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Original Research Article

Detection and Epidemiology of Vulvovaginal Candidiasis among Asymptomatic Pregnant Women Attending a Tertiary Hospital in Ogbomoso, Nigeria

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Abstract

Vulvovaginal candidiasis (VVC) is caused by the overgrowth of *Candida* species, most commonly *Candida albicans* in the vagina and is characterized by curd-like vaginal discharge, itching and erythema. This study was conducted to determine the prevalence and to emphasize the need for proper detection of vulvovaginal candidiasis among asymptomatic pregnant women. A total of 140 pregnant women aged between 20-49 years and of gestation age of 14-36 weeks were recruited for this study. High Vaginal swabs were collected under aseptic condition. Samples collected were analyzed within one hour of collection using microscopy and culture methods. The isolates were further subjected to the Germ tube test (GTT) and the chromogenic agar test (CHROM agar). The rate of *Candida* infection was found to be 25% (n=35) among the pregnant women. The peak age of infection was 20-29 years 33.8% (n=26), no woman between the age 40 to 49 years had candidiasis. Candidiasis was significantly more likely to be detected in pregnant women in the second trimester compared to those in the first (χ^2=5.952; p <0.05) and third trimesters ((χ^2=9.282; p <0.05). This study revealed a high incidence of asymptomatic vulvovaginal candidiasis among pregnant women in Ogbomoso and various *Candida spp* responsible for VVC were identified.

Keywords: Vulvovaginal candidiasis, pregnant women, Ogbomoso.

1. Introduction

The female genital tract is the point of entry for various sexually and non-sexually transmitted diseases. A number of pathogens exist that affect the female genital tract and cause vaginal discharge. Vaginal discharge is a common symptom in gynaecological clinic and is often the second most common gynaecological problem after menstrual disorders.

Vulvovaginal candidiasis (VVC) is caused by the overgrowth of *Candida* species, most commonly *Candida albicans*, in the vagina and is characterized by curd-like vaginal discharge, itching and erythema [1,2]. An estimated 70 to 75% of healthy adult women have at least one episode of VVC during their lifetimes and 50% of them suffer recurrent events [3,4]. *Candida* species are part of the lower genital tract flora in 20-50% of healthy asymptomatic women [5]. Prevalence rates are higher in pregnant women, diabetic women, women with previous history of antibiotics use and women with HIV/AIDS [6-8].

Candidiasis is the most common opportunistic fungal infection and it is responsible for 90% of the cases of infectious vaginitis [9,10]. Vaginitis is one of the principal motives that lead women to seek out an obstetrician or gynaecologist.

Pathogenic *Candida* species that cause vaginitis most often are *C. albicans*, *C.glabrata*, and *C.tropicalis*. *Candida* species that rarely cause infection include *C.parapsilosis*, *C.pseudotropicalis*, *C.krusei*, *C.guilliermondi* and *C.stellatoidea* [11].

Antifungal drugs such as Nystatin, Amphotericin B and Imidazoles are used for the treatment of VVC. However, the most effective antifungal drugs are very expensive and out of reach for many Africans who reside in the rural areas. These antifungal drugs may also be associated with some serious side effects [12]. Therefore, the use of herbal medicine such as Cinnamon, *Vernonia amygdalina* (bitter leaves), *Moringa oleifera* seeds, *Allium sativum* (garlic) cloves, which have fewer side effects and are economically cheaper, have been recently taken into consideration [13-15].

Many health practitioners believe that nylon underwear and tight insulating clothing in temperate environment predispose to vaginal candidiasis by increasing the temperature and moisture of the perineum [5,16]. A study conducted among African women revealed that, regular users of tight clothing had 88.2% of *Candida albicans* and occasional and non-wearers had 68.6% of *Candida albicans*[16]. Associated risk factors include use of the sponge, intrauterine devices (IUDs), condoms, orogenital sex, douching and intercourse, in addition to a diet with high glucose content [17-19].

