ISSN NO. 2320-5407

Journal homepage:http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Antibiotic Resistant Pattern of Some Pathogenic Bacteria and *Candida albicans* Isolated from Asymptomatic Adolescents and their Susceptibility to Four Medicinal Plant Extracts

Agu, G. C.,[1]Olatunbi, I.T.,[1] Thomas, B. T.,[1] Agu, N. C.,[2]and Abolade, O.M.[2]

1. Medical Microbiology Laboratory, Department of Microbiology, OlabisiOnabanjo University (O. O. U.), P.M.B 2002, Ago- Iwoye, Ogun State, Nigeria.

2.Parasitology Laboratory, Department of Plant Science and Applied Zoology, OOU., P. M. B. 2002, Ogun State, Nigeria.

Manuscript Info

Manuscript History:

Received: 10 July 2013
Final Accepted: 29 July 2013
Published Online: August 2013

Key words:
Asymptomaticadolescents, vaginal secretion, microorganisms, antibiotics, plant extracts, antimicrobial activity

Abstract

Sexually transmitted diseases pose severe risks to human health. This study was designed to investigate the state of cleanliness and hygienic practices of adolescents using secondary school age females as case study. One hundred vaginal samples were collected from females within the age range of 9-20years that attended an urban and a rural general Hospital in Ijebu- North East Local Government Area of Ogun State, Southwest Nigeria with the aid of sterile swab sticks. Wellstructured questionnaire was used to obtain vital information about the students. The samples were processed and identified according to the standard methods. The plant extracts tested were *Garcinia kola, Cola milleni, Vernoniaamygdalina, and Brideliaferruginea*while the antibiotics used were commercial antibiotic disk. Agar disc and well diffusion methods were employed in determining the effect of antibiotics and plant extracts respectively on the isolated organisms. Enzymatic activity was used in determining the pathogenicity of the organisms. *Staphylococcus aureus* (42%), *Lactobacillus* species (24%), *Escherichia coli* (11%), *Proteus* species (7%), *Pseudomonas aeruginosa* (3%) and *Candida albicans* (13%) were isolated. Four typed bacteria were used as control. The age groups 18-20 and 15-17 had the highest occurrence with a frequency rate of 48% and 27% respectively. The enzymatic profile revealed high enzymatic activity. Both the antibiotics and the extracts revealed highest inhibitory effects against the standard organisms than the isolated ones. Pefloxacin exhibited the highest inhibitory zone of 9.00 mm and 8.00 mm against both the control and isolated *E. coliand P. aeruginosa*respectively ($P>0.005$). *Cola milleni* had the highest inhibitory effect of 28 mm against *P. vulgaris (*$P< 0.005$). The study revealed that the studied subjects harboured pathogenic organisms, also the four plant extracts had more inhibitory effects on the organisms than conventional drugs used.

Introduction

Adolescence is the period of psychosocial development between childhood and adulthood. It begins with the start of puberty which is the onset of sexual maturity, and the stage at which the reproductive organs begins to function (Women's health, 2009). These changes are brought about by an increase in sex hormone activity due to stimulation of the ovaries and testes by pituitary hormones in female and male respectively (Fidel, 2004). The stage of psychosocial development and the level of cognitive maturation strongly influence each adolescent's response to any health concern, including those related to sexuality (Women's health, 2009).

