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Studies of biofilms and phenotypic characteristics of *Candida* fungi

E. M. Lenchenko[1], N. P. Sachivkina[2]

[1] Moscow State University of Food Production (MSUFP), Moscow, Russia

[2] Peoples' Friendship University of Russia (RUDN University), Moscow, Russia

[1] *ORCID 0000-0003-2576-2020, e-mail: lenchenko-ekaterina@yandex.ru*

[2] *ORCID 0000-0003-1100-929X, e-mail: sachivkina@yandex.ru*

SUMMARY

Yeast-like fungi of the *Candida* genus are causative agents of the infectious lesions of the mucous membrane of the gastrointestinal, respiratory, urogenital tracts and skin of mammals, sepsis, and disseminated infection in birds. The search and testing of multilevel algorithms for biofilm identification when exposed to chemotherapeutic and disinfectant drugs for blocking the synthesis or destruction of the intercellular matrix in the development of superficial, deep and systemic candidiasis of animals are relevant for developing and improving diagnostic and antiepidemic measures. It was established that the formation of biofilm heterogeneous structure comprises multiple stages implementing the processes of intercellular communication due to the synthesis of a polymer matrix composites. Optical microscopy revealed a three-dimensional structure of biofilms in the form of a dense network consisting of yeast cells, hyphal and pseudohyphalic forms surrounded by an intercellular polymer matrix. *Candida* spp. pathogenicity factors contribute to infection of susceptible species due to adhesion, invasion, secretion of hydrolases, and dimorphism. Formation of mono-species or poly-species biofilms of microorganisms, including *Candida* spp., causes the development of superficial, deep and systemic candidiasis. Detection of a large amount of yeast and micellar phases in *C. albicans* and *C. africana* isolates was a differential sign of a significant degree of colonization of the mucous membranes of the larynx, pharynx, and tonsils in case of localized and systemic lesions in pigs. The results of studies of the biofilm heterogeneous structure and phenotypic signs of yeast-like fungi can be used in a comparative study of biological characteristics and the identification of common patterns and differential signs of microorganisms, optimization of mycological diagnostics, and also in the development of antimycotic drugs.

Key words: microscopic fungi, *Candida* spp., biofilms, optical density, microscopy, phenotypic characters.

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For correspondence: Ekaterina M. Lenchenko, Doctor of Science (Veterinary Medicine), Professor, Department of Veterinary Medicine, Moscow State University of Food Production, 125080, Russia, Moscow, Volokolamskoye Shosse, 11, *e-mail: lenchenko-ekaterina@yandex.ru.*

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Исследование биопленок и фенотипических признаков грибов рода *Candida*

Е. М. Ленченко[1], Н. П. Сачивкина[2]

[1] ФГБОУ ВО «Московский государственный университет пищевых производств» (ФГБОУ ВО «МГУПП»), Москва, Россия

[2] ФГАОУ ВО «Российский университет дружбы народов» (РУДН), г. Москва, Россия

[1] *ORCID 0000-0003-2576-2020, e-mail: lenchenko-ekaterina@yandex.ru*

[2] *ORCID 0000-0003-1100-929X, e-mail: sachivkina@yandex.ru*

РЕЗЮМЕ

Дрожжеподобные грибы рода *Candida* – возбудители инфекционной патологии слизистой оболочки желудочно-кишечного, дыхательного, урогенитального трактов и кожи млекопитающих, сепсиса и диссеминированной инфекции птиц. Для разработки и совершенствования диагностических и противоэпизоотических мероприятий актуальность представляют изыскание и апробация многоуровневых алгоритмов индикации биопленок при воздействии химиотерапевтических и дезинфицирующих препаратов для блокировки синтеза или разрушения межклеточного матрикса при развитии поверхностных, глубоких и системных кандидозов животных. Установлено, что формирование гетерогенной структуры биопленок представляет собой множество этапов, реализующих процессы межклеточной коммуникации за счет синтеза полимерного матрикса. При оптической микроскопии выявлялась трехмерная структура биопленок в виде плотной сети, состоящей из дрожжевых клеток, гифальных и псевдогифальных форм, окруженных межклеточным полимерным матриксом. При инфицировании восприимчивых видов этиологическая значимость факторов патогенности *Candida* spp.

