Vulvovaginal candidiasis is the second most common cause of vaginitis worldwide (after bacterial candidiasis). Maternal vulvovaginal candidiasis is a major risk factor for Candida colonization and infection of the infant where prognosis depends on different predisposing factors. The aim of this study was to determine the incidence and the etiological structure of vulvovaginal candidiasis in pregnant women and its impact on Candida colonization of newborns.

Materials and methods: Samples of vaginal secretions from 80 healthy pregnant women who were clinically suspicious for Candida vaginitis were collected within 48 hours before delivery. Samples for probable Candida colonization from the oral mucosa and feces were collected from their newborns within 47-72 hours after birth. Samples were plated on Sabouraud agar, followed by species identification by API Candida yeast assay.

Results: Twenty-three (28.75 ± 5.06%) of the evaluated pregnant women were positive for *Candida* spp. Positive samples for *Candida* colonization were found in 18 (22.22 ± 4.62%) of the examined 81 newborns (one pair of twins) from mothers who were clinically suspicious for vaginal candidiasis. Isolates of the newborns were 100% identical to those of the mothers’ vaginal secretion. *Candida albicans* was the predominant species identified in the pregnant women (91.67 ± 0.06%) and in the neonates (83.33±8.78%).

### INTRODUCTION

Although vaginal infections are common in hospital and community acquired setting, they are often underestimated as a problem.⁴–⁶ Vulvovaginal candidiasis (VVC) is the second most common cause of vaginitis worldwide, after the bacterial one.⁴ In Plovdiv region (Bulgaria), it constitutes 23.79% of all cases of vaginosis, which makes it second in incidence to bacterial vaginosis (31.14%).¹

Apart from the negative impact on the reproductive function of the patients, a correlation between vaginal infections and perinatal morbidity and mortality in newborns has been found.¹,³ It is estimated that 75% of all women have at least one episode of vulvar and vaginal candidiasis in their live, 40-55% of them having at least another one.⁵ About 5-8% of women with vulvar and vaginal candidiasis develop the so-called recurrent vulvar and vaginal candidiasis, which is defined as presence of more than 4 episodes over the past 12 months.¹,⁵ The provoking relapse risk factor cannot be established in the majority of the cases.⁵
Age dependent are 5-30% of the cases with Candida colonization in the vulvovaginal tract, most commonly affecting the age of 21 to 30 years. Additional risk factors are hyperestrogenemia, hormonal imbalance, immune suppression after disease or psycho-emotional stress, hyperglycemia, antibiotic treatment, vaginal dysbacteriosis, oral contraceptives, intraterine device, spermicides, condoms and some habits of hygiene, clothing and sexual practices. If the risk for development of vulvovaginal candidiasis for healthy population is approximately 20%, it increases by 30% during the third trimester of pregnancy. Therefore, the mother’s vulvovaginal candidiasis is a major risk factor for Candida colonization of the newborn. Approximately 70-85% of these patients contaminate their newborns ascending during the pregnancy or transmitting, within the act of giving birth, while only in 22% of the newborns acquired colonization by the medical staff within the first days after birth. The asymptomatic Candida carriage among the medical staff is as high as 30%. Colonization of the infant with Candida yeasts can be the first step towards the development of Candida infection whose course and prognosis depend on a number of predisposing factors. It is believed that the vertical transmission of yeasts during the act of birth has a major role in the colonization of the newborns in the first days of life. Complementary role for the subsequent development of clinically manifested infection have the peculiarities of his immunity, the possibility of intestinal translocation, low and extremely low birth weight, long-term antibiotic broad-spectrum treatment, invasive procedures, long lasting parenteral nutrition, central venous catheters and previous episodes of mucous-cutaneous or chronic vaginal candidiasis during pregnancy.

