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Candida species Isolated from Aquatic Ecosystems of the Arecibo region, Puerto Rico: Beach, Estuary and River

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Abstract:

Beaches, rivers, and estuaries are exposed to diverse environmental changes due to natural disturbances and anthropogenic pollution. The waters of pier Beach, the Caño Tiburones estuary, and the Arecibo River estuary were studied for five weeks, from February to March 2023. The objectives of this study were to isolate yeasts from different bodies of water in Arecibo, identify the genus and species, estimate the number of yeasts by counting colony-forming units (CFU), and compare the isolated species among the three bodies of water. Samples were collected at three different points equidistant from each location and placed in sterile bags. One hundred mL of water was filtered in triplicate, and the membrane was placed to grow in Hardy Chrom™ Candida culture medium. The incubation period for the samples was 2 to 3 days at 30-37°C. Colonies were counted and isolated. Macroscopic and microscopic analysis was used for yeast identification. The growth of the colonies sampled from the Caño Tiburon estuary was 171 CFU, those from the Arecibo River estuary were 215 CFU, and those from the Arecibo Pier beach were 138 CFU. The yeast species identified at the three sampling points were: *Candida albicans, C. tropicalis, C. krusei, C. glabrata, C. parapsilosis*, and *C. auris*, which was only present in the Arecibo River Estuary. The majority of species isolated in this study are responsible for opportunistic fungal infections. The study indicates the patterns that are being observed in the epidemiology of Candida species, such as variations in their prevalence and antibiotic resistance according to geographic location.

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INTRODUCTION

Estuaries and coastal aquatic ecosystems, such as those found in the Arecibo region of Puerto Rico, are dynamic and biodiverse habitats that support a variety of organisms, including fungi (yeasts) of the genus Candida. These aquatic ecosystems, which include pier Beach, the Caño Tiburones estuary, and the Arecibo River estuary, are subject to a combination of unique environmental factors that influence their microbiological composition, including the presence of fungal species such as Candida. Identifying and studying Candida species in these aquatic environments not only contributes to a better understanding of the microbiota present in these habitats but also helps assess the potential risk to human health, especially in areas frequented by humans (Agnello *et al.,* 2017).

The Candida genus, which includes several opportunistic pathogenic species, is primarily known for its ability to colonize all types of environments, including those with variations in salinity and nutrients, such as estuaries. Estuaries, in particular, such as the Caño Tiburones estuary and the Río Grande de Arecibo estuary, are ecosystems where freshwater and saltwater mix, creating a favorable environment for diverse Candida species. These areas also serve as refuges for a rich microbial biodiversity and are vulnerable to pollution from human activity, which can alter the composition of microbial communities and favor the proliferation of pathogens (O'Neill *et al.,* 2018).

Previous studies have shown that Candida is commonly found in aquatic environments, where it plays a role in the decomposition of organic matter and nutrient recycling (Ghosh, 2015; Ashraf and Iqbal, 2022). However, the presence of Candida species in these aquatic ecosystems in Arecibo also raises public health concerns, given that some species of the Candida genus, such as Candida albicans, are known human pathogens that can cause infections in immunocompromised individuals (Kullberg *et al.,* 2015; Echevarría, 2022). In this sense, the waters of El Muelle Beach, as well as nearby estuaries, may be a potential reservoir for the spread of these pathogenic species.

The Caño Tiburones estuary and the Río Grande de Arecibo estuary, with their high biodiversity and environmental variability, are representative of the complex coastal aquatic systems of the Caribbean region. The estuaries, mostly protected due to their status as areas of ecological importance, offer an ideal environment for the study of fungal microbiota, including Candida fungi, which may be both part of natural ecosystem processes and potential risks to public health (Nieves-Rivera and Santos-Flores, 2005; Lin *et al.,* 2023; Qu *et al.,* 2024; Hervé *et al.,* 2025).

