CHARACTERIZATION OF YEAST ISOLATES FROM PREGNANT WOMEN IN ZARIA.

BY:

EDWARD DAVID JATAU
B.Sc. and M.ED. BUFFALO, N. YORK

A THESIS

Submitted to the Postgraduate School,
Ahaadu Bollo University, Zaria,
In partial fulfilment of the requirements
for the degree of Master of Science

in

MICROBIOLOGY.

Department of Microbiology,
Faculty of Sciences,
Ahnadu Bollo University,
Zaria.
NIGERIA.

OCTOBER, 1988.

DECLARATION:

I hereby declare that the work presented in this thesis was carried out by me under the careful supervision of Doctors A. A. Ahmad and C.S.S. Bello in the laboratory of the Department of Microbiology, Ahmadu Bello University Zaria.

No part of this work has been submitted for any degree or qualification before. The works of other workers were referred to during this work and they are duly acknowledged.

E. D. JATAU,
DEPARTMENT OF MICROBIOLOGY,
AHMADU BELLO UNIVERSITY,
Z A R I A.

OCTOBER, 1988.

CERTIFICATION.

This thesis entitled "Characterization of Yeast Isolates from Pregnant Women in Zaria" meets the regulation governing the degree of Master of Science, Ahmadu Bello University, Zaria and is approved for its Scientific knowledge and literary presentation.

DATE: 1/12/88

DR. A. A. AHMED,
CHAIRMAN, SUPERVISORY COMMITTEE,
DEPARTMENT OF MICROBIOLOGY,
AHMADU BELLO UNIVERSITY, ZARIA.

DATE: 1/12/88

REV. (DR.) C. S. S. BELLO,
MEMBER, SUPERVISORY COMMITTEE,
DEPARTMENT OF MEDICAL MICROBIOLOGY,
AHMADU BELLO UNIVERSITY,
TEACHING HOSPITAL, ZARIA.

DATE: 1/12/88

HEAD OF DEPARTMENT,
DEPARTMENT OF MICROBIOLOGY,
AHMADU BELLO UNIVERSITY, ZARIA.

DATE: 25-1-89

DEAN OF POSTGRADUATE SCHOOL,
AHMADU BELLO UNIVERSITY,
ZARIA, NIGERIA.

DEDICATION.

Dedicated to my wife Mrs. Franca H. Jatau and our four children Job D. Jatau, Gloria D. Jatau, John D. Jatau and James. D. Jatau.

ACKNOWLEDGEMENT.

I express my sincere gratitude to my mentors, Drs. A. A. Ahmed and C.S.S. Bello with whose assistance and advice this work was made successful. I also acknowledge the helps of Dr. R.N. Okagbue for his advice and help with some culture media and Dr. N.E. Gomwalk for his encouragement.

My gratitude to Mr. G.O. Anyam, Mrs. Theresa Addai, Mrs. H. Kankara all of Microbiology Department and Mr. T. Akinde of Chemical Pathology, Ahmadu Bello University Teaching Hospital, Zaria for their co-operation and technical assistance.

I am indebted to Mr. B. Abdullahi of Post-Graduate office for typing this work in its final form. My gratitude also goes to Mr. S. Garba, Mr. Silas Yashim, Miss Abigail Adekanmi and Mr. Umar Sule for all the clerical services they rendered.

Finally, I am grateful to our I. & J. Technical staff particularly Mr. Aliyu Umoru, for their various contributions towards a successful completion of this work.

May God grant us, our individual's aspiration. (Amen).

E. D. JATAU.

CONTENTS.

LIST OF TABLES.

LIST OF PLATES.

LIST OF APPENDICES.

ABSTRACT.

Three hundred pregnant women consisting of 100 each of first, second and third trimesters, were screened for genital yeast infections. Microbiological diagnosis was from High Vaginal Swabs (HVS) using wet film preparation, Gram stain technique and culture on Sabouraud Dextrose Agar (SDA), *Candida species* were isolated in 60 (20%), 115 (38.3%) and 186 (62%) respectively.

The isolates, were characterised by germ tube and chlamydospore formation tests, carbohydrates fermentation and assimilation tests and growth at 37°C and room temperature. They were speciated as follows:- *Candida albicans* (108), *C. tropicalis* (23), *C. pseudotropicalis* (9), *C. parapsilosis* (22%), *C. krusei* (12), *C. guilliermondii* (10) and *Candida stellatoides* (3). This finding is in agreement with Gardner's 1982 report that other *Candida species* are associated with vulvo-vaginal candidiasis.

Three hundred blood samples (100 each of the three trimesters) were collected from the same patients for blood glucose estimation. Only five patients were found to have blood glucose level slightly higher than the normal range of 2.5 - 7.5 m Mol./L. Out of the three hundred urine samples collected only the same five patients gave positive urine glucose test result of (+) indicating concentration of 100mg/100ml of urine. This shows that pregnancy might not be responsible for their vulvo-vaginal candidiasis.

CHAPTER ONE.

INTRODUCTION.

Vaginal discharge is one of the commonest female complaints and one that is in general poorly understood and managed. It is a condition known as vaginitis or vulvo-vaginitis that can be caused by a variety of aetiologic agents resulting in the abnormal vaginal discharge. In general, the vulvo-vaginal diseases are categorized into four major groups: fungal, bacterial, protozoal and non-specific vulvo-vaginitis (Lossick, 1984).

Yeast species (known as yeast-like-fungi) are responsible for the fungal vaginitis. (Candida albicans, an air-borne, dimorphic, Gram positive fungus with both yeast and filamentous growth phases, accounts for most of the cases of clinical vaginal yeast infections. According to Gardner (1982), other Candida species like C. krusei, C. tropicalis, C. stellatoidea, C. pseudotropicalis, and C. guillermondii have been isolated from patients with vulvo-vaginitises. In 1977, Hurley reported the association of another yeast-like-fungus-Torulopsis glabrata with clinical vaginitis. Vulvo-vaginitis is characterized by thick whitish and cheesy discharge with intense vulvo-vaginal itching (Catterall, 1974). According to Omer et al. (1982), Candida species have assumed the role of the most common cause of vaginitis with a higher frequency than trichomonas, gonococcal and non-specific bacterial infections.

Although all *Candida species* are known to be normal flora of the human intestine, skin, the mouth and vagina, *C. albicans* is reported to be responsible for about 80-95% mycotic vaginitis and *T. glabrata* 3 - 16% (Harris, 1975). Generally, mycotic vaginitis is common in compromised individuals e.g. diabetic patients, pregnant women, patients on immuno-suppressive therapy, women on oral contraceptive and broad-spectrum-antibiotics (Catterall, 1974).

Urethritis due to candida has not been well documented, but it is commonly accepted that from 1 - 3% of cases of non-gonococcal urethritis is associated with candida (Lossick, 1984). Swartz *et. al.* (1978), isolated *Candida albicans* in only 1 out of 48 patients with non-gonococcal urethritis.

In Nigeria, most of the work done on the prevalence of genital candidiasis has been on the attenders of sexually transmitted diseases clinics. Sogbetun (1977) diagnosed 23 (4.0%) cases of candidiasis out of 578 patients. Rotimi (1980) reported 29 (10%) cases out of 276. Similarly, Bello *et al.* (1983) diagnosed 304 (9.4%) cases out of 3089 patients. These reports have shown some increase in the incidences of genital candidiasis in this country. Others are those of Elegbe *et al.* (1982) and Elegbe, 1983 that incriminated the use of tight clothing and the use of Nylon under-wears for the increase in incidences of candidiasis among Nigerian women.

There has not been any report on work done on the attenders of antenatal clinics despite the fact that pregnancy is one of the predisposing factors of women to candida vulvo-vaginitis. Most workers have attributed the cause of vulvo-vaginal candidiasis to *Candida albicans* and occasionally generalized the aetiology as *Candida species* thereby avoiding the problem of speciating it. No survey has been conducted to establish the trimester at which pregnant women are more prone to genital candida infections.

The objective of this project is to carry out a survey on the prevalence of vulvo-vaginal candidiasis among the attenders of antenatal clinics in Zaria to determine the trimester that it occurs most. Hence, three hundred, 100 each from 1st, 2nd and 3rd trimesters were screened. Samples of blood and urine were also collected from each patient for glucose level estimations to determine its role in the pathogenesis of *Candida species* in vulvo-vaginitis. All the yeast isolates were inoculated into sugars and their biochemical activities were used in speciating them.

CHAPTER TWO

LITERATURE REVIEW.

2.1: The Yeasts: Cultural Characteristics and Morphology:

Yeasts are unicellular, oval or spherical, asporogenous, pseudomycelial fermenters (Black et al., (1970). Their cells and progeny sometimes form chains or pseudo-hyhae by adhering to each other. They are Gram positive fungi measuring about 3 - 5 micrometer in diameter.

Yeasts, like moulds, resemble higher plants and animals in the anatomic complexity of their cells. That is they are eukaryotic with several different chromosomes and a well-defined nuclear membrane that contains sterols. They possess mitochondria and endoplasmic reticulum. They are opportunistic fungi belonging to the class of ascomycetes consisting of many genera e.g. Cryptococcus, Histoplasma, Blastomyces, and Candida. The most frequently encountered opportunistic mycoses are candidiasis, aspergillosis, cryptococcosis, phycomycosis and torulopsis.

Candidiasis is caused by *Candida sp.* mostly as a result of some pre-disposing factors like antibiotic therapy, oral contraceptive use, diabetes, pregnancy, cytotoxic drugs, and corticosteroids therapy, leukaemia and other neoplasms. *Candida sp.* are thermo-tolerant, have higher metabolic rate at 37°C and show a degree of resistance to phagocytosis and host defenses. *Candida albicans* is the most common cause of candidal infections. It grows readily on conventional media at both room temperature and 37°C producing smooth, creamy bacteria-like colonies on Sabouraud dextrose agar. Microscopically, the young cells are spherical to oval, unicellular and multiply by budding.

2.1.1 Pathogenesis of Candida.

They are normal flora of the mucous membranes of mouth, vagina, and intestinal tract of man. When they become invasive due to the factors mentioned above, they do establish a variety of acute, chronic, localized or widely disseminated lesions (Davies et al., 1980). So very minor changes in the host defense including avitaminoses, pH changes in the vagina, or reduced bacterial competition, can lead to thrush - (an oral candidiasis that occurs during the first few days of life in the newborn due to infection during birth and in person in the terminal stage of a wasting disease e.g. carcinomatosis), vaginitis during pregnancy and diabetes, gastro-intestinal invasion or other acute conditions of candidiasis. Generalised rapidly fatal candidiasis may occur in patients with debilitating diseases such as leukaemia or in patients being treated with steroids and cytotoxic chemotherapy (Beneke, 1979).

