Introduction

Candida species, which are part of the normal flora in the vagina in small quantities but in some cases may be involved in human pathology and at least 20% of women, cause opportunistic infections. This situation happens in some circumstances that compromise host immunity (hormone replacement, uncontrolled diabetes mellitus, long-term broad-spectrum antibiotic treatment, surgical manipulation of the vagina, and immunosuppression) [1].

According to recent studies the disturbance of local host defense mechanisms limiting Candida growth also lead to vulvovaginal candidiasis (VVC) [1, 2].

VVC is a common problem worldwide and is multifactorial in origin, affecting millions of women annually, with serious impact on their quality of life and is associated with significant direct and indirect costs [3, 4].

Currently, there are official reports, studies that have shown that in Europe VVC is one of the most common causes of vaginitis, and in the United States, it is the se-
The purpose of this study was undertaken to evaluate the Candida species distribution isolated from vaginal swab and their antifungal susceptibility patterns.

Materials and methods
During the 2017–2019, after microbiological analyzes 1030 isolates were recovered from the clinical samples. Specimens were taken from the posterior fornix of the vagina with sterile swabs and transported to the microbiology laboratory for further processing.

Culture isolation and identification
For the isolation of Candida spp., specimens were inoculated on Sabouraud dextrose agar supplemented with chloramphenicol (Oxoid) and incubated at 36°C for 72 hours. Simultaneously, with the fungal strains, other associated bacterial strains were isolated. Identification to the species level was performed by MALDI-TOF MS (Bruker Daltonics) and bacterial strains by Vitek 2 automated system (BioMérieux).

Antifungal susceptibility testing
The in vitro activity of the antifungal agents against each isolate was determined by using Fungitext galleries (Bio-Rad SDP Paris, France).

Inoculum preparation
From the isolated colonies an inoculum was prepared by suspending them in 3 ml of sterile saline (aqueous solution of 0.50 % NaCl, pH 7.0). To obtain a turbidity equivalent to that of the McFarland 0.50 standard, the measurements were performed with the PhoenixSpec nephelometer (Becton Dickinson). Subsequently, the antifungal susceptibility test was performed by inoculating the Fungitext microplates and incubating at 37°C for 24–48 hours.

Interpretation of results
The results were interpreted based on interpretive susceptibility criteria for antifungal breakpoints from EUCAST (European Committee on Antimicrobial Susceptibility). Isolates with minimum inhibitory concentration (MIC):<2μg/ml - amphotericin B, <2 μg/ml - 5-flucytosine, <0.5 μg/ml - miconazole, <0.5 μg/ml - ketocazole, <0.5 μg/ml - intraconazole, <8 μg/ml - fluconazole) were considered susceptible.

Quality control of the investigation. For quality control, C. albicans (ATCC 10231) was used as reference strain and tested simultaneously with the clinical isolates.

Results and discussions
After microbiological analyzes 1030 isolates were recovered from the clinical samples. Information regarding the prevalence of VVC in Moldova is not well known and varies from one study to another. Literature evidence regarding VVC reports highlighted that this disease it is the second most common infection of the vulvovaginal area of symptomatic women accounting for about 17 % to 42 % [21, 22, 23].

Regrettably, this disease is routinely diagnosed by sign and symptom and is not confirmed with laboratory investigation when necessary. As a result, the spectrum of yeasts implicated in causing the disease, their drug susceptibility profile is not known in the country.
Studies demonstrate the necessity of identification the Candida species responsible for infections in all patients presenting with VVC especially those with recurrent infections. Therefore, identification will influence selection of antifungals and duration of therapy [23, 24].

Between 85% to 90% of yeast strains isolated from the vagina belong to the Candida albicans species; other yeasts account for up to 15% (8-10) of cases [24, 25, 26].

Assessing the prevalence of the identified subtypes, the predominance of C. albicans species was found (n=863; 83.8%), compared to non-albicans Candida species (n=167; 16.2%). The most common non-albicans species were C. glabrata (n=77; 7.5%) followed by C. robusta (n=28; 2.7%), C. krusei (n=24; 2.3%), C. kefyr (n=12; 1.2%), C. parapsilosis (n=9; 0.9%) and other species (n=17; 1.7%).

Simultaneously, compared to previous years, a trend has been observed that is an increase in the rate of isolation of non-albicans Candida species. This phenomenon could be due to the improvement of laboratory diagnostic practices regarding the identification of fungi or a real higher prevalence of these species [25, 26].

Of the total number of 1030 Candida isolates, 584 (56.6%) showed bacterial associations.

The most common microbial association was C. albicans and S. aureus - 127 combinations (26.6%), followed by C. albicans and E. coli - 99 combinations (20.8%). Bacterial associations with non-albicans Candida species or recorded less frequently.

The clinical significance of the identification of the Candida to the species level is definite and completely recognized by the majority of studies on the reason for the diversity of expression of virulence factors and their susceptibility to antifungal agents. The support of the laboratory is crucial in order to fight vaginal Candida infection with targeted therapy [27].

Antifungal susceptibility test results show that most isolates were sensitive to 5-fluorocytosine followed by amphotericin B and ketoconazole. Candida species have shown moderate indices of resistance to miconazole, fluconazole and itraconazole.