реализуется за счет адгезии, инвазии, секреции гидролаз, диморфизма. Формирование моновидовых или поливидовых биопленок микроорганизмов, в том числе и *Candida* spp., обусловливают развитие поверхностных, глубоких и системных кандидозов. Индикация в большом количестве дрожжевой и мицеллярной фаз у изолятов *C. albicans* и *C. africana* являлась дифференциальным признаком значительной степени колонизации слизистых оболочек гортани, глотки и миндалин при локальных и системных патологиях свиней. Результаты исследований гетерогенной структуры биопленок и фенотипических признаков дрожжеподобных грибов могут быть использованы при сравнительном изучении биологических свойств и выявлении общих закономерностей и дифференциальных признаков микроорганизмов, оптимизации схемы микологической диагностики, а также при разработке антимикотических препаратов.

Ключевые слова: микроскопические грибы, *Candida* spp., биопленки, оптическая плотность, микроскопия, фенотипические признаки.

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Для корреспонденции: Ленченко Екатерина Михайловна, доктор ветеринарных наук, профессор кафедры ветеринарной медицины ФГБОУ ВО «МГУПП», 125080, Россия, г. Москва, Волоколамское шоссе, 11, *e-mail: lenchenko-ekaterina@yandex.ru.*

INTRODUCTION

Of the numerous representatives of the genus *Candida*, 20 species were recognized as pathogenic for humans, of which, primarily, *C. albicans*, *C. tropicalis*, *C. krusei*, *C. kefyr*, *C. glabrata*, *C. guilliermondii*, *C. parapsilosis* [1, 2]. The etiological structure of candidiasis in farm animals is represented by *C. albicans* and *C. tropicalis* species recovered from the calves' pancreas (48.07%) and rennet (75.0%), piglets' stomach (88.26%), as well as the oral cavity, esophagus and crop of young birds with pathological legions [3–5]. Clinical signs of the disease are observed in the early postnatal period (up to 2 months of age) in particular, curd-like patches were detected in the stratified squamous epithelium of the digestive system (oral cavity, esophagus, paunch, reticulum, omasom) of calves and lambs [3]. In case of mastitis in cows, *C. krusei* ($n = 14$) and *C. parapsilosis* ($n = 6$) were more often detected in milk samples, *C. lipolytica*, *C. lusitaniae*, *C. neoformans* – less often [6]. Visceral candidiasis of piglets, which occupies the 4th place in the nosological profile of infectious lesions, reaches 13.36% occurrence frequency [4]. 13 (68.42%) *C. albicans* and 6 (31.57%) *C. africana* microorganism cultures were recovered from tissues and organs of pigs with clinical signs of candidiasis and identified [7]. In case of candidiasis in silver-black foxes, dogs and cats, both minor lesions of the skin and epidermic derivatives, as well as intense lesions in the form of ulcers, hyperkeratosis and local alopecia are reported [8]. Chickens and poults younger than one month of age are the most susceptible to this disease. Subacute or chronic candidiasis occurs in birds in the form of epidemic outbreaks. As a rule, it is characterized by formation of intense patches of yellow-gray color, tightly attached to the mucous membrane of the esophagus, crop, and when removed, ulcerative foci are exposed [9, 10].

In case of chronic infectious lesions of farm animals, direct correlations were established between the morphological and densitometric characteristics of biofilms and the effect of virulence factors of microorganisms, including yeast-like fungi [11–14]. *Candida* spp. virulence factors contribute to infection of susceptible species due to synthesis of polymeric substances, transcriptional control of adhesion, invasion, and secretion of toxins [15–20]. Heteromorphic population growth promotes the interaction between microorganisms of various taxonomic groups, which leads to virulence and protection of microbial biofilms from the immune response, as well as from the effects of chemotherapeutic drugs and disinfectants [21–24]. *Candida* spp. mRNA transport is inactivated when the so-called She3 adapter is removed, the hyphae become specifically defective, which is accompanied by atypical growth and decrease in the ability to damage the monolayer of epithelial cells due to a decrease in the production of phospholipase B [25, 26]. The search and testing of multilevel algorithms for biofilm identification when exposed to chemotherapeutic and disinfectant drugs for blocking the synthesis or destruction of the intercellular matrix in the development of superficial, deep and systemic candidiasis of animals are relevant for developing and improving diagnostic and anti-epidemic measures.

The research is aimed at studying the morphometric and densitometric characteristics of biofilms and phenotypic characteristics of reference strains and isolates of *Candida* spp. yeast-like fungi.