2.1.2 Diagnosis.

A presumptive diagnosis of candidiasis is usually made by microscopic demostration of abundant hyphae and yeast cells in scrapings of lesions, and the diagnosis is supported by isolation of the organisms in culture. Serologic tests and skin tests using aqueous extracts of C. albicans are of little or no diagnostic value due to the ubiquitous nature of Candida sp.

2.1.3 Prevalence of Vulvo-Vaginal Candidiasis.

Although the prevalence of vaginal candidiasis has not been well documented, Gardner (1982) noted a four-fold increase in cases from 1944 - 1976. According to Drake and Maibach 1973, Candida species are common inhabitants of the mouth, large intestine and vagina in 25 to 50% of apparently healthy individuals. Oriel et al. (1972) isolated candida from 25 to 50% of asymptomatic sexually active women, 25% of patients in sexually transmitted disease clinics, 33% of pregnant women and 15 to 29% of users of oral contraceptives. In addition to the physiologic, traumatic, haematologic, endocrine and iatrogenic predisposing factors to vaginal candidiasis (Joklik, Willet and Amos, 1980), recent studies have implicated tight clothing with both the prevalence and quantity of candida colonization (Elegbe et al., 1982 and Elegbe and Elegbe, 1983).

According to Thin et al. (1979) although there is little doubt that candida organisms are frequently shared by sexual partners, the role of sexual transmission of the disease is not clear. Mild abrasions associated with Vigorous sexual activity is said to be more contributory to the production of clinical disease than the simple transmission of the organism (Lossick, 1984). Data available indicate that although the majority of men who are sexual contacts of women with clinical candida vulvo-vaginitis are colonized with the agent, only a small proportion of them come down with clinical diseases serious enough to require medical attention.

Neonatal candidiasis following birth occurs in approximately 50% of infants born to mothers colonized with _Candida albicans_ (Kozinn _et al_, 1958). The infections are usually mild, involving the mouth, or skin but serious _in-utero_ candidiasis was documented (Levin _et al_., 1978).

According to Barlow (1979) the oral contraceptive pills, particularly those with high oestrogen content have effect on the vagina similar to that due to pregnancy. He also states that vulvo-vaginal candidiasis is not only more common among pill-takers and pregnant women but is also rather more difficult to eradicate, The correlation of vulvo-vaginal candidiasis with oral contraceptive has been a subject of controversry. Some early reports (Yaffee and Grots, 1965; Catterall, 1966; Porter and Lyl 1966; Walsh _et al_. 1968 and Diddle _et al_. 1969), indicated that there was high incidence of clinical candidiasis in women taking oral contraceptives while other workers (Morris and Morris, 1969; Rohatiner and Grimble, 1970) disagreed with such opinions. Reports by Barlow, 1979; O'Connor and Alder _et al_., 1979, Arya _et al_., 1980; Lossick, 1984 and Maboy _et al_., 1984) confirm that taking oral contraceptives predisposes women to vulvo-vaginal candidiasis.

The importance of yeast as actual or potential pathogens of the female genital tract has led to many studies of their distribution in various groups. A study conducted on 533 female patients at the West London Hospital, isolated yeasts from 138 (26%) of the 533 patients and identified the isolates as *Candida albicans* one hundred and eleven (111), *Candida albicans* and *Torulopsis glabrata* one (1), *Candida parapsilosis* and *T. glabrata* (22), *Saccharomyces cerevisiae* (3) (Oriel *et al.*, 1972). In 1984, Mabey *et al.*, reported the prevalence of infections with *Candida albicans* to be 35%, *T. vaginalis* 23%, *Chlamydia trachomatis* 6.9%, *N. gonorrhoeae* 6.7% among randomly selected attenders of an antenatal clinic in the Gambia.

Bello *et al.* (1983) in a study conducted between 1977 and 1981 on 3089 patients at the sexually transmitted diseases clinic in Zaria with a male-to female ratio of 6:1, reported 28 males with candidiasis as against 276 females giving prevalence of 9.4%. Similarly, in Ibadan out of 442 men with urethritis only 2% had candidiasis (Osoba 1977). Sogbetun *et al.* (1977) isolated *Candida* species from 27 (4.0% of 578 patients that were referred to the Endemic Diseases Clinic at the University College Hospital, Ibadan. At the Lagos University Teaching Hospital, Rotimi *et al.*, (1980) in a study carried out on 276 patients at sexually transmitted diseases clinic, reported 29 (10%) cases of candidiasis.

2.1.4 Therapy of Candidiasis.

Nystatin, an antibiotic with a fungicidal action against *Candida albicans*, has been regarded as the drug of choice for the treatment of candidiasis. It is prepared in vaginal pessaries each containing 100,000 units and in ointment (cream) form that also contains 100,000 units (Catterall 1974). According to Lossick (1984), the broad-spectrum anti-fungal imidazole preparation such as clotrimazole, miconazole and econazole have been gradually replacing nystatin as drugs of choice for candidiasis due to their improved clinical effectiveness and shorter treatment regimens.

Milsom and Forssman(1982), in their study, found that a single 500mg clotrimazole pessary was as effective as the 200mg clotrimazole daily for three days therapy. They also reported that such regimen removes the problem of patients compliance and offers maintained efficacy and maximum acceptability. Earlier in 1981, Hirvonen treated sixty women who presented at his clinic with vaginal mycosis with econazole 150mg vaginal pessaries (Gyno pevary 150) for three days; one pessary daily for 3 days and reported a cure rate of 96.6%. Hydrargaphen in the form of vaginal pessaries has also been mentionded as useful drug for candidal vaginitis and Amphotericin-B is used for disseminated candidiasis. The therapy for systemic candidiasis generally requires intravenous amphotericin-B either alone or in combination with other antifungal agents. The use of 5-fluorocytosine, in synergistic combination with amphotericin-B or separately with a dosage of 150mg/kg/day in 4 doses has been reported to be successful in some cases. Only about 50% of the *Candida* sp. are sensitive to 5-fluorocytosine (Beneke, 1979).

CHAPTER THREE

MATERIAL AND METHODS.

3.1.0: Materials:

3.1.1: Media:

Sabouraud dextrose agar (SDA), Sabouraud dextrose broth (SDB) and corn meal agar (CMA) were purchased from Oxoid Limited, London, in dehydrated forms and prepared as directed by the manufacturer.

3.1.2: Selective Sabouraud Dextrose agar for the isolation of pathogenic fungi:

Sixty five (65g) of dehydrated SDA was weighed into a flask containing one litre of distilled water dissolved properly by boiling it on a bunsen flame and autoclaved at 121°C for 15 minutes. It was allowed to cool down to 55°C then filter-sterilized, 40 units/ml of streptomycin and 16 microgram per millilitre of chloramphenicol were added. The selective medium was aseptically poured into sterile petri dishes, allowed to solidify and kept at 4°C refrigerator until needed for use. The addition of antibiotics makes the medium selective for the isolation of pathogenic fungi (Georg et al., 1954).

3.1.3: Sabouraud Dextrose Broth (SDB):

Ten (10g) of mycological peptone and 40 grams of dextrose were weighed into a medical bottle containing one litre of distilled water, dissolved by gentle heating and dispensed in 4ml volumes into screw-capped tubes and autoclaved at 121°C for 15 minutes. Using sterile

Pasteur pipette, 4 drops of 1N HCL were added into each tube of 4ml of broth and kept at 4°C refrigerator until needed for use.

3.1.4: <u>One Percent Tween 80 Corn Meal Agar:</u>

Seventeen (17g) of corn meal agar (CMA) were suspended in one litre of distilled water, boiled to dissolve properly, 10ml of Tween 80 was added to give 1% of it in medium and then autoclaved at 121°C for 15 minutes. It was allowed to cool down to 50°C before dispensing into sterile petri dishes that were labelled CMA after the medium had solidified. The plates were packed into 4°C refrigerator until needed for use. The addition of 1% Tween 80 to corn meal agar enhances the development of chlamydospores on the medium (Rosenthal and Furnari, 1958; Kelly and Funigiello, 1959; Walker and Huppert, 1959; Walker <u>et al</u>., 1960; and Gordon and Little, 1962). Corn meal agar preparations are examined for the presence of hyphae, blastospores and chlamydospores. The presence of pseudohyphae and blastospores identifies the unknown yeast as belonging to the Genus Candida. The production of chlamydospores is diagnostic of <u>Candida albicans</u>. All sugars (glucose, galactose, maltose, sucrose, lactose, raffinose and trehalose) used in the carbohydrates assimilation and fermentation tests were supplied by Oxoid Limited, London.

3.2.0: Yeast Nitrogen Base and Yeast Extract Broth:

These were obtained in dehydrated forms from Difco Laboratories Limited, Detroit, Michigan, U.S.A. and were prepared in accordance with the manufacturers' directions.

3.2.1: Yeast Nitrogen Base:

Yeast Nitrogen Base was prepared by dissolving 6.7 grams in one litre of distilled water, dispensed in 4.5 millilitre aliquots into screw-capped tubes, labelled accordingly and autoclaved at 121°C for 15 minutes. A 5% solution of each carbohydrate (10% in the case of Raffinose) to be tested was filter-sterilized and 0.5 millilitre was aseptically added to each appropriately labelled tube and stored at 4°C refrigerator until needed for use. These carbohydrates broth were used to test the ability of different species of yeasts to grow in various single carbohydrate substrates.

3.2.2: Yeast Extract Broth:

The broth was prepared by dissolving 2.5 grams of Yeast Extract in 500ml of distilled water to give 0.5% of the yeast extract. Fifty millilitres of 0.04% bromocresol purple was added to make up the five hundred millilitres broth - indicator. It was then dispensed in 7.0ml volume into screw-capped tubes each containing an inverted Durham tube. One millilitre of filter - sterilized 5% sugars (10% in the case of Raffinose) was added to each appropriately labelled screw-capped tube containing 7.0 millilitre yeast extract broth making 8.0 millilitre per tube. The fermentation test assesses the ability of various species of yeasts to produce gas from different carbohydrates (Kononen *et al.*, 1979). One millilitre of filter sterilized 5% sugars (10% in case of Raffinose) was added to each appropriately labelled screw-capped tube containing 7.0 millilitre yeast extract broth making 8.0 millilitre per tube. The fermentation test assesses the ability of various species of yeasts to form gas from different carbohydrates (Kononen *et al.*, 1979).

3.3.0: Trinder's Glucose Oxidase Method (Trinder, 1969).

3.3.1: Colour Reagent:

This was supplied by Hughes and Hughes Limited, Chicago, Illinois, U.S.A. Sodium dihydrogen phosphate solution (40g/L) 75 millilitre + 215 millilitre of water, 5 millilitre of Ferncozyme 653A glucose Oxidase, and 5 millilitre of peroxidase RZ 0.6 (1.0g/L. supplied by Sigma Chemical Company, St. Louis, Minnesota, U.S.A.) were mixed and 300mg of sodium azide and 100mg of 4-amino phenazone were added.