Vaginal candidiasis is common in pregnancy due to altered pH and sugar content in vaginal secretions. The acidity level of the vagina is maintained at pH 4.0- 4.5. However, any physiological changes that affect the beneficial bacteria in the vagina would alter the acidity of the vagina reducing its pH to 5.0-6.5 therefore enhancing the establishment of pathogenic organisms such as *Candida* [2,20]. Vaginal pH may increase with age, phase of menstrual cycle, sexual activity, contraception choice, pregnancy, and use of antibiotics [2]. Increased oestrogen level during pregnancy leads to the production of more glycogen in the vagina which allows for the proliferation of yeast cells on the wall of the vagina [21]. It has been estimated that up to 40% of pregnant women worldwide may have vaginal colonization by *Candida* species [22].

There is a dearth of information on the prevalence of vaginal candidiasis among pregnant women in Ogbomoso. In view of this, the present study was conducted to determine the prevalence and to emphasize the need for proper detection of vulvovaginal candidiasis among asymptomatic pregnant women attending routine antenatal clinic visits in a tertiary hospital in Ogbomoso, Southwest Nigeria.

2. Patients and Methods

2.1 Study Design

The study was a cross sectional survey of one hundred and forty (140) pregnant women aged between 20-49 years and of gestation age of 14-36 weeks who were attending routine antenatal clinic visits at the LAUTECH Teaching Hospital in Ogbomoso. The study was conducted over a period of seven months from July 2014-January 2015. Socio-demographic information and obstetric history was obtained from participants by the use of a structured questionnaire. Pre-test counseling for sexually transmitted infections (STIs) was given to each subject by a trained counselor before specimens were collected from them. The protocol was examined and approved by the institution ethics committee with Institutional Ethical Clearance (IEC) Number =LTH/OGB/EC/2014/041, and therefore the study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

2.2 Inclusion Criteria

Pregnant women on attending antenatal at the hospital were included while members of staff of the hospital, patients with previous history of preterm labour or spontaneous abortion and those that did not give their consent were excluded from the study.

2.3 Sample Collection

Samples of High Vaginal were collected under aseptic condition with the aid of a speculum. Samples collected were analyzed within one hour of collection. In case of delay, a drop of phosphate buffer saline was added to the swabs stick container and stored in the refrigerator at 4 to -8 ˚C and assayed within 1-3 hours. Positive patients were given posttest counseling and those infected were treated. It was well documented in their case note.

2.4 Wet Preparation and Culture Isolation

A sample of the exudates was transferred onto a microscope slide. A drop of sterile physiological saline was added and mixed. It was covered with a cover slip and examined under the microscope at ×10 and ×40 objective lenses. Direct Gram stain was also performed on smears of the exudates made on another slide. The High vaginal swabs collected were inoculated onto Saboraud dextrose agar plates and incubated aerobically at 37°C for 48hours [23].

2.5 Germ Tube Test (GTT) to Identify *C. albicans*

Yeast isolates from Saboraud dextrose agar were screened for germ tube production in serum broth using 0.5 ml of human serum. The broth was dispensed into small test tubes, and each tube was

inoculated with yeast colonies from the culture plate. The tube was incubated at 35–37 ºC for 3 hours. A drop of the serum containing the yeast culture was placed on a clean microscope glass slide, and covered with a cover glass. The preparation was examined using the ×10 and ×40 objective lenses. Sprouting yeast cells (tube-like outgrowths) also known as germ tubes were observed in positive samples. Germ tube negative species were regarded simply as *Candida* species. When sprouting yeast cells were seen they were reported as *C. albicans*. *Candida glabrata* ATCC 22018 was used as negative control [23].

2.6 Cultivation on the Selective Medium (CHROM agar)

Isolates from Saboraud dextrose agar (SDA) were inoculated on CHROMagar (Oxoid, Basingstoke, UK) using an inoculating loop and incubated at 37^{o}C for 48 hours. The method is based on the differential release of chromogenic breakdown products from various substrates by *Candida* species following differential exoenzyme activity [24]. Identification of yeast was done based on the colour of each colony. Using this method, the following *Candida* species were identified; *C. glabrata* (wet dark pink colonies), *C. tropicalis* and *C. dublinensis*(wet blue colonies) and*C. albicans* (wet, green colonies).