**AIM**

Our aim was to determine the incidence and the epidemiological structure of vulvovaginal candidiasis in pregnant and Candida colonization of newborns from mothers with vaginal candidiasis. We set out to (1) establish the share of pregnant women suffering from Candida vaginitis immediately before the act of birth; (2) determine the main types of isolated Candida species; (3) estimate the rate of infants developing a colonization of Candida species within the first days after their birth; (4) compare Candida isolates in newborns and in maternal vaginal tract; (5) define the distribution of maternal and neonatal candida colonization, depending on the type of delivery.

**MATERIAL AND METHODS**

Samples of vaginal secretions were collected within 48 hours before delivery from 80 healthy pregnant women who were clinically suspicious for Candida vaginitis.

The included pregnant women were healthy, without comorbidities and medication with normal proceeded pregnancy. We considered as clinically suspicious Candida Vaginitis all cases of unusual vaginal secretion, accompanied by itching. Samples for Candida colonization from the oral mucosa and feces were collected from their newborns, between 47-72 hours after birth. Samples were plated on Sabouraud agar (40 g of glucose, 10 g peptone, 20 g agar, 1 liter distilled water, pH 5.6) and annealed in a thermostat for 72 hours at 36°C under aerobic conditions, followed by species identification of the isolated spp. by API Candida test - API 20C AUX (Biomerieux, Marcy-l’Etoile, France), as every strip contains 20 wells with dehydrated substrates, which allow implementation of 19 assimilation tests, followed by software processing of the results. The cupules are inoculated with a semi-solid minimal medium. The yeasts will only grow if they are capable of utilizing each substrate as the sole carbon source. The reactions are read by comparing them to growth controls. Identification is obtained by referring to the Analytical Profile Index, followed by using identification software.

The strips are systematically quality controlled at stages of their manufacture. Part of the isolates were confirmed by AUXACOLOR (Bio-Rad, Marnes la Coquette, France) - identification system based on the principle of assimilation of sugars.

Verification of data quality and statistical processing of the results was performed using the statistical package SPSS, version 19.0. Descriptive statistics, alternative and nonparametric analyzes had been implemented.

Currently molecular techniques (PCR or pulse field electrophoresis) are considered as the most precise methods for identification, though expensive and difficult for universal application. In contrast, the API 20C AUX identification system is less expensive and widely applicable for clinical practice, being reliable and secure.

**RESULTS**

The study included 80 women between 18 and 40 years of age (mean age, 8.20 ± SD 4.85 yrs/.
The relative parts of women who delivered once or twice were 46.25 ± 5.57% and 43.75 ± 5.57. Seven women (8.75 ± 5.57) delivered three times and only one woman reported having six deliveries (1.25 ± 5.57). Almost one third (31.25 ± 5.18%) delivered naturally, while the others (68.75 ± 5.18%) delivered through cesarean section. The women with recurrent vulvovaginal candidiasis (56.25 ± 5.55%) were more than the women who got the infection for the first time (43.75 ± 5.55%).

Twenty-three (28.75 ± 5.06%) of the pregnant women were positive for Candida spp. (Table 1). 78.26 ± 8.60% of the positive pregnant (18/23) reported history of previous episodes of VVC during their pregnancy, treated with local antifungal agents. Positive samples for Candida colonization were established in 18 (22.22 ± 4.62%) of the examined 81 newborns (one pair of twins) from mothers clinically suspicious for vaginal candidiasis. Contamination of the newborns was not detected in 10 of the 23 positive mothers (43.48 ± 10.34%) (Table 2). According to the localization in the group of the infected babies 3 newborns (16.67 ± 8.78%) were positive for Candida spp. only from the oral cavity material, 12 cases (66.67 ± 11.11%) were positive only from feces material, while simultaneous positive colonization of Candida yeasts from oral cavity and feces was found in 3 newborns (16.67 ± 8.78%) (Table 1). 22.22 ± 9.80% of the newborns (4 out of 18 cases) showed positive cultures of feces with negative maternal vaginal secretions, as almost half of these mothers reported previous episodes of VVC during their pregnancy (49.12 ± 6.62%) (Table 2). Candida albicans was the predominant causative agent identified both in pregnant women – n = 22 (91.67 ± 0.06%) and in the newborns – n = 16 (83.33 ± 8.78%) (Table 1) (Fig. 1). The isolates of the newborns were 100% identical to those of the mothers’ vaginal secretion (Fig. 1). Candida famata was isolated from the feces of one of the 4 babies (5.560 ± 5.4%) whose mothers were negative, with negative history for previous episodes of VVC. We assume undiagnosed mother’s Candida vulvovaginitis during the pregnancy or infection, acquired from the staff. The evaluated newborns were healthy, with normal weight (>3000 g), with exception of the pair of twins (i.e. -2800), with normal postnatal adaptation, discharged healthy, without further therapy.