3.3.2: Standard Glucose Solution:

Ten m Mol. per litre (1.80g/L) in Benzoic acid (10g/L) was prepared as the standard to be used against the tests.

3.3.3: Protein Precipitant:

Ten (10.0g) of sodium tungstate, 9.0g of sodium chloride and 10.0g of disodium hydrogen phosphate were dissolved in 800 millilitres of distilled water and 125 millilitres of 1.0 M. HCL was added to obtain pH3. One gram of phenol was then added and the volume made up to one litre with distilled water.

3.4.0: Glucotest Strips for Urine Glucose Test:

These were supplied by Boehringer Mannhein GmbH, Mannhein, Germany. They were used for urine glucose test. The test paper strips were impregnated with enzyme glucose oxidase and peroxidase with an indicator. The reaction is specific for glucose. Other sugars present in the urine are not detected. A buffer mixture makes the reaction independent of the pH of the urine within the range from pH_{4-9}. Positive results are visible within a few seconds. Only at high concentration that a more intense colour reaction is produced after 60 seconds.

3.5.0: Study Population:

300 pregnant women consisting of 100 each of first, second and third trimesters were screened for genital yeast infections in this study.

3.6.0: Collection and Treatment of Samples.

3.6.1: (a) High Vaginal Swabs (HVS):

A HVS was collected from each patient in the study by using a sterile speculum to open the vagina to ensure accurate sampling with a sterile cotton wool swab. A wet mount preparation was made of each HVS and examined microscopically on the spot for *Trichomonas vaginalis* and yeast cells. A smear was also made of each HVS for Gram staining technique to look for yeast cells. The swab was then put into the tube of acidified Sabouraud dextrose broth, taken to the laboratory and incubated at room temperature for 24 hours to eliminate other bacterial contaminants before subculturing onto SDA for pure isolation of yeast cells.

3.6.2: Blood:

Ten millilitres of blood were collected by venepuncture from each patient into a sterile universal bottle. It was allowed to clot and the serum separated by centrifugation into a properly labelled sterile bijou bottle and stored at $-20^{o}C$ until it was needed for the glucose estimation.

3.6.3: Urine:

Each patient was given a sterile disposable plastic container to collect her urine sample into it. She was told to clean herself very well before voiding the urine sample. Each specimen on receipt was labelled with the patient's number and kept for glucose determination.

3.7.0: Methods:

3.7.1: Isolation and Identification:

A wet mount preparation was made from each HVS and examined microscopically for the presence of *Trichomonas vaginalis* and yeast cells. A smear was also made on a clean glass slide for Gram staining for the direct microscopic examination for yeast cells. Each HVS that was inoculated into an acidified Sabouraud dextrose broth (4 drops of 1N HCL into 4ml of SDB) and incubated at room temperature for 24 hours was subcultured onto a labelled Sabouraud dextrose agar (SDA) plate and incubated at 37°C for 24 hours for isolation of yeast cells. Each pure yeast isolate was subcultured on a SDA slant and kept at 4°C for further tests which included the germ tube formation, chlamydo-spore formation, carbohydrates assimilation and fermentation tests, growth at 37°C and at room temperature.

3.7.2: The Germ Tube Tests:

Sterile bovine serum supplied by Flow Laboratories, United Kingdom was aseptically dispensed in 0.5ml aliquots into sterile serologic tubes. Each tube was inoculated with a light suspension of the appropriate yeast isolate and incubated at 37°C for 3 hours after which a drop of each was transferred to a clean glass slide and examined microscopically for germ tube formation.

3.7.3: Chlamydospore Formation Test:

Isolates to be tested were streaked on sterile 1% tween 80 corn meal agar and incubated at room temperature for 24 hours. The plates were examined for chlamydospores by removing the petri dish cover and placing the bottom agar surface up to the microscope substage.

3.7.4; Carbohydrate Assimilation Test:

Suspension of the pure isolates to be tested was made with sterile distilled water in bijou bottles. The suspension was washed three times in distilled water by centrifugation at 2,000 rpm for 2 minutes. This was to wash off the residual carbohydrate on the test organisms. Using sterile pipette, 0.1ml. of each washed yeast suspension was inoculated into sterile 5% (except raffinose 10%) carbohydrate tested and incubated at 37°C for 14 days. A negative control (yeast nitrogen base with no carbohydrate added) was set up in each case.

3.7.5: Carbohydrate Fermentation Test:

Five (5) drops of yeast suspension in a sterile saline equivalent to a MacFarland No. 4 standard was added to each of the carbohydrate fermentation tubes and incubated at 37°C for 48 hours.

3.7.6: Blood Glucose Determination (Trinder's Glucose Oxidase Method):

One hundred microlitre of serum was added to 2.9ml of protein precipitant, mixed well and centrifuged.

One millilitre (1.0ml) of the clear supernatant was pipetted into a 1.6cm test tube. One millilitre of protein precipitate was put into a test tube for blank while 1.0ml of a mixture containing 100ml of the glucose standard and 2.9ml of protein precipitant was made for the standard. Three millilitre colour reagent were added to each tube and incubated at 37°C for 10 minutes. They were briefly shaken three times before the tests and standards were read against the blank at 520 nm wavelength using Corning colorimeter model 253. The tests results were calculated by using this formula:

Calculation:

Blood glucose (m Mol./L) = $\frac{\text{Reading of unknown}}{\text{Reading of standard}}$ X 10 (or X 180 as mg/100ml)

E.g. Sample 177 gave reading as follows:-

Unknown	= 30 (OD)
Standard	= 38 (OD)
Multiplication factor	= 10

Calculation = $\frac{30}{38}$ X 10 = 7.89 m Mol./L.

The principle of this method is based on the fact that the aldehyde group of glucose is oxidized by glucose oxidase to give gluconic acid with gluconolactone as intermediate i.e. glucose + H_2O + $O_2 \rightarrow$ gluconic acid + H_2O_2. The hydrogen peroxide formed in the reaction is broken down to water and oxygen by a peroxidase in the presence of an oxygen acceptor which is converted to a coloured compound that can be read colorimetrically.

3.7.7: <u>Determination of Glucose in Urine:</u>

Each patient was provided with a sterile plastic container to collect urine for the test. The test strip was dipped briefly (for one second) into urine specimen. It was withdrawn and the excess urine wiped off. After 30 seconds, the test strip was compared with the manufacturer's colour scale. The positive reaction was indicated by colour change from yellow to green and the intensity of the colour was rated as it matches the scale i.e. +, ++, +++ respectively while the negative result was indicated by no colour change. The interpretation of positive results is given as follows:-

+ Equals to concentration of up to 100mg/100ml

++ Equals to concentration of up to 100–500mg/100ml

+++ Equals to concentration of up to above 500mg/100ml.

CHAPTER FOUR.

RESULTS.

4.1.0: Microbiologic Diagnosis of High Vaginal Swabs (HVS):

The 300 High Vaginal Swabs collected (100 each from first, second and third trimesters) from the patients were microbiologically examined using, wet film preparation, Gram staining technique and cultures on Sabouraud Dextrose Agar (SDA).

4.1.1: Wet Film Preparation:

Wet film preparation was made from each HVS collected and examined, sixty cases (20%) were found to harbour *Candida species*. The direct examination also diagnosed thirty-six cases of Trichomoniasis representing 12% of the 300 patients. On trimester basis, the first trimester had 4 cases of only trichomoniasis and 9 cases of mixed trichomoniasis and candidiasis. Thus, giving the total cases of trichomoniasis as 13 out of the 100 cases examined in the trimester. The second trmester yielded 3 cases of only trichomoniasis and 5 cases of mixed trichomoniasis and candidiasis totalling eight (8) cases of trichomoniasis out of the 100 cases examined while the third trimester gave four (4) cases of only trichomoniasis and eleven (11) cases of mixed trichomoniasis and candidiasis making fifteen (15) cases also out of hundred cases examined. The total cases of only trichomoniasis is 11 (4%) out of 300 patients and that of mixed candidiasis and trichomoniasis is 25 (8%) also out of 300 patients screened. Table I shows these details.

TABLE I.

Cases of candidiasis, trichomoniasis and those of mixed candidiasis and trichomoniasis diagnosed per one hundred patients of each trimester.

Trimester	Candidiasis	Trichomoniasis	Mixed Candidiasis and Trichomoniasis
1st	50	4	9
2nd	59	3	5
3rd	52	4	11
Total No.	161	11	25
Percentage	54	4	8

4.1.2: Gram Staining Technique:

A smear was made from each HVS collected, Gram-stained and examined microscopically for yeast cells. Out of the 300 HVS examined, 115 cases representing 38.3% were positive for candidiasis.

4.1.3: Culture on Sabouraud Dextrose Agar (SDA):

Of the 300 HVS collected, treated and cultured on SDA medium 186 (62%) cases of candidiasis were diagnosed.

The total cases of candidiasis and those of trichomoniasis per trimester are shown in Table II. The grand total of both trichomoniasis out of the 300 cases examined is 36. Thus representing 12% and that of candidiasis is 186 out 300, which is 62%

TABLE II.

Percentages of cases of candidiasis and trichomoniasis per trimester. The grand total of each is also shown.

Trimester	Types of Infection	No. of Isolates	Percentage
First	Total cases of candidiasis	59	[illegible]
	Total cases of Trichomoniasis	13	[illegible]
Second	Total cases of candidiasis	64	64
	Total cases of Trichomoniasis	8	8
Third	Total cases of candidiasis	63	63
	Total cases of Trichomoniasis	15	15
Grand Total	Candidiasis	186/300	62
	Trichomoniasis	36/300	12

4.2.0: Comparing the Sensitivity of the Three Methods:

Direct microscopic examination of the wet smears yielded 20%, Gram staining technique gave 38.3% while culture technique gave recovery rate of 62%. Hence, the degree of sensitivity of the three methods varies with direct wet film examination being the least sensitive, followed by Gram staining technique and culture method being the most sensitive. Approximate sensitivity ratio is 1:2:3.

4.3.0: Speciation of Yeast Isolates:

The yeast isolates were speciated by using germ tube and chlamydospore formation, carbohydrates fermentation and assimilation tests and incubation at both 37°C and at room temperatures. The germ tube test is accepted as the most reliable test for the identification of Candida albicans (Taschdjian, Burchall and Kozinn, 1960; Mackenzie, 1962; Ahearn, Jonnach and Roth, 1966; Dolan and Ihrke, 1971; and Joshi and Gavin, 1974). Presumptive identifications of C. albicans based solely on germ-tube formation have been reported to be 95 - 100 percent accurate (Stenderup and Thomsen, 1964). Ogletree et al., 1978 found that bovine serum was more effective than defined media for the induction of germ tube formation in the majority of their isolates.