This article aims to isolate, identify, estimate, and compare the diversity and distribution of Candida species present in the waters of El Muelle Beach, the Caño Tiburones estuary, and the Arecibo River estuary. Using a mycological approach, the ecological implications of the presence of these species will be discussed, as well as the potential risks they pose to human health in these coastal areas. Furthermore, the factors that favor the proliferation of Candida in these ecosystems will be examined, in order to improve the understanding of its role in aquatic environments and its relationship to public health in the region.

MATERIALS AND METHODS

Samples were taken from February to March 2023 (five weeks) at the Arecibo pier beach, in the Caño Tiburon estuary in Arecibo, and in the Arecibo River estuary. One hundred ml of water was collected from three equidistant points on the shore in triplicate into sterile bags. In the laboratory, 100 ml of the sample was filtered, and the membrane was placed in a Petri dish containing HardyCHROM™ Candida culture medium in triplicate (Echevarría, 2022). The samples were incubated at 30 to 37°C for 2 to 3 days. To perform the positive controls, the same medium was used (HardyCHROM™ Candida inoculating *C. albicans*, this to demonstrate that the medium has the growth capacity. The

negative control is a clean plate of the uninoculated medium. Both are incubated at the same temperature as the sample and the result must be positive for the inoculated one and without growth for the negative control, guaranteeing the sterility of the medium. The colonies on each plate are counted and the average per sample is calculated (Echevarría, 2019; Echevarría and Iqbal, 2021). The different colonies are selected and isolated in HardyCHROM™ Candida, incubated for 2 to 3 days at 30 to 37 °C. The genus and species are identified.

RESULTS AND DISCUSSION

After the incubation period, growth of the positive control (*C. albicans* growth) was confirmed. The negative control did not grow. Yeasts are unicellular fungi that play a fundamental role in the decomposition of organic matter and in the biogeochemical processes of aquatic ecosystems, including estuarine zones. Within this group, species of the genus Candida are known for their wide distribution in natural environments and their ability to adapt to extreme conditions, such as variations in salinity, pH, and temperature (Sampaio *et al.,* 2016).

Figure 1 shows the sampling site on the Arecibo pier beach, the Caño Tiburon estuary, and the Arecibo Grande River estuary. Some of these species, such as *Candida albicans*, are opportunistic pathogens of medical importance in humans, generating interest in their monitoring in aquatic environments that could serve as potential reservoirs for these yeasts (Borman *et al.,* 2012).