2.7 Data Analysis

Frequencies were obtained and percentages were calculated for study variables. The results were analyzed using the χ2-test, with the level of significance set at ($p < 0.05$). Statistical Package for Social Sciences (SPSS) version 18.0 (SPSS, Inc., Chicago, Ill) was used for the analysis

3. Results

Of all the *Candida* species isolated (n=35) from the pregnant women, the germ tube test identified 21 (60.0%) as *Candida albicans* while 14 (40.0%) could not be identified. Nineteen (54.3%) of the isolated *Candida* species were identified as *Candida albicans* by CHROMagar while 9 (25.7%) were identified as *Candida glabrata*, 2 (5.7%) as *Candida tropicalis*, and 5 (14.3%) as *Candida dublinensis* as shown in Table 1.

Table 1: Frequency, Distribution and Identification of *Candida* Isolates

Isolates	Germ Tube Test (%)	CHROM agar (%)
Candida albicans	21(60)	19(54.3)
Other *Candida* spp		
Candida glabrata	0	9(25.7)
Candida tropicalis	0	2(5.7)
Candida dublinensis	0	5(14.3)
Unidentifiable spp	14(40)	0(0.0)
Total	35	35

Table 2 shows the percentage distribution of candidiasis in pregnant women of different age groups was as follows: Of the total (140) number of pregnant women recruited into the study, 26 (33.8%) who were 20-29 years, 9 (24.3%) who were 30-39 years, had candidiasis. No woman between the age group of 40 to 49 years had candidiasis.

Table 2: Distribution of Candidiasis in Relation to Age of Pregnant Women

Age (yr)	Number studied	Women with candidiasis
20 – 29	77	26 (33.8)
30 – 39	37	9 (24.3)
40 – 49	6	0 (0)
Total	140	35

The distribution in percentage of candidiasis according to the trimester of the pregnancy is shown in (Figure 1). The results showed that pregnant women in their second trimester had the highest incidence of candidiasis 19 (54.3%), followed by first trimester with 9 (25.7%) and third trimester had the least 7 (20%). The occurrence of vaginal candidiasis infection in pregnant women was significantly different in the three trimesters ($p < 0.05$). Candidiasis was significantly detected in pregnant women in the second trimester compared to those in the first (χ^2=5.952; $p < 0.05$) and third trimesters ((χ^2=9.282; $p < 0.05$).

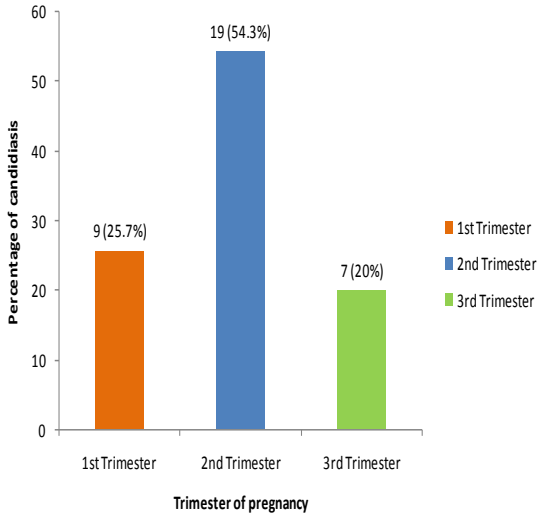

Figure 1: Distribution of candidiasis according to gestational age

4. Discussion

VVC is an important cause of morbidity in pregnant women. In pregnant women, vaginal candidiasis has been related to emotional stress and suppression of immune system which steps up the risk of *Candida* species overgrowth and become pathogenic [25]. It can cause abortion, *Candida*

chorioamnionitis and subsequent preterm delivery [26]. Transmission of *Candida* infections can occur from the vagina of the infected mother to the newborn, giving rise to congenital *Candida* infection [21]. Therefore, early detection, early diagnosis with adequate pharmacotherapy and avoidance of predisposing factors would resolve VVC in short period of time.

In the present study, the prevalence of VVC is 25%. This prevalence rate is in tandem with 26% reported in Ibadan [32]. This rate is almost double the rate reported in Burkina faso (14%) and about half the rate reported in Maroua, Cameroon 55.4% [27,28]. The prevalence reported in this study is lower than the 30.7% reported in Jamaica [29], and the rate of 30% reported in Nnewi, a town in Nigeria [30].

