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Invasive fungal infection during COVID era at tertiary care hospital

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Introduction: Invasive fungal infection is a leading cause of morbidity and mortality in COVID patients as a secondary infection. Invasive fungal infections are often rapidly life-threatening and require specialist consultation for prompt diagnosis and therapy. The reporting of these invasive fungal infections (Mucormycosis, Aspergillosis, and candidiasis) is less than its occurrence.

Objective: This study was undertaken to diagnose invasive fungal infections in COVID patients during the first, second, and third COVID-19 pandemic waves by conventional methods.

Materials and Methods: From March 2020 to December 2020 was considered the first wave pandemic, March 2021 to May 2021 was the second wave pandemic and December 2021 to February 2022 was considered the third wave pandemic era for this region. A total of 42, 168, and 87 samples were collected from patients clinically suspected to have invasive fungal infections during three pandemic waves from Covid indoor patients of RIMS, Ranchi. Among a total 24 were tissue samples, 13 BAL and 260 were nasal swabs/scrapes collected from suspected patients. KOH screening of all samples was done followed by culture on SDA media.

Results: A total of 83/297 (27.94%) cases were positive for KOH screening. In all, 91/297 (30.64%) samples showed culture positivity. A majority of growth was for *Mucorales* (48.2%), *Aspergillus* species (24.1%), *Candida* species (15%), and mixed growth (12.7%). Among *Mucorales*, *Rhizopus* was identified in 64% followed by *Mucor* (28%), *Absidia* (5%), and *Rhizomucor* (3%). Among *Aspergillus* species, majority were *A. flavus* (48%) followed by *A. niger* (37%), and *A. fumigatus*

(15%). Maximum positivity for invasive fungal infections was observed during the second wave pandemic (62%) followed by the third wave (27%), and the first wave (11%) respectively.

Discussion: Maximum cases were observed during and after the second wave COVID pandemic era due to excessive use of steroids during COVID treatment and cases were decreased during the third wave as compared with second wave timeframe due to the use of COVID vaccine, and many asymptomatic cases and home quarantine policy.

Conclusion: Early diagnosis and treatment of invasive fungal infections with antifungal therapy and surgical debridement are necessary to reduce mortality and end-organ damage.

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Functional genomics in *Candida glabrata*, new tools to study stress, pathogenesis and drug resistance

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The processes of life are dynamic and changes on a molecular level enable organisms to grow but to adapt and survive in different environments, such as the ability to cause disease within a human host. My research focuses on the human fungal pathogen, *Candida glabrata*, which can cause illnesses in humans ranging from allergic reactions, infections such as thrush which affects ~75% of women at least once, to serious diseases in patients that have impaired immune systems. These fungi are increasing in incidence and the reason for this increase is not understood. However, it is clear that the fungus can defend itself against high levels of stress and antifungal drugs used in treatment regimes. My hypothesis is that *C. glabrata* has evolved the capabilities to withstand a challenge from the combination of environmental and imposed drug stresses.

To look at *C. glabrata*, I will take advantage of my recent discovery of the sexual cycle in this fungus which offers novel methods to test hypotheses about evolution and pathogenesis. Pathogens of humans, such as *C. glabrata*, are successful because

they adapt effectively to environmental stresses encountered within the host body. Upon recognition by host immune cells, *C. glabrata* is engulfed and exposed to a combination of stresses. In contrast to other pathogenic fungi, *C. glabrata* is highly resistant to stress allowing it to survive the host immune defenses. This suggests that resistance to both antifungal drugs and natural host-induced stresses are essential for the establishment and progression of infection. The molecular mechanisms underpinning antifungal resistance and the response to individual stresses have been investigated in isolation, however, little is known about how *C. glabrata* adapts to combinatorial stresses. The mechanistic explanation of stress adaptation will yield new insights into *Candida* infection.

Using my newly discovered sexual cycle in *C. glabrata*, I have generated a series of related strains of the same fungal pathogen that have increased resistance to combinatorial and drug stresses. I will sequence their genomes to identify the critical genes involved in stress resistance and characterize the mechanisms of *C. glabrata* stress responses. My preliminary data demonstrate that the *C. glabrata* response to *in vitro* combinatorial stress is similar to that observed upon phagocyte engulfment. At the level of gene expression, there is an up-regulation of genes encoding functions related to stress adaptation and nutrient recycling overlap. Understanding this regulatory network and the role that selected components (different genes) play in stress resistance, is essential to the development of future drug regimes.

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Comparison of *Candida* colonization in intensive care unit patients with and without COVID-19: First prospective cohort study from Turkey

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Objectives: *Candida* species, as the main component of human mycobiome, are the most common cause of fungal infections in intensive care units (ICU). ICU patients with COVID-19 are more prone to fungal infections, due to various causes like mechanical ventilation, use of steroids, or long-term hospitalization. There is yet no extended prospective study examining *Candida* colonization rates, epidemiology of species, and predisposing factors in this population. This is the first prospective cohort study comparing the time-varying colonization features of *Candida* species in ICU patients with and without COVID-19.

Methods: This study was performed between March 2021-December 2021 in ICUs of Istanbul University, Istanbul Faculty of Medicine, Department of Anesthesiology and Reanimation. COVID-19 and non-COVID-19 ICU patients who were ≥ 18 years and expected to stay in the ICU for at least 7 days were included in the study.

