

Article

Diversity of Culturable Yeasts in the Feces of Mew Gulls Breeding in Natural and Urban Habitats, with Insights into the Antifungal Susceptibility of the Observed Pathogens

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Simple Summary: The study focused on the comparison of culturable yeast communities in the feces of two populations of Mew Gulls depending on the breeding site: natural and urban. It was found that the species diversity of yeasts in the feces of gulls from the natural population was significantly higher, while the diversity of pathogenic species in the feces of birds from the anthropogenic population increased. The proportion of strains of pathogens (*Candida*, *Rhodotorula*) isolated from the feces of the anthropogenic colony that were resistant to conventional antibiotics was high. Indeed, populations of migratory birds breeding in an anthropogenic area could be involved in the global spread of the “alarming” geography of antifungal resistance, which is an urgent public health problem.

Abstract: Migratory birds play an important role in the spread of yeasts in the environment over long distances and in different geographical regions. Human activities, in turn, have a major impact on the biology of wild birds and, consequently, on the microbial communities for which birds act as carriers and disseminators. We sought to assess the “response” of the diversity of culturable yeasts in the feces of Mew Gulls to the type of nesting site (natural/anthropogenic) during the breeding season from April to October 2023. We isolated and molecularly identified 26 yeast species. The species composition in the feces of birds from the natural habitat was more diverse, and the diversity increased from April to October. In contrast, the diversity in the feces of birds from the urban habitat decreased from April to October. Analysis of susceptibility to conventional antibiotics (fluconazole, voriconazole, and amphotericin B) using the CLSI BMD (Clinical and Laboratory Standards Institute broth microdilution) method in isolated strains of opportunistic *Candida* (*C. parapsilosis* and *C. tropicalis*) and strains of the emerging pathogen *Rhodotorula mucilaginosa* showed that the proportion of resistant strains was higher in strains isolated from the feces of birds from the anthropogenic population. Mew Gulls that spent their breeding season near a landfill and flew away for wintering appear to be a source for the spread of pathogenic yeasts with resistance against antifungal agents.

Keywords: seabirds; droppings; antibiotic resistance; *Candida*; *Rhodotorula*; landfill



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1. Introduction

Urban areas pose various challenges for wildlife, such as human disturbance and interaction with anthropogenic waste, among other stressors [1,2]. However, urban settlements also create new ecological niches in which some species can survive, mainly due to the availability and predictability of anthropogenic food resources [2–4]. These include, in particular, species that are generalists [5], such as gulls from the *Laridae* family, which have increased exponentially in urban areas in recent decades [2].

Gulls can nest, breed, and live in cities anywhere in the world, and the term “urban gull” is increasingly used today [6–8]. The first report of a gull nesting on rooftops dates

back to 1894, when a Herring Gull nested on a rooftop in the Black Sea region. Since then, gulls have colonized urban environments around the world, with significant populations in various cities in Europe, North America, and Australia [2,9]. They rely on man-made food resources, feed on landfills, and increasingly interact with anthropogenic wastes [2]. Some species are even more successful in urban areas than in their natural habitats, as they are very flexible and use new feeding and nesting habitats [5,10,11]. In particular, landfills create an abundant and predictable new food source [12] with huge amounts of man-made “junk” food attracting birds [2,13]. At the same time, however, foraging in landfills can weaken bird health through the ingestion of anthropogenic materials (glass, plastic) [14] or the bioaccumulation of heavy metals [15]. In addition, the quality of anthropogenic food itself is nutritionally inferior to that of natural food [16,17]. And there is a consistently high risk of interaction with various pathogens (viruses, bacteria, microfungi, and protozoa) [2,5,18,19], including potentially pathogenic yeasts and yeast-like fungi, which they can transmit via their excretions from the environment [20–23].

The isolation of potentially pathogenic yeasts (*Candida albicans*, *C. tropicalis*, *Nakaseomyces glabratus* (*C. glabrata*) from fecal samples of various gull species—Yellow-legged Gull (*Larus michahellis*), Herring Gull (*Larus argentatus*), Kelp Gull (*Larus dominicanus*), Lesser Black-backed Gull (*Larus fuscus*)—has been demonstrated in a growing number of studies [5,24–29]. At the same time, most studies refer to man-made habitats. We have not found comparative studies that have simultaneously examined yeast complexes in the feces of gulls from natural and anthropogenic habitats in their dynamics, focusing on both natural and pathogenic species and evaluating the virulent properties of the latter.

The pathogenic yeast *C. albicans* was isolated from bird droppings when gulls were fed with *C. albicans*-infected fish in captivity [26]. When they were fed with uninfected fish, *C. albicans* was gradually no longer detected in the feces. This confirms that the diet and habitat of the birds have a major influence on the diversity of yeasts in their feces.