MATERIALS AND METHODS

Strains. Biofilms and phenotypic characteristics were tested using reference strains (ATCC): *Candida albicans* ATCC 14053, *C. parapsilosis* ATCC 22019, *C. tropicalis* ATCC 750, *C. glabrata* ATCC 66032 [27]. The isolates we recovered were also used in the experiments: *C. albicans* and *C. africana* – from pig lymph nodes in case of localized or systemic lesions; *C. albicans* and *C. tropicalis* from vaginal mucus of dogs in the presence of clinical signs of candidiasis, as well as *C. humilis* from compound feed for cattle [7, 25].

Nutrient media. The microorganisms were cultured for 24 hours at 37, 42, 45 °C using liquid and dense nutrient media: cardiac broth (HiMedia, India), Sabouraud (bioMerieux, France), HiCrome Candida Agar (HiMedia, India). The presence of chlamydospores was taken into account when inoculating a 24-hour culture of microorganisms from Sabouraud's culture medium on the rice agar (API-System R.A.T., France) and cultivated for 24 hours at 25 °C.

To assess the formation of germ tubes, the microorganisms were cultured in 1.0 ml of meat-peptone broth (MPB) with addition of cattle blood serum (FSUE NPO Microgen, Russia) at 37 °C for 5 hours.

Phenotypic characteristics. The morphological, cultural, and biochemical properties of microorganisms were studied by conventional methods using differential diagnostic media and test systems [1, 2].

For recording the enzymatic features, *Candida* spp. day-old culture (optical density OD = 0.5 at a wavelength of 620 nm) was added to the wells of the HiCandida Identification Kit test system (HiMedia, India) and cultured for 48 hours at 22.5 °C.

Morphometric parameters of biofilms. Microorganisms were cultured for 48 hours at 37 °C on coverslips placed in Petri dishes with 20 ml of MPB and 5 ml of suspension of 18-hour cultures of microorganisms at a concentration of 105 CFU/ml. The preparations were fixed with a mixture of alcohol and ether (1:1) for 10 min; they were stained with a 0.5% methylene blue solution [12, 21].

The preparations were tested using optical microscopy BIOMED MS-1 Stereo (Russia).

Densitometric characteristics. When cultured in a 96-well plate (Medpolymer, Russia), the crystal violet binding (HiMedia, India) reading was performed using an Immunochem-2100 photometric analyzer (HTI, USA). The test samples (optical density OD 580, wavelength 580 nm) were added to the wells of the plates and cultured at 37 °C for 48 hours. Then, the liquid was removed, the wells were washed three times with 200 µl of phosphate-buffered solution (pH 7.3). Fixation was performed using 150 µl of 96% ethanol for 15 min. Then the plate wells were dried for 20 min at 37 °C and a 0.5% solution of crystalline violet was added, and again placed in a thermostat at 37 °C. In 5 min, the contents of the wells were removed, washed three times with 200 µl of phosphate-buffered saline (pH 7.2) and dried. The dye was eluted with 200 µl of 96% ethanol for 30 min [16, 28, 29].

The data obtained during the experiment were processed by the method of statistical analysis using Student's t-test, the results were considered reliable at $p \leq 0.05$.

RESULTS AND DISCUSSION

Candida spp. – gram-positive oval-based budding microorganisms. The yeast phase of the tested species was represented by relatively large round or oval budding cells of 2.0–6.0 µm in diameter, the micellar phase – by groups of round small cells (blastospores) and hyphae.

The microorganisms *C. albicans* and *C. humilis* had round, subspherical, elliptical cell shapes 2.0–3.0 × 3.0–5.0 µm in size.

The *C. parapsilosis* and *C. tropicalis* cells of 1.7–2.0 × 3.0–4.0 µm in size had an elliptical elongated shape; mycelium with groups of round small cells – blastospores – was also detected (Fig. 1).