The carbohydrates assimilation tests were considered positive when more growth (turbidity) was observed in the tube containing carbohydrate than in the control tube. The negative results were recorded only after 14 days (Wicherham, 1951).

The positive carbohydrates fermentation tests were indicated by the presence of (gas) in the inverted Durham tube. All negative tests were left in the incubator for 10 days before discarding as negative. Plate I shows germ tubes formed by Candida albicans in bovine serum at 3 hours incubation at 37°C and the typical chlamydospores also formed by this species on 1% tween 80 corn meal agar incubated at room temperature for 24 hours are seen in Plate II. The Candida species so identified on the basis of trimester are shown in table III. Patient number 251 had a mixed infection by Candida albicans and Candida krusei. Thus the total number of yeast isolates identified is 187 but the number of cases of candidiasis remains 186 representing 62% of the 300 patients in this survey.

Appendix I provides detailed results of both blood and urine glucose estimations, while appendices II, III and IV gave details of the Candida species isolated from each trimester respectively. The identification processes are also shown on these appendices.

TABLE III.

Candida Isolates Per Trimester Speciated by using Germ Tube and Chlamydospore Formations, Carbohydrates Fermentation and Assimilation tests and growth at both Room Temperature and 37°C.

ISOLATES	TRIMESTER 1st	2nd	3rd	TOTAL
Candida albicans	38	37	33	108
Candida tropicalis	8	6	9	23
Candida Pseudotropicalis	4	2	3	9
Candida parapsilosis	2	11	9	22
Candida krusei	2	3	7	12
Candida guilliermondii	3	5	2	10
Candida stellatoidea	2	0	1	3
Total isolates identified	59	64	64	187

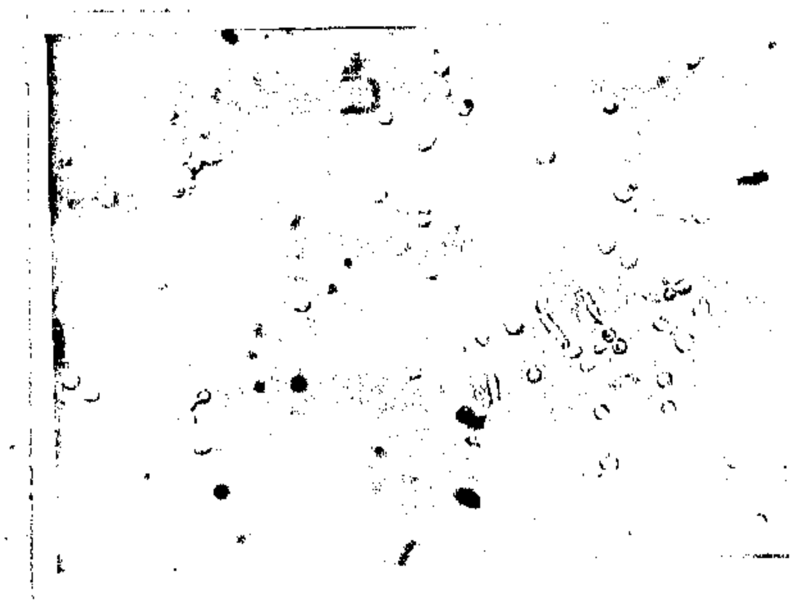

Plate I: Showing Germ-tube formed by Candida albicans in bovine serum at 3 hours incubation at 37°C.

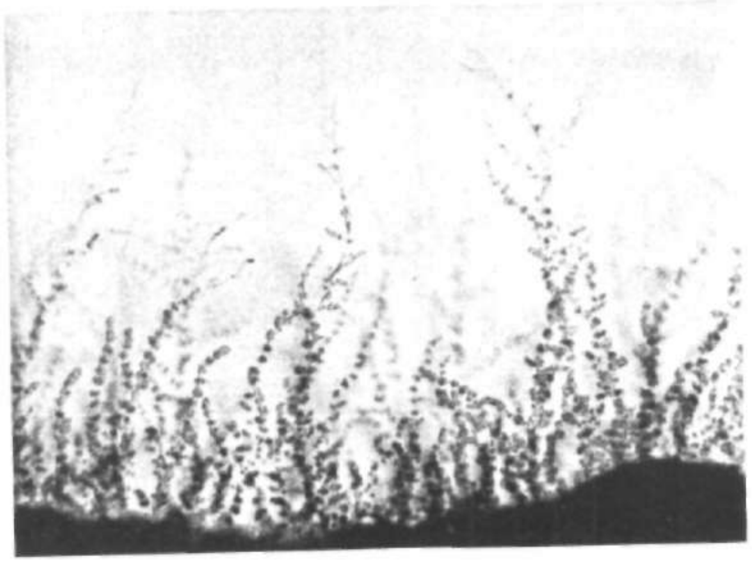

Plate II: Chlamydospores formed by <u>Candida albicans</u> on a 1% Tween 80 Corn Meal agar. The chlamydospores were identified by the appearance of thick-walled round spores formed singly and in clusters from terminal or intercalated hyphal cells.

4.4.0: Blood Glucose Determination by Trinder's Glucose Oxidase Method:

Out of the 300 blood (100 samples each from first, second and third trimesters) collected for blood glucose determination using Trinder's glucose oxidase method, only five cases (patients) were found to have higher blood glucose values than normal. That is while the normal range is between 2.5 - 7.5m Mol. per litre of blood, the cases, No. 177 had 7.9m Mol./l., No. 186 had 8.15 m Mol/l., No. 202 had 7.9 m Mol./l., No. 245 had 7.9 m Mol./l. and No. 267 had 8.07 m Mol./L. Table IV shows the cases of glucosuria and their respective trimesters with amount of glucose estimated in m Mol./L.

4.5.0: Urine Glucose Determination Using Gluco Test Strips:

The same five patients that had higher blood glucose levels also gave positive urine glucose test results. Each of the five samples matched the one plus (+) scale given by the manufacturer. The positive results were indicated by colour change of the impregnated glucose test strips. The one plus meant that the flucose content of the urine sample was equivalent to 100mg/100ml of urine.

Table IV shows the number of cases of glucosuria estimated on the trimester basis by using Trinder's glucose oxidase method for blood glucose test. The blood glucose values are reported in m Mol/L, while the urine glucose are reported in mg/100ml of urine. The ages and case numbers of the five patients are also indicated.

TABLE IV.

Blood and urine glucose levels of the five patients with glucosuria. The age and trimester of each patient are indicated.

Case No.	Trimester	Age	B/glucose in m Mol./L.	Urine glucose in mg/100ml.
177	2nd	16 years	7.90m Mol/l.	(+)=100mg/100ml.
188	2nd	17 years	8.15m Mol.	(+)=100mg/100ml.
202	2nd	17 years	7.90m Mol.	(+)=100mg/100ml.
245	3rd	20 years	7.90 m Mol.	(+)=100mg/100ml
267	1st	20 years	8.07m Mol.	(+)=100mg/100ml.

B/glucose = Blood glucose in m Mol./L.

U/glucose = Urine glucose in mg/100ml.

The normal range of blood glucose values is between 2.5 - 7.5m Mol./L.

DISCUSSION.

Out of the 300 High Vaginal Swabs (100 each from 1st, 2nd and 3rd trimesters) collected from pregnant women attending various antenatal clinics in Zaria, 186 (62%) had vulvo-vaginal candidiasis i.e. 186 yielded Candida sp. on isolation using direct microscopy, Gram staining and culture techniques. On trimester basis, 59, 64 and 63 isolates were obtained from 1st, 2nd and 3rd trimester respectively. The differences between the trimester are of no statistical significance and thus provide no clue as at which trimester that pregnant women are most susceptible to vulvo-vaginal candidiasis which was one of the objectives of the study.

The isolates so speciated by using germ tube, and chlamydospore formations, carbohydrates assimilation and fermentation tests, into various Candida species (table III) confirm the fact that C. albicans is the most common cause of vulvo-vaginal candidiasis. It can also be seen from table III that other Candida species isolated from the vaginal specimens are C. tropicalis , C.

parapsilosis, _C. krusei_, _C. guillermondii_, _C. pseudotropicalis_ and _C. stellatoidea_.

The use of wet film microscopy, Gram staining of smears and culture on Sabouraud Dextrose Agar (SDA) yielded recovery rate of _Candida species_ from the samples at 20%, 38.3% and 62% respectively. The results show that direct microscopy examination of wet preparation technique is about 50% less sensitive than Gram stained smears which in turn is approximately 25% less sensitive than the culture technique. The sensitivity of the three tests is approximately 1:2:3. Eddie (1968) showed that up to 50% and Becker and Schweisfurth (1971) reported 60% of candidal infections would be missed if microscopy alone was used. It has thus been suggested (Oriel, 1977) that culture of specimens is essential for a definitive diagnosis. Although the differences between the three techniques in sensitivity were not as high as either of the two reports, they have indeed indicated the significance of employing them in the diagnosis of candidiasis.

This study diagnosed 36 (12%) cases of trichomoniasis with 11 cases of only trichomoniasis and 25 cases of mixed candida and trichomonas infections. The diagnosis of trichomoniasis was done by microscopic examination of wet mount preparation only. The incidence rate could have been higher if culture method was employed. Feinberg and Whittington (1975) reported an additional 18% trichomonads recovery from vaginal specimens when culture techniques were used in association with microscopy. Combination of smear and culture technique is recommended for more effective diagnosis than either one alone (King and Nicol, 1975). The wet film microscopic examination (Direct Microscopy) was employed to detect cases of trichomoniasis alone and mixed trichomonas and candida infections.

It was observed that patients with mixed candida and trichomonas infections had heavier abnormal vaginal discharge-characterized by cheesy and slimy appearance with foul smell. Most of the patients denied having any vaginal discharge but were found with heavy discharge on examination. Few of them reported having experienced some vulvo-vaginal itching, particularly those with mixed trichomonal and candidal infections. There were no records of any of the patients being on antibiotic prior to or during the week of her attending the clinic. This aspect was included in the questionnaire to exclude those on broad spectrum antibiotics that are themselves predisposing factors to candidiasis.

During pregnancy the sugar content of the vaginal secretion increases and glycogen like substance is present on the vaginal mucosa thus making it acidic and conducive for the growth of osmophilic Candida species. Hence pregnancy is therefore known to be a predisposing factor to vulvo-vaginal candidiasis. This study confirms this fact: 62% in pregnant Nigerians as against 4 - 10% in non-pregnant Nigerians.

From the results of blood glucose values determination and urine glucose test, the patients that have higher blood glucose values and positive urine sugar were 3 cases from second trimester, and one each from 1st and third trimesters respectively, showing that pregnancy might not be responsible for this condition.

Some of those that had heavy vaginal discharge, when interviewed, said that they always experience such discharge whenever they are pregnant and that soon after delivery the discharge disappears without treatment. At this point one wonders if after delivery, the physiologic change of the vagina provides a cure to such candidiasis.