The yeasts *C. parapsilosis* and *C. tropicalis* are known to be globally distributed, potentially pathogenic species that have been included in the High Group in WHO fungal priority pathogens list to guide research, development, and public health action in 2022 [30]. *C. parapsilosis* is a normal part of the human microbiota and causes no harm under healthy conditions. It can cause invasive infections of the blood, heart, central nervous system, eyes, and internal organs, especially in people with weakened immune systems. Among non-*albicans* pathogens, *C. parapsilosis* is one of the most important opportunistic yeast species, with an increasing prevalence among clinical isolates [31–34]. In some regions, this species is the main cause of candidemia not caused by *C. albicans* [35–37]. *C. tropicalis* is also a common member of the human microbiota and does not cause damage under healthy conditions [34]. And like *C. parapsilosis*, it can cause invasive infections in immunocompromised individuals. Trends over the last decade indicate an increasing incidence of *C. tropicalis* [30,38]. We have already detected both species in the feces of the semi-synanthropic birds, Eurasian magpie (*Pica pica*) and Eurasian jay (*Garrulus glandarius*) [39].

Rh. mucilaginosa is a ubiquitous species that can be recovered from many sources around the world. It is widely distributed in soil, water, and phylloplane and is also frequently extracted from food and beverages [40–44]. Fungal infections in humans caused by *Rhodotorula* species have increased in recent decades [42,45–47]. *Rhodotorula* species that were previously considered non-pathogenic have emerged as opportunistic species that can colonize and infect susceptible patients [42]. While no cases of *Rhodotorula* infections were reported in the medical literature prior to 1985, the number of infections increased thereafter, most likely due to the increased use of intensive care and central venous catheters [48].

Like other migratory birds, many gulls should be considered important vectors of yeasts (including those pathogenic to humans) between wintering areas and breeding areas [20,23,49]. The distance between them can be thousands of kilometers. Due to the high mobility of gulls, these birds facilitate the spread of yeasts by depositing their feces over a large area in marine and terrestrial natural and urban environments [28]. As the

seasonal migration of gulls is a widespread phenomenon, the dispersal of yeasts by these migratory birds could contribute to biodiversity in many geographical locations and should be considered in biodiversity assessments [23]. But at the same time, it is important to note that global warming is now contributing to a change in the migratory behavior of different urban bird species (from migratory to resident), as warmer winters allow residents to survive in the season when food availability is at its lowest [50].

Apart from the fact that gulls can potentially spread pathogenic microbial species, it has also been shown that antibiotic-resistant bacteria [51–54] and yeasts [28,29] may be present among the strains isolated from bird feces. The high nutrient content of bird droppings provides an excellent haven for potentially harmful organisms [55–58]. This can pose a threat to public health, especially when these areas are in close proximity to humans [57–59]. Resistance to antifungals is one of the most important virulence factors in microorganisms [43,60]. The decreasing susceptibility of pathogenic yeasts to conventional antibiotics is known worldwide and is an important public health issue.

The aim of our study was to investigate how the yeast diversity in the feces of Mew Gulls (*Larus canus* L.) changes during the breeding season from their arrival in April to their departure to the wintering grounds in October, depending on their habitat. We hypothesized that the habitat (anthropogenic or natural) influences the culturable yeast diversity in the feces of Mew Gulls and that this influence is also affected by seasonal factors. We also predicted that in anthropogenic habitats, potentially pathogenic species with active virulent properties (resistance against antifungal drugs) should be present in bird droppings. For this purpose, two gull colonies were selected: (1) a colony nesting at a natural site along a system of Klepikovo lakes on the territory of Meshchera and Meshchera National Parks in the subzone of coniferous and deciduous forests and (2) a colony nesting on ponds near the territory of one of the largest landfills in Europe “Timokhovo” in the Moscow region. In addition, we tried to investigate the susceptibility to worldwide used antifungals (fluconazole, voriconazole, and amphotericin B) in the strains of potentially pathogenic yeasts (*Candida parapsilosis*, *C. tropicalis*, and *Rhodotorula mucilaginosa*) observed in feces of gulls in vitro.

2. Materials and Methods

2.1. Study Sites

Fecal samples were collected from two colonies of Mew Gulls (the size of each gull colony in the habitat was 40–50 pairs) at two different sites (Figure 1) during their breeding season from April to October in 2023. Mew gulls are migratory birds in the study area. The birds overwinter in the Mediterranean region, sometimes also in Iran and the Persian Gulf, on the shores of ice-free seas.

One population of birds nested at a natural site along a system of Klepikovo lakes (55°14'00" N 40°10'00" E) on the border between Vladimir, Ryazan, and Moscow regions, partly on the territory of Meshchera and Meshchera National Parks in the subzone of coniferous and deciduous forests. Another bird population nested at an urban site along ponds near the largest landfill in Europe “Timokhovo” (1.14 km²), located in the Moscow region (55°27'40" N 38°10'21" E). The maximum capacity of the landfill is 450,000 tons per year (biowastes included). According to some reports, it can hold up to 1,500,000 tons per year.

The study areas are characterized by a temperate continental climate with cold, snowy winters and moderately warm summers (<https://ru.wikipedia.org/wiki/>, accessed on 13 January 2024) (Table 1).

Birds were observed using Carl Zeiss Victory 8 × 42 SF (42 × 8) binoculars (Wetzlar, Germany), with all elements of feeding activity and defecation recorded in detail. Two populations of one bird species were selected for the study to dynamically (during the seven-month breeding season) assess the influence of habitat on fecal yeast community structure.