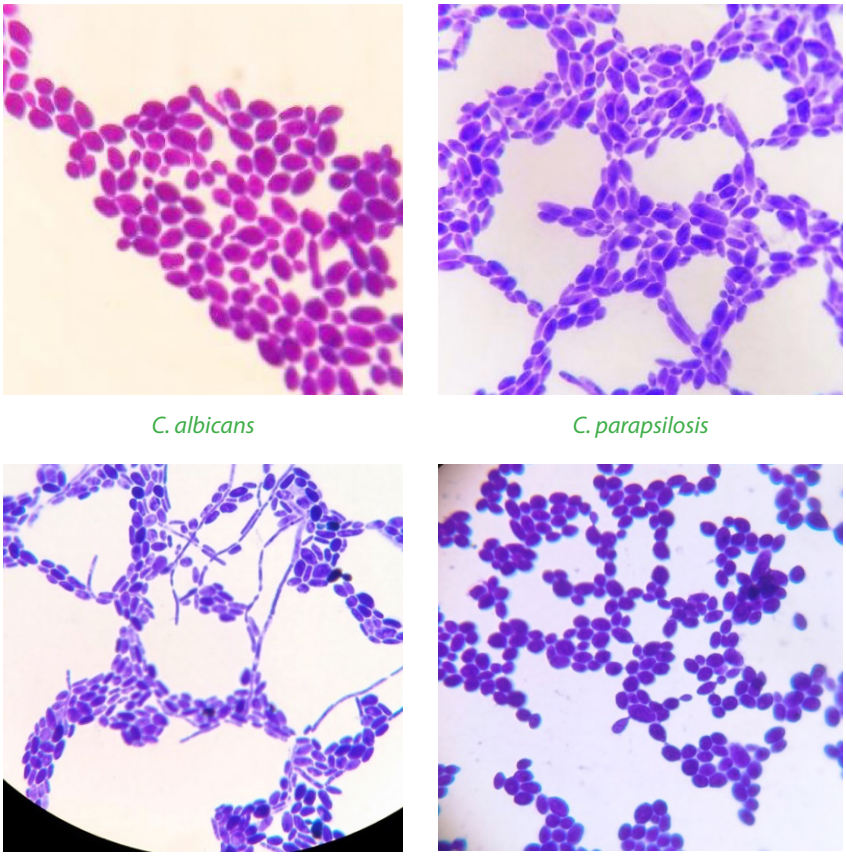

Fig. 1. Morphology of yeast-like fungi.
Growth at 25 °C for 24 h in Sabouraud medium. Methylene blue staining, oc. 10, ob. 100, immersion.

Рис. 1. Морфология дрожжеподобных грибов.
Рост при 25 °С 24 ч на среде Сабуро. Окрашивание метиленовым синим, ок. 10, об. 100, иммерсия.

Detection of a large number of yeast and micellar phases in *C. albicans* and *C. africana* isolates recovered from pig lymph nodes was a differential sign of a significant degree of colonization of the larynx, pharynx, and tonsils mucous membranes in case of localized and systemic lesions.

The population immobilization of the mature three-dimensional biofilm architectonics, in accordance with the cultivation conditions, was accompanied by co-aggregation between yeast and micellar forms combined by an exocellular matrix, the presence of long branched hyphal forms forming dense pseudomycelial structures. In their central part microcolonies had a more pronounced matrix, therefore, yeast forms and mycelium were not detected. In the peripheral part of biofilms, as a rule, the exocellular matrix gradually thinned, individual yeast cells and micellar forms were detected (Fig. 2).

When evaluating densitometric caracteristics of the microorganism culture, it was found that after 48 hours of *C. albicans* (ATCC 14053) and *C. africana* cultivation recovered from the lymph nodes of piglets, the absolute optical density values (OD_s) were in the range from 0.423 ± 0.11 to 0.510 ± 0.19, and the intensity of biofilm formation (*ID*) was ≥ 0.3–0.4.

C. albicans and *C. tropicalis* microorganisms (ATCC 750) isolated from dog vaginal mucus discharge, as well as *C. glabrata* (ATCC 66032), *C. parapsilosis* (ATCC 22019) demonstrated OD_s values ranging from 0.331 ± 0.10 to 0.350 ± 0.08, biofilm formation intensity *ID* ≥ 0.2–0.3.

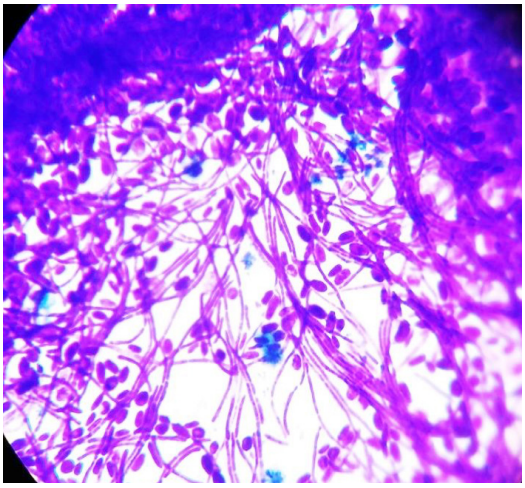

Fig. 2. Morphology of yeast-like fungi C. tropicalis. Growth at 37 °C for 48 h in Sabouraud medium. Methylene blue staining, oc. 10, ob. 100, immersion.