In the United States, 70-90% of VVC cases are caused by *Candida albicans*, while remainders of infections were caused by other *Candida* species [9]. In the present study, *C. albicans* (60%, n=21) and other *Candida* species (40%, n=14) were isolated and identified by Germ tube test. The overall carrier rates of 60% observed for *C.albicans* and 40% for other *Candida* species is in tandem with the rate reported by Alli, *et al* [5]. These prevalence rates reported for *Candida albicans* and *Candida* species in this study closely agrees with 65.4% and 34.6% respectively reported for *Candida* infection among pregnant women by Donbraye-Emmanuel, *et al* [31]. The overall carrier rate of 60% observed for *C.albicans* favourably agrees with the 60% also reported and published by Sobel, *et al* [3]. This is comparatively higher than the 22.1% reported by Anorlu, *et al* and 21.5% reported by Usanga, *et al*, among pregnant women in Calabar, Nigeria [32,33].

The overall carrier rates of 40% reported in this study for other *Candida spp* is higher than 34.6% reported by Donbraye-Emmanuel, *et al*; among pregnant women in Ibadan, Nigeria and comparatively lower than 42% reported by Nwadioha, *et al*; among pregnant women at Aminu Kano Teaching Hospital, Kano, Nigeria [31,34].

In this study, the CHROMagar test showed the highest occurrence of *Candida albicans* 54.3%, followed by *C.glabrata* 25.7%, *C.tropicalis* 5.7% and *C.dublinensis* 14.3%. Nelson, *et al* also showed *Candida albicans* followed by *C.glabrata* as the most common vaginal *Candida* species causing vaginal candidiasis among pregnant women. Another report by Oyewole, *et al* showed the highest occurrence of *C.albicans* (50%) followed by *C.glabrata* (21.4%), *C.tropicalis* (14.3%), *C.krusei*(11.9%) and *C.pseudotropicalis* (2.4%) [35,36].

The age group that represents the peak of childbearing in Nigeria society is 20-30 years, and it is among this group that significantly high prevalence of VC occurs. This study shows that candidiasis was predominantly detected in women in the age group of 20-29 years (33.8%), followed by 30-39 years (24.3%). The high infection rate in the age group of 20-29 may be due to indiscriminate drug usage and use of contraceptives [35]. No woman in the age group of 40 to 49 years had candidiasis. The high concentration of oestrogen hormone during pregnancy provides favourable environment for the growth of *Candida* [37,38]. However, the reduction in the effect of oestrogen hormone in women as they advance in age could lead to lower infection rates in pregnant women above 40.

In our study, 54.3% prevalence was observed in the second trimester followed by 25.7% in first trimester and 20% in the third trimester. Deepa, *et al* reported 54% prevalence in second trimester, 30% in third trimester and 16% in first trimester [26]. In comparison with our study, even though the orders of prevalence are different, the prevalence rates in the second trimester are the same. Similarly, the study by Oyewole, *et al* also observed the highest incidence of vaginal candidiasis among pregnant women in their second trimester (61%) [36].

5. Conclusion

This study revealed a high incidence of asymptomatic vulvovaginal candidiasis among pregnant women in Ogbomoso. This study was also able to detect other *Candida* isolates that are associated with candidiasis. The diagnosis of *Candida* in almost all the hospitals in Ogbomoso are only based on microscopy, culture and the use of GTT for identification of the isolates. These methods are inadequate for the effective diagnosis of VVC hence addition of the CHROMagar test is advised. Proper and early diagnosis and treatment can prevent the complications associated with candidiasis. We therefore recommend that a holistic screening protocol be incorporated in routine antenatal check-up for early diagnosis of candidiasis and its treatment. An appropriate health education is also very imperative, this includes avoidance of contributory factors such as wearing tight underwear by pregnant women and improved general personal hygiene are required in order to reduce the incidence of vaginal candidiasis.

References

[1] Rathod SD, Klausner JD, Krupp K, Reingold AL, Madhivanan P. Epidemiologic Features of Vulvovaginal Candidiasis among Reproductive-

Age Women in India. Hindawi Publishing Corporation, *Infect Dis Obstet Gynecol* 2012; 8.

[2] Nviriesy P. Vulvovaginal Candidiasis and bacterial vaginosis infections. *North Am J Clin Diseases* 2008; 22:637-652.

[3] Sobel JD, Faro S, Force RW. Vulvovaginalcandidosis: epidemiologic, diagnostic, and therapeutic considerations. *Am J Obstetrics and Gynecol* 1998; 178:203–211.

