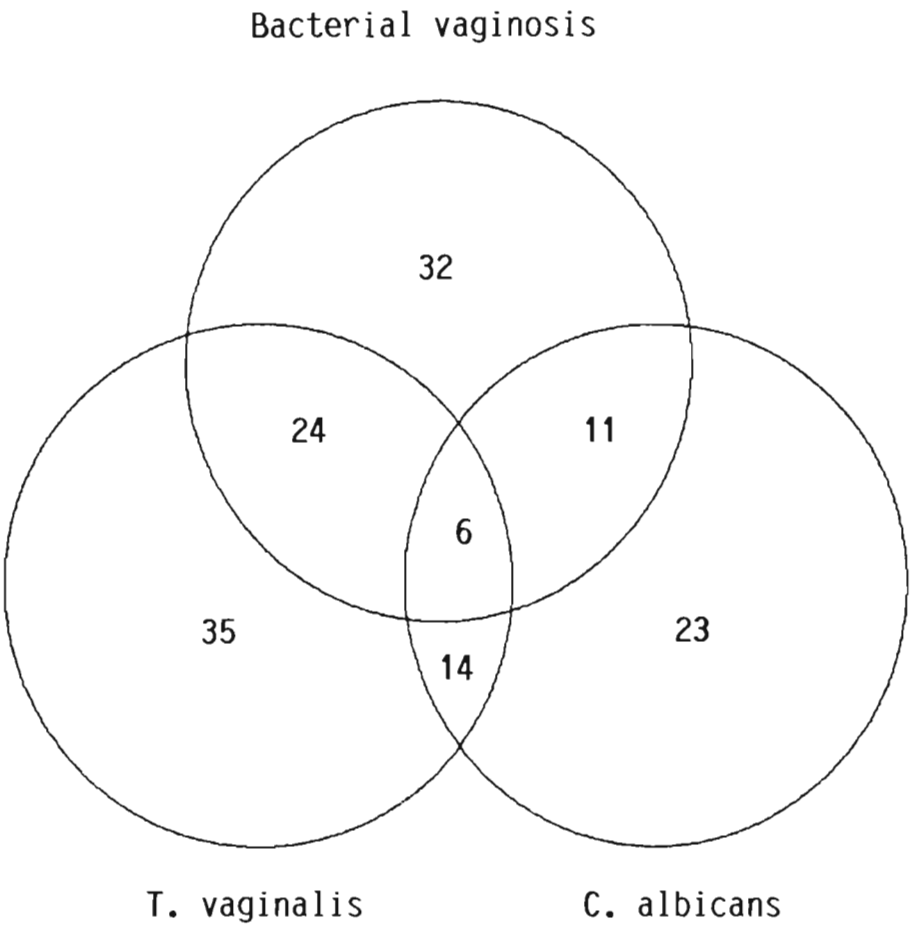


FIGURE 3 :

Diagram to shown the prevalence of single and mixed vaginal infections

TABLE XXV : VAGINAL AND ENDO-CERVICAL INFECTIONS IN WOMEN ATTENDING THE SEXUALLY TRANSMITTED DISEASE CLINIC

| | Bacterial vaginosis present n=22 | | Bacterial vaginosis not present n=29 | |
|---|---|---|---|---|
| | No | % | No | % |
| Vaginal infections: | | | | |
| T. vaginalis | 10 | 45,5 | 11 | 37,9 |
| C. albicans | 3 | 13,6 | 6 | 20,7 |
| T. vaginalis and C. albicans | 1 | 4,5 | 2 | 6,9 |
| T. vaginalis and C. albicans negative | 8 | 36,4 | 6 | 20,7 |
| Endo-cervical infections*: | | | | |
| N. gonorrhoeae | 4 | 18,2 | 4 | 13,8 |
| C. trachomatis | 4 | 18,2 | 8 | 27,6 |
| N. gonorrhoeae and C. trachomatis | 3 | 13,6 | 3 | 10,3 |
| N. gonorrhoeae and Herpes simplex virus | 1 | 4,5 | 0 | 0 |
| N. gonorrhoeae, C. trachomatis and Herpes simplex virus | 1 | 4,5 | 0 | 0 |
| No vaginal or endo-cervical pathogens | - | - | 4 | 13,8 |

* Alone or in combination with vaginal infections

TABLE XXVI : VAGINAL AND ENDO-CERVICAL INFECTIONS IN WOMEN ATTENDING THE COLPOSCOPY CLINIC

| | Bacterial vaginosis present n=18 | | Bacterial vaginosis not present n=32 | |
|---|---|---|---|---|
| | No | % | No | % |
| Vaginal infections: | | | | |
| *T. vaginalis* | 9 | 50,0 | 4 | 12,5 |
| *C. albicans* | 2 | 11,1 | 7 | 21,9 |
| *T. vaginalis* and *C. albicans* | 0 | 0 | 1 | 3,1 |
| *T. vaginalis* and *C. albicans* negative | 7 | 38,9 | 4 | 12,5 |
| Endo-cervical infections*: | | | | |
| *N. gonorrhoeae* | 0 | 0 | 0 | 0 |
| *C. trachomatis* | 3 | 16,7 | 6 | 18,8 |
| *N. gonorrhoeae* and *C. trachomatis* | 0 | 0 | 1 | 3,1 |
| No vaginal or endo-cervical pathogens | - | - | 16 | 50,0 |

* Alone or in combination with vaginal infections

TABLE XXVII : VAGINAL AND ENDO-CERVICAL INFECTIONS IN WOMEN ATTENDING THE ANTE-NATAL CLINIC

| | Bacterial vaginosis present n=13 | | Bacterial vaginosis not present n=39 | |
|---|---|---|---|---|
| | No | % | No | % |
| Vaginal infections: | | | | |
| *T. vaginalis* | 3 | 23,1 | 13 | 33,3 |
| *C. albicans* | 3 | 23,1 | 6 | 15,4 |
| *T. vaginalis* and *C. albicans* | 3 | 23,1 | 8 | 20,5 |
| *T. vaginalis* and *C. albicans* negative | 4 | 30,7 | 3 | 7,7 |
| Endo-cervical infections*: | | | | |
| *N. gonorrhoeae* | 0 | 0 | 2 | 5,1 |
| *C. trachomatis* | 1 | 7,7 | 8 | 20,5 |
| *N. gonorrhoeae* and *C. trachomatis* | 0 | 0 | 1 | 2,6 |
| No vaginal or endo-cervical pathogens | - | - | 9 | 23,1 |

* Alone or in combination with vaginal infections

TABLE XXVIII : VAGINAL AND ENDO-CERVICAL INFECTIONS IN WOMEN ATTENDING THE FAMILY PLANNING CLINIC

| | Bacterial vaginosis present n=20 | | Bacterial vaginosis not present n=35 | |
|---|---|---|---|---|
| | No | % | No | % |
| Vaginal infections: | | | | |
| *T. vaginalis* | 2 | 10,0 | 7 | 20,0 |
| *C. albicans* | 3 | 15,0 | 4 | 11,4 |
| *T. vaginalis* and *C. albicans* | 2 | 10,0 | 3 | 8,6 |
| *T. vaginalis* and *C. albicans* negative | 13 | 65,0 | 5 | 14,3 |
| Endo-cervical infections*: | | | | |
| *N. gonorrhoeae* | 3 | 15,0 | 0 | 0 |
| *C. trachomatis* | 1 | 5,0 | 6 | 17,1 |
| *N. gonorrhoeae* and *C. trachomatis* | 0 | 0 | 0 | 0 |
| No vaginal or endo-cervical pathogens | - | - | 16 | 45,7 |

* Alone or in combination with vaginal infections

STD and CC clinics. Bacterial vaginosis was diagnosed in the absence of T. vaginalis and C. albicans in 32 (43,8%) women.

In women without bacterial vaginosis (n=135), T. vaginalis alone was identified in 35 (25,9%) women and C. albicans alone in 23 (17,0%) women. Mixed infections with T. vaginalis and C. albicans occurred in 14 (10,3%). Of the vaginal infections, T. vaginalis was again the most frequent pathogen from women in the STD and ANC clinics. No vaginal infections were detected in 18 (13,3%) women.

### 4.5.2 Endo-cervical infections

Of the 41 mixed vaginal and endo-cervical infections (Fig 2), 21 (51,2%) endo-cervical infections were associated with bacterial vaginosis. N. gonorrhoeae and C. trachomatis were associated with bacterial vaginosis in 7 (9,6%) and 9 (12,3%) women respectively. Concurrent N. gonorrhoeae and C. trachomatis infections occurred in 4 women. In 2 women (Table XXV) bacterial vaginosis was associated with Herpes simplex virus and N. gonorrhoeae and/or C. trachomatis.

In women without bacterial vaginosis, N. gonorrhoeae alone was detected in 6 (4,4%) women whereas C. trachomatis alone was detected in 28 (20,7%) women.

In a total of 45 women no vaginal or endo-cervical pathogens were detected.

## 4.6 ASSOCIATION OF BACTERIAL VAGINOSIS WITH OTHER VAGINAL MICRO-ORGANISMS

The association of G. vaginalis, and other vaginal flora such as M. hominis, anaerobic bacteria, anaerobic Gram-negative curved rods and lactobacilli in women with and without bacterial vaginosis is shown in Table XXIX. Although G. vaginalis, M. hominis, anaerobic bacteria and Gram-negative curved rods were present in women without bacterial vaginosis; they were present significantly more often in women with bacterial vaginosis ($p < 0,05$). The absence of lactobacilli on Gram stain was significantly associated with bacterial vaginosis ($p < 0,05$). Although lactobacilli were present in vaginal smears of 2 (2,7%) women, the density of the organisms was less than that seen when bacterial vaginosis was diagnosed ($p < 0,05$).

## 4.7 RELATIONSHIP OF "CLUE" CELLS, POSITIVE AMINE TEST AND RAISED VAGINAL pH IN WOMEN WITH BACTERIAL VAGINOSIS AND IN WOMEN WITH RECOGNIZED GENITAL INFECTIONS WITHOUT BACTERIAL VAGINOSIS

These data are presented in Tables XXX and XXX1. "Clue" cells were observed in vaginal wet smear preparations in 67/73 (91,8%) women with bacterial vaginosis. They were found less often in women without bacterial vaginosis (19/135; 14,1%). Amine release was detected in the vaginal secretions of 56/73 (76,7%) women with bacterial vaginosis. This test was positive in only 6/135 (4,4%) women without bacterial vaginosis.

TABLE XXIX : VAGINAL MICRO-ORGANISMS OF WOMEN WITH AND WITHOUT BACTERIAL VAGINOSIS

| | Bacterial vaginosis present n=73 | | Bacterial vaginosis not present n=135 | | |
|---|---|---|---|---|---|
| | No | % | No | % | p value |
| G. vaginalis | 66 | 90,4 | 76 | 56,3 | < 0,05 |
| M. hominis | 63 | 86,3 | 61 | 45,2 | < 0,05 |
| Anaerobic bacteria | 46 | 63,0 | 22 | 16,3 | < 0,05 |
| Presence of* Gram-negative curved rods | 26 | 35,6 | 15 | 11,1 | < 0,05 |
| Presence of* lactobacilli | 2 | 2,7 | 54 | 40,0 | < 0,05 |

* Detected on Gram stained smears of vaginal secretions

TABLE XXX : CORRELATION OF "CLUE" CELLS, POSITIVE AMINE TEST, RAISED VAGINAL pH AND PRESENCE OF LACTOBACILLI ON GRAM STAIN IN WOMEN WITH AND WITHOUT BACTERIAL VAGINOSIS

| | Bacterial vaginosis present n=73 | | | | Bacterial vaginosis not present n=135 | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Positive | | Negative | | Positive | | Negative | | p value |
| | No | % | No | % | No | % | No | % | |
| +ve "clue" cells | 67 | 91,8 | 6 | 8,2 | 19 | 14,1 | 116 | 85,9 | $< 0,05$ |
| +ve amine test | 56 | 76,7 | 17 | 23,3 | 6 | 4,4 | 129 | 95,6 | $< 0,05$ |
| Vaginal pH $\geqslant$ 4,5 | 69 | 94,5 | 4 | 5,5 | 47 | 34,8 | 88 | 65,2 | $< 0,05$ |
| Lactobacilli on Gram stain | 2 | 2,7 | 71 | 97,3 | 54 | 40,0 | 81 | 60,0 | $< 0,05$ |

TABLE XXXI : VALUE OF LABORATORY CRITERIA FOR THE DIAGNOSIS OF BACTERIAL VAGINOSIS (expressed as percentages)

| | "Clue" cells on wet smear microscopy | Positive amine test | Raised vaginal pH | G. vaginalis culture |
|---|---|---|---|---|
| Sensitivity | 91,7 | 76,7 | 94,5 | 90,4 |
| Specificity | 85,9 | 95,5 | 65,2 | 43,7 |
| Predictive value of +ve test | 77,9 | 90,3 | 59,4 | 46,4 |
| Predictive value of -ve test | 95,1 | 88,3 | 95,7 | 89,4 |

An increased vaginal pH of $\geqslant$4,5 was found in 69/73 (94,5%) women with bacterial vaginosis, however 47/135; (34,8%) women without bacterial vaginosis were also found to have a raised vaginal pH.

