Putative virulence factors of *Candida* species colonising asymptomatic pregnant Jordanian women

Mohammad A Abu-Lubad, Ghada F Helaly, Amin A Aqel, Dua'a A Jarajreh, Ahlam M Al-kharabsheh, Seham M Abufraijeh, Eman M Albataineh and Nedal A Alnawaiseh

ABSTRACT

Objectives: Pregnant women are frequently exposed to yeast colonisation and infection compared to non-pregnant women. This study was undertaken to investigate carriage rates of *Candida* species and their virulence factors in high vaginal samples from asymptomatic pregnant women as a probable predisposing factor for neonatal candidiasis.

Methods: High vaginal swabs were collected from 200 pregnant women from Al-Karak Governmental Hospital, Jordan from March to December 2018. *Candida* isolates were identified by their growth on CHROMagar *Candida* and Vitek2 automated system. Some virulence factors were determined.

Results: Among 200 swabs tested, 67 (33.5%) yielded *Candida* isolates as follows: *C. ablicans*, 28 (41.8%), while non-*albicans Candida* (NAC) were 39 (58.2%) isolates (p=0.04). *C. parapsilosis* was the most prevailed NAC species isolated, 29 (74.4%) followed by *C. tropicalis*: 6 (15.4%) while *C. glabrata* and *C. krusei*, each represented 2 (5.1%). Among *C. albicans* isolates, 27 (96.4%), 18 (64.3%), and 24 (85.7%) compared to 24 (82.7%), 4 (13.8%), and 25 (86.2%) among *C. parapsilosis* were positive for proteinase, phospholipase, and haemolysin respectively. The protease activity was the highest detected (88.1% of *Candida* isolates) compared to other enzymes but did not reach statistical significance between *Candida* species (p=0.215). Phospholipase activity was significantly detected among *C. albicans* isolates compared to other species (p <0.001). Insignificant differences in haemolysin production was observed among isolates (p= 0.134).

Conclusions: In Jordanian pregnant women, NAC, mainly *C. parapsilosis,* were the most frequently isolated *Candida* species from high vaginal swabs. Routine screening and treatment are recommended for pregnant women, irrespective of symptoms.

Keywords: *Candida albicans*; virulence factors; *C. parapsilosis*; asymptomatic pregnant women; non- *Candida albicans*.

INTRODUCTION

The anatomical structure of the female genital tract provides a suitable environment for many pathogenic microbiota that colonise the vulvovaginal area. These microorganisms may either cause multiple infections or persist for years asymptomatically, especially opportunistic fungi (1, 2). *Candida* spp. are the most common opportunistic pathogens that colonise the vaginal tract in 20% of healthy asymptomatic women in childbearing age and up to 30% of pregnant women (3). Their colonisation and overgrowth causes vulvovaginal candidiasis which is associated with inflammation of the vagina and/or vulva, vaginal discharge, vaginal and vulvar pruritus, pain, burning, itching, and erythema (4). In fact, around 75% of all adult women experience at least one episode of vulvovaginal candidiasis, 50% of them are predisposed to recurrence (5) .

Candida infections impose a serious public health burden and socioeconomic challenge. Despite that more than 17 different *Candida* species are known, the vast majority of invasive infections are caused by *Candida albicans* and non-albicans *Candida* (NAC) species, including *C. glabrata*, *C. parapsilosis*, *C. tropicalis,* and *C. krusei* (6). They can asymptomatically colonise the gastrointestinal tract, oral cavity, and reproductive tract of healthy individuals. Asymptomatic colonisation of *Candida* spp., under certain conditions, may cause superficial infections such as oropharyngeal and vulvovaginal candidiasis up to systemic infections such as fungemia and invasive candidiasis(7,8). Several virulence factors are involved in establishment of *Candida* infections and permit *Candida* colonisation and invasion. These factors facilitate the adherence to target tissues, hyphal morphology, drug resistance, and cell lysis which include the exoenzymes aspartyl proteinase, phospholipase, and haemolysins (9).