Рис. 2. Морфология дрожжеподобных грибов C. tropicalis. Рост при 37 °C 48 ч на среде Сабуро. Окрашивание метиленовым синим, ок. 10, об. 100, иммерсия.

C. humilis isolated from cattle feed demonstrated OD_s in the range from 0.208 ± 0.06 to 0.288 ± 0.11, and the intensity of biofilm formation *ID* ≥ 0.1–0.2 (Table 1).

Table 1
Densitometric parameters of *Candida* spp. biofilm formation

Таблица 1
Денситометрические показатели формирования биопленок *Candida* spp.

Microorganism species	Optical density ($n = 3$)		
	OD_s*	Δ (OD_s – OD_c)**	Intensity (*ID*)***
Reference strains			
C. albicans, ATCC 14053	0.423 ± 0.11	0.325 ± 0.20	≥ 0.3–0.4
C. tropicalis, ATCC 750	0.342 ± 0.13	0.244 ± 0.22	≥ 0.2–0.3
C. parapsilosis, ATCC 22019	0.350 ± 0.08	0.252 ± 0.17	≥ 0.2–0.3
C. glabrata, ATCC 66032	0.288 ± 0.11	0.130 ± 0.20	≥ 0.1–0.2
Isolates			
C. albicans (piglets' lympnode)	0.482 ± 0.09	0.384 ± 0.18	≥ 0.3–0.4
C. africana (piglets' lympnode)	0.510 ± 0.19	0.412 ± 0.28	≥ 0.3–0.4
C. albicans (dogs' vaginal mucus discharge)	0.398 ± 0.16	0.300 ± 0.25	≥ 0.2–0.3
C. tropicalis (dogs' vaginal mucus discharge)	0.331 ± 0.10	0.213 ± 0.19	≥ 0.2–0.3
C. humilis (compound feed)	0.208 ± 0,06	0.110 ± 0,15	≥ 0.1–0.2

* OD_s – optical density of the sample (оптическая плотность образца);
** OD_c – optical density of the control sample (оптическая плотность контроля);
*** *ID* – intensity: the difference between the optical density of the test sample (ODs) and control sample (ODc)
(интенсивность: разность оптической плотности исследуемого образца (OD_s) и контроля (OD_c).

Table 2
Phenotypic characteristics of *Candida* spp.

Таблица 2
Фенотипические признаки *Candida* spp.

Microorganism species	Distinctive features in 48 hours of cultivation			
	Sabouraud medium			HiCrome Candida Agar
	37 °C	42 °C	45 °C	
Reference strains				
C. albicans, ATCC 14053	+	+	+	Green colour of the colony
C. tropicalis, ATCC 750	+	+	+	Blue colour of the colony
C. parapsilosis, ATCC 22019	+	+	–	Pale-pink colour of the colony
C. glabrata, ATCC 66032	+	+	+	Cream colour of the colony
Isolates				
C. albicans (piglets' lympnode)	+	+	+	Green colour of the colony
C. albicans (dogs' vaginal mucus discharge)	+	+	+	Green colour of the colony
C. africana (piglets' lympnode)	+	+	–	Green colour of the colony
C. tropicalis (dogs' vaginal mucus discharge)	+	+	+	Blue colour of the colony
C. humilis (compound feed)	+	+	+	Lilac colour of the colony

«+» – species characteristic growth is observed (присутствие характерного для вида роста);
«–» – species characteristic growth is not observed (отсутствие характерного для вида роста).

The reference strains and isolates of yeast-like fungi *Candida* spp., regardless of the recovery source, demonstrated growth characteristic of the species, which is quite informative in terms of sediment, film and the degree of medium turbidity. Taking into account the microorganism temperature tolerance, the distinctive feature of *C. africana* and *C. parapsilosis* species was the absence of growth on Sabouraud medium at 45 °C (Table 2).

HiCrome Candida Agar contains chloramphenicol, which inhibits the growth of concomitant microorganisms. The presence of chromogenic substrates makes it possible to differentiate *Candida* spp. colonies, which differed in size, shape, color and consistency. At the same time, the *C. albicans* and *C. africana* species formed similar green colonies on this medium.