Healthy vagina is usually colonized by a mutually symbiotic flora of microorganisms that protect it

from disease-causing microbes. The acidity of a healthy vagina which is as a result of the lactic acid secreted by symbiotic microorganisms retards the growth of many strains of dangerous microbes (Todar, 2008). Women's health (2009) reported that the vagina is self-cleansing and usually needs no special treatment and that a healthy vaginal flora aids in the prevention of yeast infections and other possible problems by occupying the chemical resources otherwise utilized by pathogen organisms. The vagina and its microflora form a balanced ecosystem which is an important health-maintaining biologic feature that provides host defense against infections. Any attempt to upset this balance may cause many undesirable outcomes, such as abnormal discharge, yeast infections, harmful bacteria or imbalance in bacteria which can lead to infections. The commonest vaginal infections in female are those of bacteria (bacterial vaginosis), Yeasts (candidiasis), *Trichomonas* (trichomoniasis). Infections with other organisms such as *Chlamydia trachomatis, Treponemapallium*and *Neisseria gonorrhoeae* may cause vaginal discharge due to cervicitis, including foreign bodies, cervical ectopy and genital tract malignancy (Health Protection Agency, 2005).

Sexually transmitted diseases (STDs) are a major health problemamong adolescents and the prevalence is highest among adolescents (10-19) and young adults (20-24), withabout 3 million adolescents in US infected with STD each year (Burstein and Murray, 2003). Psychosexual maturation, cognitive development, knowledge base, physiologic development, first sexual experience, risky behavior, perception of being sexual active and barriers to health care are some of the reasons why the risk of sexually transmitted diseases is high among adolescents (Brayerman, 2000). The highest reported rates of gonorrhea andchlamydia are found among adolescents and young adults but the most commonly reported STD in the United States is Chlamydia (Janini*et al*., 2002). Adolescent susceptibility to STDs reflects both their biologicand behavioral stages of development. The adolescent cervixis more susceptible to infection compared with the adult cervixbecause of the presence of cervical ectopy. The young femaleintroitus is small and subject to more trauma and exchange ofbody fluids during intercourse. Chlamydia and Gonorrhea co-infection is extremely common among adolescents and young adults and they are found in up to 40% of patients with pelvic inflammatory disease, a severe infection of the upper female reproductive tract with severe complications such as infertility, chronic pelvic pain, tubo-ovarian abscesses and even death (Houry and Lavely, 2001; Behrman and Michelson, 2001). Female adolescents are future mothers and leaders of tomorrow and if not properly taking care of and guided appropriately, their future will be messed up. Since most of these sexually transmitted diseases present no noticeable symptoms and women with untreated infections are at risk of infertility, increased risk of ectopic pregnancy and chronic pelvic inflammatory diseases.This study aimed at investigating the level of exposure, hygiene and cleanliness of female adolescents using senior secondary school students as case study. Also, to determine the probable ways of circumventing such resistance.

MATERIALS AND METHODS

Isolation and Identification of Isolates

One hundred vaginal samples were collected from females (with their consent) within the age range of 9-20 using sterile swab sticks. The subjects were secondary school students from Ijebu-North and Ijebu-East Local Government Area of Ogun State, Nigeria, that attended general hospitals for medical treatment other than sexually transmitted diseases. Well structured questionnaire was used to obtain information about their socio-demographic background, sexual risk factors, sexual habits, purpose while they visited the hospital, genital symptoms of the students etc. The samples were collected while they were in lithotomy position. The swab sticks well labeled were transported to the ,Medical Microbiology laboratory of the Microbiology Department of OlabisiOnabanjo University, Ago-Iwoye, Ogun State and were processed immediately. The samples after microscopic examination were cultured onto four media (MacConkey agar, chocolate agar, potato dextrose agar and nutrient agar) that were prepared according to manufactures' instructions. The media were autoclaved at 121^0C for 15 minutes after which they were allowed to cool down (45^0C) before being poured aseptically into sterile petri dishes (Cheesbrough, 2004). The plates were inoculated by streaking, MacConkey and chocolate agar plates were incubated at 37^0C for 24 hours, plates of PDA were incubated at 37^0C for 48 hours. The plates with visible growth were sub-cultured into nutrient agar plates for bacteria and PDA for yeast. The identification of both yeast and bacteria were done using standard methods (Cheesborough, 2005)