The presence of "clue" cells, positive amine test, vaginal pH of $\geqslant$4,5 and the absence of lactobacilli on Gram stain were found to be statistically significant in women with bacterial vaginosis in comparison to those without bacterial vaginosis (Table XXX). The sensitivity, specificity, predictive value of positive and negative tests of these laboratory criteria for the diagnosis of bacterial vaginosis are shown in Table XXXI. The detection of clue cells on wet smear microscopy showed good sensitivity and specificity overall. Culture for G. vaginalis and raised vaginal pH whilst being very sensitive showed poor specificity. The positive amine test showed good specificity with low sensitivity.

The above laboratory criteria were also assessed in women with specific genital infections (Table XXXII). "Clue" cells, positive amine test and vaginal pH $\geqslant$ 4,5 were found less often in specific genital infections caused by yeasts, T. vaginalis and in mixed infections of yeasts and T. vaginalis. G. vaginalis was cultured from vaginal secretions of 31/32 (96,9%) women with bacterial vaginosis, and significantly less often in women with other genital infections ($p < 0,05$).

TABLE XXXII : CORRELATION OF "CLUE" CELLS, POSITIVE AMINE TEST, RAISED VAGINAL pH AND PRESENCE OF LACTOBACILLI ON GRAM STAIN IN WOMEN WITH SPECIFIC GENITAL INFECTIONS

| | +ve "clue" cells | | +ve amine test | | Vaginal pH$\geqslant$4,5 | | G. vaginalis culture +ve | | Lacto-bacilli on Gram stain | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % | No | % |
| Bacterial vaginosis only n=32 | 31[a] | 96,9 | 25[e] | 78,1 | 28[i] | 87,5 | 31[m] | 96,9 | 0[q] | 0 |
| Yeast infections only n=35 | 9[b] | 25,7 | 3[f] | 8,6 | 6[j] | 17,1 | 15[n] | 42,9 | 15[r] | 42,9 |
| T. vaginalis infections only n=35 | 1[c] | 2,9 | 2[g] | 5,7 | 22[k] | 62,9 | 22[o] | 62,9 | 7[s] | 20,0 |
| Yeast and T. vaginalis infections n=13 | 0[d] | 0 | 1[h] | 7,7 | 3*[l] | 23,0 | 8[p] | 61,5 | 7[t] | 53,8 |

* The other 10 women had a vaginal pH of 4,0

a vs. b $p < 0,05$ i vs. j $p < 0,05$ q vs. r $p < 0,05$
a vs. c $p < 0,05$ i vs. k $p < 0,05$ q vs. s $p < 0,05$
a vs. d $p < 0,05$ i vs. l $p < 0,05$ q vs. t $p < 0,05$

e vs. f $p < 0,05$ m vs. n $p < 0,05$
e vs. g $p < 0,05$ m vs. o $p < 0,05$
e vs. h $p < 0,05$ m vs. p $p < 0,05$

## 4.8 CULTURE OF GARDNERELLA VAGINALIS

Vaginal secretions inoculated directly onto human blood (HB) and horse blood (BA) agar were examined after 48 hours incubation at 37°C. The use of selective and non-selective media enabled the assessment of G. vaginalis in relation to the total aerobic genital flora and the characteristic diffuse beta-haemolysis exhibited on HB agar aided in the rapid presumptive identification of this organism.

The density of the G. vaginalis of HB agar was graded from 1+ to 3+ (Table XXXIII). G. vaginalis was isolated from direct culture from 136/208 (65,4%) women. In comparison the organism was isolated from subcultures from modified Cary-Blair transport media (CB) in 121/208 (58,2%) women and from subcultures from modified Stuart transport media (ST) in 107/208 (51,4%) women.

A density grade of 3+ was recorded more often from the direct culture method (115/136; 84,5%) and from CB media (89/121; 73,6%) compared to ST media which yielded a 3+ density in only 54/107 (50,5%). A density of 1+ growth of G. vaginalis was found more often in subcultures of ST media and the aerobic vaginal flora frequently overgrew that of the G. vaginalis. If a selective medium had not been used this overgrowth would have masked the G. vaginalis organisms resulting in a further reduction in recovery. This problem of overgrowth was not seen with subcultures from CB transport media, the aerobic growth and G. vaginalis growth corresponded more closely to that of directly plated

TABLE XXXIII : COMPARISON OF DIRECT PLATING METHOD VERSUS TRANSPORT MEDIA FOR THE RECOVERY OF GARDNERELLA VAGINALIS FROM VAGINAL SPECIMENS

| Density of Growth | Direct | Cary-Blair | Stuart |
|---|---|---|---|
| * 1+ | 7 | 12 | 38 |
| * 2+ | 14 | 20 | 15 |
| * 3+ | 115 | 89 | 54 |
| Total +ve culture | 136[a] | 121[b] | 107[c] |
| Negative culture | 72 | 87 | 101 |

* 1+ Growth along the initial inoculum only

* 2+ Growth along the initial inoculum and along the first line of plate out

* 3+ Growth along the initial inoculum and through to the third line of plate out

a vs. b $p = 0,16$

b vs. c $p = 0,20$

a vs. c $p < 0,05$

media. The recovery of G. vaginalis was significantly lower from ST media than from the direct plating method ($p < 0,05$).

## 4.9 DISTRIBUTION AND FREQUENCY OF BIOTYPES OF GARDNERELLA VAGINALIS

The biochemical tests used in the biotyping scheme were those described by Piot et al (1984).

The distribution of the eight biotypes among G. vaginalis isolates is shown in Table XXXIV. The most common biotypes encountered were types 1, 2 and 5. Biotype 1 was encountered most frequently (42,2% overall) from vaginal, urethral and urine isolates, followed by type 5 (22,2%) and type 2 (11,4%). Biotypes 3, 4, 7 and 8 accounted for 5,4%, 7,0%, 9,6% and 2,2% of the strains respectively. Biotype 6 was not encountered amongst strains from the vagina, urethra and urine. From the urine isolates biotype 2 was not encountered.

The distribution of the biotypes of vaginal isolates from women with and without bacterial vaginosis was similar. (Table XXXV). Biotype 1 was predominant in both groups followed by type 5 and type 2. Biotypes 1 and 5 were present significantly more often in women with bacterial vaginosis than in those without ($p < 0,05$). The slight differences in the distribution of the other biotypes between the two groups were not statistically significant.

TABLE XXXIV : BIOTYPES OF GARDNERELLA VAGINALIS

| | Site | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Biotype | Vagina | | Urethra | | Urine | | Total | |
| | No | % | No | % | No | % | No | % |
| 1 | 43 | 43,0 | 29 | 40,3 | 6 | 46,2 | 78 | 42,2 |
| 2 | 11 | 11,0 | 10 | 13,9 | 0 | 0 | 21 | 11,4 |
| 3 | 6 | 6,0 | 2 | 2,8 | 2 | 15,4 | 10 | 5,4 |
| 4 | 7 | 7,0 | 4 | 5,6 | 2 | 15,4 | 13 | 7,0 |
| 5 | 22 | 22,0 | 18 | 25,0 | 1 | 7,6 | 41 | 22,2 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 10 | 10,0 | 6 | 8,2 | 2 | 15,4 | 18 | 9,6 |
| 8 | 1 | 1,0 | 3 | 4,2 | 0 | 0 | 4 | 2,2 |
| TOTAL | 100 | 100 | 72 | 100 | 13 | 100 | 185 | 100 |

TABLE XXXV : PREVALENCE OF BIOTYPES OF GARDNERELLA VAGINALIS OF VAGINAL ORIGIN IN WOMEN WITH AND WITHOUT BACTERIAL VAGINOSIS

| Biotype | Bacterial vaginosis present n=73 | | Bacterial vaginosis not present n=135 | | Total n=208 | |
|---|---|---|---|---|---|---|
| | No | % | No | % | No | % |
| 1 | 24 | 32,9[a] | 19 | 14,1[b] | 43 | 20,6 |
| 2 | 2 | 2,7 | 9 | 6,6 | 11 | 5,3 |
| 3 | 1 | 1,3 | 5 | 3,7 | 6 | 2,9 |
| 4 | 5 | 6,8 | 2 | 1,5 | 7 | 3,4 |
| 5 | 12 | 16,4[c] | 10 | 7,4[d] | 22 | 19,4 |
| 6 | 0 | 0 | 0 | 0 | 0 | 0 |
| 7 | 6 | 8,2 | 4 | 2,9 | 10 | 4,8 |
| 8 | 1 | 1,3 | 0 | 0 | 1 | 0,5 |
| Total | 51 | 69,9 | 49 | 35,3 | 100 | 48,1 |
| Culture not available | 15 | 20,5 | 27 | 20,0 | 42 | 20,2 |
| Negative culture | 7 | 9,6 | 59 | 43,7 | 66 | 31,7 |
| Total | 73 | 100 | 135 | 100 | 208 | 100 |

a vs b $p < 0,05$

c vs d $p < 0,05$

## 4.10 DISTRIBUTION AND FREQUENCY OF SEROTYPES OF GARDNERELLA VAGINALIS

The serotyping scheme used, identified a total of 27 serotypes. Tables XXXVI and XXXVII show the distribution of serotypes from vaginal and urethral isolates of G. vaginalis. Serotypes 125 and 25 were seen more frequently in both vaginal and urethral strains. A wide range of other serotypes - each in small numbers were identified. Only 7% of strains overall could not be typed with available antisera. The distribution of serotypes of G. vaginalis isolated from women with and without bacterial vaginosis were similar. The analysis of 50 strains in each group is shown in Table XXXVIII.

## 4.11 ANTIMICROBIAL SUSCEPTIBILITY OF GARDNERELLA VAGINALIS

The MICs, MIC ranges, MIC50s and MIC90s of the 93 vaginal strains of G. vaginalis tested are shown in Table XXXIX. The cumulative percentages of strains inhibited at each concentration for the various antimicrobial agents tested are shown in Figures 4 to 9.

All strains were susceptible to penicillin (MIC90:0,5mg/L), ampicillin (MIC90:0,5mg/L), erythromycin (MIC90:0,06mg/L), clindamycin (MIC90:0,03mg/L), vancomycin (MIC90:0,5mg/L) and chloramphenicol (MIC90:2,0mg/L).

LY 146032, a cyclic lipopeptide antibiotic, which has excellent activity against Gram-positive organisms showed limited activity against these strains (MIC90:8,0mg/L).

TABLE XXXVI : DISTRIBUTION OF SEROTYPES OF 100 VAGINAL ISOLATES OF *GARDNERELLA VAGINALIS*

| Serotype | Frequency | % |
|---|---|---|
| Non-typable | 7 | 7,0 |
| 1 | 5 | 5,0 |
| 12 | 3 | 3,0 |
| 123 | 1 | 1,0 |
| 1235 | 5 | 5,0 |
| 1235C | 3 | 3,0 |
| 123C | 1 | 1,0 |
| 125 | 11 | 11,0 |
| 125C | 6 | 6,0 |
| 13 | 1 | 1,0 |
| 135 | 4 | 4,0 |
| 135C | 8 | 8,0 |
| 13C | 2 | 2,0 |
| 15C | 1 | 1,0 |
| 1C | 4 | 4,0 |
| 2 | 3 | 3,0 |
| 235 | 2 | 2,0 |
| 235C | 2 | 2,0 |
| 23C | 1 | 1,0 |
| 25 | 11 | 11,0 |
| 25C | 4 | 4,0 |
| 2C | 1 | 1,0 |
| 3 | 4 | 4,0 |
| 35 | 2 | 2,0 |
| 35C | 2 | 2,0 |
| 3C | 1 | 1,0 |
| 5 | 1 | 1,0 |
| C | 4 | 4,0 |
| TOTAL | 100 | 100,0 |

TABLE XXXVII : DISTRIBUTION OF SEROTYPES OF 72 URETHRAL ISOLATES OF GARDNERELLA VAGINALIS

| Serotype | Frequency | % |
|---|---|---|
| Non-typable | 6 | 8,3 |
| 1 | 2 | 2,8 |
| 12 | 1 | 1,4 |
| 123 | 1 | 1,4 |
| 1235 | 3 | 4,2 |
| 1235C | 3 | 4,2 |
| 123C | 1 | 1,4 |
| 125 | 8 | 11,1 |
| 125C | 5 | 6,9 |
| 13 | 1 | 1,4 |
| 135 | 5 | 6,9 |
| 135C | 3 | 4,2 |
| 13C | 1 | 1,4 |
| 15C | 1 | 1,4 |
| 1C | 4 | 5,6 |
| 2 | 1 | 1,4 |
| 235 | 4 | 5,6 |
| 235C | 1 | 1,4 |
| 25 | 9 | 12,5 |
| 25C | 4 | 5,6 |
| 2C | 1 | 1,4 |
| 3 | 1 | 1,4 |
| 35C | 1 | 1,4 |
| 5C | 2 | 2,8 |
| C | 3 | 4,2 |
| TOTAL | 72 | 100,0 |

