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Research Article

Opportunistic yeasts *Candida parapsilosis* sensu stricto from soil of children's recreational areas in the city of Moscow

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Abstract: Strains of the opportunistic yeast *Candida parapsilosis* were isolated from urban topsoil on playgrounds in Moscow. Evaluation of the susceptibility profile of the isolated strains to three conventional antifungal drugs (fluconazole, voriconazole and amphotericin B) showed a high proportion of resistant strains to fluconazole. Two strains showed multiresistance, i.e. resistance to all three antifungal agents tested. Thus, urban topsoil around playgrounds can be considered a reservoir for the maintenance and spread of yeast strains that pose a potential risk to the health of children and adults.

Keywords: urban topsoil; playgrounds; resistance to antifungal compounds; *Candida parapsilosis*

Introduction

Soil yeasts are an obligatory component of the microbial communities of various undisturbed and slightly disturbed soils in natural ecosystems as well as heavily amended urban soils in urban ecosystems [1-4]. The specificity of changes in the taxonomic structure of soil yeast complexes in urban soils largely depends on the type and intensity of the prevailing anthropogenic influences and their intensity. There may be a decrease in the proportion of typical saprotrophic pedobiont basidiomycete species of yeasts (*Apiotrichum porosum, Goffeauzyma gastrica, Saitozyma podzolica, Solicoccozyma terricola, Tausonia pullulans*, etc.), an increase in the proportion of red-pigmented species (*Cystofilobasidium capitatum, Cys. infirmominiatum, Cys. macerans, Rhodotorula babjevae, Rh. glutinis, Rh. mucilaginosa*) and an increase in the share of opportunistic species of the genus *Candida* (C. *albicans, C. tropicalis, C. parapsilosis, Diutina catenulata* (C. catenulata), *Pichia kudriavzevii* (*C. krusei*), *Meyerozyma guilliermondii* (*C. guilliermondii*). The latter is particularly characteristic of urban soils in areas where household waste is stored and disposed of in the city [5-6].

Yeasts in urban soils near playgrounds have been insufficiently studied. However, the presence of opportunistic and pathogenic yeast species from the genera *Candida* and *Cryptococcus* has already been demonstrated by researchers from Poland for urban soils in the playground area of Lodz, Poland [7]. The presence of opportunistic and pathogenic yeasts here can pose a serious risk to children whose immune system is not yet sufficiently developed, as well as to people with a low immune status, the proportion of which is quite high in the urban population.

The aim of our work was to examine the topsoil (0-15 cm) of playgrounds in Moscow for the presence of opportunistic and pathogenic yeasts of the genus *Candida* and to evaluate the susceptibility profile of the isolated strains to three common antifungal drugs (fluconazole, voriconazole and amphotericin B), which are most commonly available in Russia. Better treatment of candidiasis should include echinocandins, followed by a stepdown to azoles when appropriate. Although echinocandins were included in the EML in 2021 (WHO Essential Medicines List) in 2021, they are unfortunately still not always available in the country.

Methods

Study Location and Sampling

Sampling took place from the end of August 2022 (when there are particularly many children in playgrounds) to the beginning of November 2022 (until the air temperature began to fall below zero). The samples were collected on the territory of the city of Moscow from ten playgrounds in the south-eastern residential district of the city (**Figure 1**). A total of 60 samples of topsoil were analyzed (six samples from each playground).

Figure 1. South-eastern district of Moscow where topsoil samples around playgrounds were collected.

The collected samples were packed in a sterile zip bag and provided with an accompanying label. The samples were packed in special cooling bags at a temperature of 8 °C and delivered to the laboratory for microbiological analysis within 24 hours. In the laboratory, the samples were stored in a refrigerated chamber at a temperature of no more than 4 °C for a maximum of four days. Each topsoil sample was then taken and poured with sterile water to obtain a dilution of 1:10. The suspensions were vortexed on a Multi Reax Vortexer (Heidolph Instruments, Germany) for 15 minutes at 2000 rpm. Three suspensions were prepared per sample. And each suspension was plated in four replicates on solid culture media.

The chromogenic medium "CandiSelect 4" (Bio-Rad, France) was used for the rapid presumptive identification of opportunistic yeast species of the genus Candida. The plates were incubated at 37 °C. The growth of *Candida* spp. was inspected at 24, 48, 72, 96 and 120 hours. *Candida parapsilosis* ATCC 22019 was used as a positive control and *Escherichia coli* ATCC 25922 as a negative control. The grown yeast colonies were counted and isolated in a pure culture. A total of 96 colonies were isolated.

It should be noted that our study on opportunistic *Candida* was limited by the culturing conditions used. "CandiSelect 4" allows the differentiation of opportunistic Candida species and does not favor (under simultaneous cultivation conditions of 37 °C) some other psychrophilic *Candida* species (e.g. *C. sake, C. norvegica*), which are common in urban soils in the Moscow region. At the same time, our aim was to identify only those *Candida* species that are most prevalent in clinical practice worldwide and are associated with the highest number of severe infections and the highest mortality rate in immunocompromised individuals [8-9]. We also focused our work on identifying only the *Candida* species included in the "WHO fungal priority pathogens list" in 2022 [10].