[4] Aslam M, Hafeez R, Ijaz S, Tahir M. Vulvovaginal Candidiasis in Pregnancy. *Biomedica* 2008; 24:54-56.

[5] Alli JAO, Okonko IO, Odu NN, Kolade AF, Nwanze JC. Detection and prevalence of Candida isolates among patients in Ibadan, Southwestern Nigeria. *J Microb Biotechnol Res* 2011; 1:176-184.

[6] Singh SI. Treatment of Vulvovaginal Candidiasis. *Clin J Rev* 2003; 136(9):26-30.

[7] De Leon EM, Jacober SJ, Sobel JD, Foxman B. Prevalence and risk factors for vaginal *Candida* colonization in women with type 1 and type 2 diabetes. *BMC Infectious Diseases* 2002; 2(1):1110-1186.

[8] Duerr A, Heilig CM, Meikle SF, Cu-Uvin S, Klein RS, Rompalo A, Sobel JD. Incident and persistent vulvovaginal candidiasis among human immunodeficiency virus-infected women; Risk factors and severity. *Obstetrics and Gynecology* 2003; 101(3):548-556.

[9] Hedayati T and Shafiei G. 2010. Candidiasis. eMedicine Specialties.

[10] Adad SJ, de Lima RV, Sawan ZT, Silva ML, de Souza MA, Saldanha JC, Falco VA, da Cunha AH, Murta EF. Frequency of *Trichomonas vaginalis*, *Candida sp* and *Gardnerella vaginalis* in cervical-vaginal smears in four different decades. *Sao Paulo Med J* 2001; 119(6):200-205.

[11] Cronje HS, Joubert G, Muir A, Chapman RD, Divan P, Barn RH. Prevalence ofvaginitis, syphilis and HIV infection in women in the Orange Free State. *S Atr Med J* 1994; 84:602-605.

[12] Barret D. From natural products to clinically useful antifungal. *Biochimica et Biophysica Acta* 2002; 1587:224-233.

[13] World Health Organization 2002. Traditional Medicine: Growing Needs and Potential, WHO Policy Perspectives on Medicines. World Health Organization, Geneva Swiss 2002; 1-6.

[14] Ajose F. Some Nigerian plants of dermatologic importance. *Int J Dermatol* 2007; 46:48-55.

[15] Yongabi KA, Mbacham WF, Nubia KK, Singh RM. Yeast strains isolated from HIV-seropositive patients in Cameroon and their sensitivity to extracts of eight medicinal plants. *Afr J Microbiol Res* 2009; 3(4):133-136.

[16] Nwankwo EOK, Kandakai-Olukemi YT, Shuaibu SA. Aetiologic agents of abnormal vaginal discharge among females of reproductive age in Kano, Nigeria. *Journal of Medicine and Biomedical Sciences* 2010; 12-16.

[17] Akah PA, Nnamani CE, Nnamani PO. Prevalence and treatment outcome of vulvovaginal candidiasis in pregnancy in a rural community in Enugu State, Nigeria. *Journal of Medicine and Medical Sciences* 2010; 1(10):447-452.

[18] Mardh PA, Rodrigues AG, Genc M, *et al*. Facts and myths on recurrent vulvovaginalcandidosis: a review on epidemiology, clinical manifestations, diagnosis, pathogenesis and therapy. *Int J STD AIDS* 2002; 13(8):522-539.

[19] Reed BD, Zazove P, Pierson CL, Gorenflo DW, Horrocks J. Candida transmission and sexual behaviors as risks for a repeat episode of *Candida vulvo* vaginitis. *J Women's Health* 2003; 12(10): 979-89.

[20] Akinbiyi AA, Watson R, Feyi-Waboso P. Prevalence of *Candida albicans*and bacterial vaginosis in asymptomatic pregnant women in South Yorkshire, United Kingdom. *Arch Gynecol Obstet* 2008; 278:463-466.

[21] Parveen N, Munir AA, Din I, Majeed R. Frequency of vaginal candidiasis in pregnant women attending routine antenatal clinic. *J Coll Physicians Surg Pak* 2008; 18:154-157.

[22] Alo MN, Anyim C, Onyebuchi AK, Okonkwo EC. Prevalence of asymptomatic Co-infection of Candidiasis and Vaginal Trichomoniasis among Pregnant Women in Abakaliki, South-Eastern Nigeria. *J Nat Sci Res* 2012; 2:87-91.