TABLE XXXVIII : DISTRIBUTION OF SEROTYPES OF *GARDNERELLA VAGINALIS* AMONG WOMEN WITH AND WITHOUT BACTERIAL VAGINOSIS

| | Bacterial vaginosis Present | Bacterial vaginosis Not present | |
|---|---|---|---|
| Serotype | Frequency | Frequency | Total |
| Non-typable | 3 | 4 | 7 |
| 1 | 2 | 3 | 5 |
| 12 | 3 | 0 | 3 |
| 123 | 1 | 0 | 1 |
| 1235 | 3 | 2 | 5 |
| 1235C | 0 | 3 | 3 |
| 123C | 0 | 1 | 1 |
| 125 | 7 | 4 | 11 |
| 125C | 4 | 2 | 6 |
| 13 | 1 | 0 | 1 |
| 135 | 1 | 3 | 4 |
| 135C | 3 | 5 | 8 |
| 13C | 1 | 1 | 2 |
| 15C | 0 | 1 | 1 |
| 1C | 1 | 3 | 4 |
| 2 | 3 | 0 | 3 |
| 235 | 2 | 0 | 2 |
| 235C | 0 | 0 | 2 |
| 23C | 1 | 0 | 1 |
| 25 | 5 | 6 | 11 |
| 25C | 3 | 1 | 4 |
| 2C | 0 | 1 | 1 |
| 3 | 1 | 3 | 4 |
| 35 | 2 | 0 | 2 |
| 35C | 0 | 2 | 2 |
| 3C | 1 | 0 | 1 |
| 5 | 1 | 0 | 1 |
| C | 1 | 3 | 4 |
| TOTAL | 50/73 | 50/135 | 100 |

TABLE XXXIX : IN VITRO SUSCEPTIBILITY OF 93 STRAINS OF GARDNERELLA VAGINALIS TO 25 ANTIMICROBIAL AGENTS

| Antimicrobial agents | Number of isolates with MIC's (mg/L) of : | | | | | | | | | | | | | | | MIC50 (mg/L) | MIC90 (mg/L) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 0,007 | 0,015 | 0,03 | 0,06 | 0,12 | 0,25 | 0,5 | 1,0 | 2,0 | 4,0 | 8,0 | 16,0 | 32,0 | 64,0 | 128,0 | | |
| Metronidazole | | | | | | | | | 4 | 36 | 34 | 14 | 3 | 1 | 1 | 8,0 | 16,0 |
| 2-Hydroxymetabolite* | | | | | | 14 | 16 | 33 | 19 | 2 | 3 | 5 | | | 1 | 1,0 | 4,0 |
| Tinidazole | | | | | | | | 3 | 11 | 32 | 36 | 10 | 0 | 0 | 1 | 8,0 | 8,0 |
| Penicillin G | | 2 | 25 | 13 | 12 | 24 | 17 | | | | | | | | | 0,12 | 0,5 |
| Ampicillin | | | 5 | 18 | 8 | 14 | 43 | 5 | | | | | | | | 0,5 | 0,5 |
| Cefamandole | | | | | 2 | 18 | 6 | 28 | 39 | | | | | | | 1,0 | 2,0 |
| Cefoxitin | | | | 2 | 7 | 7 | 16 | 21 | 30 | 10 | | | | | | 1,0 | 4,0 |
| Cefuroxime | | | | 2 | 9 | 7 | 22 | 15 | 25 | 13 | | | | | | 1,0 | 4,0 |
| Cefotaxime | | | | | | 12 | 20 | 9 | 45 | 7 | | | | | | 2,0 | 2,0 |
| Ceftriaxone | | | | 8 | 10 | 9 | 25 | 28 | 12 | 1 | | | | | | 0,5 | 2,0 |
| Aztreonam | | | | | | | | | | 3 | 9 | 9 | 72 | | | 32,0 | 32,0 |
| Imipenem | | | | 8 | 10 | 35 | 10 | 30 | | | | | | | | 0,25 | 1,0 |
| Tetracycline | | | | | | | | | 34 | 5 | | | | 45 | 9 | 64,0 | 64,0 |
| Minocycline | | | | | 1 | 2 | 13 | 18 | 17 | 8 | 1 | 33 | | | | 2,0 | 16,0 |
| Erythromycin | 16 | 28 | 33 | 16 | | | | | | | | | | | | 0,03 | 0,06 |
| Clindamycin | 21 | 42 | 29 | | | | | | | | | | | | | 0,01 | 0,03 |
| Vancomycin | | | | | 8 | 42 | 43 | | | | | | | | | 0,25 | 0,5 |
| LY 146032 | | | | | | | 3 | 19 | 32 | 22 | 17 | | | | | 4,0 | 8,0 |
| Chloramphenicol | | | | | | | 7 | 71 | 15 | | | | | | | 1,0 | 2,0 |
| Amikacin | | | | | | | | | | 3 | 7 | 11 | 43 | 20 | 12 | 32,0 | 128,0 |
| Rifampicin | | | | | | | 14 | 42 | 37 | | | | | | | 1,0 | 2,0 |
| Ciprofloxacin | | | | | | | | 72 | 17 | 3 | | | | | | 1,0 | 2,0 |
| Sulphamethoxazole | | | | | | | | | | | | | | | 93 | 128,0 | 128,0 |
| Trimethoprim | | | | | | | 6 | 21 | 55 | 11 | | | | | | 2,0 | 4,0 |
| Co-trimoxazole** | | | | | | | | | | 2 | 8 | 1 | 11 | 71 | | 64,0 | 64,0 |

* 2-Hydroxymetabolite of metronidazole ([1-(2-hydroxyethyl)-2-hydroxymethyl-5-nitroimidazole])

** Sulphamethoxazole/trimethoprim in 19:1 ratio

MIC50 - minimal inhibitory concentration at which 50% of isolates were inhibited

MIC90 - minimal inhibitory concentration at which 90% of isolates were inhibited

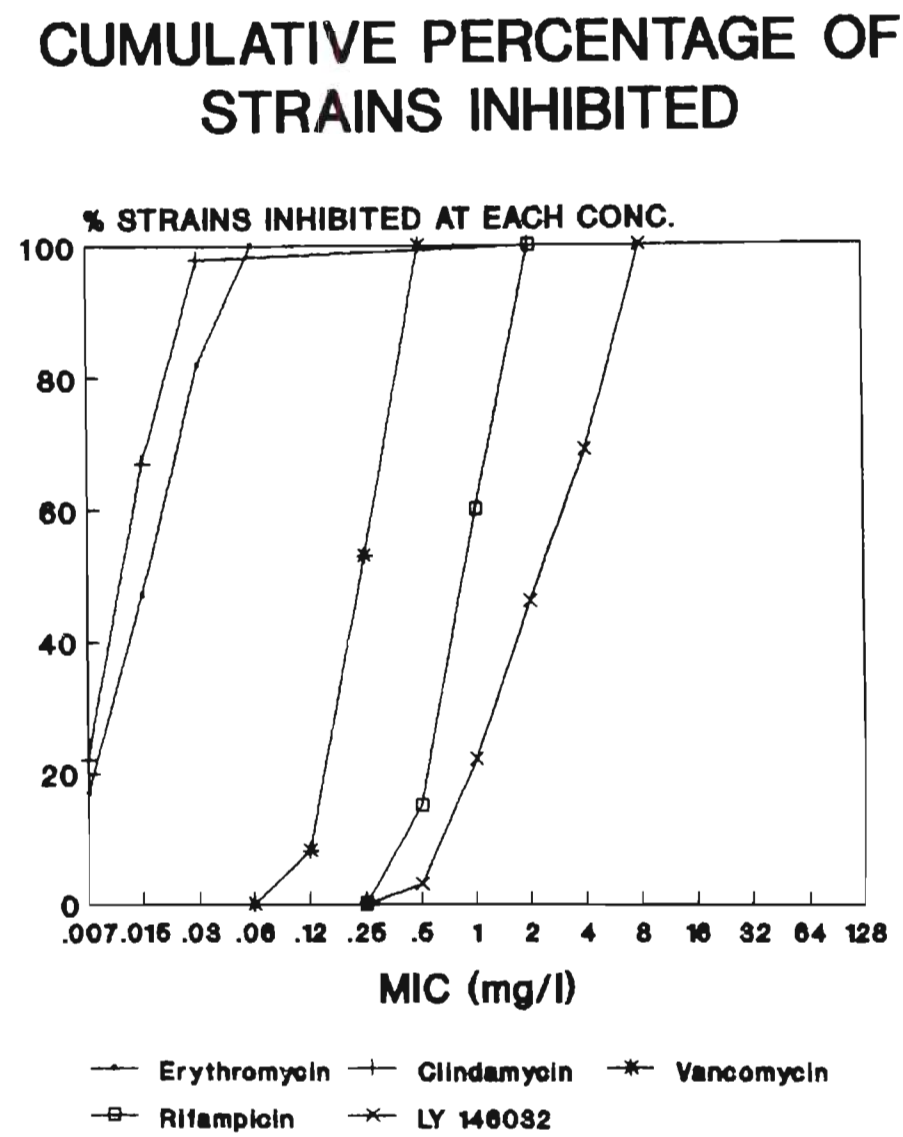


FIGURE 4 :

Cumulative % minimum inhibitory concentrations for Gardnerella vaginalis to erythromycin, clindamycin, vancomycin, rifampicin and LY 146032

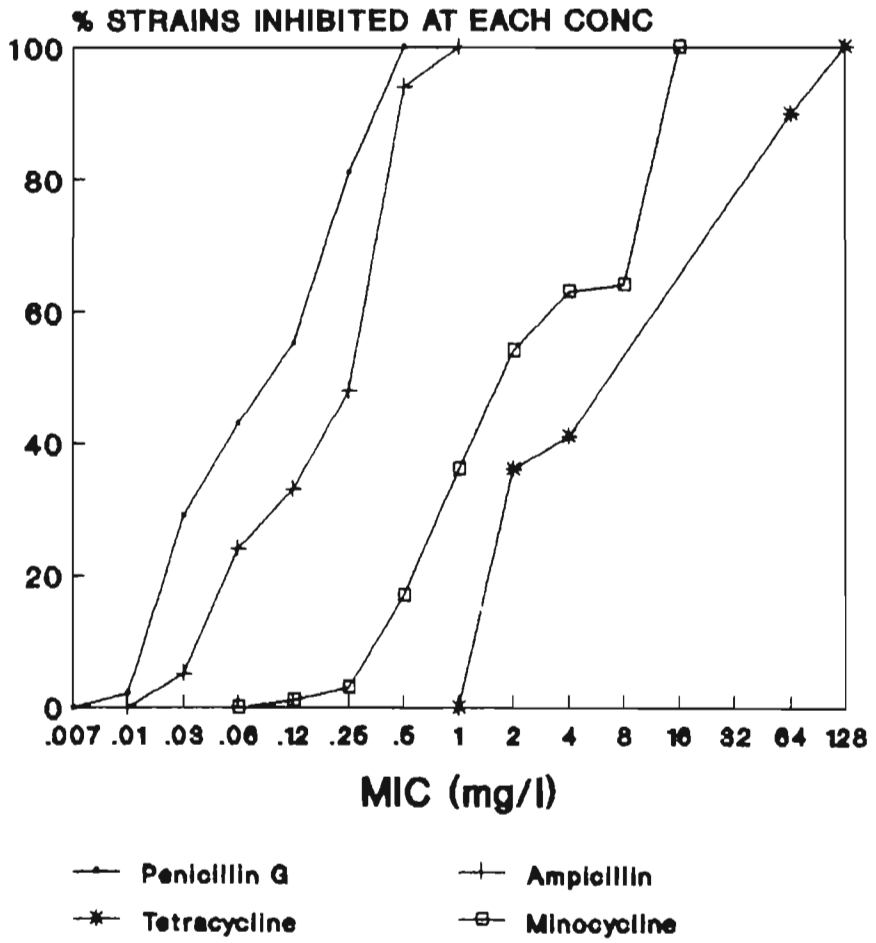


FIGURE 5 :

Cumulative % minimum inhibitory concentrations for Gardnerella vaginalis to penicillin G, ampicillin, tetracycline and minocycline

