https://doi.org/10.46798/ijam.2019.v22i02.001

Indian Journal of Applied Microbiology
ISSN (Online): 2454-289X, ISSN (Print): 2249-8400
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Volume 22 Number 2
July - September 2019, pp. 1-11

Prevalence of Vaginal Candidiasis among pregnant women attending Ganesh Das Government Maternity and Child Health hospital, Shillong, Meghalaya, India

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Abstract: Vaginal Candidiasis infections are highly frequent in developing countries and their prevalence varies according to the geographical area and environmental conditions. **Background:** The study was undertaken to study the prevalence of vaginal candidiasis among pregnant women attending a tertiary care hospital. **Materials & Methods:** A total of 152 clinical samples were collected from patients attending antenatal care. A portion of each sample was examined microscopically and the remaining samples were cultured on Sabouraud's dextrose agar plates and identified using HiCrome™ Candida Differential Agar (HiMedia). An antifungal sensitivity test was performed using Mueller Hinton agar supplemented with 2% dextrose with 0.5 ug/ml methylene blue and the antifungal drugs used were Amphotericin B, Fluconazole and Ketoconazole. **Results:** Out of the 152 samples, 110 (72.37%) were culture positive for Candida, 85 (55.92%) by KOH. Among the Candida species isolated, *Candida albicans* was the most recurrent (64.55%) cause of infection. The antifungal sensitivity profile was found to be satisfactory. **Conclusion:** Proper diagnosis of vaginal *Candida* infections should be made with laboratory confirmation followed by antifungal sensitivity tests to reduce antimicrobial resistance.

Keywords: Vaginal Candidiasis, *Candida*, prevalence, pregnancy

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Introduction

Vaginal Candidiasis is a fungal infection caused by the *Candida* species. *Candida* is a fungus known to be a normal inhabitant of the skin, urinogenital and gastrointestinal tracts, which is asymptomatic. However due to many risk factors such as pregnancy, stress, diabetes, hormonal imbalance, antibiotic drug abuse, unhealthy dietary practices, underlining diseases such as HIV/AIDS which weaken the immune system and tight clothing can lead to vaginal candidiasis. Until recently, the incidence of vaginal candidiasis was often taken ignored as an insignificant pathology among the female population [1]. The commonest organism implicated is Candida albicans, and the predisposing factors include; prolonged or repeated use of antibiotics, steroids hormone medication, hormone replacement therapy (HRT), contraceptive and change in the mucous lining of the vagina could encourage Candida to flourish [2].

Pregnancy is a dynamic state that normal foetal development needs the availability of essential nutrients such as glucose, free fatty acids, long-chain polyunsaturated fatty acids, amino acids, minerals, and vitamins are to be continuously supplied to the growing fetus despite intermittent maternal food intake [3,4] Pregnant women are more vulnerable to VVC than healthy women with chronic recurrent candidiasis [5]. The infection can be acute, chronic, superficial or deep and has a broad clinical spectrum. The increased estrogen 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 [6].

There is a balance between *Candida*, normal bacterial flora, and body immune defense mechanisms. When this balance is disturbed, normal colonization is replaced by pathological infection. There may be multiple mechanisms by which *Candida* can cause cell damage and lead to a direct invasion of hyphae in epithelial tissues. During vaginal candidiasis, the vagina is in the normal pH range (pH 4- 4.5), as opposed to mixed infections (bacterial, *Trichomonas*), where pH levels rise [7].

Due to variable clinical presentations of *Candida* infections, it becomes very important to identify these pathogens from all the clinical specimens received at the laboratory irrespective of symptoms and clinician's suspicion. *Candida* species differ in their antifungal susceptibility and virulence factors [8]. Thus identification of *Candida* up to species level along with antifungal susceptibility becomes very essential in treatment modalities. Recent studies show an increase in the number of cases resulting from infections with non-Candida *albicans* (NCA) species and an increase in antifungal resistance [9]. The three most common types of vaginal infections in adult women are; vaginal candidiasis, trichomoniasis, and non- specific vaginitis [10]. Moreover, candidiasis may be caused by different species of Candida which include; *Candida albicans, C. glabrata, C. tropicalis, C. krusei*. *Candida albicans* is both the most frequent colonizer and responsible for many cases of Vaginal Candidiasis. Nevertheless, over the last decades, there have been reports stating an increase in the frequency of cases caused by non-albicans species. The most prominent is *Candida glabrata*. The only well-proven factors are diabetes mellitus, pregnancy and the use of broad-spectrum antibiotics as well as oral contraceptives with high estrogen content [11].