Figure 1. Photos taken in the second half of the summer showing striking visual differences in the habitat of two Mew Gull colonies: Natural ((A,B) were taken from <https://gnazarkin456.wixsite.com/natureryazan/landscapes>, accessed on 9 April 2024) and Anthropogenic ((C,D) were taken from <https://regnum.ru>, accessed on 9 April 2024).

Table 1. Average monthly values of temperature, humidity, and wind speed at the locations (natural/urban) during the sampling period (April–October 2023). (<http://pogoda-service.ru/archive> (accessed on 9 April 2024)).

Month	Temperature, Day (°C)	Temperature, Night (°C)	Wind Speed, (m/s)	Air Humidity, (%)
April	13/14	4/6	2.1/1.0	62/62
May	17/16	8/8	1.7/0.9	59/63
June	19/21	9/12	1.8/1.0	59/61
July	21/21	14/15	2.3/0.9	75/74
August	24/23	15/16	1.5/0.7	71/74
September	19/20	10/12	1.7/0.7	74/72
October	5/7	3/5	3.3/0.6	83/81

2.2. Sample Collection and Processing

Fresh feces were collected to avoid possible bias in the selection of yeast species that resist desiccation. Bird excrements were collected using sterile gloves and sterile cotton swabs. Each swab was dipped in 3 mL of sterile saline (0.9% NaCl) supplemented with 0.5 g/L chloramphenicol transport medium and delivered to the laboratory for analysis. The samples were labeled according to origin and date. The samples were processed within 24 h from the moment of collection. Each swab was shaken in the transport medium on the Multi Reax Vortex (Heidolph Instruments, Schwabach, Germany) for 15 min at 2000 rpm and then allowed to sediment for 15 min. After the swab was removed, the supernatant was transferred to a sterile conical tube. Subsequently, 100 µL of the supernatant was inoculated onto (GPY) agar (20 g/L glucose, 10 g/L peptone, 5 g/L yeast extract, 20 g/L agar) supplemented with chloramphenicol (500 mg/L). The samples were taken at two locations from Monday to Friday morning between 10:00 and 11:00 GTM for seven months (a total of 40 samples from both areas per month). A total of 280 excretions were collected from two populations (from natural and anthropogenic habitats) of Mew Gulls (140 samples per population). Each sample was plated in three replicates. The plates were incubated at 22 °C for 9–12 days. The colonies were differentiated into macromorphological types using a dissecting microscope, counted, and 3–5 representatives of each colony type were transferred to pure culture and then molecularly identified. A control examination of the

waste samples for yeast diversity by plating on the same medium as the fecal samples was performed monthly (data not included in the article).

2.3. Molecular Identification of Pure Cultures

The yeasts were molecularly identified using the ITS rDNA region as a universal DNA-barcoding for fungi [61]. The nuclear ribosomal ITS1-5.8S-ITS2 region was amplified and sequenced using ITS5 primer. The criteria described in Vu [62] were used to separate the yeast species. DNA isolation and PCR were performed according to the procedure described previously [63,64]. 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, Russia). For sequencing, the ITS5 primer (5'-GGA AGT AAA AGT CGT AAC AAG G) was used [64]. For species identification, nucleotide sequences were compared with those in public databases, using the BLAST NCBI (www.ncbi.nlm.nih.gov (accessed on 24 February 2024)) and the MycoID (www.mycobank.org (accessed on 24 February 2024)) tools. The ITS regions of the strains studied were 99.5–100% similar to the type strains. Sequences obtained in the present study for yeast species were deposited in the GenBank database (OR582606; OR582608–OR582609; PP481694–PP481716, Table 2). All the purified and sequenced yeast strains isolated in this study 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/>, accessed 20 June 2024). Isolates of *C. parapsilosis* and *C. tropicalis* (*C. parapsilosis*—48 strains; *C. tropicalis*—30 strains) were further identified by growth on chromogenic medium “Brilliance Candida Agar” (Thermo Scientific™, Waltham, MA, USA). The plates were incubated aerobically at 30 °C. *Candida albicans* ATCC 10231, *Candida parapsilosis* ATCC 22019, and *Candida tropicalis* ATCC 750 were used as the positive control, and *Escherichia coli* ATCC 25922 was used as the negative control.

Table 2. Species list and relative abundance of yeast taxa isolated from the feces of two populations of Mew Gulls during their breeding season (April–October 2023).