A diagnostic test for the presence of germ tubes, the precursors of true hyphae, makes it possible to differentiate fungi in 5 hours of microorganism cultivation in MPB at 37 °C with the addition of cattle blood serum. The tested types of microorganisms were able to grow in the presence of cycloheximide, no urease activity was observed. *C. africana*, unlike other species, fermented sucrose and raffinose and did not ferment maltose, *C. humilis* and *C. glabrata* species did not ferment galactose and xylose, *C. parapsilosis* did not ferment trehalose (Table 3).

In general, the formation of a heterogeneous structure of *Candida* spp. fungi reference strain and isolate biofilms represents many stages of intercellular communication processes due to the synthesis of a polymer matrix. The results of studying biofilm heterogeneous structure as well as phenotypic characteristics and virulence factors of the microorganisms expand the boundaries of general and special mycology, and the applied aspects – the identification of common patterns and differential characteristics of saprophytes, potentially pathogenic and pathogenic microorganisms – have potential for optimization of the infectious lesion diagnostics and the development of antimycotic preparations.

CONCLUSION

When studying the morphometric parameters of *Candida* spp. fungi reference strain and isolate biofilms the three-dimensional structure of biofilms was detected in the form of a dense network consisting of yeast cells, hyphal and pseudohyphal forms surrounded by an intercellular polymer matrix. Detection of a large number of yeast and micellar phases in *C. albicans* and *C. africana* isolates was a differential characteristics of a significant degree of colonization of the larynx, pharynx, and tonsils mucous membranes in case of localized and systemic lesions of pigs.

REFERENCES

1. Moroz A. F., Snegireva A. E. Methodical recommendations. Fungi of the genus *Candida* (methods of isolation, identification at the species level and determination of sensitivity to antifungal drugs) [Metodicheskie rekomendacii. Griby roda *Candida* (metody vydeleniya, identifikacii

Table 3
Differential diagnostic characteristics of *Candida* spp.

Таблица 3
Дифференциально-диагностические признаки *Candida* spp.

Species	Carbonhydrates											
	Urease	Melibiosis	Lactose	Maltose	Suchrose	Galactose	Cellobiose	Inositol	Xylose	Dulcite	Raffinose	Trehalose
Reference strains												
C. albicans, ATCC 14053	–	–	–	+	+	+	–	–	+	–	–	+
C. tropicalis, ATCC 750	–	–	–	+	+	+	+	–	+	–	–	+
C. parapsilosis, ATCC 22019	–	–	–	+	+	–	–	–	+	–	–	–
C. glabrata, ATCC 66032	–	–	–	+	–	–	–	–	–	–	–	+
Isolates												
C. albicans (piglets' lympnode)	–	–	–	+	+	+	–	–	+	–	–	+
C. albicans (dogs' vaginal mucus discharge)	–	–	–	+	+	+	–	–	+	–	–	+
C. africana (piglets' lympnode)	–	–	–	–	+	+	–	–	+	–	+	+
C. tropicalis (dogs' vaginal mucus discharge)	–	–	–	+	+	+	+	–	+	–	–	+
C. humilis (compound feed)	–	–	–	+	–	+	–	–	+	–	–	+

«+» – positive test result (положительный тест);
«–» – negative test result (отрицательный тест).

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2. Sutton D., Fothergill A., Rinaldi M. Identifier of pathogenic and opportunistic fungi [Opredelitel' patogennyh i uslovno patogennyh gribov]: trans. from English K. L. Tarasova, Yu. N. Kovaleva; ed. by I. R. Dorozhkova. M.: Mir; 2001. 468 p. (in Russian)

3. Agoltsov V. A. Candidiasis, aspergillosis and animal mucorosis (diagnosis and control measures) [Kandidoz, aspergillez i mukoroz zhivotnyh (diagnostika i mery bor'by)]: Author's abstract. thesis ... Doctor of Sciences (Vet. Med.). N. Novgorod; 2006. 50 p. Available at: http://disus.ru/r-raznoe/272146-1-kandidoz-aspergillez-mukoroz-zhivotnih-diagnostika-meri-borbi.php. (in Russian)

4. Lysuho T. N. Visceral candidiasis of piglets: epizootic features, clinical and pathomorphological manifestations, treatment and prophylactic measures [Visceral'nyj kandidamikoz porosyat: epizooticheskie osobennosti, klinicheskie i patomorfologicheskie proyavleniya, lechebno-profilakticheskie meropriyatiya]: Author's abstract. thesis ... Cand. of Sciences (Vet. Med.). Stavropol; 2001. 18 p. Available at: https://rusneb.ru/catalog/000200_000018_RU_NLR_bibl_560584/viewer. (in Russian)