[23] Cheesebrough, M., 2006. District laboratory practice in tropical countries. Part 2. Cambridge University Press, pp: 434.

[24] Baker F. Handbook of bacteriological techniques. 2nd ed. Butterworth Co. ltd London; 1967.

[25] Nelson M, Wanjiru W, Margaret MW. Prevalence of vaginal Candidiasis and Determination of the Occurrence of Candida Species in Pregnant Women Attending the Antenatal Clinic of Thika District Hospital, Kenya. *Open J Med Microbiol* 2013; 3:264-272.

[26] Deepa VK, Vashe KV, Rashmi K, Harish S, Aparna R. Prevalence of Vaginal Candidiasis in Pregnancy among Coastal South Indian Women. *J Womens Health, Issues Care* 2014;3:6 DOI: http://dx.doi.org/10.4172/2325-9795.1000168

[27] Vroumsia T, Moussa D, Bouba G, Daniel EM, Ebot AC, Eneke T, Vikumitsi VA, Thomas N. Prevalence of Vulvovaginal Candidiasis amongst pregnant women in Maroua (Cameroon) and the sensitivity of *Candida albicans*to extracts of six locally used antifungal plants. *International Research Journal of Microbiology* 2013; 4(3):89-97.

[28] Meda N, Sangaré L, Lankoandé S, Sanou PT, Compaore PI, Catraye J, Cartoux M, Soudré RB. Pattern of sexually transmitted diseases among pregnant women in Burkina Faso, West Africa: potential for a clinical management based on simple approaches. *Genitourin Med* 1997; 73(3):188–193.

[29] Kamara P, Hylton-Kong T, Brathwaite A, Del Rosario GR, Kristensen S, *et al*. Vaginal infections in pregnant women in Jamaica: prevalence and risk factors. *International Journal of STD and AIDS* 2000; 11(8):516-20.

[30] Okonkwo NJ, Umeanaeto PU.Prevalence of Vaginal Candidiasis among Pregnant Women in Nnewi Town of Anambra State, Nigeria. *An International Multi Disciplinary Journal, Ethiopia* 2010; 4(4):539-548.

[31] Donbraye-Emmanuel OOB, Donbraye E, Okonko IO, Alli JA, Ojezele MO, Nwanze JC. Detection and prevalence of Candida among pregnant women in Ibadan, Nigeria. *World Applied Science Journal* 2010; 10(9):986-991.

[32] Anorlu R, Imosemi D, Odunukwe N, Abudu O, Otuonye M. Prevalence of HIV among women with vaginal discharge in a gynecological clinic. *Natl Med Assoc* 2004; 96(3): 367–371.

[33] Usanga VU, Abia-Bassey L, Inyang-etoh PC, Udoh S, Ani F, Archibong E. Prevalence Of Sexually Transmitted Diseases In Pregnant And Non-Pregnant Women In Calabar, Cross River State, Nigeria. *The Internet Journal of Gynecology and Obstetrics* 2010; 14(2).

[34] Nwadioha S, Egesie JO, Emejuo H, Iheanacho E. *Asian Pacific Journal of Tropical Medicine* 2010;3(6):483-485.

[35] Nelson M, Wanjiru W, Margaret MW. Prevalence of vaginal Candidiasis and Determination of the Occurrence of Candida Species in Pregnant Women Attending the Antenatal Clinic of Thika District Hospital, Kenya. *Open J Med Microbiol* 2013; 3: 264-272.

[36] Oyewole OA, Okoliegbe IN, Alkhalil S, Isah P. Prevalence of Vaginal Candidiasis among Pregnant Women Attending Federal University of Technology, Minna, Nigeria, Bosso Clinic. *Res J Pharm Biol Chem Sci* 2013; 4:113-120.

[37] Garcia HM, García SD, Copolillo EF, Cora EM, Barata AD, Vay CA, *et al*. Prevalence of vaginal candidiasis in pregnant women. Identification of yeasts and susceptibility to antifungal agents**.** *Revista Argentinade Microbiología* 2006; 38(1):9-12.

[38] Glatthaar E, Cartwright RY, Kunz J. Vulvovaginitis-congresses. Vulvovaginitis-Round Table Discussion: Conference Proceedings, National Library of Australia, 1982; pp37.