Yeast Species	GenBank Accession No.	Mew Gulls (Natural Habitat)							Mew Gulls (Anthropogenic Habitat)						
		1 *	2	3	4	5	6	7	1	2	3	4	5	6	7
<i>Aureobasidium pullulans</i>	PP481694	1.2	1.1	1.4	0.9	3.1	3.5	3.6	1.5	4.2	10.8	15.8	20.6	20.8	20.5
<i>Candida boleticola</i>	PP481695	0	0	5.1	4.4	4.2	3.1	2.2	0	0	0	0	0	0	0
<i>Candida cetoniae</i>	PP481696	0	5.7	1.2	0	0	0	0	0	0	0	0	0	0	0
<i>Candida parapsilosis</i>	PP481697	0	0	0	0	1.1	0	0	0	1.1	2.4	3.2	2.1	2.5	2.4
<i>Candida santamariae</i>	PP481698	5.2	1.4	2.2	2.1	1.9	2.4	2.1	6.1	4.2	2.1	2.1	1.8	1.6	1.9
<i>Candida tropicalis</i>	PP481699	0	0	0	0	0	0	0	0	0	0.8	2.2	3.3	2.1	2.1
<i>Candida zeylanoides</i>	PP481700	20.8	21.4	25.7	22.3	20.1	18.5	19.6	22.8	20	21.7	19.8	18.1	19.5	18.4
<i>Cyberlindnera misumaensis</i>	PP481701	0	0	1.7	1.1	1.2	0.8	1.5	1.1	0	0	0	0	0	0
<i>Debaryomyces hansenii</i>	PP481702	42.4	40.4	40.2	40.6	36.4	35.3	37.8	44.2	39	37.1	35.2	28.2	30.1	29.1
<i>Hanseniaspora uvarum</i>	PP481703	7.2	5.6	3.1	2.5	2.1	2.4	2.6	10.2	5.6	2.4	1.2	0	0	0
<i>Metschnikowia colchici</i>	PP481704	0	1.2	0	0	0.9	1.1	1.1	0	5.2	5.1	5.1	4.2	2.1	0
<i>Metschnikowia pulcherrima</i>	PP481705	3.2	3.1	1.1	1.5	2.2	2.1	1.8	1.2	6.8	3.8	5.1	3.5	3.2	3.2
<i>Meyerozyma guilliermondii</i>	PP481706	5.2	4.8	3.4	2.8	3.2	3.5	2.8	2.1	4.2	4.1	1.2	1.5	0	0
<i>Priceomyces vitoshaensis</i>	PP481707	0	0	0	0	1.2	1.4	0.9	0	0	0	0	0	0	0
<i>Yarrowia alimentaria</i>	PP481708	0	0	0	2.4	2.1	0.9	1.2	0	0	0	0	0	0	0
<i>Yarrowia lipolytica</i>	PP481709	4.5	3.2	3.1	2.6	2.4	2	2.1	0	0	0	0	0	0	0
<i>Yarrowia</i> sp.	OR582608	0	0	0	2.2	2	2.1	2.1	0	0	0	0	0	0	0
<i>Wickerhamia</i> sp.	OR582606	0	0	0	0	0	0	1.2	0	0	0	0	0	0	0
<i>Wickerhamomyces</i> sp.	OR582609	0	0	0	0	0	0	1.4	0	0	0	0	0	0	0
<i>Cutaneotrichosporon moniliforme</i>	PP481710	0	2.2	1.8	1.5	1	1.1	1.5	1.5	0	0	0	0	0	0
<i>Goffeauzyma gastrica</i>	PP481711	3.7	1.5	1.2	2.3	2.4	2.2	2.1	0	0	0	0	0	0	0
<i>Naganishia adeliensis</i>	PP481712	0	1.1	1.2	1.1	2.1	2.4	2.1	1.1	0	0	0	0	0	0
<i>Rhodotorula mucilaginosa</i>	PP481713	4.4	3.2	3.5	3.3	5.6	6.2	8.1	5.6	8.8	9.1	9.1	16.7	20.1	22.4
<i>Tausonia pullulans</i>	PP481714	2.2	4.1	3.5	4.3	3.2	3.5	1.1	1.5	1.1	0.6	0	0	0	0
<i>Vanrija albida</i>	PP481715	0	0	1.1	2.1	1.6	5.5	0.5	1.1	0.5	0	0	0	0	0
<i>Tausonia</i> sp.	PP481716	0	0	0	0	0	0	0.6	0	0	0	0	0	0	0

* 1—April; 2—May; 3—June; 4—July; 5—August; 6—September; 7—October. Background color highlights patterns and trends in the relative abundance data.

Isolates of potentially pathogenic yeasts *Rh. mucilaginosa*, *C. parapsilosis*, and *C. tropicalis* found in the feces of natural and anthropogenic populations of Mew Gulls were tested in vitro for virulence potential using antifungal assays.

2.4. Antifungal Susceptibility Testing

The antifungal susceptibility profiles of *C. parapsilosis*, *C. tropicalis*, and *Rh. mucilaginosa* strains from feces were tested in vitro for susceptibility to fluconazole, voriconazole, and amphotericin B using the Clinical and Laboratory Standards Institute broth microdilution method (CLSI BMD). The following equivalent MIC breakpoints ($\mu\text{g/mL}$) or epidemiological cut-off values (ECV) were used to categorize these yeast strains as sensitive (S), intermediate (I) or resistant (R) [65–67]: FLZ (fluconazole) (S MIC $\leq 2 \mu\text{g/mL}$, I MIC = $4 \mu\text{g/mL}$, R MIC $\geq 8 \mu\text{g/mL}$); VRZ (voriconazole) (S MIC $\leq 0.125 \mu\text{g/mL}$, I MIC = $0.250\text{--}0.5 \mu\text{g/mL}$, R MIC $\geq 1 \mu\text{g/mL}$); and AmB (amphotericin B) (ECV S MIC $\leq 2 \mu\text{g/mL}$, R MIC $> 2 \mu\text{g/mL}$). The reference powders were obtained from the following manufacturers: Fluconazole and Amphotericin B from HiMedia (Mumbai, India) and Voriconazole from Tocris Bioscience (Cambridge, UK). Quality control was performed using *C. parapsilosis* ATCC 22019 and *C. tropicalis* ATCC 750. A total of 178 isolates (*C. parapsilosis*—48 strains; *C. tropicalis*—30 strains; *Rh. mucilaginosa*—100 strains) were tested. Each of the 178 strains was tested in three plate replicates for each antifungal agent.