Collection of Plant Materials

Fresh leaves of *G. kola*, *V. amygdalina*, *C. mellenii*, *T, glaucescens* and the stem bark of *B. ferruginea* were collected from the bush in Ago-Iwoye and its environs. They were identified at the Forestry Research Institute of Nigeria, Ibadan. Voucher

specimens of the plants were deposited at the Department of Plant Science and Applied Zoology Herbarium. The plant parts were air-dried in the laboratory at ambient temperature (28 ± 5^0C) for 14 days, the stem bark of *B.ferruginea* was pulverized using pestle and wooden mortar. The leaves were powdered using an electric blender (Philips, BolmixerMelangeur HR, 2846, Brazil), the obtained powders were stored until further use

EXTRACTION PROCEDURE

Equivalent amounts of the crushed samples (10 g) of the leaves and bark were soaked in 50 ml of methanol (70%) and sealed properly to prevent evaporation. The suspended solutions were left to stand for 2 days and then first filtered with 8 layers of sterile muslin cloth before using Whatman No 1 filter paper. The individual filtrate was first evaporated using a rotary evaporator. The residues were then concentrated to dryness using water bath. The final products which were gelatinous were weighed using Gibertini analytical balance . The stock solution was obtained by reconstituting 1.3 g, 6.3 g, 1.1 g and 8.6 of *V. amygdalina, G. kola, C. milleni* and *B. ferruginea* with 10 ml, 20 ml and 15 ml of 60% methanol respectively. Each of the extracts was made up to 100 ml with sterile distilled water. From the stock solutions, concentrations (mg/ml) of 100, 50, 25, 12.5, and 6.25, (working solution) were obtained by serial dilution and were used for the antibacterial activity.

Conventional Drugs

The following commercially available standard antibiotic disks were used: amoxacillin (30 µg), ciprofloxacin (10 µg), ampiclox (30 µg), erythromycin (20 µg), gentamicin (10 µg), Pefloxacin (10 µg), zinnacef (20 µg), rocephin (30 µg), streptomycin (30 µg), septrin (30 µg), chloramphenicol (30 µg), sparfloxacin (10 µg), augmentin (30 µg) and tarivid (10 µg).

Test Organisms

The isolates used for the sensitivity tests were those isolated and identified from adolescents. *Staphylococcus aureus, Lactobacillus species, Escherichia coli, Proteus vulgaricus,Pseudomonas aeruginosa, Candida albicans and* four standard organisms: *S. aureus* (ATCC 2999), E. coli (ATCC 24822), Proteus species (ATCC 13315) and P. aeruginosa (ATCC 27853)) were used for the antibiotic disks. Whereas *S. aureus,E. coli*, *Proteus vulgaricus, Pseudomonas aeruginosa*and *E. coli* (ATCC 24822) were used for the plant extracts. The standard organisms which served as control were obtained from the Culture Collection Centre of Medical Microbiology Department of the University College Hospital (UCH) Ibadan, Nigeria

Evaluation of Antibacterial Activity

The antibacterial sensitivity testing of the plant extracts was determined using the agar-well diffusion method as described by NCCLS (2002). Agar diffusion method was used for the standard antibiotic discs. A loopful of the bacterial isolates that were stored on slants was grown in nutrient broth (Lab M) (10 ml) for 18 h before use. Suspensions of the isolates were adjusted to the 0.5 McFarland's standard and 0.2ml of $1.0x10^8$ CFU ml was aseptically used to seed a molten nutrient agar which had been cooled to about 45^0C. Mixed gently and poured into sterile Petri dishes and allowed to set, after which 3 wells were bored on each plate using a standard sterile cork borer of 5 mm diameters. The extracts were tested at 12.5 mg/m concentrations and equal volumes of the extracts (100 µl) were dropped into each well using micropipette. The experiments were carried out in duplicate and the plates were allowed to stand for an hour for pre-diffusion of the extracts to occur. For the antibiotic discs, the organisms were seeded as described above and the agar was allowed to solidify in the Petri plates. Sterile forceps was used to aseptically place the discs on the surface of the medium. All the plates were incubated for 24 h at 37^0C after which they were observed and zones of inhibition measured using transparent meter rule. The average zones of inhibition were recorded (Si *et al.,* 2006).