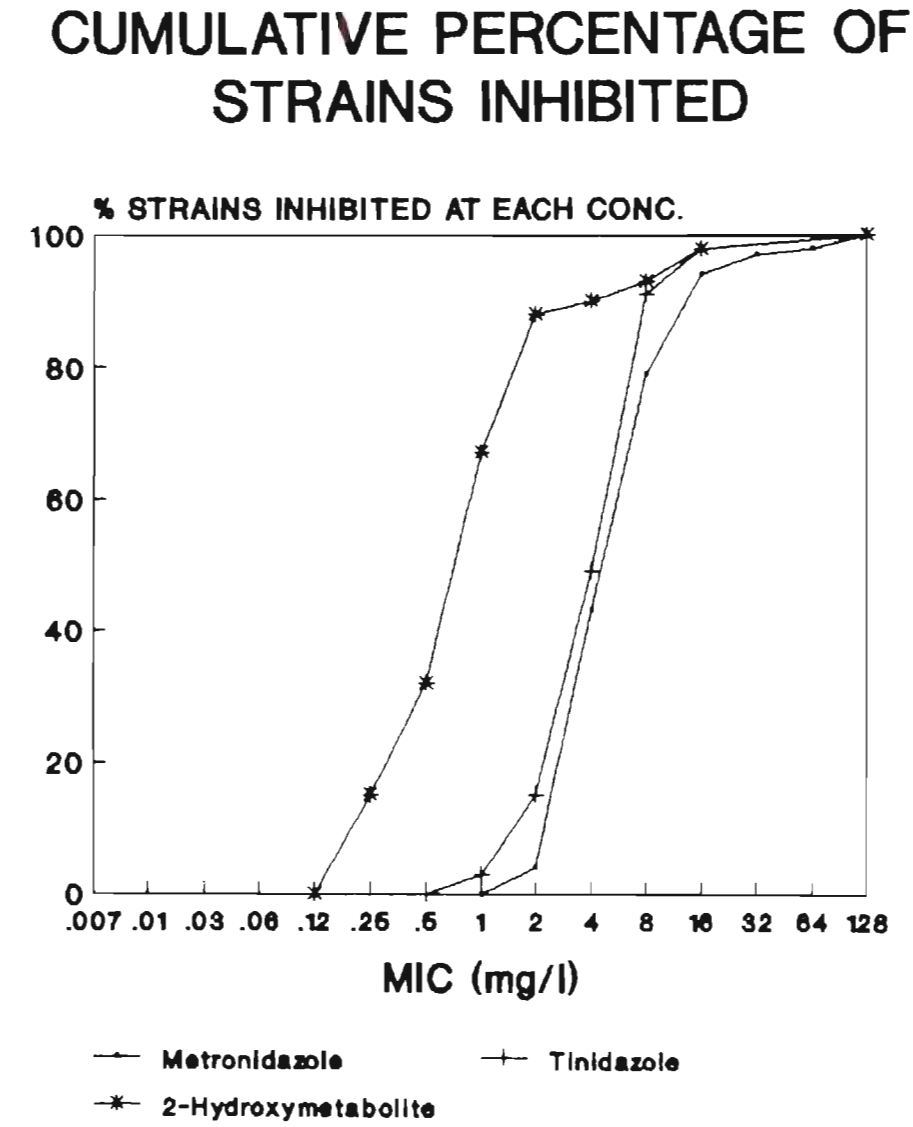


FIGURE 6 :

Cumulative % minimum inhibitory concentrations for Gardnerella vaginalis to metronidazole, tinidazole and 2-hydroxymetabolite of metronidazole

# CUMULATIVE PERCENTAGE OF STRAINS INHIBITED

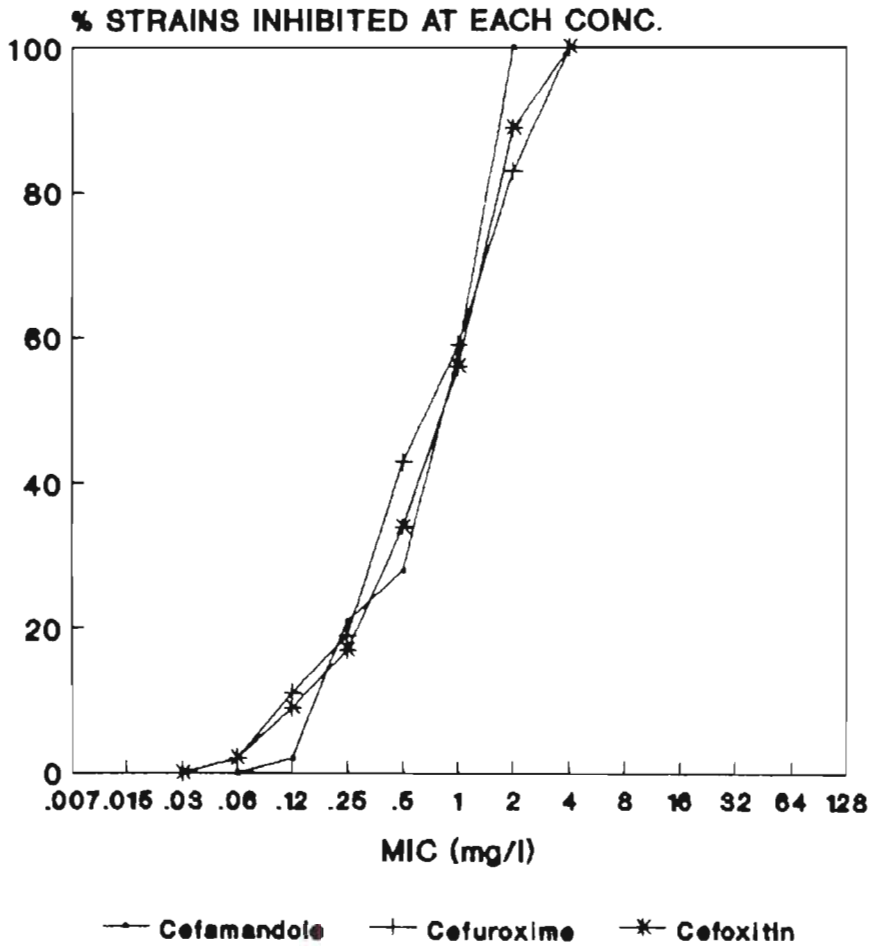


FIGURE 7 :

Cumulative % minimum inhibitory concentrations for Gardnerella vaginalis to cefamandole, cefuroxime and cefoxitin

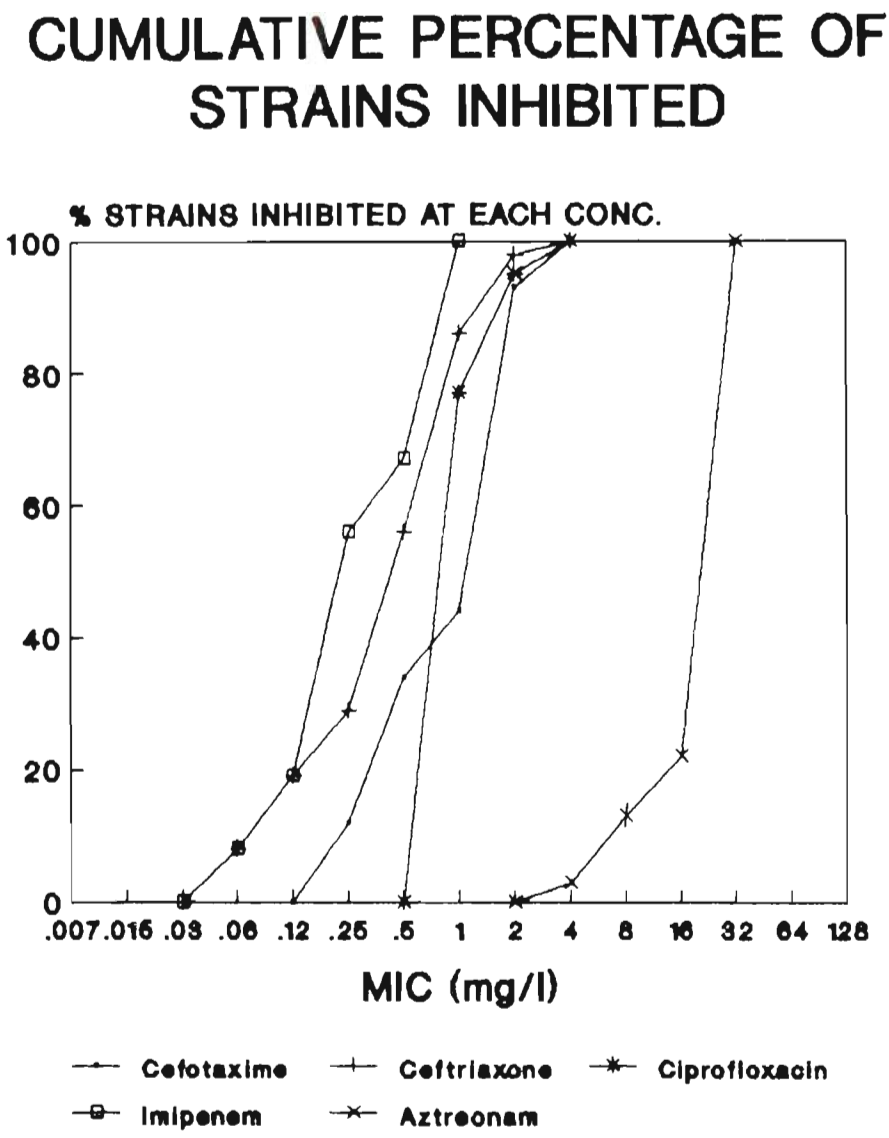


FIGURE 8 :

Cumulative % minimum inhibitory concentrations for Gardnerella vaginalis to cefotaxime, ceftriaxone, ciprofloxacin, imipenem and aztreonam

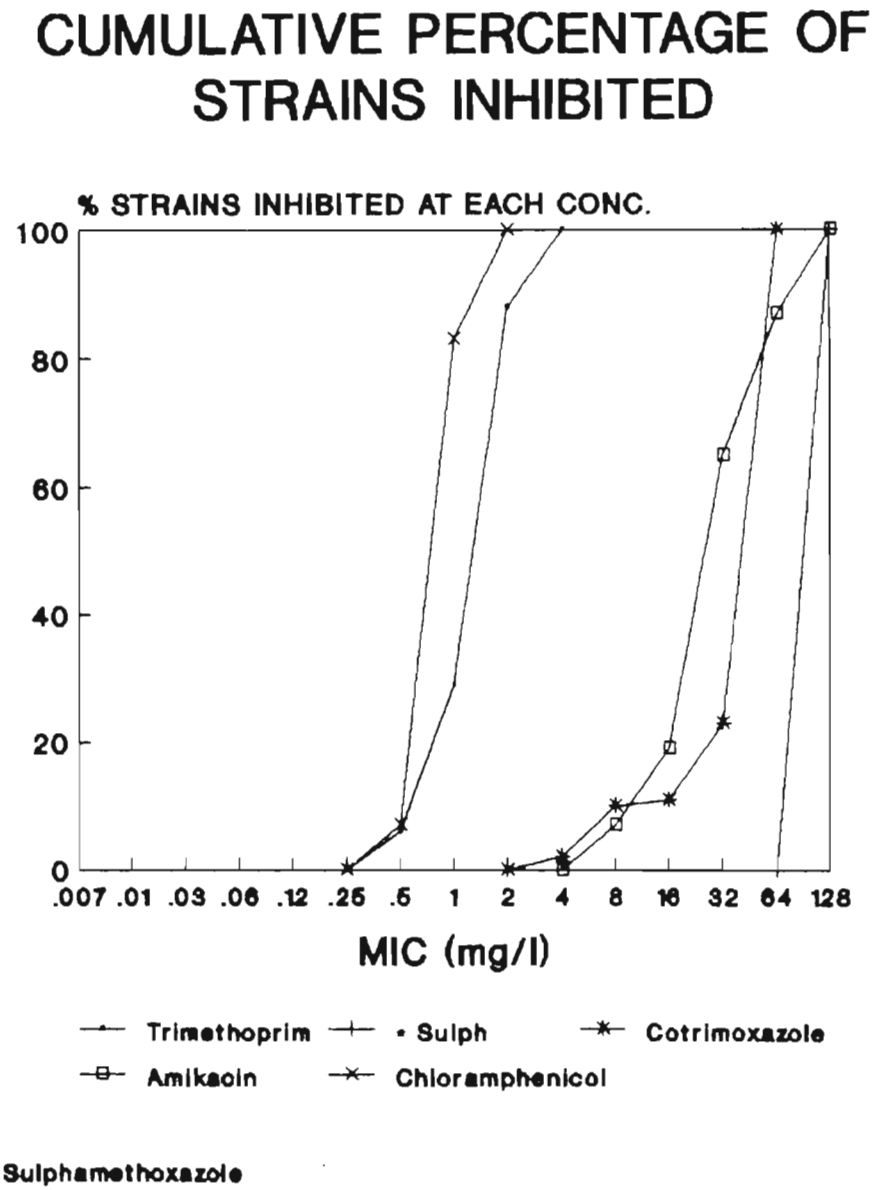


FIGURE 9 :

Cumulative % minimum inhibitory concentrations for Gardnerella vaginalis to trimethoprim, sulphamethoxazole, cotrimoxazole, amikacin and chloramphenicol

Tetracycline MICs were bimodal in distribution with many strains having an MIC of 2,0 to 4,0mg/L, whilst the majority of the strains had MICs of 64mg/L or more. More strains were sensitive to minocycline, although there was some resistance to minocycline with 33 (35,5%) of the isolates having an MIC of 16,0mg/L. The MICs for metronidazole were variable and paralleled those for tinidazole. The hydroxymetabolite of metronidazole was more active than both the parent compound and tinidazole, with MICs being at least two dilutions less than those for metronidazole (Fig 7). One strain which showed marked resistance to metronidazole (MIC = 128,0mg/L) was also resistant to tinidazole (MIC = 128,0mg/L) and the hydroxymetabolite (MIC = 128,0mg/L).