Samples were taken at certain time intervals from different body parts of the patients [mouth, skin (axilla), rectal, and urine] (Table 1) and evaluated at Istanbul University, Istanbul Faculty of Medicine, Department of Medical Microbiology, Mycology Laboratory. All specimens were inoculated on CHROMagar Candida media (CHROMagar Candida, France) to detect mixed growth and CHROMagar Candida Plus media (CHROMagar Candida Plus, France) to avoid missing *Candida auris*.

Cultures were incubated at 35-37⁰C for 48 h and phenotypically different colonies on primary media were subcultured on corn meal-tween-80 agar for determining their morphology. All strains were identified to the species level using MALDI-TOF MS (Version 4.1.80; Biotyper Bruker) in Yeditepe University, Faculty of Engineering, Genetics and Bioengineering Department. Patient groups were compared statistically in terms of isolated *Candida* species and distribution according to regions.

Results: The study consisted of 122 ICU patients including 62 COVID-19 (25 female; 37 male; mean age:63.29 years) and 60 non-COVID-19 (24 female; 36 male; mean age:63.9 years).

A total of 1464 samples (756 COVID-19 and 708 non-COVID-19 patients) were taken (Table 1) and fungi grew in 340 (23.2%) samples. Mixed growth was observed in 108 cultures; was more frequently in COVID-19 patients ($P < .05$), and significantly higher in oral specimens ($P < .05$).

Out of a total of 471 strains that were obtained from fungal cultures, *C. albicans* (42.25%) and *C. glabrata* (24.2%) were most frequently isolated. *Candida auris* was not observed in this period (Table 2).

Patients with COVID-19 were found more frequently colonized in oral ($P < .001$), rectal ($P < .05$) regions and urine ($P < .001$) compared with non-COVID-19 patients. There was no growth in the axillary region in any of the patients.

Non-albicans *Candida* strains were found significantly more frequent in patients with COVID-19 in oral ($P < .001$) and rectal regions ($P < .05$).

CONCLUSION: In this study, we found significantly higher oral, rectal, and urine *Candida* colonization rates in COVID-19 ICU patients compared with non-COVID-19 individuals. Increased oral *Candida* colonization can be the result of insufficient oral care application to these patients in the ICUs due to infection control anxiety, and also mechanical ventilation.

Because non-*albicans Candida* strains were found significantly more frequent in COVID-19 patients, intrinsically resistant isolates should be kept in mind before administering antifungals.

The high mixed growth rate detected in all individuals and especially in COVID-19 patients will affect the antifungal therapy and therefore emphasized the importance of using chromogenic media for routine evaluation.

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Contamination of wind instruments: A potential threat to musicians

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Research shows that wind instruments are often contaminated with various bacteria and mould, creating a potential threat to musicians. These microorganisms are partially transferred from the players to the instrument during playing. Since the moist and dark inner surface of the instruments create the perfect environment for the microorganisms, they can easily nestle and grow. Then, they are retransferred back to the players as they inhale during playing. Current literature reports many cases of Hypersensitivity Pneumonia caused by contaminated instruments.

Objective: The objective of the current research is to explore the amount and variety of microorganisms inside brasswind instruments as well as testing antibacterial properties of different materials used in instrument production.

Method: In this three-part study, first of all, with a face-to-face survey, information such as instrument cleaning habits, cleaning products, and methods they use and etc. are collected from the subjects. Then, with samples taken from two-three different parts of the subjects' instruments, the amount and types of microorganisms are detected. Lastly, three instruments made of three different materials (gold, silver, brass) are tested for differences in the growth rate of the pathogens, and their advantage/disadvantages for health purposes are evaluated.

Results: When obtained metagenomic data are analyzed, it is found that the tested instruments were highly contaminated with various bacteria, many of which are opportunistic pathogens, and mould. Furthermore, the tests done on instruments made of different materials reveal that silver instruments have antibacterial characteristics. On the other hand, gold and brass instruments do not have antibacterial characteristics.

Conclusion: To conclude, the brass instruments harbor a vast amount of microorganisms and need to be regularly cleaned with an effective hygiene protocol by the players. Also, due to its antibacterial properties, the use of silver plating in brass instruments would be preferable for health purposes.

Table 1. Sampling times and numbers of specimens taken from COVID-19 (+) and COVID-19 (-) patients

COVID STATUS	REGIONS	SAMPLES					Total Samples
		1st Samples[a] (n)	2nd Samples[b] (n)	3rd Samples[c] (n)	4th Samples[d] (n)	5th Samples[e] (n)	
Positive Patients (n:62)	Oral	62	62	60	4	1	189
	Skin (Axilla)	62	62	60	4	1	189
	Rectal	62	62	60	4	1	189
	Urine	62	62	60	4	1	189
Negative Patients (n:60)	Oral	60	60	56	1	0	177
	Skin (Axilla)	60	60	56	1	0	177
	Rectal	60	60	56	1	0	177
	Urine	60	60	56	1	0	177
Total Samples		488	488	464	20	4	1464

n: Numbers of the patients

a: 1st samples were taken on the 1st day at ICU

b: 2nd samples were taken on the 7th day at ICU

c: 3rd samples were taken on the last day at ICU [(COVID-19 (+): mean 16.06, days (8-42); COVID-19 (-): mean 12.56, days (8-32)]

d: 4th samples were taken on the 30th day (for patients stayed ≥30 days)

e: 5th samples were taken on the 42nd day