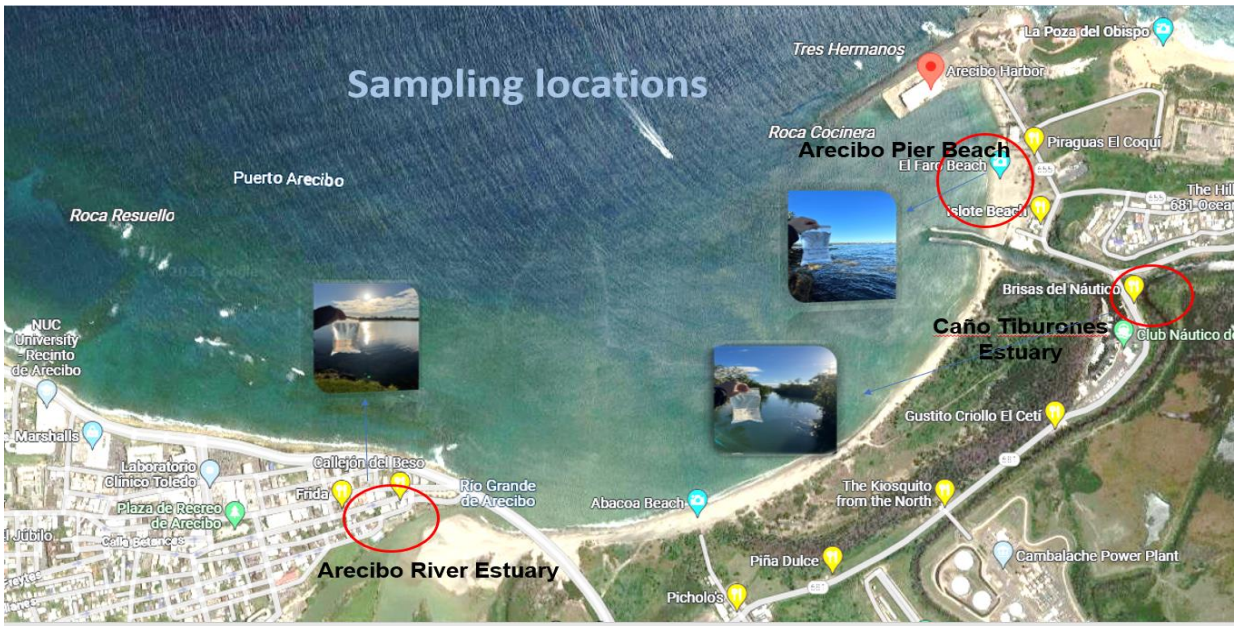

Fig. 1. Sampling location https://www.google.com

Table 1 summarizes all the Candida species found in beach and estuarine waters. It includes their habitat in the aquatic ecosystem, the diseases they cause, and their effects on both the ecosystem and human health.

Table 1. Candida species, habitat, diseases, ecosystem effects, and references.

Candida species	Habitat on Beaches and Estuaries	Diseases it Causes	Effects on the Ecosystem Human Health	References
Candida albicans	Common in coastal aquatic environments, including beaches and estuaries	Oral, vaginal, invasive, esophageal candidiasis	Present in aquatic environments, where it can be part of the microbiota. As an opportunistic pathogen, it can cause serious infections in immunocompromised individuals. In the ecosystem, it can be involved in decomposition processes.	Ghosh, 2015
Candida tropicalis	Beaches, coastal estuaries and freshwater bodies	Invasive candidiasis, fungal, in the blood, peritoneum	Commonly found in estuarine environments, it is an opportunistic pathogen that primarily affects immunocompromised individuals. In estuaries, it contributes to nutrient cycling as a decomposer of organic matter.	Agnello *et al.,* 2017
Candida krusei	Beaches, estuaries, salt and fresh water bodies	Systemic infections, invasive candidiasis	Resistant to some antifungals, making it a challenging pathogen. Its presence in estuarine and coastal waters could alter the biodiversity of the aquatic microbiome.	Tamo, 2020
Candida parapsilosis	Estuaries, coastal waters, in contact with organic materials	Invasive candidiasis, fungemia, skin infections	Frequently associated with nosocomial infections. In aquatic environments, its presence can influence biogeochemical cycles and the decomposition of organic matter.	O'Neill *et al.,* 2018
Candida glabrata	Beaches and coastal estuaries, especially in areas with pollution	Vaginal, invasive, urinary candidiasis	Common in saline and estuarine aquatic environments. Its ability to generate antifungal resistance makes it a species of public health concern. Its presence can also alter the balance of the aquatic microbiome.	Ghosh, 2015
Candida auris	Estuaries, areas with salt water, often in polluted conditions	Systemic infections, fungemia, invasive infections	An emerging species of hospital pathogen. It adapts well to estuarine and coastal environments, where it can be a reservoir. It poses a threat to public health due to its resistance to antifungal treatments.	Agnello *et al.,* 2017

Figure 2 shows that the fastest-growing species, *Candida parapsilosis*, only grew on three dates. No growth of C*. albicans, C. glabrata*, or *C. auris* was observed in the water at the Arecibo pier. Some studies have reported the presence of Candida species in marine and brackish aquatic environments, particularly *Candida albicans*, *Candida glabrata*, and *Candida krusei*, among others. These species have been found not only in water, but also in sediments and marine organisms that inhabit these ecosystems (Lee *et al.,* 2015).