2.5. Statistical Data Analyses

The structure of the yeast community was determined for each sample. The relative abundance was calculated as the proportion of a particular species in the sample and was based on the number of colonies. The species diversity of the yeasts was estimated using the Shannon index [68]. Simpson's diversity index ($1 - D$) was used to assess the dominance of yeast species [69]. Species evenness was assessed using the Pielou index [70]. Statistical analyses were performed using Statistica 8 (StatSoft Inc., Tulsa, OK, USA). The similarities between the yeast groups in the feces of gulls from different habitats during the breeding season were estimated using the UMPGA clustering technique based on the qualitative Sørensen and Bray–Curtis indices and performed with PAST 4.04 (Oslo, Norway). Beta diversity estimated using Bray–Curtis was also visualized using principal coordinates analysis (PCoA) using PAST 4.04 (Oslo, Norway). Effects were considered statistically significant at the $p \leq 0.05$ level.

3. Results

3.1. Main Features of Observed Yeast Diversity

A total of 26 yeast species were isolated and identified in this study. They belong to four lineages of fungi: *Pezizomycotina* (1 species), *Saccharomycotina* (18 species), *Agaricomycotina* (6 species), and *Pucciniomycotina* (1 species) (Table 2). The observed species richness in the feces of Mew Gulls varied between the minimum number of 8 species (in October, the last month of the breeding season, in the anthropogenic habitat) and the maximum number of 23 species (also in October, but in the natural habitat). The median species richness values differed significantly between the urban (11 species) and natural (19 species) populations of Mew Gulls (Mann–Whitney U Test, $p = 0.007$). On average, eight more yeast species were found in the feces of gulls from the natural population. The median species diversity values (Shannon index) also differed significantly between the urban (median value = 1.87) and natural (median value = 2.08) populations (Mann–Whitney U Test, $p = 0.008$).

The indices of yeast species richness, diversity, and community evenness changed in the feces of gulls from both colonies during the breeding season (Figure 2).

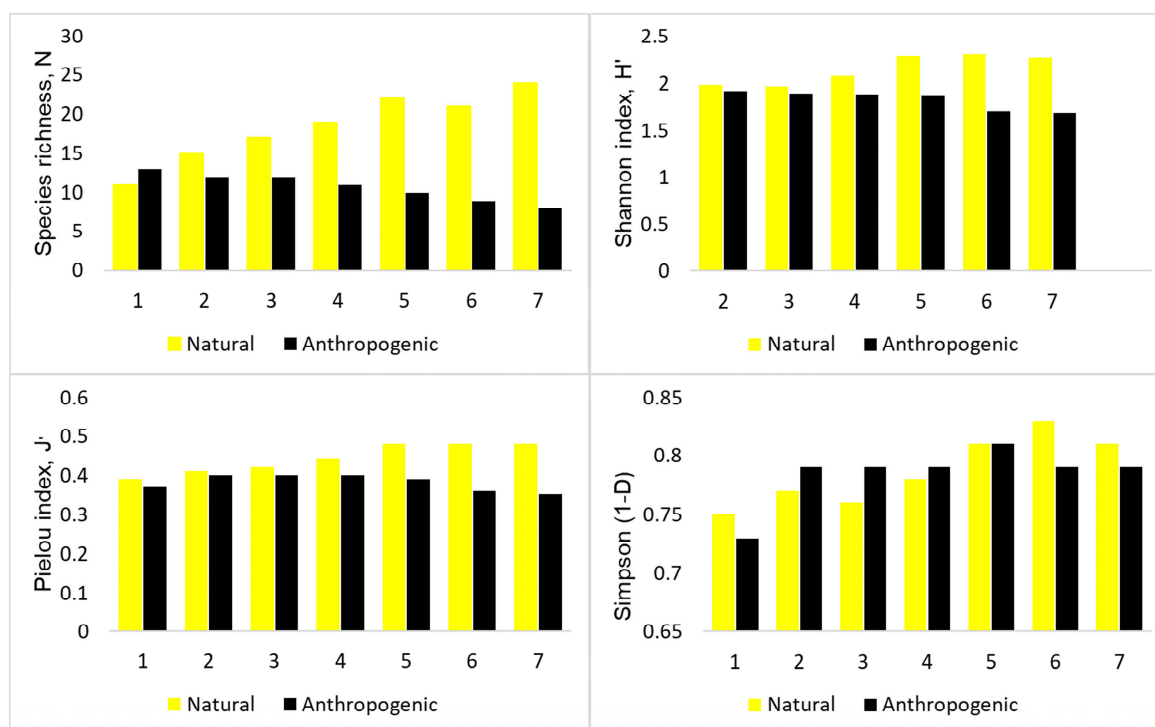


Figure 2. Changes in yeast species alpha diversity found in Mew Gull feces from natural and urban habitats during the seven-month breeding season from April to October 2023. 1—April; 2—May; 3—June; 4—July; 5—August; 6—September; 7—October. Yellow colour—natural habitat; Black colour—anthropogenic habitat.