5. Sebryakov Ye. V. Structural and functional characteristics of fungi of the genus *Candida* and pathogenetic features of candidiasis in farm animals [Strukturno-funkcional'naya harakteristika gribov roda *Candida* i patogeneticheskie osobennosti kandidamikoza sel'skohozyajstvennyh zhivotnyh]: Author's abstract. thesis … Doctor of Sciences. (Vet. Med.). M.; 1991. 51 p. Available at: https://rusneb.ru/catalog/000199_000009_000347294/viewer/?page=8. (in Russian)

6. Du J., Wang X., Luo H. Epidemiological investigation of non-albicans *Candida* species recovered from mycotic mastitis of cows in Yinchuan, Ningxia of China. *BMC Vet. Res*. 2018; 14:251. DOI: 10.1186/s12917-018-1564-3.

7. Sachivkina N., Lenchenko E. M., Lyseizev A. V. Diagnostic of swine candidiasis. *Veterinariya*. 2018; 11: 26–30. DOI: 10.30896/0042-4846.2018.21.11.26-30. (in Russian)

8. Apanasenko N. A. Specific therapy and prevention of cutaneous candidiasis of carnivores [Specificheskie terapiya i profilaktika kozhnogo kandidoza plotoyadnyh zhivotnyh]: Author's abstract, thesis ... Cand. of Sciences (Vet. Med.). M.; 2010. 24 p. Available at: https://rusneb.ru/catalog/000199_000009_004613111/viewer. (in Russian)

9. Bessarabov B. F., Vashutin A. A., Voronin Ye. S., et al. Infectious diseases of animals [Infekcionnye bolezni zhivotnyh]. Ed. by A. A. Sidorchuk. M.: KolosS; 2007. 671 p. Available at: https://www.studmed.ru/view/bessarabov-bf-vashutin-aa-voronin-es-i-dr-infekcionnye-bolezni-zhivotnyh_30a18b3684f.html?page=1. (in Russian)

10. Asfaw M., Dawit D. Review on major fungal disease of poultry. *British J. Poultry Sci*. 2017; 6 (1): 16–25. DOI: 10.5829/idosi.bjps.2017.16.25.

11. Laffey S. F., Butler G. Phenotype switching affects biofilm formation by *Candida parapsilosis*. *Microbiology*. 2005; 151 (Pt 4): 1073–1081. DOI: 10.1099/mic.0.27739-0.

12. Lenchenko E. M., Blumenkrants D. A. Study of biofilms of enterobacteria appeared in animals with alimentary diseases. *Veterinariya*. 2020; 1: 25–29. DOI: 10.30896/0042-4846.2020.23.1.25-29. (in Russian)

13. Ruiz A. C., Calderón D. M., Gómez J. E. Molecular variation of *Candida* spp. isolates using Random Amplified Polymorphic DNA (RAPD), from women in Armenia, Colombia. *Infectio*. 2009; 13 (1): 21–35. DOI: 10.1016/S0123-9392(09)70140-7.

14. Kaneko Y., Miyagawa S., Takeda O., Hakariya M., Matsumoto S., Ohno H., et al. Real-time microscopic observation of *Candida* biofilm development and effects due to micafungin and fluconazole. *Antimicrob. Agents Chemother*. 2013; 57 (5): 2226–2230. DOI: 10.1128/AAC.02290-12.

15. Lenchenko E. M., Mansurova E. A., Motorygin A. V. Characterization of toxigenic *Enterobacteriaceae* from farm animals with gastrointestinal diseases. *Agricultural Biology* [*Sel'skokhozyaistvennaya biologiya*]. 2014; 2: 94–104. DOI: 10.15389/agrobiology.2014.2.94rus. (in Russian)

16. Lenchenko E., Lozovoy D., Strizhakov A., Vatnikov Yu., Byakhova V., Kulikov Eu., et al. Features of formation of *Yersinia enterocolitica* biofilms. *Vet. World*. 2019; 12 (1): 136–140. DOI: 10.14202/vetworld.2019.136-140.

17. Lenchenko E., Blumenkrants D., Sachivkina N., Shadrova N., Ibragimova A. Morphological and adhesive properties of *Klebsiella pneumoniae* biofilms. *Vet. World*. 2020; 13 (1): 197–200. DOI: 10.14202/vetworld.2020.197-200.

18. Mannapova R. T., Shajhulow R. R. Dynamics of *Lactobacillus* spp. against the backdrop of candidiasis in the digestive tract of geese. *Materials of the International Conference: Scientific Research of the SCO Countries: Synergy and Integration*. Beijing: PRC; 2018. 248–252.