In North-eastern India, very few studies have been implicated in the prevalence and therapeutic consideration of VVC. Studies have reported that vaginal discharge is one of the few problems that one in three women wants a consultation [12]. However, there has been a drastic increased in opportunistic yeast infections, especially in immune-compromised patients [13]. Therefore, the current study was focused to understand the importance of agents causing VVC and helped to understand the prevalence of this disease among pregnant women in Meghalaya.

Materials and Methods

Source of data and Setting

The study data was collected from pregnant women attending Ganesh Das Hospital, Shillong from December 2018 to February 2019. Ganesh Das Mother and Child Hospital in Shillong is a state government-run women and child hospital under the Directorate of Health Services (DHS), Government of Meghalaya.

Sample Population

A total 152 patients of suspected cases of vaginal discharge, attending Obstetrics & Gynaecology OPD (ANC unit) were included in the study. A detailed history was taken with particular reference to name, age, and address, OPD no., presence of predisposing factors, onset and duration of complaints, treatment taken. All patients were asked about a standard questionnaire on their symptoms (vaginal discharge, vulvovaginal itching, vulvovaginal burning sensation).

Ethical Consideration

Informed verbal consent was obtained from the participants before proceeding to the questionnaire and specimen collection and ethical approval was taken from the hospital ethical committee of Ganesh Das Hospital, Directorate of Health Services (MI), Reference Number:No.29(B)/work-exp/GDH/2018/14315 dated 10/12/12018)

Collection of clinical samples and processing

Two vaginal swabs were collected from posterior formix of each pregnant women with the help of gynaecologists from the antenatal unit of the hospital. The first swab was used for Wet mount and Amine Test. The second swab was used for culture on Sabouraud dextrose agar (SDA). The collected samples were immediately brought to the laboratory and processed according to standard methods at the Department of Clinical Microbiology, Pasteur Institute, Shillong.

The first swab was rolled on the clean, dry, grease-free new slide and a drop of 10% KOH was added to it. The slide was held close to the nose to detect the amine odour. If fishy smell noticed then the sample was considered as positive. The specimen would be quickly become odourless upon standing so the odour should be noted as soon as possible. Using the same swab, vaginal secretions were taken on a clean glass slide and a drop of normal saline added, mounted with a coverslip. The number of pus cells and clue cells were counted in microscopy. The remaining portion of each clinical sample was cultured on Sabouraud Dextrose Agar (SDA) plate. The plates

were then incubated at 37ºC for 24 – 48 hours. The significant growth was observed and colony morphology was noted. Grams staining were performed. If the yeast cells were seen in staining then the identification was carried out.

Isolates from Sabouraud Dextrose Agar was inoculated on HiCrome^{TM} Candida Differential Agar (HiMedia) and incubated at 37^{o}C for 48 hours. Identification was done based on the colour of each colony as instructed by HiMedia Company. Using this method, the *Candida* species were identified as *C. glabrata* (cream to white colonies), *C. tropicalis* (blue colonies), *C. albicans* (light green colonies) *and C. krusei* (purple colonies).

Antifungal susceptibility testing was done by the disk diffusion method, was performed according to the standard guidelines. The medium used for the disk diffusion test was Mueller-Hinton Agar supplemented with 2% dextrose and 0.5 ug/ml methylene blue. The antifungal discs used in the test were Amphotericin B (20ug), Fluconazole (100ug) and Ketoconazole (10ug). Sensitivity is based on the zone of inhibition. Interpretation on Susceptibility and Resistance of the Antifungal discs used were determined based on the Hi-media charts.

Results:

In the present study, a total of 152 clinical samples were collected from pregnant women of which the prevalence rate of positive isolates of Candida among pregnant women was found to be (110/152) 72.368%.