Aspartyl proteinase facilitate adhesion, invasion and tissue damage, while phospholipase mediates host cell membrane damage (10). Lysis of red blood cells by the haemolysin enzyme plays a role in fungal survival and facilitates hyphal invasion during disseminated candidiasis which is coupled with iron uptake (11).

The predisposing factors that might increase establishment of vulvovaginitis are consumption of contraceptives and broad spectrum antibiotics, uncontrolled diabetes, immuno-suppressive drugs, and pregnancy (1,12). The increase in development of vulvovaginal candidiasis in asymptomatic pregnant women, in contrast to non-pregnant women, is influenced by alteration of vaginal pH and decrease in antibody secretion in the vagina by gestational hormones (i.e. estrogen) which result in higher glycogen content in the vagina that serves as a carbon source for *Candida* species colonisation (12,13). Furthermore, stage of pregnancy and number of gravid influence the development of infection(14). During pregnancy, untreated vaginal colonisation of *Candida* is associated with certain complications, including delivery complications, preterm birth, low birth weight, chorioamnionitis, congenital cutaneous candidiasis, and systemic infections in the neonates leading to 25-35% mortality rates (13,15).

To date, two studies have focused on isolation of yeasts colonising the vagina of females residing in the central part of Jordan (16,17), without determining the associated virulence factors. Our study is the first one that addressed the determination of the carriage rates of *Candida* species in high vaginal samples from asymptomatic pregnant women, visiting prenatal clinic in AL-Karak Hospital in South Jordan. In addition, production of putative virulence factors by isolated *Candida* species was investigated. Our study aimed to provide

grounds to establish prenatal screening protocols for candidiasis in asymptomatic pregnant women and development of treatments regimes that reduce the rates of abortions and pre-term birth among vulvovaginal candidiasis infected women.

MATERIALS AND METHODS

Study design and sample collection
A cross-sectional study was conducted from March to December 2018.A total of 200 samples were collected from asymptomatic pregnant women during their regular antenatal visit to the gynaecologic clinic of the Al-Karak governmental hospital, south of Jordan. Vaginal samples were collected by the gynaecologist from pregnant women using a sterile cotton swab moistened with normal saline that were inserted and rotated gently to pick up the specimen. The age groups of included women and samples numbers were 20-26 years (65 samples, 32.5%), 27-33 years (75 samples, 37.5%), and 34-45 years (60 samples, 30%). Socio-demographic data, antenatal visits, and pregnancy complications were obtained from the study participants using structured questionnaires after informed consent was obtained from each participant.

Inclusion criteria
Inclusion criteria for the participants were all at the 35th week of gestation, healthy, not diagnosed with vulvovaginal candidiasis, gestational diabetes mellitus, any kind of immune deficiency, no history of previous preterm labour or spontaneous abortion, and not under medications or currently on treatment for antifungal therapy.

Ethical approval
This study approved by the Ethics Committee at the Faculty of Medicine, Mutah University, Jordan according to the institutional ethical considerations and guidelines.

Culture and identification of *Candida* species:
Vaginal swabs were inoculated on Sabouraud Dextrose Agar (SDA) (Oxoid, UK), and incubated at 37°C for 48 h. The grown creamy colonies were selected, re-cultured on SDA plates for purification, and identified by the automated VITEK2 compact system (bioMérieux, Marcy-l'Etoile, France). A suspension of each purified colony was plated on CHROMagar *Candida* (Oxoid, Ltd, Basingstoke, UK), a selective medium for *Candida* species. Isolation and identification was based on colony colour and morphology. Reference standard strains of *C. albicans* (ATCC 60193), *C. glabrata* (ATCC 22553), *C. parapsilosis* (ATCC 22019), *C. krusei* (ATCC 34135), and *C. tropicalis* (ATCC 1369) were included and cultured on CHROMagar medium as positive controls (Figure 1).