Ascomycetous yeasts were more prevalent in gull feces from both habitats. Out of 26 yeast species observed, 19 taxa were ascomycetes, including two potentially pathogenic *Candida* yeasts: *C. parapsilosis* and *C. tropicalis*. In the samples from the natural habitat, 18 of the 19 detected ascomycetous species were found, with the exception of the opportunistic yeast *C. tropicalis*—which was isolated only in the feces of the anthropogenic population. The emerging pathogen *Rh. mucilaginosa* was isolated from the feces of gulls from both populations throughout the breeding season. In the population from the natural habitat, however, their proportion did not increase greatly from April (time of arrival) to October (time of departure for wintering) and averaged 4.9%. In the anthropogenic population, the relative abundance of *Rh. mucilaginosa* in feces increased from 5.6% upon arrival in April to 22.4% upon departure in October, and the average proportion of the species was 13.1% (Table 2).

Differences were found in the structure of yeast complexes and the number of yeast species by habitat and season. Only in the fecal samples collected in April, immediately after the arrival of the birds (at the beginning of the nesting season), were the yeast complexes similar in both habitats (Figure 3).

Beta diversity (Bray–Curtis) and principal coordinate analysis (PCoA) also demonstrated that at the end of the nesting season (August–October), the yeast complexes in the feces of Mew Gulls from the anthropogenic population were maximally dissimilar to the yeast complexes in the feces of Mew Gulls from the natural population (Figure 4).

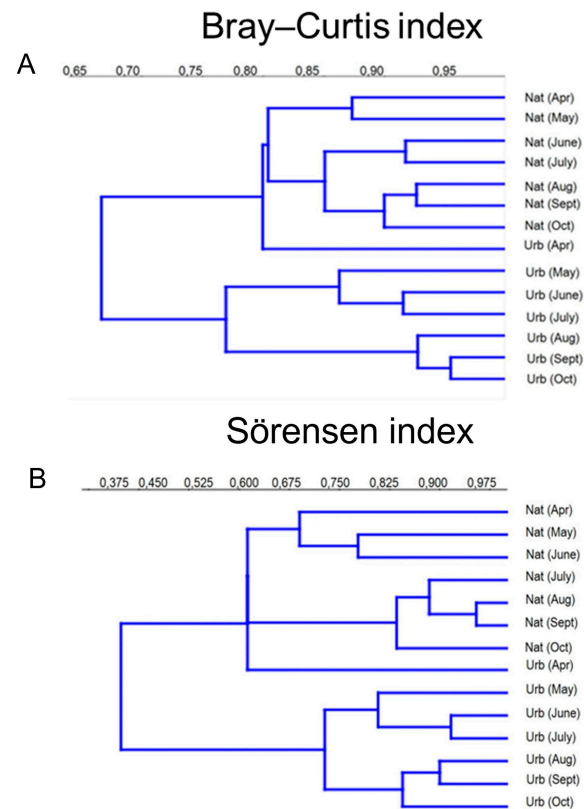


Figure 3. Comparison of studied yeast groups in fecal samples of Mew Gulls from natural and urban habitats during the breeding season by clustering analysis using the Bray–Curtis (A) and Sørensen (B) measures based on relative abundances and lists of species. Urb—urban; Nat—natural.