RESULTS

The biochemical tests is shown in table 1 below. All the 100 samples collected showed visible growth, as such 100 isolates comprised five genera of bacteria and a genus of fungi were recovered. These organisms include: *Staphylococcus aureus*(42%),*Lactobacillus* species (24%), *Candida albicans*(13%), *Escherichia coli* (11%), *Proteus* species (7%) and *Pseudomonas aeruginosa*(3%) (Table2).

Table 1: Parameters used in identifying the isolates

ISOLATES	BIOCHEMICAL TESTS								
	Citrate	Catalase	Indole	Urease	Coagulase	DNase	Oxidase	Motility	Germtube
S. aureus	-	+	-	-	+	+	-	-	-
L. species	-	-	-	-	-	-	-	-	-
C. albicans	-	-	-	-	-	-	-	-	+
E. coli	-	-	+	-	-	-	-	+	-
P. species	-	-	-	+	-	-	-	+	-
Pseudomonas aeruginosa	-	-	-	-	-	-	+	+	-

Table 2: Prevalence of the isolates and their occurrence according to age

Organism	**Age-Group (Years)**				**Prevalence**
	9-11	12-14	15-17	18-20	(%)
Staphylococcus aureus	5	8	13	16	42
Lactobacillus species	4	5	4	11	24
Candida albicans	-	-	4	9	13
Escherichia coli	-	2	2	7	11
Proteus species	-	1	4	2	7
Pseudomonas aeruginosa	-	-	1	2	3
Total	9	16	28	47	100

Table 3: The inhibitory effects of antibiotics on Gram positive organisms

ISOLATES	ZONES OF INHIBITIONS (mm)									
	PEF	CN	APX	Z	AM	R	CPX	S	SXT	E
Staphylococcus aureus	5.0	4.0	0.0	0.0	0.0	1.0	4.0	4.0	0.0	0.0
S. aureus (control)	5.0	4.0	0.0	0.0	0.0	2.0	6.0	5.0	0.0	0.0
Lactobacillus species	0.0	2.0	0.0	0.0	0.0	3.0	0.0	2.0	3.0	0.0
Candida albicans	5.0	3.0	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0

Key:

PEF - Pefloxacin
CN -Gentamycin
APX -Ampliclox
Z - Zinnacef
AM -Amoxacillin
R - Rocephin
CPX -Ciprofloxacin
S -Streptomycin
SXT - Septrin
E - Erythromycin

Table 4: Antibacterial activity of Gram negative bacteria

ISOLATES	ZONES OF INHIBITIONS (mm)									
	SXT	CH	SP	CPX	AM	AU	CN	PEF	OFX	S
Escherichia coli	0.0	0.0	3.0	7.0	0.0	0.0	0.0	8.0	0.0	4.0
E. coli ATCC 24822 (control)	0.0	0.0	0.0	5.0	0.0	0.0	0.0	9.0	0.0	2.0
Proteus species	0.0	0.0	0.0	4.0	0.0	0.0	0.0	3.0	0.0	1.0
*P.*species ATCC 13315 (control)	0.0	0.0	0.0	4.0	0.0	0.0	0.0	5.0	0.0	6.0
Pseudomonas aeruginosa	0.0	0.0	0.0	6.0	4.0	0.0	0.0	8.0	0.0	2.0
P. aeruginosa ATCC 27835 (control)	0.0	0.0	1.0	7.0	0.0	0.0	0.0	9.0	0.0	4.0

Key:
SXT - Septrin
CH - Chloramphenicol
SP - Sparfloxacin
CPX - Ciprofloxacin
AM - Amoxacillin
AU - Augmentin
CN - Gentamycin
PEF - Pefloxacin
OFX - Tarivid
S -Streptomycin