The fact that ignorance is high among most of these classes of women, there is the need for health personnel to educate them on the principles of personal hygine just to avert the potential health hazards that they pose for their neonates and husbands. i.e. oral thrush of their neonates and urethritis and balanitis in their male contacts.

REFERENCES.

AHEARN, D.G., JANNACH, R.R. AND ROTH, F.J. JR. (1966). Speciation and densities of yeasts in human urine specimens. Sabouraud 5:110-119.

ARYA, O., OSOBA, A.O. AND BENNETT, F.J. (1980). Tropical Venereology: pp. 179-182. Published by Churchill Livingstone, London.

BARLOW, D. (1979). Sexually Transmitted Diseases: "THE FACTS". pp. 54-57. Oxford University Press, London.

BECKER, H. AND SCHWEISFURTH, R. (1971). A Medium for the diagnosis of Vaginal candidiasis. Mycosen, 14: 127-130.

BELLO, C.S.S., ELEGBA, O.Y. AND DADA, J.D. (1983). Sexually Transmitted Disease in Northern Nigeria. Br. J. Vener. Dis., 59: 202-205.

BENEKE, E.S. (1979). Human Mycoses (7th Edition). pp. 44-55. A scope Publication (UP JOHN).

BLACK, A. P., KEIRIN, M.A., SMITH, J.J., DYKIS, G.M. AND HABAN, W.E. (1970). "The disinfection of Swimming Pool water Part II". A field study of the disinfection of Public Swimming Pools. Am. J. Pub. Hlth., 69: 740-750.

CATTERALL, R.D. (1966). Candida albicans and the contraceptive pill. Lancet, 2: 830-831.

CATTERALL, R.D. (1974). *A short Text Book of Venereology:* The Sexually Transmitted Diseases (2nd Ed.). pp. 64-68. English University Press, London.

DAVIES, B.D., DULBECCO, R., RISEN, H.N. AND GINSBERG, H.S. (1980). *Microbiology* (3rd Ed.) pp. 818-85[illegible]. Harper & Row, Publishers, INC.

DIDDLE, A.W., GARDNER, W.H., WILLIAMSON, P.J. AND O'CONNOR, K.A. (1969). Oral Contraceptive medications and vulvo-vaginal candidiasis. *Obstet. and Gyne.*, 34: 373 - 377.

DOLAN, C.T. AND IHRKE, D.M. (1971). Further studies of germ tube tests for *Candida albicans* identification *Am. J. Clin. Pathol.*, 55: 733-734.

DRAKE, T.E. AND MAIBACH, H.I. (1973a). Candida and Candidiasis. *Postgrad. Med.*, 53: 83.

DRAKE, T.E. AND MAIBACH, H.I. (1973b). Candida and Candidiasis. *Postgrad. Med.*, 53: 120.

EDDIE, D.A. (1968). The laboratory diagnosis of vaginal infection caused by Trichomonas and Candida species *J. Med. Microbiol.*, 1: 153-159.

ELEGBE, I.A. AND BOTU, M. (1982). A Preliminary Study on dressing patterns and incidence of candidiasis. *Am. J. Pub. Hlth.*, 72: 176-177.

ELEGBE, I.A. AND ELEGBE, I. (1983). Quantitative relationship of Candida albicans infections and dressing patterns in Nigerian Women. Am. J. Pub. Hlth., 73: 450-452.

FEINBERG, J.G. AND WHITTINGTON, M.J. (1957). A culture Medium for Trichomonas vaginalis and species of Candida. J. Clin. Path., 10: 327-329.

GARDNER, H.L. (1982). Infectious vulvo-vaginitis: In Monif, G.R.F. (ed.) Infectious Diseases in Obstetrics and Gynecology, Philadelphia, Harper and Row.

GEORG, L.K., AJELLO, L. AND PAPAGEORGE, C. (1954). Selective medium for Isolation of Pathogenic fungi. J. Lab. Clin. Med., 44(3): 422-428.

GORDON, M.A. AND LITTLE, G.N. (1962). Identification of Candida albicans. Sabouraudia 2: 171-175.

HARRIS, J.R.W. (1975). Introduction, epidemilogy and Social aspects of candidiasis. In Morton, R.S. and Harris, J.R.W. (eds). Recent Advances in sexually Transmitted Disease. Edinburgh Churchill Living Stone.

HIVVONEN, E. AND KAROUSSOS, K. (1981). Three-day Treatment of vaginal mycosis with econazole (Gyno-Pevary 150). Clin. Trials Jour., 18(3): 180-186.

HURLEY, R. (1977). Candidal vaginitis. Proc. R. Soc. Med. (suppl. 4): 70: 1.

JOKLIK, W.K., WILLET, H.P. AND AMOS, D.B. (1980). Zinsser Microbiology (17th ed.). pp.1409-1416. Appletoncentury-crofts; A publishing Division of Prentice-Hall Inc.

JOSHI, K.R. AND GAVIN, J.B. (1974). A simple Laboratory Method for the rapid identification of Candida albicans. *Pathology,* 6: 231-233.

KELLY, J.P. AND FUNIGIELLO, F. (1959). *Candida albicans.* A study of media designed to promote chlamydospore production. *J. Lab. Clin. Med.* 53: 807-809.

KING, A. J. AND NICOL, C.S. (1975). In *Veneral Disease.* Balliere Tindall and Cassel: London.

KONEMAN, E.W., ALLEN, S.D., DOWELL, V.R. AND SOMMERS, A.M. (1979). *Color Atlas and Text-book of Diagnostic Microbiology,* pp. 413-421 Published by J.B. Lippincott Company, Toronto.

KOZINN, A.J., TASCHDJIAN, C.L. AND WIENER, H. (1958). Incidence and Pathogenesis of neonatal candidiasis, *Pediatrics,* 21: 421-429.

LEVIN, S., ZAIDEL, L. AND BERNSTEIN, D. (1978). Intra-uterine infection of foetal brain by candida. *Am. J. Obstet. & Gyneco.,* 130: 597-599.

LOSSICK, J.G. (1984). Sexually Transmitted Vaginitis. Urologic Clinics of North America. 11(1): 141-15[illegible].

MABEY, D.C.W., LLOYD - EVANS, N.E., CONTEH, S. AND FORSEY, T. (1984). Sexually Transmitted Diseases among randomly selected attenders at an antenatal clinics in the Gambia. Br. J. Ven. Dis., 60:331-336.

MACKENZIE, W.R. (1962). Serum tube identification of Candida albicans. J. Clin. Path., 15: 563-565.

MILSOM, I. AND LARS FORSSMAN (1982). Treatment of vaginal candidiasis with a single 500mg clotrimazole Pessary. Br. J. Vener. Dis., 58: 124-126.

MORRIS, C.A. AND MORRIS, D.F. (1969). Vaginal candidiasis. Br. Med. Jour., 1: 319.

O'CONNOR, B.H. AND ADLER, M.W. (1979). Current approaches to the diagnosis, treatment, and reporting of trichomoniasis and candidiasis. Br. J. Vener. Dis., 55: 52-57.

OGLETREE, F.F., ABDELLAL, A.T. AND AHEARAN, D.G. (1978). Germ-tube formation by a typical strains of Candida albicans. Antonis van leeu-wenhoek, 44: 15-24.

OMER, E.E., HAG, A.M. AND ERWA, H.H. (1982). Study of Sexually Transmitted Diseases in Sudanese Women. Am. J. Obstet. & Gynecol., 140:2.

ORIEL, J.D., PARTRIDGE, B.M., DENNY, M., JAJIL AND COLEMAN, J.C. (1972). Genital yeast infections. Br. Med. Jour., 4: 761-764.

ORIEL, J.D. (1977). Clinical over view of candidal vaginitis. Proc. Roy. Soc. Med., 70, (supplement 4), 7-9.

OSOBA, A.O. (1972). Epidemiology of urethritis in Ibadan. Bri. J. Vener. Dis., 48: 116-120.

PORTER, P.S. AND LYLE, J.S. (1966). Yeast vulvovaginitis Due to Oral Contraceptives. *Arch. Dermat.*, 93: 402-403.

ROHATINER, J.J. AND GRUBLE, A. (1970). Genital candidiasis and Oral Contraceptives. *The J. Obstet. & Gyne. Brit. Commonwlth.*, 77: 1013-1015.

ROSENTHAL, S.A. AND FURNARI, D. (1958). Slide Agglutination as a Presumptive Test in the Lab. Diagnosis of *Candida albicans*. *J. Invest. Derm.*, 31: 251-25[illegible].

ROTIMI, V.C. AND SOMORIN, A.O. (1980). Sexually Transmitted Diseases in clinic patients in Lagos. *Br. J. Vener. Dis.*, 56: 54-56.

SOGBETUN, A.O., ALAUSA, K.O. AND OSOBA, A.O. (1977). Sexually Transmitted Diseases in Ibadan. *Br. J. Vener. Dis.*, 53: 155-160.

STENDORUP, A. AND THOMSEN, J.B. (1964). Identification of *Candida albicans*. *Acta. Pathol. Microbiol. Scand.*, 62: 303-304.

SWARTZ, S.L., KRAUS, S.J. AND HERMANN, K.L. (1978). Diagnosis and etiology of non-gonococcal urethritis. *J. Infect. Dis.*, 138: 445.

TASCHDJIAN, C.L., BURCHALL, J. AND KOZINN, P.J. (196[illegible]). Rapid Identification of *Candida albicans* by filamentation on serum and serum substitutes. *Am. J. Dis. Child.*, 99: 212-215.

THIN, R.N., RENDELL, P. AND WADSWORTH, J.C. (1979). How often are gonorrhea and genital yeast sexually transmitted? Br. J. Vener. Dis., 55: 278-280.

TRINDER, P. (1969). Glucose Oxidase Method. Using Phenol and 4-aminophenazone. Ann. Clin. Biochem., 6: 24.

WALKER, L. AND HUPPERT, M. (1959). Rapid Reliable Technique for the identification of *Candida albicans*. Am. J. Clin. Path., 31: 551-558.

WALKER, L., HUPPERT, M. AND WOODS, A. (1960). Corn Meal Tween Agar: An improved medium for the identification of *Candida albicans*. Am. J. Clin. Path., 33: 190-194.

WALSH, H., HILDEBRANDT, R. J. AND PRYSTOWSKY, H. (1968). Oral progestational agents as a cause of Candida vaginitis. Am. J. Obstet. & Gynecol., 101: 991-993.

WICKERHAM, I.J. (1951). Taxonomy of yeasts. U.S. Department of Agriculture Tech. Bull. 1029. U.S. Department of Agriculture, Washington, D.C.

YAFFEE, H.S. AND GROTS, I. (1965). Moniliasis Due to Novethynodrel with mestranol. New Eng. J. Med., 272: 647.