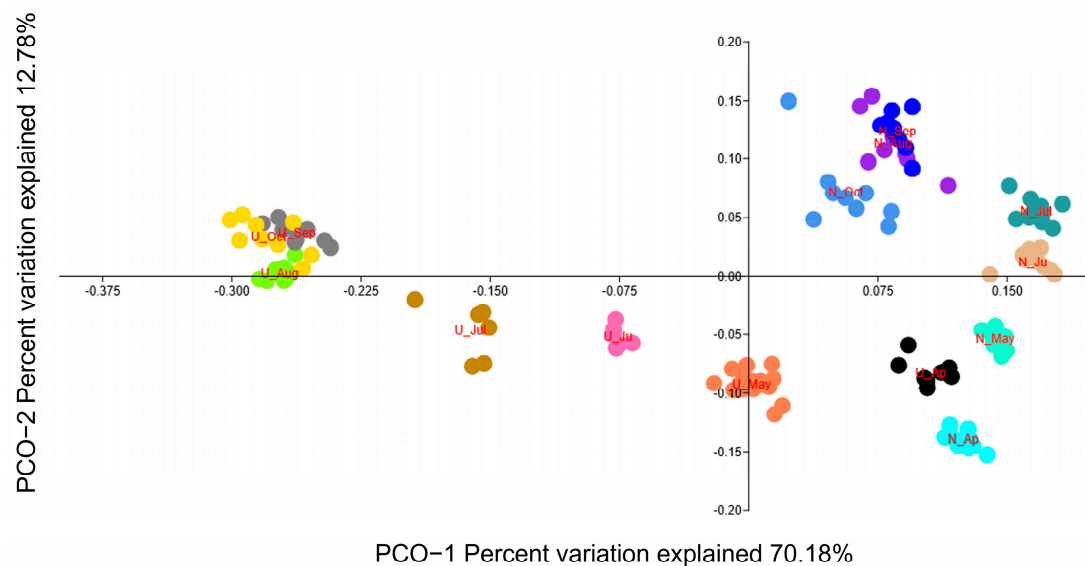


Figure 4. Principal Coordinates Ordination (PCoA) of natural and urban habitats and months of the breeding season according to species relative abundance based on Bray–Curtis similarity. Different colours demonstrate groups of samples from different habitats and months. U—urban; N—natural; Ap—April; May; Ju—June; Jul—July; Aug—August; Sep—September; Oct—October.

3.2. Susceptibility of *Rh. mucilaginosa*, *C. parapsilosis* and *C. tropicalis* to Conventional Antifungal Agents

The results of the tests for the strains are shown in Table S1 (Table S1a–c).

Rh. mucilaginosa

Resistance to fluconazole was detected in 4% of *Rh. mucilaginosa* strains isolated from the feces of Mew Gulls nesting in the natural habitat and in 18% of strains isolated from the feces of gulls nesting near the landfill; no resistance against voriconazole was detected in the strains from the feces of the natural gull population, while 10% of the strains from the anthropogenic population showed resistance against this drug; all strains isolated from the feces of gulls from the natural habitat were sensitive to amphotericin B, 6% of the strains isolated from the feces of the anthropogenic population showed resistance.

C. parapsilosis

No resistance against fluconazole, voriconazole, and amphotericin B was detected in *C. parapsilosis* strains isolated from the feces of gulls from the natural population. The proportion of strains resistant to fluconazole from the feces of gulls nesting near the landfill was 8%, and to voriconazole and amphotericin B—4%.

C. tropicalis

The resistance of the tested strains of *C. tropicalis* isolated from the feces of gulls from the urban population against fluconazole was 100%, against voriconazole—5%, and against amphotericin B—49%.

4. Discussion

4.1. Main Features of Yeast Communities in the Feces of Mew Gulls from Natural and Urban Colonies

Differences were found in the diversity of yeast complexes by habitat and season. Only in the fecal samples collected in April immediately after the arrival of the birds (at the beginning of the nesting season) were the yeast complexes similar in both habitats. The proportion of pathogenic species in their feces was minimal (no pathogenic *Candida* was found; the proportion of *Rhodotorula* was low). This remains the biggest mystery for us and a requirement for additional research. At the moment, we cannot answer this question precisely because we do not know from where the studied gull colonies flew, stopped, and fed during migration. This is one of the major limitations of our work. It is also possible that the proportion of *Candida* species was low, and we were unable to isolate them using conventional microbiological methods of plating on solid culture media (the use of metabarcoding is required). However, even if the anthropogenic impact extends only to the nesting period, it is not short (7–8 months in our region) to successfully transmit antifungal-resistant strains of yeast pathogens to human or animal hosts via environmental contamination.

The higher diversity of yeasts in the feces of gulls from the natural population was primarily due to yeast species regularly found in entomophilous flowers, on the surface and inside fruit tissues, in fruit seeds and observed in association with insect pollinators (*H. uvarum*, *Met. pulcherrima*, *Met. colchici*, *Y. lipolytica*) [63,71–75], soil insects and topsoil (*C. boleticola*, *C. cetoniae*, *G. gastrica*, *Pr. vitoshaensis*, *T. pullulans*, *Y. alimentaria*) [71,76–78] and also due to eurybionts (*Aur. pullulans*, *C. zeylanoides*, *D. hansenii*, *M. guilliermondii*) [71,79–81]. The Mew Gulls of the natural population fed mostly on fish, invertebrates, and plant parts. Each of the natural substrates is associated with different yeast species whose undigested cells remained viable in the bird droppings. Yeast diversity increased from spring to fall, which is consistent with the known trend of increasing yeast abundance and diversity in natural substrates during the fall season in temperate climates [82–84]. In the urban area of the landfill, the birds consumed plant parts, but mainly “junk” food from waste (which was actively brought to the landfill in April after the winter break). It contains fewer yeast species diversity, mostly pathogenic yeasts, and eurybiontic species adapted to the

negative anthropogenic influences (*C. parapsilosis*, *C. tropicalis*, *D. hansenii*, *Rh. mucilaginosa*, *Aur. pullulans*).

Although the basidiomycetous emerging pathogenic yeast species *Rh. mucilaginosa* was found in the feces of gulls from both populations, its occurrence in the feces of the urban population increased steadily (Table 2), and on average, over the entire study period, its relative abundance was two and a half times higher than in the feces of gulls from the natural population. The yeast *Rh. mucilaginosa* has a polysaccharide-rich microcapsule containing compounds such as carotenoids that confer high resistance against various environmental stresses [85–87]. Thus, this species has properties that give it a competitive advantage when it develops in different ecological niches under the negative influence of anthropogenic factors.