TABLE 5: Antibacterial activity of extract of *Garcina kola, Cola milleni, Vernoniaamygdalina* and *Brideliaferruginea* at 12.5 mg/ml

Isolates	zone of inhibition (mm)			
	GK	CM	VA	BF
ATCC 24822 *Escherichia coli*	27	25	0	22
Staphylococcus aureus	12	9	9	15
Proteus vulgaricus	25	0	17	18
Klebsiellapneumoniae	18	29	19	22
Pseudomonas aeruginosa	23	21	7	11

KEY: GK= Garcina kola, CM= Cola milleni, VA= Vernoniaamygdalina, BF= Brideliaferrruginea, 0= resistance.

TABLE 6: Antimicrobial activity of extract of *Garcina kola, Cola milleni, Vernoniaamygdalina* and *Brideliaferruginea* at 6.25 mg/ml

Isolates	zone of inhibition (mm)			
	GK	CM	VA	BF
ATCC 24822 *Escherichia coli*	7	7	0	5
Staphylococcus aureus	5	5	7	6
Proteus vulgaricus	6	0	3	6
Klebsiellapneumoniae	4	7	4	7
Pseudomonas aeruginosa	5	4	6	5

KEY: GK= Garcina kola, CM= Cola milleni, VA= Vernonia

DISCUSSION

The result revealed that *Staphylococcus aureus* had the highest percentage, followed by *Lactobacillus* species, *Candida albicans*, *Escherichia coli*, *Proteus* species and *Pseudomonas aeruginosa*respectively. This findings corroborated that of Joanne*et al.*(2008) who reported that *Staphylococcus aureus* have increasingly been associated as opportunistic pathogen which causes various supparative, or pus-forming diseases and also nosocomial infections in people whose defensive mechanisms have been compromised, such as those in the hospitals.

Candida albicans being the only fungal organisms showed that bacterial organisms are the most common microorganisms associated with vaginal swab. This is attributed to the fact that candida exists as part of the normal flora of the vagina. Nwokedi and Omole (2007) stated that the overgrowth of the fungus give rise to pathological conditions at the vagina vulva and the perineum with resultant inflammation, discharge and itching. Symptomatic *Candidaalbicans* infections arises when there is an excessive proliferation of this microorganism in the vaginal flora, ceasing colonization and starting to achieve outright adherence to the vaginal cells, consequently causing infection (Fatemeh and Monsour, 2006). Lopes *et al.*(2004) showed in their studies that 20 to 25 of healthy and totally asymptomatic women exhibit positive vaginal secretion cultures for *Candida albicans*.

The result revealed that the percentages of the isolated organisms from Ijebu Ode are higher except *Lactobacillus* species that has the highest percentage of 19% in Ijebu Igbo. This observation may be attributed to the differences in the behavioural attributes of the subject studied. Most strains of *Lactobacillus* produce hydrogen peroxide which serves as a mechanism by which there is inhibition of other genital microorganisms (Fidel, 2004).

Pseudomonas aeruginosa was isolated in just three samples from Ijebu Ode and this may be due to the status of the immune system of the studied populations. This bacterium is a notorious organisms infecting majorly immunocompromised tissues (Nelson *et al*., 2007). From the results, adolescent with twenty years of age have high percentage (24% out of 100%) and also have all the isolated microorganisms present in their samples. This may be due to their more exposure than other age range. Although most of the organism isolated are vaginal normal flora and/or part of the body normal flora, there are also infectious microorganisms which may be as a source of infection. The antimicrobial sensitivity result of the isolates shows little or no difference when compared with the control samples results. It was observed that all the isolates were much more sensitive to pefloxacin and ciprofloxacin except *Lactobacillus* species that is resistant, than other antibiotics. Most of the antibiotics are not effective and this leads to the resistance of the microorganisms to them. Insusceptibility of the isolated microorganisms is due to ability of a particular mutant to destroy a given antibiotics or may be that the receptor sites of the microorganism for antibiotics developed less affinity for it (Furuya and Lowy, 2006). In conclusion, the investigated adolescent harbor array of organisms, most of which are normal vaginal microflora.