All strains were susceptible to the cephalosporins tested. No individual cephalosporin was found to be superior (Fig 8). For cefoxitin and cefuroxime the MIC90s were 4,0mg/L, whereas for cefamandole, cefotaxime and ceftriaxione these were 2,0mg/L.

Ciprofloxacin had an MIC90 of 2,0mg/L and imipenem an MIC90 of 1,0mg/L (Fig 9). All strains were susceptible to trimethoprim and resistant to sulphamethoxazole, and the combination of these two did not demonstrate any synergistic activity on the strains tested. Resistance to aztreonam and amikacin was marked with MIC90s of 32,0mg/L and 128,0mg/L respectively.

## 4.12 VAGINAL YEASTS

Vaginal yeasts were cultured from 77/208 (37%) women (Table XL). Of these, C. albicans was the commonest isolate (55/77 ; 70,1%)). Four of these were present together with C. lipolytica, C. krusei and/or T. glabrata. T. glabrata alone was present in 12 (15,8%) women. Other strains were also encountered infrequently

## 4.13 VAGINAL TRICHOMONIASIS

T. vaginalis was detected in 79 (37,9%) vaginal specimens, either by wet smear microscopy or by culture in modified Diamond's medium. Wet smears and cultures were positive in 60 and 77 women respectively. Motile trichomonads were detected in wet smears of 2 women in whom cultures were negative. In an additional 19 women the organism was detected by culture only (Table XLI).

## 4.14 ASSOCIATION OF SEXUALLY TRANSMITTED PATHOGENS IN WOMEN WITH CERVICAL INTRA-EPITHELIAL NEOPLASIA (CIN)

The prevalence of the human papilloma virus (HPV) and cervical intra-epithelial neoplasia (CIN) in women attending the different clinics is shown in Table XLII. In the CC group, HPV was detected in 26 (52%) and CIN in 28 (56%) women by colposcopic examination and histology of biopsied specimens.

TABLE XL : YEASTS ISOLATED FROM THE VAGINA

| Yeast | No | % |
|---|---|---|
| C. albicans | 54* | 70,1 |
| T. glabrata | 12 | 15,6 |
| S. cerevisiae | 5 | 6,5 |
| T. maris | 1 | 1,3 |
| C. lipolytica | 1 | 1,3 |
| C. tropicalis | 1 | 1,3 |
| C. guilliermondii | 1 | 1,3 |
| C. paratropicalis | 1 | 1,3 |
| C. parapsilosis | 1 | 1,3 |
| TOTAL | 77 | 100 |

* 4 cultures were mixed with either C. lipolytica, C. krusei or T. glabrata

TABLE XLI : WET SMEAR MICROSCOPY AND CULTURE FOR THE DIAGNOSIS OF TRICHOMONAS VAGINALIS INFECTION IN ALL WOMEN (n=208)

| | No | % |
|---|---|---|
| Total number of patients positive by either wet smear microscopy and/or culture | 79 | 37,9 |
| Positive wet smear microscopy and culture | 58 | 73,4 |
| Positive wet smear microscopy only | 2 | 2,5 |
| Positive culture only | 19 | 24,1 |
| Total number of patients negative by either wet smear microscopy and/or culture | 129 | 62,1 |

TABLE XLII : PRESENCE OF HUMAN PAPILLOMA VIRUS INFECTION AND CERVICAL INTRA-EPITHELIAL NEOPLASIA (CIN) IN CLINIC ATTENDERS

| | STD patients n=51 | | CC patients n=50 | | ANC patients n=52 | | FPC patients n=55 | |
|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % |
| HPV | 1° | 1,9 | 26*° | 52,0 | 1° | 1,9 | 0 | 0 |
| CIN | 1° | 1,9 | 28*° | 56,0 | 0 | 0 | 2° | 3,6 |

° Diagnosed on microscopy of Papanicolaou stained cervical smears

* Confirmed histologically

The distribution of various sexually transmitted pathogens in women with and without CIN attending CC clinic is shown in Table XLIII. Bacterial vaginosis was found significantly more often in women with CIN than in those without CIN (p=0,04). With regard to other agents, prevalences were similar for both groups.

## 4.15 SEROLOGICAL RESULTS

The results of the serological tests performed on patients' sera are shown in Table XLIV. In a total of 47 (22,6%) women, there was evidence of reactive syphilis serology which was confirmed by specific treponemal tests; the Treponema pallidum haemagglutination test (TPHA) and fluorescent treponemal antibody absorption test (FTA-Abs). In an additional 35 (16,8%) women, either the TPHA and/or FTA-Abs tests were positive, suggesting past exposure to syphilis.

Hepatitis B surface antigen (HBsAg) was detected in 16 (7,7%) women with the majority (9/52; 17,3%) in pregnant women.

Antibody to human immunodeficiency virus (HIV) was detected in 4 women attending the CC clinic, and not in any of the women from the other clinics. However, these 4 women were known HIV antibody positive, detected on routine screening of blood donors by the Natal Blood Transfusion Service and were referred to the CC clinic for colposcopic examination.

TABLE XLIII : ASSOCIATION OF SEXUALLY TRANSMITTED PATHOGENS/INFECTIONS IN WOMEN WITH AND WITHOUT CERVICAL INTRA-EPITHELIAL NEOPLASIA (CIN)

| | CIN n=28 | | NON CIN n=22 | |
|---|---|---|---|---|
| | No | % | No | % |
| *N. gonorrhoeae* | 1 | 3,6 | 1* | 4,5 |
| *C. trachomatis* | 6 | 21,4 | 5 | 22,7 |
| Human papilloma virus | 13 | 46,4 | 13 | 59,1 |
| *G. vaginalis* | 20 | 71,4 | 14 | 63,6 |
| *T. vaginalis* | 11[a] | 39,3 | 3[b] | 13,6 |
| *C. albicans* | 7 | 25,0 | 9 | 40,9 |
| *M. hominis* | 18 | 64,3 | 16 | 72,7 |
| *U. urealyticum* | 15 | 53,6 | 15 | 68,2 |
| Bacterial vaginosis | 14[c] | 50,0 | 4[d] | 18,2 |

* Rectal isolate only

a vs. b p = 0,09

c vs. d p = 0,04

TABLE XLIV : RESULTS OF THE SEROLOGICAL TESTS

| | STD patients n=51 | | CC patients n=50 | | ANC patients n=52 | | FPC patients n=55 | | Total patients n=208 | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No | % | No | % | No | % | No | % | No | % |
| Syphilis | | | | | | | | | | |
| RPR | 15 | 29,4 | 6 | 12,0 | 14 | 26,9 | 12 | 21,8 | 47 | 22,6 |
| Total number of women with specific tests* positive | 27 | 52,9 | 15 | 30,0 | 22 | 42,3 | 18 | 32,7 | 82 | 39,4 |
| HBsAg positive | 1 | 1,9 | 2 | 4,0 | 9 | 17,3 | 4 | 7,2 | 16 | 7,7 |
| HIV antibody positive | 0 | 0 | 4 | 8,0 | 0 | 0 | 0 | 0 | 4 | 1,9 |

RPR Rapid plasma reagin test

* Treponema pallidum haemagglutination (TPHA) test and/or Fluorescent treponemal antibody absorption test (FTA-Abs)

HBsAg Hepatitis B surface antigen

HIV Human immunodeficiency virus

# 5.0 DISCUSSION

Vaginal discharge is a common clinical condition and is one of the most frequent problems encountered in clinical practice. The aetiology of vaginal infections in sexually active women is attributed to trichomoniasis, candidiasis and the recently recognised syndrome of bacterial vaginosis. The pathogenesis of bacterial vaginosis in particular, has received considerable attention and G. vaginalis has been implicated as an important aetiological agent.

Studies conducted in developed communities have shown the increasing importance of bacterial vaginosis (Fleury 1981). However, local studies in developing communities have been few (Fehler et al, 1984; Pochee et al, 1986) and not much information on the incriminating organism is available.

In this study, the age distribution of all women recruited at the four clinics was similar, however, women attending the colposcopy (CC) clinic were slightly older (mean age of 31,2 years). The prevalence of sexually transmitted pathogens was high (Tables XXII and XXIII). These findings were probably due to the fact that all women were sexually active and unmarried with multiple lifetime sexual partners.

Pelvic inflammatory disease and vaginal discharge have been associated with the use of intra-uterine contraceptive devices (IUD). Bacterial vaginosis specifically, has been associated with the use of IUD's (Amsel et al, 1983) and oral contraception (McCormack, 1977). In this

study only 4 and 27 women used an IUD or oral contraceptive respectively. As the preferred method of contraception was parenteral progesterone, IUD's and oral contraception could not be evaluated as possible predisposing factors. The paucity of use of barrier methods of contraception may have played a role in the common occurrence of vaginal infections.

The majority of the women in this study did not attend with a presenting complaint of vaginal discharge; however, on direct enquiry 54,8% of women admitted to the presence of a discharge. Many considered it to be part of their "normal" status; in particular, women attending the ante-natal clinic (ANC) considered the increased discharge to be normal for pregnancy, thus failing to seek medical advice. The colour and odour of the discharge was difficult to assess. On clinical examination vaginal discharge was present in 95,1% of women.

Of the 208 women examined, a vaginal and/or endo-cervical infection was diagnosed in 163 (78,4%). A total of 104 (50%) women had vaginal infection only, 18 (8,6%) had an endo-cervical infection only, with 41 (19,7%) having concurrent vaginal and endo-cervical infections. In only 45 (21,6%) women, no recognised genital pathogens were detected. These figures are somewhat disturbing as the majority of women did not attend with a presenting complaint of vaginal discharge. Furthermore, the impact of this high prevalence of lower genital tract infections on the outcome of pregnancy and future fertility is not known. In view of this, it would be important that future studies be directed to determine the long-term consequences of these infections.

The endo-cervical pathogens detected were N. gonorrhoeae, C. trachomatis, Herpes simplex virus and the human papilloma virs (HPV). N. gonorrhoeae was cultured from 11,1% of women overall. The prevalence of 31,4% among the STD patients is higher than the 26,0% recorded in Nairobi by Mirza et al (1983) and much higher than the 14% and 18% recorded by Fehler et al (1984) and Petersen et al (1986) respectively, in southern Africa. N. gonorrhoeae isolation among ANC and FPC patients was lower, being cultured from 5,5% and 5,8% respectively. This prevalence in pregnant women is similar to that shown by O'Farrell et al (1989) who reported 5,7% in women attending a rural ante-natal clinic but lower than that reported by Hoosen et al (1981) who found a prevalence of 9% in pregnant women with a vaginal discharge and 10% in randomly selected pregnant women attending an urban ante-natal clinic.

The overall prevalence of C. trachomatis (22,1%) was higher than that of N. gonorrhoeae. The highest prevalence being recorded in the STD group (37,3%). A prevalence of only 7% was found among STD clinic attenders in Nairobi (Mirza et al, 1983). South African studies have shown prevalences of 14% and 18% (Fehler et al, 1984; Pochee et al, 1986). The prevalence of chlamydial infections in the ANC and FPC groups was 19,2% and 12,7% respectively. It appears that both C. trachomatis and N. gonorrhoeae are important pathogens locally. N. gonorrhoeae, however, appears to be the dominant aetiological agent in symptomatic patients. In the asymptomatic patients, ie those attending the ANC, FPC and CC clinics, C. trachomatis was found more frequently than N. gonorrhoeae, suggesting a role for this organism in subacute and/or latent infections.

The isolation of the Herpes simplex virus from the endo-cervices of two STD clinic patients who had no vesicular or ulcerative lesions of the genitalia confirms the asymptomatic shedding of the virus in high risk patients (Miller, 1985).

Vaginal trichomoniasis diagnosed in 37,9% of women in this study is much higher than that shown in asymptomatic (Willcox, 1960) and symptomatic (Fleury, 1981) women in developed communities. It was present in 51,9% of ANC, 47,0% of STD, 28,0% of CC and 25,5% of FPC patients. South African studies have shown 25% of urban black women (Fehler et al, 1984) and 49% of unselected ante-natal clinic attenders (Hoosen et al, 1981; O'Farrell et al, 1989) to harbour the organism. A prevalence of 51,9% among pregnant women is much higher than that of the studies mentioned above. This finding confirms the high incidence of this pathogen in pregnant African patients. Although vaginal trichomoniasis has not been associated with adverse perinatal outcome, it has been linked to low birth weight (Ross, 1982).