Of the two pathogenic *Candida* (*C. tropicalis* and *C. parapsilosis*) detected in the feces, *C. parapsilosis* was found in the feces of gulls from both populations. In the natural population, however, this species was only detected in August, and its relative abundance was within 1%. In the anthropogenic population, however, it was isolated from feces in six of the seven months of the breeding season (average relative abundance was about 2%), and the strains had pathogenic properties (antifungal resistance).

The weather conditions in both habitats were very similar throughout the breeding season (Table 1). Therefore, their influence on yeast diversity can probably be neglected.

4.2. Susceptibility Profiles of Potentially Pathogenic Yeast Species Observed in Feces

Strains of potentially pathogenic yeast species with insensitivity to antifungal drugs are regularly found in the intestines, cloaca, and feces of synanthropic birds (pigeons), domesticated birds/pets, and wild migratory birds [88–90]. We also have already found high resistance against conventional antifungals in strains of two *Candida* species from the feces of semi-synanthropic birds: 68.8% and 77.2% of *C. parapsilosis* and *C. tropicalis* isolates were resistant to azoles, respectively; 14.1% of *C. parapsilosis* and 4.3% of *C. tropicalis* were resistant to amphotericin B [39]. Other pathogenic yeasts of the genus *Candida* (*C. albicans*, *C. glabrata*, and *C. krusei*) have been found more frequently in gull feces by researchers, for which the presence of resistant strains to the most common azole antifungals (itraconazole, fluconazole) has been demonstrated [28,29]. In our work, these more common pathogenic *Candida* were not detected. However, for the *Candida* species (*C. parapsilosis* and *C. tropicalis*) that we observed in the feces of the anthropogenic gull population, resistance against fluconazole was also the highest among the resistant strains. Strains of the emerging pathogen *Rh. mucilaginosa* isolated from the feces and oropharynx of various birds (*Agapornis* birds, *Rupornis magnirostris*, *Caracara plancus*) have been shown to be resistant to fluconazole and sensitive to amphotericin B [89,91]. In our study, all strains from gull feces from the natural population were also sensitive to polyene (amphotericin B). However, among the strains from the anthropogenic population, there was already a percentage of resistant strains. The pathogenic *Candida* strains demonstrated the highest resistance against azoles (fluconazole).

It is likely that most of these resistant strains were of anthropogenic (perhaps clinical) origin in the waste consumed by the birds with “junk” food and spread via droppings. Mew Gulls nesting in urban areas can thus cross regional boundaries and spread antimycotic resistance over long distances and on a large scale, which could be a crucial link between the spread of antibiotic resistance in urban and natural environments [92].

The main limitations of our work: (1) the bird colonies are small and only two colonies were studied; (2) we do not know if all the birds flew away or if some pairs stayed to overwinter; (3) we do not know where exactly the birds flew to overwinter; (4) we did not study another widely used group of antifungals—echinocandins (they are quite effective against many pathogenic yeasts, but they are almost unavailable in our region, mainly because of the high cost); (5) we have not considered a special group of lipophilic pathogenic yeasts of the genus *Malassezia* for the selective detection wherein special glucose-free microbiological media are required.

In the future, we plan to investigate the pathogenic yeast complexes in the feces of 13–15 species of the most common urban birds in our region using culturable methods and metabarcoding. In addition to antibiotic resistance, we will also evaluate the virulence of the strains *in vivo*. We want to use the larva of *Tenebrio molitor* (Coleoptera) as a well-known model for the assessment of the pathogenicity of fungal strains.

5. Conclusions

During the breeding season, the diversity of the culturable yeast complexes in the feces increases or decreases depending on the habitat of the breeding colony of the Mew Gulls—natural or anthropogenic. The diversity of pathogenic yeast species and the proportion of resistant strains among them to conventional antimycotics was greater in the feces of the anthropogenic colony.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/birds5030036/s1>, Table S1a. Minimum inhibitory concentration (MIC, µg/mL) values of *Rh. mucilaginosa* strains isolated from the feces of two populations of Mew Gulls (from natural and urban habitats) to antifungal agents; Table S1b. Minimum inhibitory concentration (MIC, µg/mL) values of *C. parapsilosis* strains isolated from the feces of two populations of Mew Gulls (from natural and urban habitats) to antifungal agents; Table S1c. Minimum inhibitory concentration (MIC, µg/mL) values of *C. tropicalis* strains isolated from the feces of Mew Gulls (from urban habitat) to antifungal agents.

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Institutional Review Board Statement: The study was approved by the Ethics Committee of Lomonosov Moscow State University (No. 144-5/3/2008-2020, <https://bioethics.msu.ru> (accessed on 9 April 2024)). Additional ethical review and approval were waived for this study because the study did not involve the capturing or manipulation of birds, only their observation.

Informed Consent Statement: Not applicable.

Data Availability Statement: The datasets used and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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