Figure 3 shows the results from the Arecibo River estuary. We also observed that *C. parapsilosis* was the species with the greatest growth. However, unlike the pier sample, all Candida species were present here. The species found were *C. albicans*, *C. tropicalis*, *C. krusei, C. parapsilosis*, *C. glabrata*, and *C. auris*.

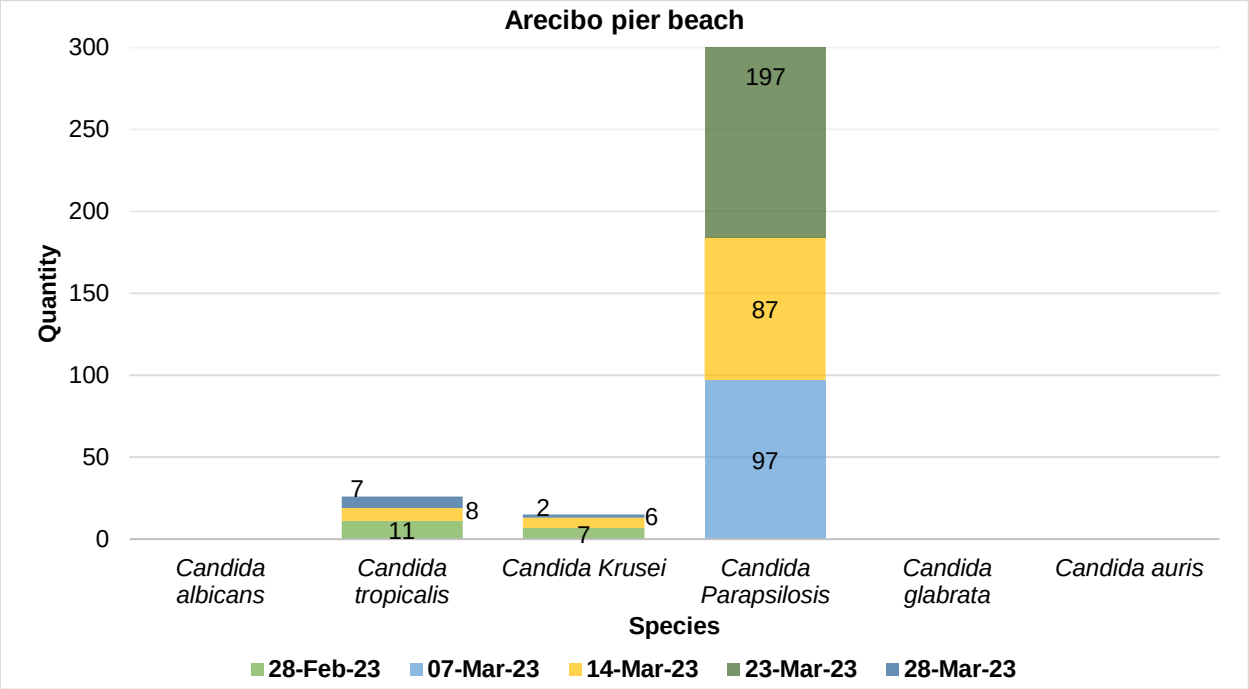

Fig. 2. Arecibo Pier Beach Number of species by date.