None of the infants with proven Candida colonization showed clinical manifestation or laboratory evidence for active Candida infection.

The distribution of Candida colonization formed

Table 1. Epidemiological structure of Candida colonization of the newborns from mothers with proven VVC

<table>
<thead>
<tr>
<th>Patient/total count</th>
<th>Material</th>
<th>Positive samples</th>
<th>Negative samples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnant women</td>
<td>Vaginal secretion</td>
<td>Positive – n = 23 (28.75 ± 5.06%)</td>
<td>Negative – n = 57 (71.25 ± 5.06%)</td>
</tr>
<tr>
<td>Total 80</td>
<td></td>
<td>Candida albicans – n = 22 (91.67 ± 0.06%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Candida krusei – n = 1 (8.33 ± 0.06%)</td>
<td></td>
</tr>
<tr>
<td>81 newborns</td>
<td>Oral cavity only</td>
<td>Positive – n = 3 (3.70 ± 2.10%)</td>
<td>Negative – n = 78 (96.30 ± 2.10%)</td>
</tr>
<tr>
<td>(1 pair of twins)</td>
<td></td>
<td>Candida albicans – n = 3 (100%)</td>
<td></td>
</tr>
<tr>
<td>Feces only</td>
<td></td>
<td>Positive – n = 12 (14.81 ± 3.95%)</td>
<td>Negative – n = 69 (85.19 ± 3.95%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Candida albicans – n = 9 (75.00 ± 12.50%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Candida famata – n = 1 (8.83 ± 7.98%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Candida krusei – n = 2 (16.67 ± 10.76%)</td>
<td></td>
</tr>
<tr>
<td>Oral cavity and feces simultaneously</td>
<td>Positive – n = 3 (3.70 ± 2.10%)</td>
<td>Negative – n = 78 (96.30 ± 2.10%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Candida albicans – n = 3 (100%)</td>
<td></td>
</tr>
<tr>
<td>Total count of positive infants</td>
<td>n = 18 (22.22 ± 4.62%)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
three groups: group I - positive infants with positive mothers - 14 infants (+) (one pair of twins) with 13 mothers (+); Group II - positive infants with negative mothers 4 infants (+) with 4 mothers (-); III group - negative infants with positive mothers - 10 infants (-) with 10 mothers (+) (Table 2). A statistically significant association was identified between the presence of Candida infection in mothers and the presence of that infection in newborns ($\chi^2 = 25.25$, $P<0.001$). Candida infection in mothers is an important risk factor for the development of such infection in newborns. The risk for Candida infection in newborns is 18 times higher in infected compared to not contaminated mother (OR = 18.20).

### Table 2. Distribution of the Candida colonization

<table>
<thead>
<tr>
<th>Group</th>
<th>Positive infants with positive mothers</th>
<th>14 infants (+) (one pair of twins) with 13 mothers (+)</th>
</tr>
</thead>
<tbody>
<tr>
<td>II group</td>
<td>Positive infants with negative mothers</td>
<td>4 infants (+) with 4 mothers (-)</td>
</tr>
<tr>
<td>III group</td>
<td>Negative infants with positive mothers</td>
<td>10 infants (-) with 10 mothers (+)</td>
</tr>
</tbody>
</table>

We divide the patients in two groups, depending on the type of delivery. From a total of 23 mothers positive for Candida yeasts, 21 gave birth by cesarean section (91.30 ± 5.88%) and 2 (8.70%

### Table 3. Distribution of maternal and neonatal Candida colonization, depending on the type of delivery (SC-Cesarean Section; PN-Per Vias Naturalis).