Figure 1. The appearance of different reference *Candida* species on the CHROMagar: green colonies of C. *albicans* (1), metallic blue colonies of *C. tropicalis* (2), fuzzy pink of C. *krusei* (3), mauve brown colonies of C. *glabrata* (4), and cream to white smooth colonies of *C. parapsilosis* (5).

Detection of virulence factors
Inoculum preparation: A suspension of 0.5 ml of McFarland yeast cells was made from SDA and incubated for 18 hours at 37ºC in brain heart fusion broth (BHI). The cells were standardised to approximately 10^6 CFU/ml at 530 nm wavelength according to the Clinical and Laboratory Standards Institute (CLSI) criteria (18) .

Production of aspartyl proteinases: The ability of collected *Candida* species to form a clear halo zone around their grown colonies on bovine serum albumin (BSA) agar was indicative on the extracellular production of aspartyl protease (19) . Briefly, 5µl yeast suspension from an 18hr culture containing 10^6 cells/ml were spot inoculated onto 1% BSA agar plate and incubated for five days at 37ºC. After which the plates were flooded with 1.25% naphthalene black solution in 90% aqueous methanol for 15 min and decolorised for a further 36 hr with several changes using the latter solution. Each isolate was tested in triplicate. The halo zone (Hz) value was scored which represents the ratio of the colony alone to the diameter of the colony plus clear zone. Based on Hz values, aspartyl proteinase protein activities were classified into four categories: Hz=1(negative); 0.9-0.99 (+1), very low; 0.80-0.89 (+2), low; 0.70-0.79 (+3), high; and < 0.70 (+4), very high according to Price *et al.*(20) Reference strains of *C. albicans* (ATCC 10231) served as a positive control for aspartyl proteinase protein activity.

Production of haemolysin: The haemolysin activity was evaluated as previously described (10,11) . In brief, Sabouraud dextrose-enriched sheep blood agar was spot inoculated with 10µl of 10^8 cells/ml yeast suspension to yield a circular inoculation site of about 5 mm in diameter. Each isolate was tested in triplicate. The haemolytic activity was determined by the presence of a translucent halo zone around the inoculum. The haemolytic index (HI) was used to represent the intensity of the haemolysin production by dividing the total diameter of the colony plus the translucent halo zone over the diameter of the colony. The strains were classified according to the HI as a negative (HI=1.00), positive (1.00<HI<1.5), or strongly positive (HI>1.5). Reference strains of *C. albicans* (ATCC 32354) served as a positive control for the haemolysin activity.

Production of phospholipase: Production of phospholipase by *Candida* species was assessed by the egg yolk agar method (20). Egg yolk agar plates comprising 65g SDA, 1M NaCl, 0.005 M $CaCl_2$, and 8% sterile egg yolk emulsion (Oxoid) were spot inoculated with 1µl containing 10^5 CFU/ml and incubated at 37°C for five days. Each isolate was tested in triplicate. The diameter of the colony and a total diameter of colony and precipitation zone (Pz) were measured, and phospholipase activity was scored as previously in the aspartyl proteinase protein test. *C. albicans* (ATCC 90028) served as a positive control for phospholipas production.

Data analysis
All data were analysed using the Statistical Package for the Social Sciences (SPSS) software, version 18.0. Study variables (frequencies and percentages) were statistically analysed using chi-square test ($\chi2$). *p*-values <0.05 were considered statistically significant.