APPENDIX I:

RESULTS OF BLOOD AND URINE SUGAR ESTIMATIONS (BLOOD SUGAR IN m Mol. AND URINE SUGAR AS POSITIVE OR NEGATIVE)

Specimen No.	OD	Results in m Mol.	Urine Sugar Tests	Specimen No.	OD	Results in m Mol.	Urine Sugar Tests
1	17	4.5	-ve	36	17	4.5	-ve
2	10	2.6	-ve	37	19	4.9	-ve
3	16	4.2	-ve	38	15	3.9	-ve
4	13	3.4	-ve	39	16	4.2	-ve
5	12	3.2	-ve	40	16	4.6	-ve
6	15	3.9	-ve	41	28	7.4	-ve
7	19	5.0	-ve	42	17	4.5	-ve
8	12	3.2	-ve	43	17	4.5	-ve
9	14	3.7	-ve	44	16	4.2	-ve
10	12	3.2	-ve	45	17	4.5	-ve
11	13	3.4	-ve	46	17	4.5	-ve
12	15	3.9	-ve	47	16	4.2	-ve
13	16	4.2	-ve	48	11	2.8	-ve
14	15	3.4	-ve	49	17	4.5	-ve
15	12	3.2	-ve	50	10	2.6	-ve
16	16	4.2	-ve	51	13	3.4	-ve
17	13	3.4	-ve	52	52	15	-ve
18	12	3.2	-ve	53	10	2.6	-ve
19	15	3.9	-ve	54	15	3.9	-ve
20	16	4.2	-ve	55	15	3.9	-ve
21	11	2.8	-ve	56	14	3.7	-ve
22	13	3.4	-ve	57	13	3.4	-ve
23	14	3.7	-ve	58	14	3.7	-ve
24	12	3.2	-ve	59	16	4.2	-ve
25	18	4.7	-ve	60	14	3.7	-ve
26	16	4.2	-ve	61	11	2.8	-ve
27	16	4.2	-ve	62	18	4.7	-ve
28	10	2.6	-ve	63	14	3.7	-ve
29	16	4.2	-ve	64	17	4.5	-ve
30	28	7.4	-ve	65	12	3.2	-ve
31	27	7.1	-ve	66	9.5	2.5	-ve
32	16	4.2	-ve	67	13	3.4	-ve
33	26	6.8	-ve	68	16	4.2	-ve
34	18	4.7	-ve	69	10	2.6	-ve
35	16	4.2	-ve	70	15	3.9	-ve

APPENDIX I:

Specimen No.	CT	Results in m Mol.	Urine Sugar Tests	Specimen No.	CT	Results in m Mol.	Urine Sugar Tests
71	14	3.7	-ve	107	19	5.0	-ve
72	9.5	2.5	-ve	108	13	3.4	-ve
73	16	4.2	-ve	109	17	4.5	-ve
74	17	4.5	-ve	110	13	3.4	-ve
75	16	4.2	-ve	111	15	3.9	-ve
76	14	3.7	-ve	112	24	6.1	-ve
77	15	3.9	-ve	113	15	3.9	-ve
78	9.5	2.5	-ve	114	27	7.1	-ve
79	9.5	2.5	-ve	115	11	2.9	-ve
80	17	4.5	-ve	116	18	4.7	-ve
81	16	4.2	-ve	117	11	2.9	-ve
82	16	4.2	-ve	118	13	3.4	-ve
83	17	4.5	-ve	119	9.5	2.5	-ve
84	17	4.5	-ve	120	13	3.4	-ve
85	13	3.4	-ve	121	20	5.3	-ve
86	9.5	2.5	-ve	122	15	3.9	-ve
87	14	3.7	-ve	123	26	6.8	-ve
88	17	4.5	-ve	124	9.7	2.6	-ve
89	12	3.2	-ve	125	13	3.4	-ve
90	13	3.4	-ve	126	12	3.2	-ve
91	13	3.4	-ve	127	20	5.3	-ve
92	11	2.8	-ve	128	16	4.2	-ve
93	12	3.2	-ve	129	10	2.6	-ve
94	13	3.4	-ve	130	9.5	2.5	-ve
95	14	3.7	-ve	131	17	4.5	-ve
96	14	3.4	-ve	132	22	5.8	-ve
97	14	3.7	-ve	133	10	2.6	-ve
98	14.	3.7	-ve	134	18	4.7	-ve
99	10	2.6	-ve	135	12	3.2	-ve
100	12	3.2	-ve	136	10	2.6	-ve
101	11	2.8	-ve	137	17	4.5	-ve
102	13	3.4	-ve	138	15	3.9	-ve
103	16	4.2	-ve	139	9.5	2.5	-ve
104	17	4.5	-ve	140	11	2.9	-ve
105	13	3.4	-ve	141	9.5	2.5	-ve
106	12	3.2	-ve	142	9.5	2.5	-ve

Specimen No.	OD	Results in m Mol.	Urine Sugar Tests	Specimen No.	OD	Results in m Mol	Urine Sugar Tests
143	17	4.5	-ve	180	17	4.5	-ve
144	19	5.0	-ve	181	18	4.7	-ve
145	14	3.7	-ve	182	16	4.2	-ve
146	11	2.9	-ve	183	11	2.8	-ve
147	18	4.7	-ve	184	14	3.7	-ve
148	9.5	2.5	-ve	185	15	3.9	-ve
149	18	4.7	-ve	186	12	3.2	-ve
150	16	4.2	-ve	187	19	5.0	-ve
151	17	4.5	-ve	188	31.0	8.15	+ve(+)
152	15	3.9	-ve	189	14	3.7	-ve
153	9.5	2.5	-ve	190	15	3.9	-ve
154	12	3.2	-ve	191	13	3.4	-ve
155	15	3.9	-ve	192	10	2.6	-ve
156	12	3.2	-ve	193	12	3.2	-ve
157	10	2.6	-ve	194	16	4.2	-ve
158	17	4.5	-ve	195	17	4.5	-ve
159	10	2.6	-ve	196	14	3.7	-ve
160	14	3.7	-ve	197	12	3.2	-ve
161	13	3.4	-ve	198	15	3.9	-ve
162	11	2.9	-ve	199	11	2.8	-ve
163	16	4.2	-ve	200	16	4.2	-ve
164	17	4.5	-ve	201	17	4.5	-ve
165	15	3.9	-ve	202	30.0	7.9	+ve(+)
166	14	3.7	-ve	203	18.0	4.7	-ve
167	11	2.8	-ve	204	14	3.7	-ve
168	13	3.4	-ve	205	15	3.9	-ve
169	12	3.2	-ve	206	13	3.4	-ve
170	14	3.7	-ve	207	12	3.2	-ve
171	10	2.6	-ve	208	17	4.5	-ve
172	15	3.9	-ve	209	16	4.2	-ve
173	16	4.2	-ve	210	14	3.7	-ve
174	14	3.7	-ve	211	16	4.2	-ve
175	17	4.5	-ve	212	13	3.4	-ve
176	16	4.2	-ve	213	14	3.7	-ve
177	30	7.9	+ve(+)	214	17	4.5	-ve
178	13	3.4	-ve	215	15	3.9	-ve
179	12	3.2	-ve	216	16	4.2	-ve

APPENDIX I:

Specimen No.	CB	Results in m Mol.	Urine Sugar Test	Specimen No.	CB	Results in m Mol	Urine Sugar Tests
217	11	2.8	-ve	254	15	3.9	-ve
218	18	4.7	-ve	255	16	4.2	-ve
219	17	4.5	-ve	256	18	4.7	-ve
220	13	3.4	-ve	257	17	4.5	-ve
221	14	3.7	-ve	258	20	5.3	-ve
222	12	3.2	-ve	259	14	3.7	-ve
223	19	5.0	-ve	260	12	3.2	-ve
224	20	5.3	-ve	261	13	3.4	-ve
225	10	2.6	-ve	262	11	2.8	-ve
226	15	3.9	-ve	263	19	5.0	-ve
227	14	3.7	-ve	264	21	5.5	-ve
228	17	4.5	-ve	265	15	3.9	-ve
229	21	5.5	-ve	266	10.75	2.8	-ve
230	18	4.7	-ve	267	30.7	8.07	+ve(+)
231	13	3.4	-ve	268	12	3.2	-ve
232	14	3.7	-ve	269	14	3.7	-ve
233	17	4.5	-ve	270	13	3.4	-ve
234	18	4.7	-ve	271	17	4.5	-ve
235	16	4.2	-ve	272	19	5.0	-ve
236	12	3.2	-ve	273	11	2.8	-ve
237	11	2.8	-ve	274	20	5.3	-ve
238	19	5.0	-ve	275	18	4.7	-ve
239	20	5.2	-ve	276	16	4.2	-ve
240	14	3.7	-ve	277	17	4.5	-ve
241	13	3.4	-ve	278	15	3.9	-ve
242	15	3.9	-ve	279	13	3.4	-ve
243	10	2.6	-ve	280	12	3.2	-ve
244	16	4.2	-ve	281	20	5.3	-ve
245	30.0	7.9	+ve(+)	282	15	3.9	-ve
246	14	3.7	-ve	283	17	4.5	-ve
247	18	4.7	-ve	284	13	3.4	-ve
248	13	3.4	-ve	285	16	4.2	-ve
249	19	5.0	-ve	286	10	2.6	-ve
250	12	3.2	-ve	287	19	5.0	-ve
251	17	4.5	-ve	288	14	3.7	-ve
252	14	3.7	-ve	289	18	4.7	-ve
253	13	3.4	-ve	290	17	4.5	-ve

APPENDIX I:

Specimen No.	OD	Results in m Mol.	[illegible]
291	19	5.0	-ve
292	13	3.4	-ve
293	12	3.2	-ve
294	15	3.9	-ve
295	18	4.7	-ve
296	16	4.2	-ve
297	15	3.9	-ve
298	11	2.8	-ve
299	14	3.7	-ve
300	18	4.7	-ve
STD	38		

Normal range is 2.5 - 7.5 m Mol./L.