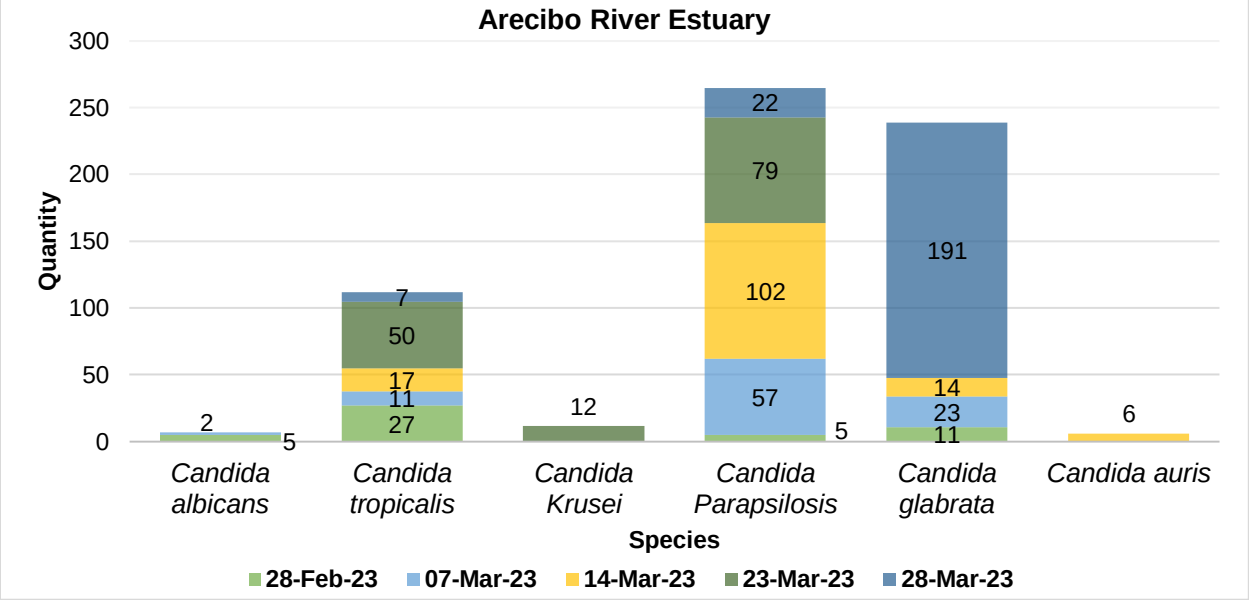

Fig. 3. Arecibo river estuary number species by date.

Figure 4 shows that *C. parapsilosis* was the species with the greatest growth in the samples from the Arecibo River estuary. No *C. auris* was identified in this sample. Despite the ecological and medical relevance of Candida, few studies have explored the diversity and ecology of these yeasts in coastal and estuarine ecosystems of Puerto Rico, an area that hosts vulnerable ecosystems due to pollution and climate change.

Species of the genus Candida are eukaryotic organisms that, in addition to being known human pathogens, play a relevant ecological role in aquatic ecosystems. In particular, species found on beaches and estuaries in various coastal regions, such as *Candida albicans*, *Candida tropicalis*, *Candida krusei*, *Candida parapsilosis*, *Candida glabrata*, and *Candida auris*, are part of the natural microbiota in these habitats, but also have significant implications for human health. The presence of Candida in estuarine and coastal environments can be a consequence of both natural processes and anthropogenic pollution. The identification of these species in estuarine waters and beaches poses a public health challenge, given their pathogenic potential and the increasing antifungal resistance observed in species such as *Candida auris* and *Candida krusei* (Agnello *et al.,* 2017; Kullberg *et al.,* 2015).

Figure 5 shows a summary of the total Candida species found at the three sampling points of the study. As seen in the previous graph, *Candida parapsilosis* was the most frequently isolated species. It is widely distributed in nature.

Fungal infections caused by yeasts of the Candida genus, especially *Candida albicans*, are significant complications in immunosuppressed patients. In many hospitals, Candida is the fourth most frequently isolated pathogen in blood cultures and is one of the leading causes of mortality. Candida is recovered in less than 70% of blood cultures from patients with invasive candidiasis (Arendrup, 2014; Al-Hakami *et al.,* 2016).