As regards the laboratory diagnosis of trichomoniasis, microscopic observation of the motile protozoa in vaginal secretions mixed with saline is widely used. This method requires minimal equipment and is specific for the diagnosis of trichomoniasis. However, since the protozoa are sensitive to cold and tend to lose their distinctive motility rapidly on cooling, facilities for microscopy and experienced personnel should be readily available to perform microscopic examinations without delay on site in the clinic settings. As the sensitivity of the wet smear examination is considered to be relatively

low by some (Krieger et al, 1988), culture is also advocated and is considered by some workers, to be the standard for the diagnosis of T. vaginalis (Diamond, 1957).

In this study modified Diamond's medium was used for the culture of T. vaginalis. In view of relatively high prevalence of gentamicin-resistance in Gram-negative bacteria at KEH, the Diamond's medium was modified by replacing streptomycin and penicillin with amikacin, ampicillin and amphotericin B. These antimicrobial agents suppress the growth of vaginal Gram-negative and positive bacteria, and yeasts without inhibiting the growth and motility of T. vaginalis.

Using the culture as a standard for diagnosis, T. vaginalis infections were diagnosed in 77 women, of these 58 were also diagnosed on wet smear microscopy. The sensitivity of the culture method was 97% and that of the wet smear microscopy 75%. No individual test detected all positive patients, however when both tests were used in combination, this substantially improved the accuracy of the diagnosis of patients infected with T. vaginalis (79/208; 37,9%).

Although culture improved the diagnosis, there are limitations in that culture requires at least 1 to 7 days incubation. In one woman the culture was positive on day 3 and in three women culture was positive on day 7. If patients are asymptomatic, negative on wet smear microscopy and positive on culture only, it would take at least one repeat visit to the clinic for appropriate treatment. During this

period, they may remain sexually active and may not return for treatment at all.

The prevalence of genital yeasts was 37,0% overall, with 55,8% in ANC, 40,0% in CC, 27,2% in FPC and 25,5% in the patients attending the STD clinic. The results of the STD and FPC patients are similar to those obtained by Mirza et al (1983) and Fehler et al (1984). However, the prevalence 55,8% for the ANC patients is higher than the 23% and 27% reported by Hoosen et al (1981) and O'Farrell et al (1989) respectively. As expected the prevalence of yeast in these pregnant women was much higher than the prevalence in other clinics. The commonest yeast isolated in all four groups was C. albicans (70,1%), followed by T. glabrata (15,6%). Yeasts other than C. albicans and T. glabrata were present in 14,3% of women.

The genital mycoplasmas, especially M. hominis, have been associated with vaginal infections. U. urealyticum was present in 48,1% and 43,3% and M. hominis in 59,6% and 47,1% of vaginal and endo-cervical specimens respectively. Factors which may contribute to the high colonisation rates of M. hominis in this study, may be related to sexual experience with multiple lifetime partners and the use of no or non-barrier methods of contraception (McCormack et al, 1986).

With the use of sensitive culture media, G. vaginalis has been isolated from women with and without vaginal infections. In this study it was cultured from vaginal specimens of a total of 136/208 (65,4%) women. It was most commonly found in the FPC patients (72,6%), whilst in the STD

patients it was present in 66,6%. Similarly, Fehler et al (1984) in Johannesburg isolated G. vaginalis from 55% and 47% of FPC and STD patients respectively.

With regard to the association of G. vaginalis with bacterial vaginosis, in this study it was cultured from 66/73 (90,4%) women with bacterial vaginosis. In another South African study in symptomatic Black women attending a general practice, Pochee et al (1986) made a diagnosis of bacterial vaginosis in 61%. However, they did not culture for the organism.

The criteria for diagnosing bacterial vaginosis as proposed by Amsel et al (1983) are well accepted. These criteria do not exclude other infections and there is no clear distinction between bacterial vaginosis based on the accepted criteria and a clinical diagnosis of bacterial vaginosis which excludes recognised pathogens such as T. vaginalis, C. albicans, N. gonorrhoeae and C. trachomatis. Many researchers have diagnosed bacterial vaginosis in the absence of these recognised pathogens (Spiegel et al, 1980; Krohn et al, 1989), whereas others have reported the occurrence of these pathogens in the presence of bacterial vaginosis. Hallen et al (1987) found bacterial vaginosis in association with C. albicans (8%), C. trachomatis (57%), T. vaginalis (71%) and N. gonorrhoeae (78%). In this study bacterial vaginosis was associated with T. vaginalis (75,0%), C. albicans (34,4%), C. trachomatis (17,8%) and N. gonorrhoeae (16,4%). It is important that Amsel's criteria be assessed not only in respect of

bacterial vaginosis alone, but also in the context of concurrent infections.

With regard to vaginal infections in developed communities bacterial vaginosis is reported as occurring more commonly than T. vaginalis and C. albicans (Fleury, 1981), whereas in this study T. vaginalis was found more frequently than bacterial vaginosis or C. albicans. In this respect T. vaginalis appears to be the most important cause of vaginal discharge locally.

Cervical intra-epithelial neoplasia (CIN) has been considered to behave as a sexually transmitted disease. Single or multiple microbial aetiologies have been implicated in the initiation of CIN. These are usually acquired through coitus at an early age or through multiple sexual partners (Guijon et al, 1985). This study also looked at infections in women who presented to the CC clinic for follow-up of abnormal cervical cytology (Table XLIII). Of the various micro-organisms detected in this group of patients, there were no significant differences between those isolated from women with and without CIN. However, as regards the syndrome of bacterial vaginosis, this was associated more often in women with CIN than in those without CIN. No correlations were made of the colposcopic patterns of the uterine cervix with any specific type of microbial pathogen which may have been present.

G. vaginalis together with M. hominis, anaerobic bacteria and anaerobic Gram-negative curved rods (Mobiluncus species) have been implicated in

the pathogenesis of bacterial vaginosis. However, these organisms may also be present as normal flora in women without bacterial vaginosis (McCormack et al, 1977; Levison et al, 1978; Ison et al, 1982). It has been suggested by Osborne et al (1982) that a combination of host and microbial factors could initiate the development of signs and symptoms of bacterial vaginosis or the presence of these organisms may be transient. The results of this study also confirm the close association of these micro-organisms with bacterial vaginosis. G. vaginalis was present significantly more often with bacterial vaginosis than without (90,4% vs 56,3%; $p < 0,05$). Likewise M. hominis (86,3% vs 45,2%; $p < 0,05$), anaerobic bacteria (63,0% vs 16,3%; $p < 0,05$) and anaerobic Gram-negative curved rods (Mobiluncus species) (35,6% vs 11,1%; $p < 0,05$) were also present significantly more often in women with bacterial vaginosis. Similar findings have been reported by Pheifer et al (1978); Spiegel et al (1980) and Amsel et al (1983).

The application, at the bedside, of simple laboratory techniques such as estimation of vaginal pH, the detection of amines and the microscopic examination of vaginal wet smears can be used for the diagnosis of vaginal infections.

In this study, a vaginal pH of $\geqslant$ 4,5 was found in 80,0% of women with bacterial vaginosis, however, a raised pH was also found in women with trichomoniasis (62,9%). The vaginal pH, whilst having the highest sensitivity, had a low specificity for the diagnosis of bacterial vaginosis. The predictive value for a positive diagnosis was only 59,4%, but the predictive value for a negative test was 95,7%.

Furthermore, in only 3 of the 13 women with concurrent trichomonas and candida vaginitis the pH was $>$ 4,5; in the remaining 10 women the pH was 4,0, thus decreasing the reliability of the pH in women with mixed infections. If only vaginal pH were to be used as an aid to rapid diagnosis, these infections would not have been recognised since they were within normal vaginal pH values.

The amine test was positive in 76,7% of women, and showed a high specificity of 95,5%. These data support the findings of Pheifer et al (1978) and Chen et al (1979) as to the usefulness of this test in predicting the clinical diagnosis of bacterial vaginosis. This test serves as a useful adjunct to microscopy in making a bedside diagnosis of bacterial vaginosis.

Using the vaginal wet smear preparation, "clue" cells were observed in 91,8% of women with bacterial vaginosis. "Clue" cells were found significantly less often in yeast infections (25,7%) and trichomoniasis (2,9%). The presence of "clue" cells in women with bacterial vaginosis had a sensitivity of 91,7% and a specificity of 85,9%. The predictive value for a positive test was 77,9% and that of a negative test was 95,1%. Of all the rapid tests the presence of "clue" cells was most closely associated with the diagnosis of bacterial vaginosis.

Blackwell and Barlow (1984) suggest that the presence of "clue" cells should be confirmed by microscopic examination of Gram stained smears of vaginal secretions. In this study, the Gram stain assisted in interpreting changes in vaginal flora, (ie from lactobacilli to mixed

type, the presence of gardnerellae and the presence of curved Gram-negative rods). However, the Gram stain was found to be unreliable for the detection of "clue" cells. "Clue" cells were easily seen on Gram stained smears only when the technique of using a Pasteur pipette to place a drop of saline suspension of vaginal secretions on a glass slide was employed. When smears were prepared using a swab, "clue" cells were not seen at all. It is suggested that when applying such swab specimens, may result in distortion and damage of cells. Dunkelberg (1967) and Spiegel et al (1983) and more recently Eschenbach et al (1988) have suggested the use of the Gram stain for the detection of changed/altered vaginal flora, rather than for the confirmation of the presence of "clue" cells.

Mobiluncus spp are fastidious anaerobic bacteria and are difficult to culture from vaginal secretions. These organisms appear as curved Gram-negative rods on Gram stained smears and their presence was assessed on smears only. These were observed in vaginal smears of 35,6% of women with and 11,1% of women without bacterial vaginosis ($p < 0,05$). Although several reports have appeared on the presence of Gram-negative curved rods in vaginal secretions implicated in bacterial vaginosis (Hjelm et al, 1981; Sprott et al, 1982; Phillips and Taylor, 1982; Skarin and Mardh, 1982; Thomason et al, 1984), the pathogenic role of these organisms is uncertain. It has been suggested that they may be present in a symbiotic relationship with G. vaginalis, anaerobic bacteria and M. hominis to produce symptoms of bacterial vaginosis.

Another technique used for the rapid diagnosis of bacterial vaginosis has been gas liquid chromatography (GLC). The GLC has been suggested to be a reliable indicator of infection as it detects short chain fatty acids, which result from biochemical changes occurring in bacterial vaginosis.

It is relevant to note that Spiegel et al (1980) first reported on the use of GLC for direct analysis of vaginal secretions for bacterial vaginosis. Since then, Ison et al (1983) and Hill et al (1985) have confirmed GLC to be a reliable indicator of bacterial vaginosis. However, Ison et al (1983) also found it to be significantly positive in women with gonorrhoea (82%) and trichomoniasis (69%), thus reducing its reliability. Krohn et al (1989) found that approximately 30% of specimens analysed were not interpretable and concluded that a positive test by this method had a poor predictive value (48%).

GLC was performed initially on vaginal secretions of women attending the STD clinic. The succinate lactate (S/L) ratio was increased in 77,0% of women with bacterial vaginosis and in 48,2% without bacterial vaginosis. The limitations of this technique are that equipment is available only at large central laboratories and requires skilled personnel. As this pilot study revealed no advantage with this technique in distinguishing between bacterial vaginosis and non-bacterial vaginosis groups, and in view of the restricted availability of GLC as a routine diagnostic technique, the test was not pursued further in this study.

The laboratory media for the culture of G. vaginalis used in most studies have included either human blood agar without antibiotics (Greenwood and Pickett, 1977) or human blood bilayer medium containing colistin, nalidixic acid and amphotericin B (Totten et al, 1982). In this study excellent results were obtained using human blood agar in a single layer with gentamicin and colistin. The colonies of G. vaginalis were easily recognisable since they produced diffused beta-haemolysis on human blood agar which was absent on horse blood agar.

Furthermore, with regard to the identification of G. vaginalis, all strains were Gram-variable with negative catalase and oxidase reactions. The criteria used for confirmation such as the alpha-glucosidase activity and zones of inhibition around trimethoprim and metronidazole with no zone of inhibition around the sulphathiazole disc were found to be rapid, reliable and reproducible with no discrepancies.

A variety of media have been utilized as transport media for the recovery of G. vaginalis and these have included difco proteose peptone (Dunkelberg et al, 1970), Stuart transport media (Akerlund and Mardh, 1974; Jolly, 1983), heart infusion broth (Bailey et al, 1979) and Amies medium (Josey et al, 1976; Ison et al, 1982). Furthermore, using heart infusion broth supplemented with proteose peptone as transport media, Bailey et al (1979) showed no significant differences in isolation rates after a time delay of up to 6 hours when compared with the direct plating method.