- 23 (+) mothers (sample of vaginal secretion)
  - 21 cases SC 91%
  - 2 cases PN 9%
- 4 (-) mothers 4 infants with (+) samples of feces
  - 2 SC 50%
  - 2 PN 50%
  - 10 mothers (+) infants (-)
  - 11 mothers (+) infants (+)
  - 1 mother (+) infants (-)
  - 1 mother (+) infants (+)
± 5.88%) per Vias Naturalis (Table 3). There is no statistically significant association between the delivery type and the presence of Candida infection in newborns ($\chi^2 = 0.13, P>0.05$).

**DISCUSSION**

Candida species are commonly isolated pathogens from the female genital tract, while in pregnancy their incidence increases up to 43.50%. Every third pregnant women manifests Candida vulvovaginitis at least one time during the pregnancy. Elevated secretion of estrogen enhances the adhesion and penetration of the yeast in the vaginal mucosa. The hormone influence changes the cervix texture, while the hypertrophied glands increase their mucus production. As highly alkaline, cervical mucus temporarily decreases the acidity of the vaginal content and with glycochen creates optimal environment for development of pathogenic Candida spp. Another favorable condition for Candida overgrowth and vaginal infection during pregnancy is the decrease of IgG and IgA levels by estrogens. Despite the general discomfort, VVC can lead to complicated course of pregnancy, including water abundance, placental insufficiency, premature birth, premature detachment of the placenta, inflammatory complications in the infant and high risk of clinical manifested infections within the postpartum period.

Infection of the newborn with Candida spp can be acquired in two ways: 1) vertically – in cases of a vaginal infection of the mother, and 2) horizontally (as nosocomial infections) - from the hospital environment, staff and the use of various medical devices. The subsequent development of clinically manifested candidiasis is a result from the immature immune system of the newborn. It is believed that the vertical transmission of yeasts from the vulvovaginal track during the act of birth is the main source for the colonization of the newborn. Candida colonization of the newborn has the major role in the development of Candida-infection of the newborn in the first days of life. Infection can occur dramatically as Candida sepsis, clinically indistinguishable from bacterial sepsis, with failure of all organs and systems, aggravation of the respiratory function, rhythm problems, breathing complications (apnea), cardiac instability, thrombocytopenia, as well as localized multiple organ manifestations, including mucocutaneous rash or “diaper dermatitis”; nerve system involvement with meningitis, causing high lethality rate and frequency up to 64%; Candida endophthalmitis with a frequency of about 50%; Candida endocarditis and urinary infections. Manifestation of acute renal insufficiency may be the first symptom of invasive candidiasis of the newborn.

Some authors considered VVC as a potential source of ascending infection during pregnancy, where Candida colonization of the infant is conducted via placenta. Besides the direct intrauterine fetal colonization, VVC has been identified as a risk factor for premature birth as a result from premature rupture of the fetal membrane and numerous other complications. Intrauterine infection of the newborn with Candida, can manifest itself clinically in two forms: 1) Congenital mucocutaneous candidiasis, which occurs usually within 12 hours after birth with macular rash on the skin of the body, which progresses to vesicles, papules and pustules on an erythematous base with severe desquamation; 2) Congenital systemic candidiasis – a severe systemic infection of the newborns with very low birth weight.