Isolates of C. albicans have been shown to be more resistant to these medications than non-albicans Candida. The resistance rate of C. albicans isolates to miconazole, fluconazole and itraconazole was 25.5%, 17.0% and 11.4%, compared to non-albicans Candida strains with a resistance rate of 10%, 14.4% and 10%.

Finally, the variety of non-albicans Candida species involved in human pathology, their rising contribution to fungal infections and the antifungal susceptibility profiles makes their identification at the species level essential for epidemiological investigations, optimizing therapy and patient management.

Conclusions

Candida albicans was the most common isolated fungal species among patients with vulvovaginitis, and there is an increasing tendency of isolation of Candida non-albicans species, a phenomenon found by other similar research.

Analyzing the clinical isolates in this research, associations of Candida spp. with other microorganisms were also observed. Candida albicans with S. aureus was the most common, followed by Candida albicans and E. coli.

Based on the antifungal susceptibility test results, it was found that most isolates were susceptible to 5-fluorocytosine and amphotericin B, ketoconazole and moderately resistant to miconazole, fluconazole and itraconazole. C. albicans have been shown to be more resistant than non-albicans Candida.

Additional studies on a larger sample and evaluating results of antifungal susceptibility testing are of great importance for optimizing therapy and patient management to fighting vaginal Candida infections with targeted therapy.

Conflicts of interests. Authors declare the absence of any conflicts of interests and their own financial interest that might be construed to influence the results or interpretation of their manuscript.

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казали помірну стійкість до протигрибкових препаратів. Рівень стійкості ізолятів C. albicans до міконазолу, флуконазолу та ітраконазолу становив 25,5 %, 17,0 % і 11,4 % відповідно, у порівнянні зі штамами Candida non-albicans, які показали рівень стійкості 10,8 %, 14,4 % і 10 %, відповідно.

Висновки. C. albicans є найбільш часто ізольованим видом серед пацієнтів з ВВК, але також спостерігається підвищена частка видів Candida non-albicans, таких як C. glabrata. Ізоляти C. albicans показали більш високі показники лікарської стійкості, ніж Candida non-albicans.

Ключові слова: Candida non-albicans, Candida albicans, ВВК, протигрибкові препарати.

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АНАЛИЗ РАСПРОСТРАНЕНИЯ ВИДОВ CANDIDA, ВЫДЕЛЕННЫХ У ЖЕНЩИН С КАНДИДОЗНЫМ ВУЛЬВОВАГИНИТОМ, И ПРОФИЛИ ИХ ЧУВСТВИТЕЛЬНОСТИ К ПРОТИВОГРИБКОВЫМ ПРЕПАРАТАМ

Резюме. Актуальность. Обычно виды Candida - это комменсальный грибок, обитающий во влагалище примерно 30–50% здоровых взрослых женщин, который является наиболее частым возбудителем, выделенным из клинических образцов пациенток с диагнозом вульвовагинальный кандидоз (ВВК). Культуральный метод не рекомендуется для всех пациентов на регулярной основе, но он информативен для исключения non-albicans инфекций, устойчивых к азолам, или рецидивов ВВК. Определение видов Candida и тестирование на чувствительность к противогрибковым препаратам являются ключевыми элементами в правильном ведении рецидивирующего ВВК. Авторы данной статьи ставили перед собой цель изучить спектр патогенных видов Candida. Также предъявлена картина противогрибковой чувствительности выделенных штаммов за 2017-2019 годы. Материалы и методы. Проведено описательное исследование распространения видов Candida и их чувствительности к противогрибковым препаратам. Идентификацию изолированных видов Candida проводили с помощью MALDI-TOF масс-спектрометрии, штаммов — с помощью автоматизированной системы Vitek 2 (BioMérieux), а профили противогрибковой чувствительности - с помощью Fungitest (Bio-Rad). Результаты. Из 1030 обработанных штаммов преобладали виды C. albicans – 83,8 %, Candida non-albicans – 16,2 %. Наиболее распространенным видом Candida non-albicans была C. glabrata (7,5 %). Также было замечено, что в 56,6 % случаев C. albicans представляла часть бактериальной ассоциации. Наиболее частыми микробными ассоциациями были C. albicans и S. aureus (26,6 %), затем следовали C. albicans и E. coli (20,8 %). Виды Candida показали умеренную устойчивость к противогрибковым препаратам. Уровень устойчивости изолятов C. albicans к миконазолу, флуконазолу и итраконазолу составлял 25,5 %, 17,0 % и 11,4 % соответственно, по сравнению со штаммами Candida non-albicans, которые показали уровень устойчивости 10,8 %, 14,4 % и 10 % соответственно. Выводы. C. albicans является наиболее часто изолированным видом среди пациенток с ВВК, но также наблюдается повышенная доля видов Candida non-albicans, таких как C. glabrata. Изоляты C. albicans показали более высокие показатели лекарственной устойчивости, чем Candida non-albicans.

Ключевые слова: Candida non-albicans, Candida albicans, ВВК, противогрибковые препараты.