The need to assess different transport media is important especially in large busy hospitals, where time delay in the transport of specimens is inevitable. However, data comparing different transport media are scarce. King Edward VIII Hospital is a large teaching hospital using Stuart (ST) transport media routinely, whilst another regional hospital for the area (Addington Hospital) uses Cary-Blair (CB) transport media. The direct plating method was considered as gold standard and both CB and ST transport media were compared to this. CB medium was shown to be the superior transport media because the yield of isolates as well as the density of growth was comparable to the direct plating method. The growth from ST medium was reduced compared to the CB medium, and significantly reduced when compared to the direct plating method ($p < 0,05$). Furthermore, the ST media allowed the overgrowth of aerobic Gram-negative organisms which tended to mask the growth of G. vaginalis.

Since G. vaginalis has been isolated from women with and without bacterial vaginosis the need to differentiate strains arises. A biotyping scheme has been suggested by Piot et al (1984) for epidemiological studies. This study demonstrated that the majority of the strains belonged to either biotype 1, 2 or 5. Strains isolated from the vagina, urethra and urine were of similar biotypes. Similarly, biotypes 1, 2 and 5 were also predominant among strains tested from Antwerp, Seattle and Nairobi by Piot et al (1984) and in strains tested from the UK by Ison et al (1987).

A significant finding of this study was that among the strains tested, biotypes 1 and 5 were associated with bacterial vaginosis. In women whose urine isolates were typed the biotypes corresponded to those of the urethral and vaginal origin.

The serotyping scheme used was similar to the one originally described by Ison et al (1987). This scheme made use of polyclonal antisera which were directed against proteins of differing molecular weights. Subsequently, using specific monoclonal antibodies an improved scheme was developed. Using Ison's monoclonal antibodies this study identified 27 serotypes. Of these, serotypes 125 and 25C were present more frequently than others. In the UK, serotype 125C has been found to be the predominant serotype in women with recurrent bacterial vaginosis (Ison, personal communication). However, in this study women were investigated for the current episode of vaginal discharge only and were not subsequently followed up. There were no differences in the serotypes of strains of G. vaginalis isolated from women with and without bacterial vaginosis.

The choice of immunizing strains is crucial for any typing scheme. So far the antisera produced against G. vaginalis have not always given clear differentiation among strains. Weak serological reactions have been obtained with a large number of strains. Negative reactions were obtained with 13 of the strains tested suggesting that the antigens selected for antisera production did not provide cover for these strains. These antisera may be more useful for the identification

rather than the typing of G. vaginalis, or there may be other as yet unrecognised serotypes.

Antimicrobial agents belonging to the nitroimidazole group are recommended as drugs of choice for treating bacterial vaginosis. The MICs for both metronidazole and tinidazole against G. vaginalis ranged from 1mg/L to 128mg/L, with the majority of the strains having MICs of 16mg/L. However, the hydroxymetabolite of metronidazole was more active than the parent compound. The corresponding MICs of the hydroxymetabolite were in general at least two dilutions lower than that of metronidazole itself. One strain however, had an MIC of 128mg/L for metronidazole, tinidazole as well as the hydroxymetabolite of metronidazole. Similar results for these antimicrobial agents were also obtained by Ralph and Amatnieks (1980).

Antimicrobial agents active against Gram-positive organisms eg penicillin, ampicillin, erythromycin, clindamycin, vancomycin, rifampicin and chloramphenicol showed significantly higher MICs than those normally expected for Gram-positive organisms. These results are consistent with those of McCarthy et al (1979). Moreover, the lipopeptide, LY 146032, which is excellent for Gram-positive organisms, also showed markedly decreased activity against G. vaginalis.

The activity of the cephalosporins, ciprofloxacin and imipenem was relatively reduced for G. vaginalis compared to those expected for most gentamicin sensitive Gram-negative bacteria. The monobactam, aztreonam, which has activity only against Gram-negative organisms, demonstrated

very little activity, with MICs of 4mg/L or higher. G. vaginalis was found to be sensitive to trimethoprim but resistant to sulphamethoxazole. However, when tested in combination these agents did not show any synergy.

The results of the MICs further conflict with the reports on the cell wall analysis of G. vaginalis. On the basis of the MICs to antimicrobial agents known to act at the cell wall level (ie penicillins and cephalosporins), the cell wall of the organism appears to be neither strictly Gram-positive or negative. It has been suggested that the organism is Gram-negative based on the presence of low mucopeptide, absence of techoic acid, the presence of 11 to 14 amino acids in the cell wall (Criswell et al, 1971) and a "lipopolysaccharide-like" fraction (Greenwood and Pickett, 1980). However, the MICs for Gram-negative agents were high in this study. This is particularly the case with aztreonam which is specifically active against Gram-negative organisms.

The original suggestion that G. vaginalis forms septa (Reyn et al, 1966) and more recent reports based on the absence of diaminopimelic acid (Harper and Davis, 1982) and the absence of lipopolysaccharide (Sadhu et al, 1989) suggest the organism to be Gram-positive. However, the MICs to antimicrobial agents such as penicillin G, vancomycin and to LY 146032 which have activity restricted specifically to Gram-positives were high, suggesting that G. vaginalis is not a "typical" Gram-positive organism. These results emphasize the

uniqueness of G. vaginalis in being neither "typically" Gram-positive or Gram-negative. .

For the therapy of bacterial vaginosis many antimicrobial agents have been used with varying success. Erythromycin although active against G. vaginalis and some anaerobic bacteria exhibits reduced activity in an acidic environment such as the vagina. Ampicillin which shows in vitro activity against G. vaginalis may be limited in its clinical use because of the production of beta-lactamases by the vaginal anaerobes.

Bacterial vaginosis and pelvic inflammatory disease (PID) are characterised by a polymicrobial aetiology. The micro-organisms that are common in the aetiology of both these syndromes include M. hominis and anaerobic bacteria. It is plausible that the presence of a lower genital tract infection such as bacterial vaginosis may lead to the development of an upper genital tract infection such as PID. Therefore adequate therapy for bacterial vaginosis would be recommended for preventing ascending genital tract infections.

Sexually transmitted diseases that are diagnosed by serological tests alone include syphilis, hepatitis B and human immunodeficiency virus infection. Serological tests for these infections were performed on all patients. The prevalence of syphilis remains high in South Africa. Studies from different centres have shown a prevalence of up to 18% in asymptomatic pregnant women (Naicker et al, 1983; Mahomed et al, 1984; O'Farrell et al, 1989). This study recorded a surprisingly high prevalence of 26,9% in ANC women, a figure close to the 29,4% recorded in the STD clinic attenders. Furthermore, the high background prevalence of syphilis could be gauged by the fact that specific treponemal tests were positive in nearly 40% of all women examined.

These data strongly suggest that any woman attending an outpatient clinic at this hospital should be routinely screened for syphilis.

Hepatitis B is important in South Africa where horizontal transmission is said to be predominant. The overall finding of HBsAg in 7,7% of women, is in keeping with South African figures ranging between 2,1% (Botha et al, 1984) and 15,8% (Bersohn et al, 1974). As with syphilis serology there was a high prevalence of HBsAg (17,3%) in the ANC group. This could also suggest a role for vertical transmission.

Human immunodeficiency virus infections in Africa are said to be predominantly due to heterosexual transmission. This is corroborated by the local figures for blood donors and STD clinic attenders (male to female ratio being similar). At the time of this study, four women referred for colposcopy tested positive for HIV antibody. In none of the other clinic attenders was the antibody detected.

As most lower genital tract infections in women have been shown to be sexually transmitted, it would have been of interest to simultaneously investigate the sexual contacts of the women studied. However, past local experience has shown such studies to be very difficult, if not impossible to carry out because of poor patient and sexual partner compliance. Furthermore, patients are unaware that they have a sexually transmitted disease where a sexual partner is involved.

# 6.0 CONCLUSIONS AND RECOMMENDATIONS

## 6.1 CONCLUSIONS

6.1.1 This study clearly demonstrates the high prevalence of vaginal, endo-cervical and concurrent vaginal with endo-cervical infections locally.

6.1.2 The prevalence of syphilis as determined by reactive syphilis serology was high.

6.1.3 Although N. gonorrhoeae is an important pathogen in symptomatic women locally, a higher prevalence of C. trachomatis infection was diagnosed in asymptomatic women.

6.1.4 In developed communities, bacterial vaginosis is the most common vaginal infection. However, in this study trichomoniasis was more commonly detected than either bacterial vaginosis or candidiasis.

6.1.5 With regard to bacterial vaginosis:

6.1.5.1 It frequently occurred concurrently with other recognised vaginal pathogens;

6.1.5.2 it was significantly associated with the presence of G. vaginalis, M. hominis, anaerobic bacteria and anaerobic Gram-negative curved rods and

6.1.5.3 the detection of "clue" cells by vaginal wet smear microscopy was the single most useful procedure for making a diagnosis.

6.1.6 With regard to G. vaginalis:

6.1.6.1 Its isolation was significantly associated with the syndrome of bacterial vaginosis;

6.1.6.2 the Cary-Blair medium was found to be the superior transport medium tested;

6.1.6.3 biotypes 1 and 5 were significantly associated with bacterial vaginosis;

6.1.6.4 the antimicrobial susceptibility pattern of isolates was not typical of either Gram-positive or Gram-negative bacteria and

6.1.6.5 the MICs indicate that the majority of strains tested were sensitive to the nitroimidazoles.

## 6.2 RECOMMENDATIONS

6.2.1 All women with symptoms and signs of an abnormal vaginal discharge should be screened for lower genital tract infections (ie vaginitis and cervicitis).

6.2.2 Vaginal wet smear microscopy, being the single most reliable bedside test, should be performed on all women attending the sexually transmitted diseases, colposcopy, ante-natal and family planning clinics.

6.2.3 In view of the high prevalence of syphilis, serological screening should be undertaken on all women attending the various clinics at this hospital.

6.2.4 For the transport of specimens from which G. vaginalis is to be cultured, the Cary-Blair medium is recommended.

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# 8.0 APPENDIX

## APPENDIX A

Formulae and preparation of media used.

1 Human Blood agar (HB) adapted from Greenwood et al (1977)

Basal Medium

44 g of Columbia blood agar (Difco) containing:

| | |
|---|---|
| Bacto-Pantone | 10g |
| Bacto-Bitone | 10g |
| Tryptic digest of beef heart | 3g |
| Corn starch | 1g |
| Sodium chloride | 5g |
| Bacto agar | 15g |

with Proteose peptone No 3 (Difco) 10g

Human blood: expired, approximately 3 weeks after collection from the Natal Blood Transfusion service (NBTS) was used. It was tested for antibacterial activity and thereafter aliquoted in 25mℓ amounts into sterile universal containers and stored at 4°C.

The medium was dissolved in 950mℓ distilled water, the pH adjusted to 7,3 and autoclaved at 121°C for 15 minutes. Thereafter it was cooled to 48°C and 50mℓ sterile human blood was added aseptically and mixed. Approximately 20mℓs of medium was poured into each 9cm petri dish.

2 Selective human blood agar (SHBA)

This medium was used for the primary isolation of G. vaginalis.

Human blood agar was prepared as in (1) and stock antibiotic solutions added to give a final concentration of gentamicin (Scherag) 5ug/mℓ and colistin (Sigma) 10ug/mℓ.

Antibiotic stocks were prepared as follows:
100mg gentamicin + 20mℓ sterile distilled water;
200mg colistin + 20mℓ sterile distilled water

The stock antibiotics were distributed in 1mℓ amounts separately and stored at -20°C. One aliquot of each was added to 1 litre of agar to give the final required concentration.

3 Horse blood agar (BA)

Horse blood agar was prepared using Columbia blood agar base as in (1) and 5% horse blood (v/v).
Horse Blood was otbained from the South African Institute of Medical Research (SAIMR), Johannesburg and used as for human blood.

4 Wilkins-Chalgren Anaerobe Agar (WC).

This medium was used for the primary isolation of anaerobic bacteria.

Basal medium

43g of Wilkens-Chalgren anaerobe agar (Oxoid) containing:

| | |
|---|---|
| Tryptone | 10g |
| Gelatine peptone | 10g |
| Yeast extract | 5g |
| Dextrose | 1g |
| Sodium chloride | 5g |
| L-arginine | 1g |
| Sodium pyruvate | 1g |
| Menadione | 0,0005g |
| Haemin | 0,005g |
| Agar | 10g |

The medium was dissolved in 950mℓ distilled water, the pH adjusted to 7,1 and autoclaved at 121°C for 15 minutes. Thereafter it was cooled to 48°C and 50mℓ horse blood added. Approximately 20mℓ of medium was poured into each 9cm petri dish.