Our results suggest that VVC was found in 23 out of a total of 80 (28.75 ± 5.06%) clinically suspected pregnant women, as half of them reported at least one episode of VVC during the pregnancy, most commonly detected within the second trimester, similarly to literature data, widely varying from 5.6% to 69.2%. From the total of 81 newborns (22.22 ± 4.62%), 18 were positive for Candida spp. Most of them were contaminated only in feces (66.67 ± 11.11%), followed by the equal relative parts of the babies infected only in oral cavity (16.67 ± 8.78%) and those infected simultaneously in oral mucosa and feces (16.67 ± 8.78%). According to other authors, Candida spp. colonizes the gastrointestinal tract in 4.8-10% of newborns as Candida albicans is the predominant isolated type. The major transmission route of the contamination of newborns is probably the ascending one. 7.82 ± 8.60% (21 cases from total count of 23 (+) mothers) gave births by SC, as 11 infants from these mothers were (+) for Candida, in at least one location (61.11 ± 11.49%). Candida albicans was the predominant causative agent identified both in the pregnant women and in the newborns. The isolates from newborns were 100% identical to the maternal vaginal secretion. In 10 of the cases of positive mothers, contamination of the newborn was not established, which in fact confirms the role of additional predisposing factors that may facilitate the transmission.
Candida colonization of the infant was confirmed only on laboratory studies, as all of the infants were discharged healthy on the 3rd or 5th day, with no clinical signs of manifestation of early muco-cutaneous or systemic candida infection.

CONCLUSION

The incidence and the clinical manifestation of Candida colonization of newborns from mothers with Candida vaginosis have not been previously studied in Bulgaria. Clarifying the current etiological structure of VVC in pregnant women and prevalence of Candida species determines the most appropriate subsequent therapeutic approach. The statistically significant correlation between the presence of Candida infection in mothers and newborns outlines Candida infection in pregnancy is an independent risk factor for the development of such infection in newborns. Vertical transmission has a major role in neonatal colonization by Candida in the first days of life. We found that early neonatal colonization by Candida albicans is mainly due to the vertical transmission while the horizontal transmission was not the main mode of colonization within the first days of life.

Conducting targeted screening among gravid women at risk could decrease complications during pregnancy. Examination of infants, whose mothers have chronic Candida vaginitis, would help the diagnosis and the treatment of these patients, decreasing the risk of postnatal complications.

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Вульвовагинальный кандидоз у беременных женщин и его значение при колонизации грибка Candida у новорожденных

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Вульвовагинальный кандидоз является второй самой распространенной причиной возникновения вагинита в мировом масштабе. Вульвовагинальный кандидоз у матери является основным фактором, создающим риск колонизации и инфекционного заражения новорожденного ребенка грибком Candida, причем прогноз зависит от разных предрасполагающих факторов. Целью настоящего исследования является определение частоты появления и этиологической структуры вульвовагинального кандидоза у беременных женщин и ее воздействия на колонизацию грибка Candida у новорожденных.

Материалы и методы: Пробы вагинального секрета, взятые у 80 здоровых беременных женщин в течение 48 часов до родов. У их новорожденных детей взяты пробы из слизистой оболочки полости рта и пробы испражнений на возможное наличие колонизации грибка Candida в рамках 47-72 часов после рождения. Пробы были помещены в среду Агар Сабуро, после чего была осуществлена идентификация видов посредством API анализа дрожжей Candida.

Результаты: Среди обследованных беременных женщин установлено 23 (28.75 ± 5.06%) положительных результатов, подтверждающих наличие спор грибка Candida, составило 23 (28.75 ± 5.06%) в числе протестированных беременных женщин. Наличие положительных проб на колонизацию Candida было установлено у 18 (22.22 ± 4.62%) младенцев из 81 (одна пара близнецов), рожденных матерями, являющимися клинически подозрительными в плане наличия вагинального кандидоза. Взятые у новорожденных изоляты были полностью идентичны взятым у их матерей пробам вагинального секрета.

Превалирующим идентифицированным видом является Кандида альбиканс (Candida albicans), обнаруженная как у беременных женщин (91.67 ± 0.06%), так и у новорожденных (83.33 ± 8.78%).