RESULTS

Distribution of *Candida* species within different age groups of the studied asymptomatic pregnant women
Among a total number of the 200 vaginal swabs, 67 (33.5%) samples tested positive for *Candida species*. *C. albicans* was detected in 28 samples (41.8%), while NAC species were isolated from 39 samples (58.2%). *C. parapsilosis* accounted for 43.3% of the total isolated *Candida* species and 74.4% of the NAC isolates. Most of the vaginal *Candida* species were isolated from the pregnant women at the age group 34-45 years with a total of 30 (44.8%) isolates, followed by 24 (35.8%)

isolates at the age group 27- 34 years, while the least number of *Candida* isolates were at the age group 20 - 27. *C. albicans* was the most isolated vaginal *Candida* species in the age group 27-34 years (66.7.1%), while *C. parapsilosis* dominated among other species within the age group 34-45 years (60%). *C. Krusei* and *C. glabrata* were the least isolated *Candida* species within the different age groups (Table 1).

Aspartyl-proteinase and phospholipase activity among isolates of different *Candida* species

Proteinase and phospholipase activities were detected in 59 (88.1%) and 26 (38.8%) out of 67 *Candida* spp. isolates respectively (Table 2). Out of the 28 tested isolates of *C. albicans*, 27 (96.4%) showed proteinase activity, while among the 39 tested NAC species, 32 (82.1%) positive results were found as follows: 24/29 *C. parapsilosis* isolates (82.7%), 4/6 *C. tropicalis (*66.7%), and four isolates of *C. glabrata* and *C. krusei* were found to be proteolytic. The *C. albicans* isolates were the main producers of phospholipase18/28 (64.3%). Half of isolates of *C. krusei* (1/2 cases) and *C. tropicalis* (3/6 cases) were phospholipase producers. *C. parapsilosis* showed positive activity for phospholipase but with a lower percentage, 4/29 (13.8%). No phospholipase activities were detected in *C. glabrata* isolates. The activity scoring of the produced proteinase and phospholipase virulence factors is shown in Table 2.

Considering the scoring of proteinase activity (*Hz), a large number of *C. albicans* (25/27) and *C. parapsilosis* (21/24)

isolates exhibited an enzymatic activity considered very high (+ + + +). Furthermore, all the *C. krusei, C. tropicalis,* and *C. glabrata* producers showed very high proteinase activity. Considering the scoring of phospholipase activity (*Pz), a considerable number of *C. albicans* (11/18) isolates exhibited an enzymatic activity considered very high (+ + + +). The protease activity was the highest detected (88.1% of *Candida* isolates) compared to the other enzymes but did not reach statistical significance between *Candida* species (χ^2=5.6, *p*=0.215). On the other hand, phospholipase activity was significantly detected among *C. albicans* isolates compared to other species (p <0.001).

Haemolysin activity among isolates of different *Candida* species

Among the 67 isolates of *Candida* spp., 54 (80.6%) were haemolysin producers (Table3). Regarding the 28 *C. albicans* isolates, 24 (85.7%) showed strong haemolysin activity compared to 4 (14.3%) isolates that were haemolysin negative. On the other hand, haemolysin production among the NAC species was detected in 30/39 (76.9%). Twenty-five (86.2%) out of 29 isolates of *C. parapsilosis* and 50% (3/6) of *C. tropicalis* showed strong haemolysin activity. One case each from C. glabrata and *C. krusei* revealed haemolysin activity to a lesser extent. This difference in haemolysin activity among *Candida* species did not reach the statistical significance ($\chi2$=7.03; p= 0.134).

Table 1. Distribution of *Candida* species within different age groups of the studied asymptomatic pregnant women.

Candida species	Age groups (years)			Total N (%)
	20 - 27	27- 34	34-45	
C. albicans	5 (38.5%)	16 (66.7%)	7 (23.3%)	28 (41.8)
C. krusei	1 (7.7%)	1 (4.2%)	0 (0%)	2 (3)
C. tropicalis	1 (7.7%)	2 (8.3%)	3 (10%)	6 (8.9)
C. parapsilosis	6 (46.1%)	5 (20.8%)	18 (60%)	29 (43.3)
C. glabrata	0 (0%)	0 (0%)	2 (6.7%)	2 (3)
Total	13	24	30	67(100)

Table 2. The distribution of proteinase and phospholipase activity and their scoring of the precipitation zones among isolates of different *Candida* species.