Molecular species identification

The yeast species were molecularly identified using ITS rDNA region as a universal DNA-barcoding for fungi [11]. The nuclear ribosomal ITS1-5.8S-ITS2 region was amplified and sequenced using ITS5 primer. The criteria described in Vu [12] were used to separate the yeast species. DNA isolation and PCR were performed according to the procedure described previously [13-14]. DNA sequencing was performed using the Big Dye Terminator V3.1 Cycle Sequencing Kit (Applied Biosystems, Waltham, MA, USA) with subsequent analysis of the reaction products on an Applied Biosystems 3130xl Genetic Analyzer at the facilities of Evrogen (Moscow). For sequencing, the ITS5 primer (5'-GGA AGT AAA AGT CGT AAC AAG G) was used [13]. For species identification, nucleotide sequences were compared with those in public databases, using the BLAST NCBI (www.ncbi.nlm.nih.gov) and the MycoID (www.mycobank.org) tools. The ITS regions of the strains studied were 99.5–100% similar to the type strains. All the sequenced cultures were cryopreserved in 10% (v/v) glycerol in water solution at -80 °C in the yeast collection of the Soil Biology Department at Lomonosov Moscow State University (WDCM CCINFO number: 1173; catalog: https://depo.msu.ru/).

Susceptibility to antifungal compounds

The isolated strains were examined for antifungal susceptibility to three conventional antifungal drugs. The antifungal susceptibility was tested using Mueller-Hinton agar (HiMedia Laboratories Pvt. Ltd., India), which is a standard medium for the disk diffusion method [15-16]. Disks with three widely applicated antifungal agents (HiMedia Laboratories Pvt. Ltd., India) were used: Amphotericin B (100 μg), Fluconazole (25 μg), Voriconazole (1 μg). The following equivalent MIC breakpoints (μg/mL) or epidemiological cut-off values (ECV) were used to categorize these strains as sensitive (S) or resistant (R) [17-19]: FLZ (fluconazole) (S MIC ≤ 2 μg/mL, R MIC ≥ 8 μg/mL); VRZ (voriconazole) (S MIC ≤ 0.125 μg/mL, R MIC ≥1 μg/mL); and AmB (amphotericin B) (ECV S MIC ≤ 2 μg/mL, R MIC > 2 μg /mL). The reference strain of *C. parapsilosis* ATCC 22019 was used as a control. A total of 96 isolates were tested in triplicate for each antifungal.

Results

During the study period, a total of 96 strains of an opportunistic species, *Candida parapsilosis* sensu stricto, were isolated from the topsoil of ten playgrounds in the city. The largest number of isolates was detected in August (68), the smallest in November (11). Opportunistic yeasts *Candida parapsilosis* sensu stricto were found in the topsoil of all ten playgrounds examined.

Evaluation of the susceptibility profile of all isolated strains to three commonly used antifungals (fluconazole, voriconazole and amphotericin B) using the disc diffusion method showed that there were strains resistant to at least one of the antifungals tested. The percentage of strains resistant to fluconazole was 46%, to voriconazole 21% and to amphotericin B 3% (**Figure 2**).

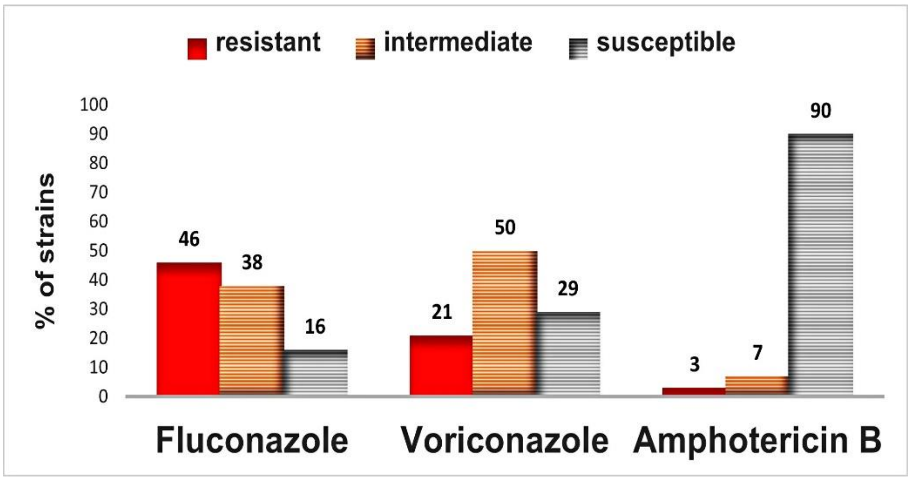

Figure 2. Susceptibility pattern of strains of *Candida parapsilosis* sensu stricto isolated from playground topsoil to fluconazole, voriconazole and amphotericin B.

Candida parapsilosis is a globally distributed commensal yeast with pathogenic potential. It is a normal component of the human body and causes no harm under healthy conditions. However, it can cause invasive infections (invasive candidiasis) of the blood (candidemia), heart, central nervous system, etc. in sick and immunocompromised people. Trends over the last 10 years show an increase in invasive *C. parapsilosis*. In some regions, this species is the main causative agent of non-*C. albicans* candidaemia [10]. In general, its antifungal resistance is moderate. However, resistance rates to azoles have been reported to have increased across multiple regions [10,20].

In late 2022, the World Health Organization (WHO) published the first fungal priority pathogens list — the WHO FPPL [10] in which *C. parapsilosis* was included in the High Group. We hope that the first published "WHO fungal priority pathogens list to guide research, development and public health action", based on the success of bacterial priority pathogens list (the WHO BPPL 2017), will prove to be a real game-changer in practice [21] and draw more attention both locally and globally to such neglected public-health issues as the accumulation and dispersal of opportunistic yeasts in urban ecosystems.

Conclusion

Thus, urban topsoil around playgrounds can be considered a reservoir for the maintenance and spread of yeast strains that pose a potential risk to the health of children and adults. The pilot study has shown that further research is needed to increase the number of sampling sites, increase the number of samples analyzed, expand the geography of the studies, expand the list of conventional antibiotics tested, additionally consider the seasonality factor, etc.

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Conflicts of Interest: The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

Ethics statement: The study was approved by the Ethics Committee of Lomonosov Moscow State University.

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