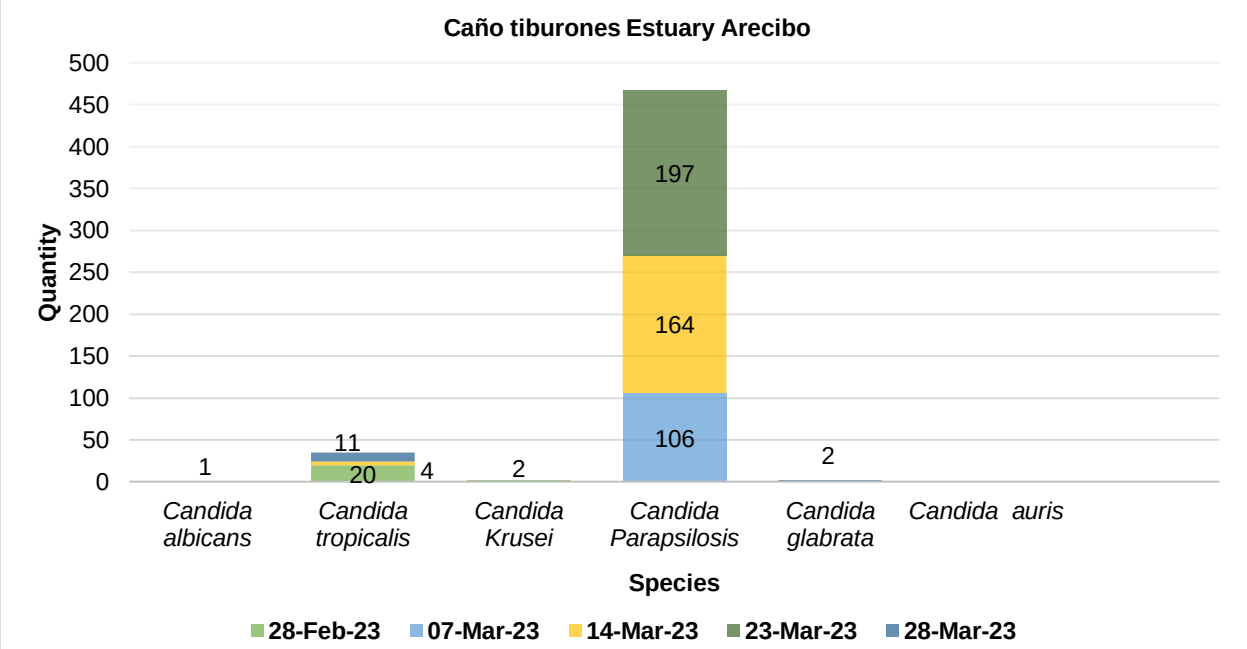

Fig. 4. Caño tiburones estuary Arecibo number species by date.