Table 1: Percentage of isolated *Candida* among pregnant women

Number	Positive	Negative
	110	42
%	72.4	27.6

Frequency distribution of Candida isolates

A total of 152 samples were processed of which 110 isolates were isolated and identified as *Candida* isolates and the rest of them were not identified due to insignificant growth and bacterial colonies were not included in the present study. Of 110 Candida isolates, 71 (64.55%) were *C. albicans*, 28 (25.45%) were *C. glabrata*, 7 (6.36%) were *C. tropicalis* and 4 (3.64%) were *C. krusei.* Among all Candida isolates, *C. albicans* was found to be a predominant organism to cause candidiasis followed by *C. glabrata.*

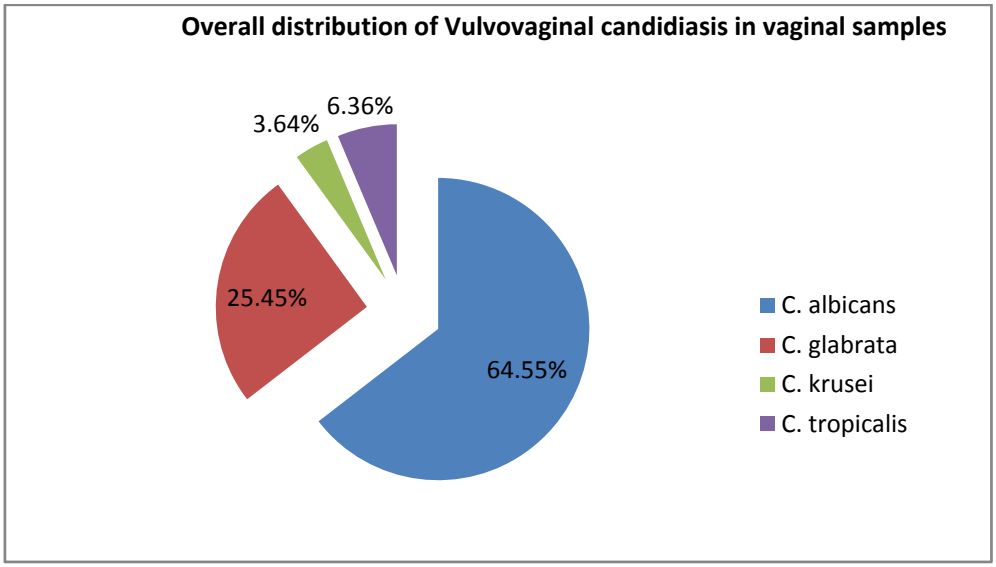


Figure 2: Distribution frequency of various Candida species among vaginal specimens.

Table 2: Percentage of *Candida* species isolated from vaginal swabs.

Isolated species	No. of Candida sp. positives	%
Candida albicans (light green colonies)	71	64.55%
Candida glabrata (cream to white colonies)	28	25.45%
Candida tropicalis (blue colonies)	7	6.46%
Candida krusei (purple colonies)	4	3.64%
Total	110	

Table 3: Percentage of *Candida* isolates among different Age groups

Age group	Total No. of vaginal samples	Number of samples positive *with Candida sp.*	Percentage (%)
16-25	82	60	73.17
26-35	60	45	75
36-45	10	5	50

Antifungal susceptibility testing:

Overall, all the *Candida species* demonstrated the highest susceptibility towards Amphotericin B 110 (100%), followed by Fluconazole 102 (92.73%), Ketoconazole 98 (89.09%). The resistance was seen for Ketoconazole and Fluconazole each with 1 (0.91%)

Antifungal tested		***Candida species*** (no.)								Total	
		Candida albicans (71)		***Candida glabrata (28)***		***Candida krusei (4)***		***Candida tropicalis (7)***			
		No.	***%***	***No.***	***%***	***No.***	***%***	***No.***	***%***	***No.***	***%***
Amphotericin B	S	71	100%	28	100%	4	100%	7	100%	110	100%
	DD	0	0%	0	0%	0	0%	0	0%	0	0%
	R	0	0%	0	0%	0	0%	0	0%	0	0%
Ketoconazole	S	64	90.14%	23	82.14%	4	100%	7	100%	98	89.09%
	DD	6	5.71%	5	17.86%	0	0%	0	0%	11	10%
	R	1	0.95%	0	0%	0	0%	0	0%	1	0.91%
Fluconazole	S	66	92.96%	25	89.29%	4	100%	7	100%	102	92.73%
	DD	5	4.76%	2	7.14%	0	0%	0	0%	7	6.36%
	R	0	0%	1	3.57%	0	0%	0	0%	1	0.91%