5 Modified Diamond's Medium

This medium was used for the culture of T. vaginalis

Basal Medium

| | |
|---|---|
| Trypticase (BBL) | 20g |
| Yeast extract | 10g |
| Maltose | 5g |
| L-cysteine hydrochloride | 1g |
| L-ascorbic acid | 1g |
| Agar | 0,5g |
| distilled water | 900mℓ |

Supplements

| | |
|---|---|
| Sterile horse serum (SAIMR) | 100mℓ |
| Antibiotics | |
| Amikacin (The B-M group Pty) | 100ug/mℓ |
| Amphotericin B (Squibb) | 5ug/mℓ |
| Ampicillin (Beechams) | 1mg/mℓ |

The dry ingredients were dissolved in distilled water and the pH adjusted to 6,0. Sterilised at 121°C for 10 minutes. Once cooled to 48°C the supplements were added. Approximately 9mℓ of the medium was dispensed in 15mℓ tubes (Falcon 2099, Becton Dickinson, USA) and kept at 4°C. When required the medium was allowed to warm up to room temperature before being inoculated.

6 Shepard's A7 Agar

Basal medium

Ureaplasma basal agar medium (Gibco) consisting per litre of the following ingredients:

| | |
|---|---|
| Peptone 220 (yeast casein polypeptone) | 17g |
| Peptone 140 (Pancreatic digest of casein) | 17g |
| Peptone 110 (Papaic digest of soy protein) | 3g |
| Dextrose | 2,5g |
| Manganous sulphate ($MnSO4.H_2O$) | 0,2g |
| Sodium chloride | 5,0g |
| Potassium phosphate dibasic | 2,5g |

| | |
|---|---|
| Agar | 10,0g |
| **Supplements** | |
| Unheated normal horse serum | 40,0mℓ |
| CVA enrichment (Gibco) | 1,0mℓ |
| 20% Yeast extract solution (Gibco) | 2,0mℓ |
| 10% urea solution | 2,0mℓ |
| 1% L-Cysteine-HCL solution | 0,5mℓ |
| 0,5% Phenol red | 2,0mℓ |
| **Antibiotics** | |
| Ampicillin (Beechams) | 1000ug/mℓ |
| Amphotericin B (Squibb) | 5ug/mℓ |

The basal agar medium (6,6 grams) was dissolved in 165mℓ of distilled water, pH adjusted to 5,5 and sterilized at 121°C for 15 minutes. Thereafter it was cooled to 48°C and sterile supplements and antibiotics were added to the molten agar. The complete medium was mixed thoroughly and poured into sterile 6cm petri dishes. The prepared medium was stored at 4°C and used within 2 weeks.

7 U9 - Broth

Basal Medium

| | |
|---|---|
| Tryptic digest broth (Difco) | 0,75g |
| Sodium chloride | 0,5g |
| Potassium acid phosphate | 0,02g |
| Distilled water | 100,0mℓ |

Supplements

| | |
|---|---|
| Unheated normal horse serum | 5,0mℓ |
| 10% urea solution | 0,5mℓ |
| 2% L-cysteine HCl solution | 0,5mℓ |
| 1% Phenol red | 0,1mℓ |

Antibiotic

Penicillin G potassium (Novo Industries) 100,000 units/mℓ

The ingredients for the basal medium were mixed thoroughly in distilled water and the pH adjusted to 5,5 with 1N HCl. Thereafter it was sterlised at 121°C for 15 minutes and cooled. To 95mℓ of basal broth the supplements and antibiotic were added and the complete medium was aseptically dispensed in 2mℓ volumes into bijou bottles. The prepared medium was stored at -20°C and used within one week.

8 Sabouraud dextrose agar

Basal Medium

65g of Sabouraud dextrose agar (Difco) consisting of :

| | |
|---|---|
| Neopeptone | 10g |
| Bacto dextrose | 40g |
| Bacto agar | 15g |
| Distilled water | 1000mℓ |

The medium was dissolved in distilled water and pH adjusted to 5,6. It was sterilized at 121°C for 15 minutes. Approximately 20mℓ of medium was poured into each 9cm petri dish.

9 Modified Stuart Transport Medium

Basal Medium

16g of Modified Stuart Transport Medium (Oxoid) consisting of

| | |
|---|---|
| Sodium glycerophosphate | 10,0g |
| Sodium thioglycollate | 0,5g |
| Cysteine hydrochloride | 0,5g |
| Calcium chloride | 0,1g |
| Methylene blue | 0,001g |
| Agar No 1 | 5,0g |
| Distilled water | 1000mℓ |

The medium was suspended in distilled water and boiled to dissolve completely, pH adjusted to 7,4 and dispensed into screw-capped bijou bottles. The bottles were filled to the brim, the caps tightened and sterilized at 121°C for 15 minutes.

10 Modified Cary-Blair Transport Media

Basal medium consisting of

| | |
|---|---|
| Sodium thioglycollate | 1,5g |
| Calcium chloride | 0,1g |
| Disodium phosphate | 0,1g |
| Sodium chloride | 5,0g |
| Sodium meta bisulphite | 0,1g |
| Purified agar | 10,0g |
| Resazurin solution | 5mℓ |
| Distilled water | 1000mℓ |
| L-cysteine HCL | 1,0g |

The ingredients were dissolved in water and steamed for 45 minutes and then cooled with $CO_2$. L-cysteine HCL was added and the pH adjusted to 7,5. The medium was dispensed into universal bottles in 25mℓ amounts in an anaerobic chamber. These were sterilized at 121°C for 15 minutes. All bottles were checked for tightness after autoclaving.

11 Modified New York City Medium

Basal medium

18g of Gonococcal (GC) agar base (Oxoid) consisting of per litre:

| | |
|---|---|
| Special peptone | 15,0g |
| Corn starch | 1,0g |
| Sodium Chloride | 5,0g |
| Dipotassium hydrogen phosphate | 4,0g |
| Potassium dihydrogen phosphate | 1,0g |
| Agar No 1 | 10,0g |
| Distilled water | 425mℓ |

Supplement

Sterile yeast autolysate supplement (Oxoid) consisting of

| | |
|---|---|
| Yeast autolysate | 5,0g |
| Dextrose | 0,5g |
| Sodium bicarbonate | 0,075g |

Reconstituted with sterile distilled water, 15mℓ

LCAT antibiotic Supplement (Oxoid) consisting of

| | |
|---|---|
| Lincomycin | 0,5mg |
| Colistin | 3,0mg |
| Amphotericin B | 0,5mg |
| Trimethoprim lactate | 3,25mg |

Reconstituted with sterile distilled water 10mℓ

Defibrinated lysed horse blood

50mℓ of horse blood (SAIMR) was lysed with 0,5% (by vol) saponin

The basal medium was dissolved in distilled water and sterilised at 121°C for 15 minutes, thereafter cooled to 50°C. Aseptically the lysed defibrinated horse blood; reconstituted yeast autolysate and LCAT antibiotic supplement were added to the cooled GC agar base. The contents were mixed gently and poured into sterile petri dishes.

Antibiotic stock solutions were prepared as follows:

a 100mg gentamicin (Scherag) dissolved in 20mℓ distilled water

b 2000mg amikacin (The B-M Group) dissolved in 20mℓ distilled water

c 200mg colistin (Sigma) dissolved in 20mℓ distilled water

d 100mg amphotericin B (Squibb) dissolved in 2mℓ of dimethyl sulphoxide (DMSO) and the volume made up with 18mℓ of distilled water

e 20000mg ampicillin (Beechams) dissolved in 20mℓ of distilled water.

The stock antibiotic solutions were distributed in 1mℓ amounts separately and stored at -20°C. One aliquot of each was added to 1 litre of medium where required to give the final concentration.

APPENDIX B

Formulae and preparation of biochemical substrates

1 Substrates used for the biochemical identification of G. vaginalis.

The determination of alpha and beta-glucosidase activity.

Buffer :

Sorensons phosphate buffer pH 8,0 was prepared as follows:

Solution A

9,08g monopotassium phosphate ($KH_2 PO_4$) (BDH 10203)

per litre of distilled water giving a concentration of 67mmoℓ

Solution B

11,88g disodium phoshate ($Na_2 HPO_4 2H_2O$) (BDH 10249)

per litre of distilled water giving a concentration of 67mmoℓ

To make up buffer

x of solution A

+

(100-x) mℓ of solution B

or x mℓ of solution A titrated with solution B to a final pH of 8,0.

Substrates

4-nitrophenyl-alpha-D-glucopyranoside (Sigma N1377)

4-nitrophenyl-beta-D-glucopyranoside (Sigma N7006)

To make up substrates

0,1% (w/v) of each substrate was made up in 67mmoℓ Sorensons phosphate buffer, pH 8,0. The substrates were aliquoted in 20mℓ portions and stored at -20°C until required.

A loop of bacterial suspension from an HB agar plate incubated for 24 hours was added to 0,5mℓ of both these solutions. These were incubated for 18 hours at 37°C in a water bath. A positive reaction was indicated by the formation of a yellow colouration.

2 Substrate and media for biotyping of G. vaginalis

a Hippurate hydrolysis

The substrate consisted of:

0,67 mol/L potassium dihydrogen phosphate 73,2mℓ (BDH 10203)

0,67 mol/L disodium hydrogen phosphate 26,8mℓ (BDH 10249)

Sodium hippurate (Sigma H9380) 1g

The pH was adjusted to 6,4 and the substrate filter sterilised. This was aliquoted into 20mℓ amounts and stored at -20°C until required. Each tube containing 0,5mℓ of the test medium was inoculated with a large loopful of bacterial growth and incubated for 4 hours at 37°C in a waterbath. The reaction was visualised by the addition of 2 drops of ninhydrin (API ≠7049). A blue colouration after 5 minutes at 37°C was indicative of a positive reaction.

b Beta galactosidase activity

The buffer was prepared as follows:

Sodium dihydrogen phosphate 6,9g

Distilled water 40mℓ

The buffer was adjusted to pH 7,0 with NaOH and made up to a final volume of 50mℓ.

The buffer was used to make the substrate

Buffer 25mℓ

Distilled water 75mℓ

2-nitrophenyl-beta-D-galactopyranoside (Sigma N1252) 0,4g

The substrate was filter sterilised and stored at -20°C until required. The test organisms were inoculated into 0,5mℓ of substrate and incubated for 18 hours at 37°C in a water bath. A yellow colouration indicated a positive reaction.

c Lipase Activity

| | |
|---|---|
| Basal medium consisting of | |
| Proteose peptone No 3 (Difco) | 8g |
| Disodium hydrogen phosphate | 1g |
| Sodium chloride | 0,4g |
| Glucose | 0,4g |
| Bacto agar | 5g |
| 5% (w/v) magnesium sulphate solution | 0,04mℓ |
| Distilled water | 170mℓ |

| | |
|---|---|
| Supplement | |
| Egg yolk emulsion (Oxoid) | 20mℓ |
| Horse serum (SAIMR) | 10mℓ |

The basal medium was dissolved in distilled water and sterilised at 121°C for 15 minutes. After cooling to 50°C the egg yolk emulsion and horse serum were added aseptically. Approximately 20mℓ of medium was filled in 9cm petri dishes.

Organisms to be tested were spot inoculated onto the medium and the plates were incubated at 37°C in an anaerobic jar (BBL) for 3 days. Using an oblique light the growth was examined for the appearance of an oily irridescent sheen, indicating a positive reaction.

3 Nitrocefin (Chromogenic cephalosporin compound) (Glaxo, England)

5mg nitrocefin was added to 0,5mℓ of dimethyl sulphoxide (Merck 2937). Once the compound dissolved well, 9,5mℓ of 0,1M phosphate buffer pH 7,0 was added. This was shaken well to mix. The prepared solution was stored in the dark at 4°C and used within 14 days.

APPENDIX C

Reagents for Immune Dot Blot Assays

1 Enzyme Linked Immunosorbent Assay (ELISA) buffer

| | |
|---|---|
| Sodium chloride | 9,0g |
| Tris (2-amino-2-(hydroxymethyl) propane -1,3 diol, (tris) (BDH 10315) | 1,21g |
| Distilled water | 1000mℓ |

Only 800mℓ of distilled water was added and adjusted to pH 7,4. The solution was then made up to a final volume of 1000mℓ.

2 Preparation of washing buffer

| | |
|---|---|
| 30% bovine serum albumin | 3,3mℓ |
| ELISA buffer pH 7,4 | 96,7mℓ |

3 Preparation of blocking buffer

| | |
|---|---|
| 30% bovine serum albumin | 13,3mℓ |
| ELISA buffer pH 7,4 | 86,7mℓ |

4 Alkaline phosphatase substrates for Dot Blotting

| Reagents | Volume |
|---|---|
| 5-bromo-4-chloro-3-indolyphosphate (Sigma B-0766) 4mg/mℓ in a mixture of methanol:acetone 2:1 v/v | 2,25mℓ |
| Nitro blue tetrazolium (Sigma N6876) (1mg/mℓ) | 15,0mℓ |
| Magnesium chloride 1mol/L | 0,6mℓ |
| Diethanolamine-HCL (BDH-28612) buffer pH 9,6 0.1 mol/L | 82,15mℓ |