APPENDIX II:

[illegible] CHARACTERISTICS OF YEAST ISOLATES

	[illegible]								CARBOHYDRATE FERMENTATIONS					[illegible]			
TEST NO.	DEXTROSE	MALTROSE	SUCROSE	LACTOSE	GALACTOSE	XYLOSE	RAFFINOSE	TREHALOSE	DEXTROSE	MALTROSE	SUCROSE	LACTOSE	GALACTOSE	TREHALOSE	CHLAMYDO-SPORE	GERM TUBE	GROWTH AT 37°	ISOLATE IDENTIFIED
118	+	+	+	-	+	+	-	+	AG	AG	AG	-	AG	AG	-	-	+	C. tropicalis
144	+	-	+	+	+	-	+	-	AG	-	AG	AG	AG	A	-	-	+	C. pseudotropicalis
169	+	-	-	-	-	-	-	-	AG	-	-	-	-	-	-	-	+	C. Krusei
230	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
231	+	+	+	-	+	+	-	+	AG	AG	AG	-	AG	AG	-	-	+	C. tropicalis
232	+	+	+	-	+	+	+	+	AG	-	AG	-	AG	AG	-	-	+	C. guillermondii
234	+	-	+	+	+	-	+	-	AG	-	AG	AG	AG	A	-	-	+	C. pseudotropicalis
259	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	-	-	+	C. tropicalis
260	+	+	+	-	+	+	-	+	AG	-	-	-	AG	AG	-	-	+	C. parapsilosis
261	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
262	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
263	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
276	+	+	+	-	+	+	-	+	AG	AG	A		A	AG	+	+	+	C. albicans
274	+	+	+	-	+	+	-	+	AG	AG	A		AG	A	+	+	+	C. albicans
223	+	+	+	-	+	+	-	+	AG	AG	A		A	AG	+	+	+	C. albicans
225	+	+	+	-	+	+	-	+	AG	AG	A		AG	AG	+	+	+	C. albicans
238	+	+	+	-	+	+	-	+	AG	AG	AG	-	AG	AG	-	-	+	C. albicans
239	+	-	-	-	-	-	-	-	AG	-	-	-	-	-	-	-	+	C. Krusei
280	+	+	+	-	+	+	-	+	AG	AG	AG	-	AG	AG	-	-	+	C. tropicalis

1ST TRIMESTER

CHARACTERISTICS OF YEAST ISOLATES

APPENDIX II

TEST NO.	[illegible]								CARBOHYDRATE FERMENTATIONS						[illegible]			ISOLATE IDENTIFIED
	DEXTROSE	MALTOSE	SUCROSE	LACTOSE	GALACTOSE	XYLOSE	RAFFINOSE	TREHALOSE	DEXTROSE	MALTOSE	SUCROSE	LACTOSE	GALACTOSE	TREHALOSE	CHLAMYDO-[illegible]	GERM [illegible]	GROWTH [illegible]	
231	+	+	+	−	+	+	−	+	AG	AG	A		A	AG	+	+	+	C. albicans
283	+	+	−	−	+	+	−	+	AG	AG	−	−	−	[illegible]	−	+	+	C. stellatoidea
285	+	−	+	+	+	+	+	−	AG	−	AG	AG	AG	AG	−	+	+	C. pseudotropicalis
286	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	AG	+	+	+	C. albicans
288	+	+	+	−	+	+	−	+	AG	AG	A		AG	AG	+	+	+	C. albicans
291	+	+	+	−	+	+	+	+	AG	−	−	−	AG	AG	−	−	+	C guillermondii
292	+	+	+	−	+	+	−	+	AG	−	−	−	AG	AG	−	−	+	C. parapsilosis
297	+	+	+	−	+	+	+	+	AG	−	AG	−	AG	AG	−	−	+	C. guillermondii
257	+	+	+	−	+	+	−	+	AG	AG	A	−	A	AG	+	+	+	C. albicans
295	+	+	+	−	+	+	−	+	AG	AG	A	−	A	AG	+	+	+	C. albicans
294	+	+	+	−	+	+	−	+	AG	AG	A		AG	AG	+	+	+	C. tropicalis
235	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	A	+	+	+	C. albicans
256	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	AG	+	+	+	C. albicans
120	+	+	+	−	+	+	−	+	AG	AG	A	−	A	A	+	+	+	C. albicans
127	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	AG	+	+	+	C. albicans
133	+	+	[illegible]	−	+	+	−	+	AG	AG	−	−	−	−	−	+	+	C. stellatoidea
159	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	AG	+	+	+	C. albicans
161	+	+	+	−	+	+	−	+	AG	AG	A		AG	AG	+	+	+	C. albicans
163	+	+	+	−	+	+	−	+	AG	AG	AG	−	AG	AG	−	−	A	C. tropicalis
136	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	A	+	+	+	C. albicans
178	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	AG	+	+	+	C. albicans

1st TRIMESTER

CHARACTERISTICS OF YEAST ISOLATES

APPENDIX II

TEST NO.	CARBOHYDRATE ASSIMILATIONS: DEXTROSE	MALTOSE	SUCROSE	LACTOSE	GALACTOSE	CARBOHYDRATE FERMENTATIONS: XYLOSE	RAFFINOSE	TREHALOSE	DEXTROSE	MALTOSE	SUCROSE	LACTOSE	GALACTOSE	TREHALOSE	OTHER REACTIONS: CHLAMYDOSPORE	GERM TUBE	GROWTH AT 37°	ISOLATE IDENTIFIED
170	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
219	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	A	+	+	+	C. albicans
216	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
212	+	+	+	-	+	+	-	+	AG	AG	A	-	A	A	+	+	+	C. albicans
217	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
236	+	+	+	-	+	+	-	+	AG	AG	A	-	A	AG	+	+	+	C. albicans
258	+	-	+	+	+	+	+	-	AG	-	AG	AG	AG	AG	+	+	+	C. pseudotropicalis
272	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
270	+	+	+	-	+	+	-	+	AG	AG	A	-	A	A	+	+	+	C. albicans
269	+	+	+	-	+	+	-	+	AG	AG	AG	-	AG	AG	-	-	+	C. tropicalis
268	+	+	+	-	+	+	-	+	AG	AG	A	-	A	AG	+	+	+	C. albicans
269	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
266	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
273	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
165	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
164	+	+	[illegible]	-	+	+	-	+	AG	AG	A	-	A	A	+	+	+	C. albicans

Candida albicans	=	38
Candida tropicalis	=	8
Candida pseudotropicalis	=	4
Candida parapsilosis	=	2
Candida krusei	=	2
Candida guillermondii	=	3
Candida stellatoidea	=	2
Total	=	59

2ND TRIMESTER

APPENDIX III

CHARACTERISTICS OF YEAST ISOLATES

TEST NO.	CARBOHYDRATE ASSIMILATION								CARBOHYDRATE FERMENTATIONS						OTHER REACTIONS			
	DEXTROSE	MALTROSE	SUCROSE	LACTOSE	GALACTOSE	XYLOSE	RAFFINOSE	TREHALOSE	DEXTROSE	MALTOSE	SUCROSE	LACTOSE	GALACTOSE	TREHALOSE	CHLAMYDO-STORE	GERM TUBE	GROWTH AT 37°	
116	+	*	*	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
117	+	*	*	-	+	+	-	+	AG	AG	AG	-	AG	AG	-	-	+	C. tropicalis
128	+	*	*	-	+	+	-	+	AG	AG	A	-	A	AG	+	+	+	C. albicans
13[illegible]	+	*	*	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
136	+	*	*	-	+	+	-	+	AG	-	-	-	AG	AG	-	-	+	C. parapsilosis
142	+	*	*	-	+	+	-	+	AG	AG	A	-	AG	AC	+	+	+	C. albicans
14[illegible]	+	*	*	-	+	+	+	+	AG	-	AG	-	AG	AG	-	-	+	C. guillermondii
150	+	-	[illegible]	-	-	-	-	-	AG	-	-	-	-	-	-	-	+	C. krusei
155	+	*	*	-	+	+	-	+	AG	-	-	-	-	AG	-	-	+	C. parapsilosis
191	+	*	*	-	+	+	-	+	AG	-	-	-	AG	AG	-	-	+	C. parapsilosis
192	+	*	*	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
106	+	*	*	-	+	+	+	+	AG	-	AG	-	AG	AG	-	-	+	C. guillermondii
147	+	*	*	-	+	+	-	+	AG	AG	A	-	A	AG	+	+	+	C. albicans
205	+	*	*	-	+	+	-	+	AG	-	-	-	AG	AG	-	-	+	C. parapsilosis
73	+	*	*	-	+	+	-	+	AG	AG	AG	-	AG	AG	-	-	+	C. tropicalis
5	+	*	*	-	+	+	-	+	AG	-	-	-	AG	AG	-	-	+	C. parapsilosis
13	+	*	*	-	+	+	-	+	AG	-	-	-	AG	A	-	-	+	C. parapsilosis
102	+	*	*	-	+	+	-	+	AG	-	-	-	AG	A	-	-	+	C. parapsilosis
109	+	*	*	-	+	+	-	+	AG	AG	A	-	A	A	+	+	+	C. albicans
110	+	*	*	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
111	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	-	-	+	C. parapsilosis

2ND TRIMESTER APPENDIX III CHARACTERISTICS OF YEAST ISOLATES

TEST NO.	CARBOHYDRATE ASSIMILATION					CARBOHYDRATE FERMENTATIONS									OTHER REACTIONS			
	DEXTROSE	MALTOSE	SUCROSE	LACTOSE	GALACTOSE	XYLOSE	RAFFINOSE	TREHALOSE	DEXTROSE	MALTOSE	SUCROSE	LACTOSE	GALACTOSE	TREHALOSE	CHLAMYDO-SPORE	GERM TUBE	GROWTH AT 37°	
113	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
114	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
115	+	+	+	-	+	+	-	+	AG	AG	AG	-	AG	AG	-	-	+	C. tropicalis
121	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
123	+	+	+	-	+	+	-	+	AG	AG	A	-	A	A	+	+	+	C. albicans
124	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
131	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	A	+	+	+	C. albicans
12	+	+	+	-	+	+	-	+	AG	AG	A	-	A	AG	+	+	+	C. albicans
18	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
32	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
41	+	+	+	-	+	+	-	+	AG	-	AG	-	AG	AG	-	-	+	C. guillermondii
44	+	+	+	-	+	+	-	+	AG	AG	AG	-	AG	AG	-	-	+	C. tropicalis
45	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	A	+	+	+	C. albicans
59	+	+	+	-	+	+	-	+	AG	AG	A	-	A	A	+	+	+	C. albicans
60	+	+	+	-	+	+	-	+	AG	-	-	-	AG	AG	-	-	+	C. parapsilosis
65	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
71	+	+	+	-	+	+	-	+	AG	AG	A	-	A	A	+	+	+	C. albicans
72	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
77	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
97	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
174	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
137	+	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	AG	AG	AG	-	AG	AG	[illegible]	[illegible]	[illegible]	C. albicans