Candida species (n)	Scoring of proteinase activity (*Hz) No of positive isolates (%)					Scoring of phospholipase activity (*Pz) No of isolates (%)				
	4+	3+	2+	1+	Negative	4+	3+	2+	1+	Negative
C. albicans (28)	25 (89.3)	0 (0)	2 (7.1)	0 (0)	1 (3.6)	11 (39.3)	3 (10.7)	3 (10.7)	1 (3.6)	10 (35.7)
C. krusei (2)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (50)	0 (0)	0 (0)	0 (0)	1 (50)
C. tropicalis (6)	4 (66.7)	0 (0)	0 (0)	0 (0)	2 (33.3)	1 (16.7)	0 (0)	2 (33.3)	0 (0)	3 (50)
C. parapsilosis (29)	21 (72.41)	0 (0)	2 (6.9)	1 (3.45)	5 (17.24)	2 (6.9)	1 (3.45)	0 (0)	1 (3.45)	25 (86.2)
C. glabrata (2)	2 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100)
Total	59 (88.1)				8(11.9)	26(39.0)				41(61.0)

*The value representing the ratio of the colony alone to the diameter of the colony plus formed zone. Score value=1 (negative); 0.9–1 (1+); 0.89–0.80 (2+); 0.79–0.70 (3+); ≤ 0.69 (4+).

Table 3. Distribution of haemolysin and its scoring among isolates of different *Candida* species.

Candida species (n)	Scoring of haemolysin activity (*HI) No of isolates (%)		
	Strongly positive	**Positive**	**Negative**
C. albicans (28)	24 (85.7)	0 (0)	4 (14.3)
C. krusei (2)	0 (0)	1 (50)	1 (50)
C. tropicalis (6)	3 (50)	0 (0)	3 (50)
C. parapsilosis (29)	24 (82.8)	1 (3.4)	4 (13.89)
C. glabrata (2)	0 (0)	1 (50)	1 (50)
Total	51 (76.1%)	3 (4.47%)	13 (19.4%)

*HI: The strains were classified according to the HI as negative (if HI=1.00), positive (if 1.00<HI<1.5) or strongly positive (when IH>1.5). The experiments were performed in triplicate and the results given as the mean of the values obtained.

DISCUSSION

Candida colonisation of pregnant women genital tract without association of any signs of infection (asymptomatic) can predispose neonates to invasive candidiasis with mortality rates of 25-35%. Therefore, implementation of screening strategies for early detection of *Candida* species and treatment of vaginally colonised women by yeast will reduce vulvovaginal candidiasis predisposing factors and facilitates early resolution of infection. Intriguingly, detection of *Candida* colonisation of the vagina in asymptomatic women is failed when relying merely on vulvovaginal candidiasis symptoms and microscopic examination of vaginal secretions, credibility of colonisation detection is highly dependent on positive culture of yeasts (21). In Jordan, very limited studies focused on the association of vulvovaginal candidiasis with *Candida* species; however, none of these studied the production of virulence factors. Therefore, the present study was carried out to evaluate the carriage rate of *C. albicans* and other NAC species among asymptomatic Jordanian pregnant women. Herein, the carriage rate of *Candida* species among asymptomatic pregnant women was 33.5%. Although it was assumed that colonisation with *Candida* species is 20% in asymptomatic adult women and might reach up to 30% during pregnancy (2,3,22,23), our finding is comparable with *Candida* prevalence rates reported in two previous studies from Jordan in 1997 and 2017 (22-40%) (16,24), 31.4%, in Italy (13), and 20-56% in different states of Nigeria (4,5) . On the contrary, it was relatively higher than reported from Argentina (21), Ethiopia (14), Australia (25), Bulgarian (26), and New York (27) with prevalence rates of 13.4%, 18.7%, 19.6%, 29%, and 29.4%, respectively.