References

Belland, R., Ojcius, D. and Byrne, G. (2004).Chlamydia.*Journal of Microbiology***2**(7): 530 - 531.

Boris, S.A. and Barbes, C. (2000). Role played by Lactobacilli in controlling the population of vaginal pathogens. *Journal of Microbes Infection***2**: 543 - 546.

Bradshaw, C.S., Morton, A.N. and Hocking, J. (2006). High recurrence rates of bacterial vaginosis over the course of 12 months after oral metronidazole therapy and factors associated with recurrence. *Journals of Infections Disease***193** (11): 1478 - 1486.

Centers for Disease Control and Prevention (CDC).(2006). SexuallyTransmitted Diseases Treatment.*Department of Health and Human Services***55**: 11.

Centers for Diseases Control and Prevention (CDC).(2007). Sexually Transmitted Diseases Surveillance.Atlanta (GA); CDC.Retrieved Mar. 18, 2009 from http:www.cdc.gov/std/stats o7/surv2007FINAL.pdf.

Cheesbrough, M. (2004).District Laboratory Practice in Tropical Countries 2nd Edition. United States of America: Cambridge University Press, New York.

Christian, J. (2007). Bacterial vaginosis and vagnitis. Retrieved Apr. 20, 2010 from www.vaginosis.com

Clinical Effectiveness Group (CEG), (2006). National guideline for the management of bacteria

vaginosis.British Association for Sexual Health and HIV (BASHH).Pp 24.

Egan, M.E. and Lipsky, M.S. (2000).Diagnosis of Vagnitis.*American Family Physician***62** (5): 1095 – 1104

Faculty of Family Planning and Reproductive Health Care Clinical Effectiveness Unit (FFPRHC) (2006).*Journal of family Planning and Reproductive Health Care* **32** (1): 33 - 42.

Fatemeh, S. and Mansour, N. (2006).A prospective study of genital infections in Hamedam Iran.*Obstetrics of Medical Sciences***37** (3): 174 - 177.

Fawole, M.O. and Oso, B.A. (2001).Laboratory Manual of Microbiology, Spectrum Book Limited, Ibadan.Pp 6 - 17.

Fidel, P.L. (2002). Immunity to Candida.*Oral Disease***8**: 69 - 75.

Fidel, P.L. (2004). Vaginal Lactobacilli in Adolescents: Presence and Relationship to Local and Systemic Immunity, and to Bacterial Vaginosis. *Journal of Sexually Transmitted Diseases***31** (7): 393 - 400.

Free, M.D. (2009). Vaginitis Definition.Retrieved Apr.20, 2010 from http://en.wikipedia.org/wiki/vagina.

Furuya, E.Y. and Lowy, F.D. (2006).Antimicrobial resistant bacteria in the community setting.*National Revolution Microbiology***4**: 36 - 45.

Health Protection Agency (HPA). (2005). Diagnosis of vagina discharge or vaginitis.Guidelines-summarizing clinical guidelines for primary care.Pp 25 - 214.

Hook, E.W. (2000). *Trichomonasvaginalis*- no longer a minor STDs. *Sexually Transmitted Diseases* (STDs) **26**: 388 – 389.

Hudson, T. (2007).Vaginitis. Women's Encyclopedia of Natural Medicine. New York: McGraw Hill.

Jannini, E., Simonelli, C. and Lenzi, A. (2002).Sexological approach to ejaculatory dysfunction. *International Journal of Andrology***25** (6): 317 - 323.