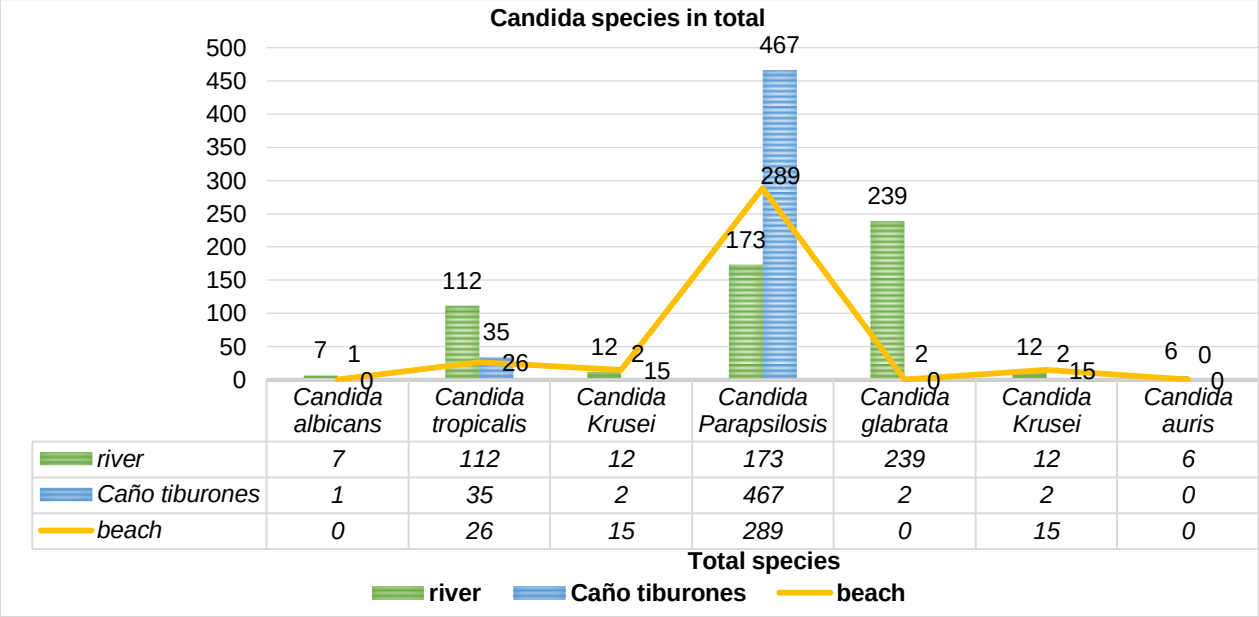

Fig. 5. Candida species in total at the three sampling sites.

Figure 6 shows an average of the Colony Forming Units (CFU). As can be seen, growth depends on the date. Candida species are distributed globally and have been identified in diverse aquatic sources, including coastal lagoons, which offer favorable habitats due to their variable ecological characteristics, such as salinity, temperature, and nutrient availability (Lozano *et al.,* 2018).

Figure 7 shows the number of species per week, noting the differences in some samples by date. This genus is a highly diverse group and can adapt to different conditions, and its role in nosocomial infections and opportunistic diseases in humans is increasingly high (Alhamzi and Al.Maqtari, 2018; Carvalho *et al.,* 2023; Macias-Paz *et al.,* 2023).

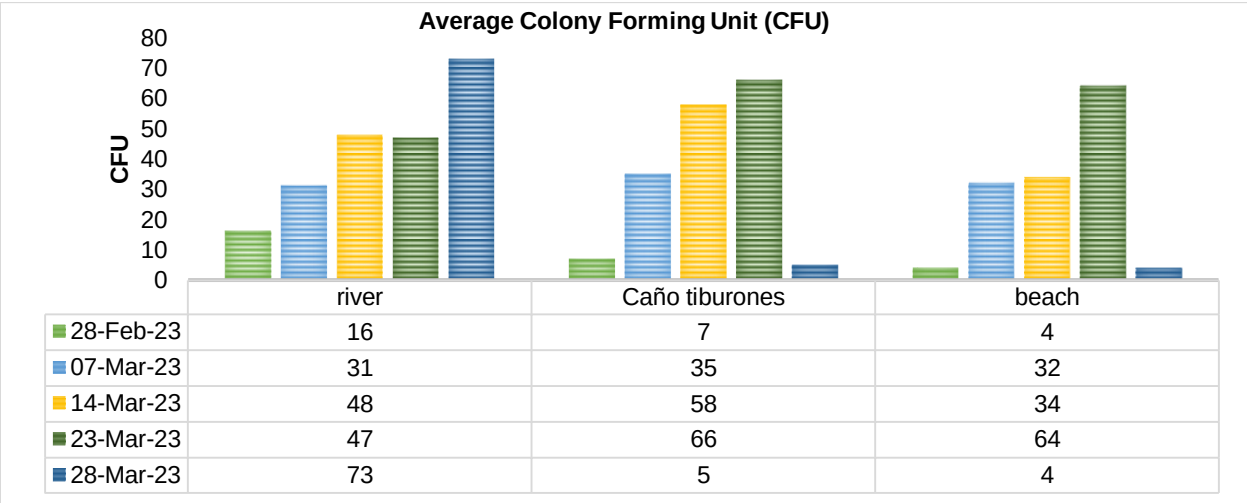

Fig. 6. Average colony forming unit (CFU).