19. Manoyan M. G., Sokolov V. V., Gursheva A. S., Gabuzyan N. A., Panin A. N. Assessment of the risks of antimycotic drug resistance [Ocenka riskov vozniknoveniya rezistentnosti k antimikoticheskim sredstvam]. *Advances in Medical Mycology.* 2019; 20: 706–711. eLIBRARY ID: 38171260. (in Russian)

20. Khaitovich A. B., Gaffarova A. S. Pathogenicity factors of *Candida albicans* and determination of their genes. *Tavricheskiy Mediko-Biologicheskiy Vestnik*. 2016; 19 (3): 121–126. eLIBRARY ID: 27687028. (in Russian)

21. Lenchenko E. Morphofunctional properties and population variability of *Yersinia* affecting farm animals, depending on the temperature factor [Morfofunkcional'nye svojstva i populyacionnaya izmenchivost' iersinij, porazhayushchih sel'skohozyajstvennyh zhivotnyh, v zavisimosti ot temperaturnogo faktora]. *Agricultural Biology* [*Sel'skokhozyaistvennaya biologiya*]. 1996; 6: 88–95. (in Russian)

22. Lenchenko E. M., Vatnikov Yu. A., Kulikov E. V., Lozovoy D. A., Gavrilov V. A., Gnezdilova L. A., et al. Aspects of Salmonellosis pathogenesis using chicken models. *Bali Med. J*. 2019; 8 (1): 206–210. DOI: 10.15562/bmj.v8i1.920.

23. Yelinov N. P. News in *Candida* species taxonomy (lecture). *Problems in Medical Mycology*. 2010; 12 (3): 3–9. Available at: http://www.rusmedserv.com/mycology/html/mapo_3_2010.pdf. (in Russian)

24. Treviño-Rangel R. de J., González G. M., Martínez-Castilla A. M., García-Juárez J., Robledo-Leal E. R., González J. G., et al. *Candida parapsilosis* complex induces local inflammatory cytokines in immunocompetent mice. *Med. Mycol.* 2015; 53 (6): 612–621. DOI: 10.1093/mmy/myv021.

25. Sachivkina N., Lenchenko E., Strizakov A., Zimina V., Gnesdilova L., Gavrilov V., et al. The evaluation of intensity of formation of biomembrane by microscopic fungi of the *Candida* genus. *Int. J. Pharmaceutical Res.* 2018; 10 (4): 738–744. DOI: 10.31838/ijpr/2018.10.04.128.

26. Wu Y., Min F., Pan J., Wang J., Yuan W., Zhang Yu., et al. Systemic *Candida parapsilosis* infection model in immunosuppressed ICR mice and assessing the antifungal efficiency of Fluconazole. *Vet. Med. Int.* 2015; 2015:370641. DOI: 10.1155/2015/370641.

27. ATCC: The Global Bioresource Center. Available at: https://www.lgcstandards-atcc.org.

28. Chandra J., Mukherjee P. K. *Candida* Biofilms: Development, Architecture, and Resistance. *Microbiol. Spectr.* 2015; 3 (4). DOI: 10.1128/microbiolspec. MB-0020-2015.

29. Chandra J., Kuhn D. M., Mukherjee P. K., Hoyer L. L., McCormick T., Ghannoum M. A. Biofilm formation by the fungal pathogen *Candida albicans*: development, architecture, and drug resistance. *J. Bacteriol.* 2001; 183 (18): 5385–5394. DOI: 10.1128/jb.183.18.5385-5394.2001.

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INFORMATION ABOUT THE AUTHORS / ИНФОРМАЦИЯ ОБ АВТОРАХ

Ekaterina M. Lenchenko, Doctor of Science (Veterinary Medicine), Professor, Department of Veterinary Medicine, Moscow State University of Food Production, Moscow, Russia.

Nadezda P. Sachivkina, Candidate of Science (Biology), Associate Professor, Department of Microbiology and Virology, Medical Institute, Peoples' Friendship University of Russia, Moscow, Russia.

Ленченко Екатерина Михайловна, доктор ветеринарных наук, профессор кафедры ветеринарной медицины Московского государственного университета пищевых производств, г. Москва, Россия.

Сачивкина Надежда Павловна, кандидат биологических наук, доцент кафедры микробиологии и вирусологии медицинского института Российского университета дружбы народов, г. Москва, Россия.