TABLE 3: Antifungal susceptibility pattern of the *Candida species* isolated

S= susceptible, DD= dose-dependent, R= resistant

Table 3: Sensitivity of different methods used to detect *Candida* species

Method	Positive samples	Percentage
Wet mount	85	77.27
Gram stain	109	99.09
Culture	110	100

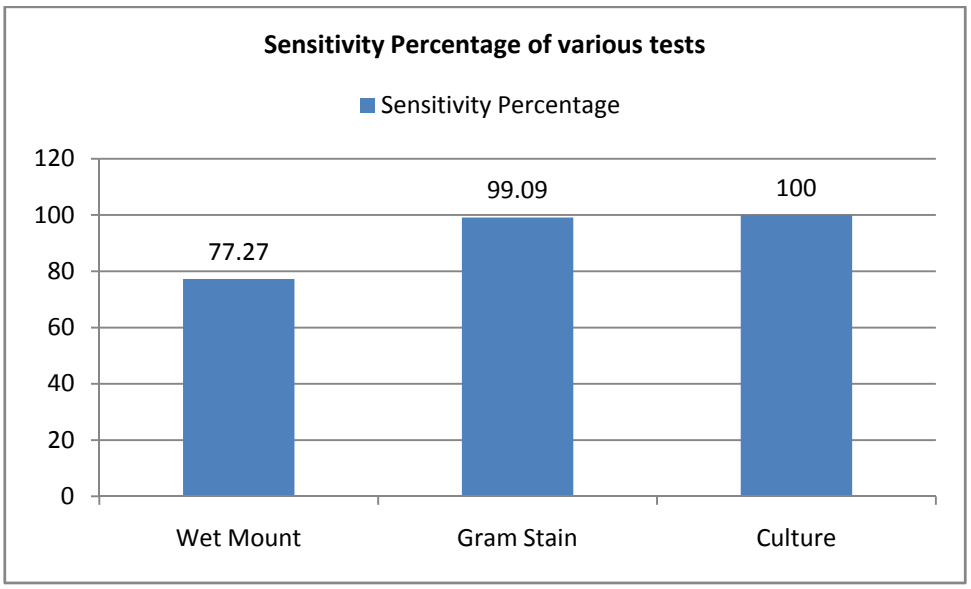


Figure 3: Sensitivity Percentage of various tests

Figure 4: Candida on SDA

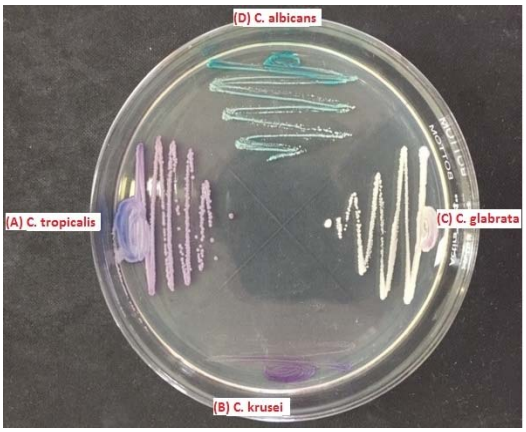
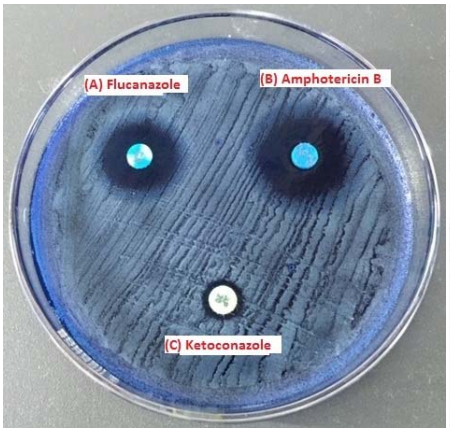


Figure 5: Candida on Candida Differential agar



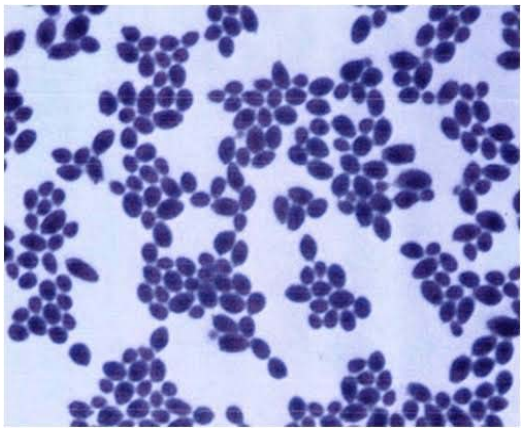


Figure 6: Antifungal Sensitivity Test **Figure 7: Gram staining of Candida**

Discussion:

Vulvovaginal candidiasis in pregnant women is usually ignored in our country. The data on vaginal *Candidiasis* and the identification of different species among pregnant women in 11 developing countries is very scanty [14]. Out of the total 110 positive samples analyzed for Wet mount examination, a total of 85 (77.27%) were found to be positive for *Candida* species. Certain bacteria such as Lactic acid bacteria and other normal flora were also seen but they are excluded from the study. No *Trichomonas* parasite was seen in all the 152 samples. This can be due to the late processing of a few samples due to transportation delay from Ganesh Das Hospital to the Pasteur Institute Microbiology laboratory. No clues cells were seen which indicates the absence of bacterium *Gardnerella.*

The present study had revealed the high prevalence rate of vaginal candidiasis among the appropriate symptomatic patients with an incidence rate of 72.37% (110/152). The observation in this study is consistent with reports that are reported in Saudi Arabia (70.2%) [17] but more than that in Nigeria (62.2%) [11], Tanzania (42.9%), [15], Libya (43.8%) [16]. This high frequency was attributed to suppression in the immunity of the body as a result of pregnancy which leads to disturbance in the balance between yeast and lactobacillus bacteria and the resultant proliferation of yeast leading to the occurrence of the disease [18,19]. It has been reported in the current study that the highest frequency of vaginal candidiasis cases was among the age group 26-35 years (75%). Among all *Candida* isolates, *C. albicans* (64.55%) was found to be the predominant fungal organism to cause candidiasis followed by *C. glabrata* (25.45%).

C. albicans was the most frequent species isolated in 64.55% from the pregnant women in this study while the rest were NAC species 35.45%. Studies from Northern Nigeria, have reported prevalence rate of *C. albicans* in 41% and 60% of high vaginal smears, respectively [20, 21]. Studies from India indicated that *C. albicans* was present in 74.4% while 25.6% were NAC

species [22]. *C. albicans* adheres to vaginal epithelial cells in significantly higher numbers than do other Candida species [23, 24]. This could explain the relative infrequency of the later in vaginal candidiasis. Another study from India have reported a rising trend in the isolation of NAC vaginitis, which was attributed to the indiscriminate use of anti-mycotic agents which eliminates the more sensitive *C. albicans* and selects resistant NAC species [25].

Conclusion

There is a high prevalence of VVC (72.37%) among pregnant women in our institution and found to be common in the age group of 26–35 years. Considering this high prevalence of 72.37% of VVC among pregnant women in this study, there is a need to educate the women on personal hygiene, and incorporate routine screening for candidiasis in our ANC unit to identify and treat symptomatic women.

Acknowledgment

I wish to express my deep sincere gratitude to Pasteur Institute for providing me the facilities to carry out this project work successfully in the Department of Clinical Microbiology and also to Assam Don Bosco University for their continuous support.

Conflict of Interest: NIL

References:

1. Sobel J D, Faro S, Force R W (1998): Epidemiologic diagnostic and therapeutic considerations, American Journal of Obstetrics and Gynaecology; 198: 203-11.
2. Jombo G T A, Opajobi S O, Egah D Z, Banwat E B, and DenenAkaa, P. (2010). Symptomatic Vulvovaginal Candidiasis and Genital Colonization by Candida species in Nigeria Public Health and Epidemiology. 2(6): 147-151.
3. Butte NF (2000). Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. American Journal of Clinical Nutrition; (71): 1256-1261.
4. Prakash S, Yadav K, Bhardwaj B, Chaudhary S (2015). Incidence of Anaemia and its Socio-demographic determinants among pregnant women attending for antenatal care: A cross sectional study. Int. J. Med. Health Res.1(3): 12-17.
5. Mitchell H (2004). Vaginal discharge- Causes, Diagnosis and Treatment. Journal of Clinical Pathology, BMJ; 328(7451): 1306-1308.
6. Parveen N, Munir A A, Din I, Majeed R (2008). Frequency of vaginal candidiasis in pregnant women attending routine antenatal clinic. Journal of College of Physicians and Surgeon Pakistan; (18): 154-157.