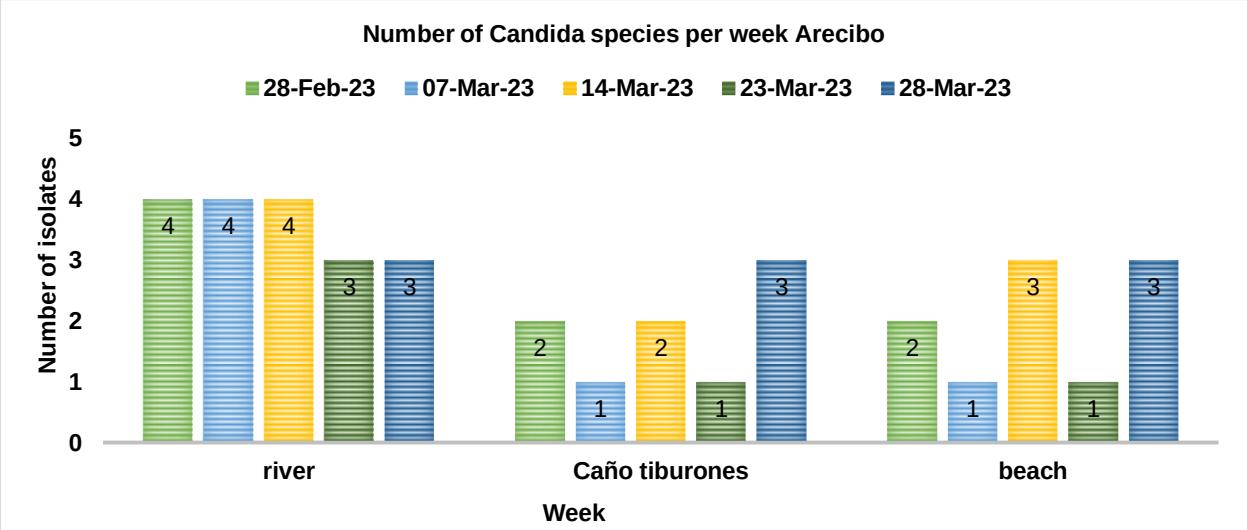

Fig. 7. Number of candida species per week Arecibo.

Furthermore, Candida species play a crucial role in nutrient recycling and the decomposition of organic matter in these aquatic ecosystems. Although their presence is natural, the alteration of these environments due to pollution can favor the proliferation of these species, increasing the risk of infection (Iqbal *et al.,* 2021). Species such as *Candida albicans* and *Candida glabrata* are particularly worrisome due to their ability to cause invasive infections and their increasing resistance to conventional antifungal treatments (Ghosh, 2015; Al-Hakami *et al.,* 2016).

In Puerto Rico, the coastal lagoons in the north of the island are critical ecosystems that address both local biodiversity and water filtration processes. These lagoons, however, also face environmental pressures from pollution, climate change, and human activities, which can alter their microbiota and favor the proliferation of opportunistic species such as Candida (Serrano *et al.,* 2020).

Despite the ecological importance of these ecosystems, the literature on the distribution and ecology of yeasts in Puerto Rico's coastal lagoons is still limited.

The diversity of Candida on beaches and estuaries highlights the need for continuous monitoring of aquatic ecosystems, especially in vulnerable regions such as coastal areas, to better understand microbial dynamics and prevent infectious outbreaks.

CONCLUSION

This study documented Candida colony growth in the samples ranged from 4 to 73 CFU/mL. Taxonomic analysis revealed six species: *Candida albicans, C. tropicalis, C. krusei, C. parapsilosis, C. glabrata*, and *C. auris*. The species found in greatest abundance was *Candida parapsilosis*. *Candida auris* was isolated only in the sample from the Arecibo River estuary. The Arecibo River estuary was the location where all six Candida species and the largest number of colonies were found. The results of this study highlight the need to monitor the mycological quality of water in estuaries, rivers, and beaches for public health safety (Brandão *et al.,* 2021). Studies indicate the resistance of these species to antibiotics depending on the geographical location (De Bedout and Gómez, 2010).

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CONFLICT OF INTEREST

The author declares this article content has no conflict of interest.

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