Joanne, M.W., Linda, M.S. and Christopher, J.W. Eds (2008).Microbiology.McGraw Hill Education Asia.

Lopes, M.E., Aline, A.T., Shizue, C.Y., Marina, P.R. and Inez,E.S. (2004). Correlation of *Candida* species and symptoms among patients with vulvovaginal candidiasis in Maringa, Parana, Brazil.*RevistaIberoamericanade Micologia***21**: 202-205.

Lisa, F.L., Spencer, R.H. and Jane, R.S. (2000).Detection of Trichomoniasis in vaginal and urine specimens from women by culture and PCR.*Journal of Clinical Microbiology* **38** (10): 3585 - 3588.

Moosa, M.Y., Sobel, J.D., Elhalis, H.W. and Akins, R.A. (2004).Fungicidal activity of fluconazole against *Candida albicans* in a synthetic vagina – simulative medium.*Antimicrobial Agents Chemoteraphy***48** (1): 161 - 167.

Mpiga, P. and Ravaorinoro, M. (2006).*Chlamydia trachomatis*persistence.*Microbiology Resolution***161** (1): 9 - 19.

Nansel, T.R., Riggs, M.A. and Adrews, W.W. (2006). The association of Psychosocial stress and bacterial vaginosis in a longitudinal cohort. *American Journal of Obstetrics and Gynecology***194** (2): 381 - 386.

Nelson, C.A., Reginald, A.O., Okoro, N. and Janet, K. (2007).Antibacterial activity of *Allium cepa* (Onions) and *ZingiberOfficinale* (Ginger) on *Staphylococcus aureus* and *Pseudomonas aeruginosa* from high vaginal swab.*The internal Journal of Tropical Medicine***3** (2): 1 - 7.

Nwokedi, E.E. and Omole, O.A. (2007).Current Clinical Review of Vulvovaginal Candidiasis (VVS).*Journal of Medicine and Rehabilitation* **1** (1): 28 - 32.

Oduyebo, O.O., Anorlu, R.L. and Ogunsola, F.T. (2009).The effects of antimicrobial therapy on bacterial vaginosis in non pregnant women.*Cochrance Database SystemicReview* **3:** 6055.

Rein, F.M. and Bryam, A.L. (2000). Diagnosis and Treatment of Infections Vaginitis.Hospital Physician Editorial Board.Pp 46 - 58.

Ryan, K.J. and Ray, C.G. (2004).*Candida albicans.*Sherries Medical Microbiology 4th Edition.McGraw Hill.

Spence, D. and Melville, C. (2007).Vaginal Discharge.*Biological Medical Journal***335** (7630): 1147 - 1151.

Stamm, W.E. (2000*). Chlamydia trachomatis* infections of the adult. In: sexually Transmitted Diseases 3rd Edition. Holmes, K., Sparling, P. and Mardh (eds). New York: McGraw-Hill; 407 - 422.

Taylor, B.N., Staib, P., Binder, A. and Lina, R.V. (2005). Profile of *Candida albicans*secreted aspartic proteinase elicited during vaginal infection. *Infectious Immunity* **73**: 1828 - 1835.

Todar, K. (2008). The nature of bacterial/host-parasite relationships in humans.*Online Textbook of Bacteriology.*

Vorvick, L.D. (2008). Vaginitis test-wet mount. Retrieved Mar. 12, 2010 from http://en.wikipedia.org/wiki/vaginal wet mount categories: Gynecology.

Weinstock, H., Berman, S. and Cates, W. (2004). Sexually transmitted disease among American youth: incidence and prevalence estimates. *Perspectives on Sexualand Reproductive Health***36**: 6-10.

Wilson, J. (2005). Managing recurrent bacterial vaginosis.*Sexually TransmittedInfections***80**: 8 - 11.

Women's Health, (2009). Vagina.retrieved Mar. 2, 2010 from http:/www.women's health.gov/glossary/vagina.

****