2ND TRIMESTER A

CHARACTERISTICS OF YEAST ISOLATES

APPENDIX III

TEST NO.	CARBOHYDRATE ASSIMILATIONS								CARBOHYDRATE FERMENTATIONS						OTHER REACTIONS			ISOLATE IDENTIFIED
	DEXTROSE	MALTOSE	SUCROSE	LACTOSE	GALACTOSE	XYLOSE	RAFFINOSE	TREHALOSE	DEXTROSE	MALTOSE	SUCROSE	LACTOSE	GALACTOSE	TREHALOSE	CHLAMYDOSPORE	GERM TUBE	GROWTH AT 37°	
95	+	+	+	-	+	+	-	+	AG	-	-	-	AG	AG	-	-	+	C. parapsilosis
103	+	+	+	-	+	+	+	+	AG	-	AG	-	AG	AG	-	-	+	C. guilliermondii
175	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
180	+	+	+	-	+	+	-	+	AG	AG	A	-	A	A	+	+	+	C. albicans
181	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
167	+	+	+	-	+	+	-	+	AG	-	-	-	AG	AG	-	-	+	C. parapsilosis
188	+	+	+	-	+	+	-	+	AG	AG	A	-	A	A	+	+	+	C. albicans
190	+	+	+	-	+	+	-	+	AG	AG	A	-	A	AG	+	+	+	C. albicans
194	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
196	+	+	+	-	+	+	-	+	AG	AG	AG	-	AG	AG	-	-	+	C. tropicalis
199	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
200	+	+	+	-	+	+	-	+	AG	AG	A	-	A	A	+	+	+	C. albicans
201	+	+	+	-	+	+	-	+	AG	-	-	-	AG	AG	-	-	-	C. parapsilosis
202	+	-	-	-	-	-	-	-	AG	-	-	-	-	-	-	-	+	C. krusei
203	+	+	+	-	+	+	-	+	AG	AG	A	-	A	A	+	+	+	C. albicans
204	+	-	+	+	+	+	+	-	AG	-	AG	AG	AG	AG	-	-	+	C. pseudotropicalis
208	+	+	+	-	+	+	-	+	AG	AG	A	-	A	AG	+	+	+	C. albicans
139	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
145	+	-	+	+	+	+	+	-	AG	-	-	-	-	-	-	-	+	C. pseudotropicalis
205	+	-	-	-	-	-	-	-	AG	-	-	-	-	-	-	-	+	C. krusei

2ND TRIMESTER [illegible]

Candida albicans	=	37
Candida tropicalis	=	6
Candida peudotropicalis	=	2
Candida krusei	=	3
Candida parapsilosis	=	11
Candida stellatoidea	=	0
Candida guillermondii	=	5
Total isolates identified	=	64

3RD TRIMESTER

APPENDIX IV: CHARACTERISTICS OF YEAST ISOLATES

TEST NO.	CARBOHYDRATE ASSIMILATIONS								[illegible]						OTHER REACTIONS			ISOLATE IDENTIFIED
	DEXTROSE	MALTROSE	SUCROSE	LACTOSE	GALACTOSE	XYLOSE	RAFFINOSE	TREHALOSE	DEXTROSE	MALTROSE	SUCROSE	LACTOSE	GALACTOSE	TREHALOSE	CHLAMYDO-STORE	GERM TUBE	GROWTH AT 37°	
2	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	AG	+	+	+	C. Albicans
3	+	+	+	−	+	+	−	+	AG	AG	A	−	A	A	+	+	+	C. albicans
4	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	AG	+	+	+	C. albicans
20	+	−	−	−	−	−	−	−	AG	−	−	−	−	−	−	−	+	C. krusei
14	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	A	+	+	+	C. albicans
15	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	A	+	+	+	C. albicans
9	+	+	+	−	+	+	−	+	AG	AG	A	−	A	A	+	+	+	C. albicans
8	+	+	+	−	+	+	−	+	AG	−	−	−	AG	AG	−	−	+	C. parapsilosis
6	+	+	+	−	+	+	−	+	AG	AG	A		AG	AG	+	+	+	C. albicans
26	+	+	+	−	+	+	−	+	AG	−	−	−	AG	AG	−	−	+	C. parapsilosis
27	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	AG	+	+	+	C. albicans
28	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	AG	+	+	+	C. albicans
29	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	AG	+	+	+	C. albicans
19	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	AG	+	+	+	C. albicans
36	+	+	+	−	+	+	−	+	AG	AG	AG	−	AG	AG	−	−	+	C. tropicalis
37	+	+	+	−	+	+	−	+	AG	AG	A	−	A	AG	+	+	+	C. albicans
40	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	AG	+	+	+	C. albicans
42	+	+	+	−	+	+	−	+	AG	−	−	−	AG	AG	−	−	+	C. parapsilosis
43	+	−	+	+	+	+	+	−	AG	−	AG	AG	AG	AG	−	−	+	C. pseudotropicalis
66	+	+	+	−	+	+	−	+	AG	AG	AG	−	AG	AG	−	−	+	C. tropicalis
70	+	+	+	−	+	+	−	+	AG	AG	A	−	AG	AG	+	+	+	C. albicans
63	+	+	+	−	+	+	−	+	AG	−	[illegible]	[illegible]	AG	AG	−	−	+	C. parapsilosis

3RD TRIMESTER APPENDIX IV: CHARACTERISTICS OF YEAST ISOLATES

TEST NO.	[illegible]								CARBOHYDRATE FERMENTATIONS						OTHER REACTIONS			ISOLATE IDENTIFIED
	DEXTROSE	MALTOSE	SUCROSE	LACTOSE	GALACTOSE	XYLOSE	RAFFINOSE	TREHALOSE	DEXTROSE	MALTOSE	SUCROSE	LACTOSE	GALACTOSE	TREHALOSE	CHLAMYDO-SPORES	[illegible]	GROWTH AT 37°C	
64	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
56	+	+	+	-	+	+	-	+	AG	-	-	-	AG	AG	-	-	+	C. parapsilosis
58	+	+	+	-	+	+	-	+	AG	AG	A	-	A	A	+	+	+	C. albicans
57	+	+	+	-	+	+	-	+	AG	AG	AG	-	AG	AG	-	-	+	C. tropicalis
53	+	+	+	-	+	+	-	+	AG	AG	AG	-	AG	AG	-	-	+	C. tropicalis
52	+	+	+	-	+	+	-	+	AG	AG	AG	-	AG	AG	-	-	+	C. tropicalis
51	+	+	+	-	+	+	-	+	AG	-	-	-	AG	AG	-	-	+	C. parapsilosis
49	+	-	+	+	+	+	+	-	AG	-	AG	AG	AG	AG	-	-	+	C. pseudotropicalis
46	+	+	+	-	+	+	-	+	AG	AG	AG	-	AG	AG	-	-	+	C. tropicalis
47	+	+	+	-	+	+	-	+	AG	AG	A	-	A	AG	+	+	+	C. albicans
92	+	-	-	-	-	-	-	-	AG	-	-	-	-	-	-	-	+	C. krusei
78	+	-	+	+	+	+	+	+	AG	-	AG	AG	AG	A	-	-	+	C. Pseudotropicalis
79	+	-	-	-	-	-	-	-	AG	-	-	-	-	-	-	-	+	C. krusei
101	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
100	+	+	+	-	+	+	-	+	AG	AG	A	-	A	A	+	+	+	C. albicans
98	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
96	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
89	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
90	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
93	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
94	+	+	+	-	+	+	-	+	AG	AG	A	-	AG	AG	+	+	+	C. albicans
63	+	[illegible]	[illegible]	-	+	+	-	+	AG	AG	A	-	AG	A	+	[illegible]	[illegible]	C. albicans

3rd TRIMESTER APPENDIX IV: CHARACTERISTICS OF YEAST ISOLATES

TEST NO.	CARBOHYDRATE ASSIMILATION: DEXTROSE	MALTOSE	SUCROSE	LACTOSE	GALACTOSE	XYLOSE	RAFFINOSE	TREHALOSE	CARBOHYDRATE FERMENTATION: DEXTROSE	MALTOSE	SUCROSE	LACTOSE	GALACTOSE	TREHALOSE	OTHER REACTIONS: CHLAMYDOSPORE	GERM TUBE	GROWTH AT 37°	
86	+	–	–	–	–	–	–	–	AG	–	–	–	–	–	–	–	+	C. krusei
105	+	+	+	–	+	+	–	+	AG	AG	AG	–	AG	AG	–	–	+	C. tropicalis
122	+	+	+	–	+	+	–	+	AG	AG	A	–	AG	AG	+	+	+	C. albicans
237	+	+	+	–	+	+	–	+	AG	–	–	–	AG	AG	–	–	+	C. parapsilosis
240	+	+	+	–	+	+	+	+	AG	–	AG	–	AG	AG	–	–	+	C. guillermondii
222	+	+	+	–	+	+	–	+	AG	AG	A	–	AG	AG	+	+	+	C. albicans
215	+	+	+	–	+	+	–	+	AG	AG	A	–	AG	AG	+	+	+	C. albicans
213	+	+	+	–	+	+	–	+	AG	AG	A	–	A	A	+	+	+	C. albicans
210	+	+	+	–	+	+	–	+	AG	AG	[illegible]	–	A	A	+	+	+	C. albicans
241	+	–	–	–	–	–	–	–	AG	–	–	–	–	–	–	–	+	C. krusei
242	+	–	–	–	–	–	–	–	AG	–	–	–	–	–	–	–	+	C. krusei
245	+	+	+	–	+	+	–	+	AG	AG	AG	–	AG	AG	–	–	+	C. tropicalis
249	+	+	+	–	+	+	–	+	AG	–	AG	–	AG	AG	–	–	+	C. guillermondii
251A	+	–	–	–	–	–	–	–	AG	–	–	–	–	–	–	–	+	C. krusei
251B	+	+	+	–	+	+	–	+	AG	AG	A	–	AG	AG	+	+	+	C. albicans
253	+	+	–	–	+	+	–	+	AG	AG	–	–	–	–	–	+	+	C. stellatoidea
31	+	–	–	–	–	–	–	–	AG	–	–	–	–	–	–	–	+	
252	+	+	+	–	+	+	–	+	AG	AG	A	–	AG	A	+	+	+	C. albicans
283	+	+	+	–	+	+	–	+	AG	AG	AG	–	AG	AG	–	–	+	C. tropicalis

Candida albicans = 32   Candida Krusei = 7
Candida tropicalis = 9   Candida Stellatoidea = 1
[illegible] = 2   Candida guillermondii = 2

RESEARCH PROFORMA

Study No. 188

Date: 30/6/86 seen by Dr. ____

Clinic No. 2321/86

Surname: Ibrahim

History of Pregnancy

First name(s) Binta

1st Trimester ____

Age: 17 years

2nd Trimester 5/12

Tribe: Hausa

3rd Trimester ____

Marital Status M M/S

Occupation: House wife

History of Discharge: ~~Yes~~/No

Religion: Muslim

Duration of discharge ____

Nationality: Nigerian

Observation of discharge ✓Yes/No

Address: Zaria City

History recent antibiotic ingestion ($^1/_{12}$) ~~Yes~~/No ←

If yes, name the antibiotic: ____

Specimen(s) collected:

1. Vaginal swab: ✓
2. Urine: Glucose test (+)
3. Blood: 8.15 mMol/L
4. Other: —