Considering *Candida* species distribution in our study, *C. albicans* represented 41.8% and NAC species accounted for 58.2% of the total identified *Candida* species. *C. parapsilosis* accounted for 43.3% of all detected *Candida spp.* (representing 74.4% of NAC), 8.9% for *C. glabrata*, and *C. tropicalis* and *C. krusei* represented by 3%. In a similar study of asymptomatic pregnant women in Australia(25), a higher colonisation rate was observed for *C. albicans* (73%) and *C. glabrata* (14.3%), similar rates were observed for the prevalence *C. krusei* and *C. tropicalis* (3.1%). On the other hand, a much lower prevalence than in our study was observed for *C. parapsilosis* 5.1%). The prevalence in our study was also lower than that by Nurat *et al.* with 54.3% positive cases for *C. albicans*, 25.7% for *C. glabrata*, and 5.7% for *C. tropicalis* (5).

Compared to our study results, many discrepancies in *Candida* species detection and distribution were reported by previous epidemiological studies, including pregnant women, both symptomatic and asymptomatic. *Candida* were detected in 33.1% of Argentinean pregnant women, *C. albican* represents 86.4% and NAC 13.6% (21). In China 15% were *Candida*-positive, 79.9% were *C. albicans,* and 20.1 were NAC (3). 30-60.7% of Nigerian pregnant women were colonised with *Candida,* 73.8% were *C. albicans* and NAC accounts for 26.2% (4, 28). *Candida* species were isolated from 42% of pregnant women in Libya, *C. albicans* were isolated from 92% of patient samples and NAC were isolated from 8% of samples (29). 39-45% of Lebanese pregnant women were colonised with *Candida* species, 42-43% were *albicans* while 57-58% were NAC (2,22). in Egypt, the vaginal swab cultures revealed prevalence of *Candida* in 50.4% of patients with 60.3% being *C. albicans* (30). In a previous study from Jordan, *Candida* species were isolated from 68.2% of pregnant women, 60.7% were *C. albicans,* 14.3% were each of *C. glabrata* and *C. tropicalis*, 7.1% *C. krusei*, 3.6% *C. guilliermondii, and no C. parapsilosis* was detected (16) .

Compared to our study, *C. albicans* from vaginal samples in most of the above literate reports was the highly dominant isolated species followed by *C. glabrata*, *C. tropicalis*, *C. parapsilosis*, and *C. krusei*. Nevertheless, epidemiological data showed a mycological shift in regards to the vaginal colonisation toward the NAC species with incidence rate greater than 50% (31) . Variation in *Candida* species distribution between countries and increase in colonisation of NAC might be attributed to differences in geographic regions, sexual behaviours, culture, customs of different nations, differences in study design, target populations, risk factors, hygiene, disease history, contraceptives, prolonged antibiotic uses, and diagnostic methods (1,12,22).

Intriguingly, the higher incidence rates of the dominant *Candida* species in our study were for *C. albicans* and *C. parapsilosis* in the age groups of 27-33 and 34-45 years, representing 66.7 % and 60% of the totally isolated *Candida* species in each age group respectively. This is consistent with most studies that indicated that dominant *Candida* isolates were detected in the active reproductive and childbearing age periods of 26-45 years. (2,4,14)

The production of certain virulence factors determines the ability of *Candida* species to colonise and persist in the infection site. These virulence factors are hydrolytic enzymes that provide *Candida* species with nutrients through their extraction from the host cells (14,16,17,26,31,32) or facilitate their adhesion, tissue invasion, and blood dissemination; the most important hydrolytic enzymes being aspartyl proteinase, phospholipase, and haemolysin 32). Aspartyl proteinase protein makes colonisation easier through disrupting the integrity of the mucosal surfaces and interfering with the components of the immune response (33). Phospholipases aid in tissue damage