7. Mahmoudi R M, Zafarghandi S, Abbasabadi B (2011). The epidemiology of Candida species associated with vulvovaginal candidiasis in an Iranian patient population, European Journal of Obstetrics Gynaecology and Reproductive Biology; 155: 199–203.

8. Manchanda V, Agarwal S, Verma N (2011): Yeast identification in routine clinical Microbiology laboratory and its clinical relevance. Indian Journal of Medical Microbiology; 29(2):172-7.

9. Jones J M (1990) Laboratory Diagnosis of Invasive Candidiasis. Clinical Microbial Review. 3:32-45.

10. Nwankwo, E.O.K., Y.T. Kandakai-Olukemi, and S.A. Shuaibu (2010). Aetiologic agents of abnormal Vaginal Discharge among females of reproductive agein Kano, Nigeria. Journal Med Biomed Sci 3: 12-16.

11. Akah, P A., Nnamani, C E. and Nnamani, P O. (2010). Prevalence and treatment outcome of Vulvovaginal Candidiasis in pregnancy in a rural community in Enugu State, Nigeria Medicine and Medical Sciences. 1(10); 447-452.

12. Sobel J D (1985). Epidemiology and pathogenesis of recurrent vulvovaginal candidiasis. American Journal of Gynaecology; (152): 924-935.

13. Richardson M and Warnock D (2003). Fungal Infection, Diagnosis and treatment, 3rd edition, Blackwell Publishing, London, pp. 38-43.

14. Kanagal DV, Vineeth VK, Kundapur R, Shetty H, Rajesh A (2014). Prevalence of Vaginal Candidiasis in Pregnancy among Coastal South Indian Women. Journal of Women's Health; 3(6):1-3.

15. Feyi P and Amadi A (2001). "The Prevalence and Pattern of Vaginal Candidiasis in Pregnancy in Abia," Journal of Medical Investigation and Practice; 2; 25- 27.

16. Altayyar I A, Alsanosi A S & Osman N A (2016).Prevalence of vaginal candidiasis among pregnant women attending different gynaecological clinic at South Libya .European Journal of Experimental Biology; 6(3):25-29.

17. Khadijah Y A, (2015). Prevalence of Vaginal Candidiasis among pregnant women attending Al-Hada Military Hospital, western region, Taif, Saudi Arabia. International Journal of Science and Research; 4(5): 1736-1743.

18. CDC, Centres for Disease Control and Prevention (2010): Sexually Transmitted Diseases Treatment Guidelines; 59(RR12):1-110.

19. Corsello, S, Spinillo, A, Osnengo, G et al (2003): An epidemiological survey of vulvovaginal candidiasis in Italy. European Journal Obstetrics Gynaecology Reproduction Biol; 110: 66–72.

20. Nwadioha S I, Egah DZ, Alao OO, Iheanacho E. (2010) Risk factors for vaginal Candidiasis among women attending primary health care centres of Jos, Nigeria. Journal of Clinical Medical Research; 2: 110-3.

21. Ibrahim SM, Bukar M, Mohammed Y, Audu BM, Ibrahim HM. (2013): Prevalence of vaginal candidiasis among pregnant women with abnormal vaginal discharge in Maiduguri. Niger J Med; 22: 138-42.

22. Jindal N, Gill P, Aggaarwal A (2007): An epidemiological study of VVC in women of childbearing age. Indian J Med Microbiol; 25: 175-6.

23. Simoes JA, Giraldo PC, Faundes A. (1998): Prevalence of cervicovaginal infections during gestation and accuracy of clinical diagnosis. Infect Dis Obstet Gynecol; 122-33.

24. Payne MS, Cullinane M, Garland SM, Tabrizi SN, Donalth Sm, Bennett CM, et al. (2016): Detection of Candida spp. In the vagina of a cohort of nulliparous pregnant women by culture and molecular methods: Is there an association between maternal vaginal and infant oral colonization? ANZJOG; 5: 179-84.

25. Pirotta MV, Gunn JM, Chondros P. (2003) “Not thrush again!” Women’s experience of post-antibiotic vulvovaginitis. Med J Aust; 179: 43-6.