and invasion through their ability to hydrolyse host cell-membrane phospholipids to fatty acids, which help in exposing receptors to facilitate adherence of *Candida* (32,33). Aspartyl proteinase and phospholipase were produced by 88.1% and 38.8% of all *Candida* isolates, respectively. They were identified in 96.4% (27/28) and 64.3% (18/28) of *C. albicans* and in 82.7% (24/29) and 13.8% (4/29) of *C. parapsilosis*, respectively. The difference in protease production did not reach statistical significance among different *Candida* species. The phospholipase among the *C. albicans* isolates showed significant activity compared to the other species. These findings were higher than those in analogous reports from several countries(31,32) . However, haemolytic enzyme was found in 80.6% of all isolated species; 85.7% (24/28) and 86.2% (25/29) of *C. albicans* and *C. parapsilosis* were haemolysin positive respectively with no significant difference among *C. albicans* and NAC species. These results indicate the ability of all isolated *Candida* species in colonising their hosts through the ability to derange the host cell membrane and to extract iron from lysed red blood cells.

Altogether, our results revealed an increase in incidence rate of NAC species (58%) over *C. albicans* (42%) in Jordanian asymptomatic pregnant women. *C. parapsilosis* was the second most detected species with a comparable incidence rate as *C. albicans* (43%); and represented 72% of NAC species. This high detection rate of *C. parapsilosis* was inconsistent with most epidemiological studies, including Jordanian reports that indicated an increase in the incidence of NAC with *C. glabrata* as the second prevailed *Candida* species after *C. albicans* in asymptomatic pregnant women and women with vulvovaginal candidiasis and recurrent vulvovaginal candidiasis infections (2,3,14,16,17) . However, the high rate of *C. parapsilosis* incidence in asymptomatic pregnant women in our study unveil that its colonisation in the vagina is rarely associated with symptomatic vaginitis (5) .

A limitation of our study should be kept in mind, which is the lack of data about antifungal susceptibility profiles of the isolated strains as the different classes of antifungal drugs were not available during the research period. Consequently, the clinical relevance of our findings points out the need for further study for antifungal susceptibility testing for prompt treatment. Antifungal susceptibility testing could pave the way for further molecular studies to determine the origin of resistance, if any.

In conclusion, the significant isolation of *C. albicans* and NAC from Jordanian asymptomatic pregnant women raises the risk of the development of *Candida* associated infections with increase in the possibility of pre-term birth and neonatal abnormalities. Therefore, we recommend a screening program for all pregnant women to monitor and treat probable vaginal candidiasis. The implementation of early screening, detection, and adequate antifungal treatment could help in improving pregnancy outcomes, minimise the health-care budget, and the burden on the health sector.

AUTHOR INFORMATION

Mohammad A Abu-Lubad, PhD, Associate Professor and Head of Department[1]
Ghada F Helaly, PhD, Professor[1,2]
Amin A Aqel, PhD, Professor and Vice Dean of Basic Affairs[1]
 Dua'a A Jarajreh, MBiol, Laboratory Supervisor[1]
Ahlam M Al-kharabsheh, MD, Assistant Professor and Head of Department[3]
Seham M Abufraijeh, MD, Assistant Professor[3]
Eman M Albataineh, PhD, Associate Professor[1]
Nedal A Alnawaiseh, PhD, Associate Professor[4]

[1]Medical Microbiology and Pathology Department, Faculty of Medicine, Mutah University, Al-Karak, Jordan
[2]Microbiology Department, Medical Research Institute, Alexandria University, Alexandria, Egypt
[3]Obstetrics and Gynecology Department, Faculty of Medicine, Mutah University, Al-Karak, Jordan
[4]Community Medicine and Public Health Department, Faculty of Medicine, Mutah University, Al-Karak, Jordan

Corresponding author: Dr. Mohammad Abu-Lubad.
Email: abu_lubbad@yahoo*.com* or abu_lubbad@mutah.edu.jo

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