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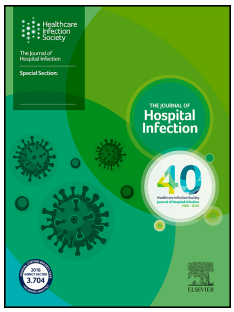

ICU ENVIRONMENTAL SURFACES ARE A RESERVOIR OF FUNGI: SPECIES DISTRIBUTION IN NORTHERN ITALY

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ICU ENVIRONMENTAL SURFACES ARE A RESERVOIR OF FUNGI: SPECIES DISTRIBUTION IN NORTHERN ITALY

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Running title: Fungi on ICU environmental surfaces

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SUMMARY

Background: Preventing and reducing nosocomial infections is a public health goal. Concern about healthcare-associated fungal infections has increased in recent years, due to the emergence and spread of new pathogens, increasing antifungal resistance and outbreaks in hospital settings.

Aim: This study investigated the presence of medically-relevant fungal species on environmental surfaces in 12 intensive care units of 8 hospitals in Milan, Italy.

Methods: Environmental samplings, using contact plates on surfaces near bed stations and medical workstations, were conducted between November 2019 and January 2020. Fungi isolated were identified and some were tested in vitro for antifungal susceptibility.

Findings: A total of 401 environmental samples were collected from 61 bed stations and 17 medical workstations. Positive samples were found in all hospitals except one, with positivity rates ranging from 4% to 24.2%. Filamentous fungi were found mainly on infusion pumps (23.2%) and patient tables (21.2%), whereas yeasts were mainly on computers (25%) and floors (10.9%). Fungi were isolated from 12% of total samples. Filamentous fungi, mainly *Aspergillus fumigatus*, grew in 70.8% of positive samples, and yeasts in 27.1%, mainly *Candida parapsilosis* (42.8%) and *C. glabrata* (28.6%). Fungi were detected both near patients' beds and on surfaces at workstations, indicating potential for environment-to-patient, patient-to-patient and healthcare workers-to-patient transmission

Conclusions: This study highlights that surveillance in hospital settings through environmental sampling may be an important component of fungal infection prevention.

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INTRODUCTION

During the last decades, concern has increased about the emergence and spread of antimicrobial resistance. Antimicrobial-resistant microorganisms are especially important as a cause of healthcare associated infections (HAIs). HAIs are an important public health consideration in respect of their impacts on mortality and morbidity, and on health economics. The European Center for Disease Prevention and Control (ECDC) estimated an annual incidence of 4.2 million HAIs in 2013, higher than that estimated previously [1]. The high number of healthcare workers (HCWs), and devices used for patient care are important risk factors in spreading pathogens.

Recently, the World Health Organization (WHO) reported [2,3] that one of the main goals in the next years will be to strengthen knowledge of HAI and antimicrobial resistance through efficient surveillance programmes in healthcare systems worldwide. In Europe and in other countries, surveillance programmes concerning meticillin-resistant *Staphylococcus aureus* (MRSA) [4] or surgical site infections are widely performed routinely. However, in recent years, fungal HAIs have gained greater attention, especially related to a rapid increase of antifungal resistance.

Fungal infections have traditionally been regarded as being associated with specific high-risk populations, such as patients with neutropenia or on ICUs. Nevertheless, many recent studies have reported fungal HAIs in a wider range of categories of hospitalized patients [5].

The most important fungal species associated with HAIs iare *Candida* spp. and *Aspergillus* spp. Candidaemia and invasive candidiasis are the most common fungal HAI, with an incidence ranging from 0.17 to 2.7 episodes per 1,000 discharges, and from 0.30 to 4.9 per 10,000 patient/days [6]. In the last decade, *C. auris* has emerged as an important pathogen, characterized by high transmissibility in hospital settings, [7] its multidrug-resistance [8,9] and a consequent high mortality rate (from 28% to 78%) [10-12]. The potential of fungi to spread in hospital wards, e.g. on HCWs' hands or through air conditioning systems, and to cause life-threatening invasive diseases, suggests that greater surveillance of fungal infections is required, especially in ICUs [13].

The aim of this study was to detect, and identify the species of, medically-relevant fungi on different surfaces in the ICU environment.

METHODS

During the period November 2019 - January 2020, environmental samplings were performed in 12 ICUs of 8 hospitals (named H1-H8) in Milan, Italy. The sampled ICUs included general (n=6), paediatric (n=3), neonatal (n=1), post-surgical (n=1), and neurosurgical ICUs (n=1). A second sampling was conducted in July 2020 only in two different ICUs of the same hospital (H3) and in one general ICU (H4) already involved in the first phase of the study.

Sampling was performed using Rodac contact plates (65 mm diameter) containing Sabouraud dextrose agar (SDA, Biolife, Milan, Italy) supplemented with 0.5 g/L chloramphenicol (Sigma-Aldrich, St. Louis USA) pressed on the different surfaces for 15 seconds. Plates were incubated at 37°C for 5 days, and any isolates sub-cultured on SDA for identification.

Yeasts were identified using CHROMagar colorimetric media (CHROMagar, Paris, France) and biochemical tests (API ID 32C, bioMérieux, Marcy l'Etoile, France). Filamentous fungi were identified out by macroscopic and microscopic analysis. *Aspergillus* spp. isolates were sub-cultured on Czapek agar (CA, Becton Dickinson, Franklin Lakes, NJ, USA) at 37°C and 25°C.

Molecular identification was performed to confirm the identification of both yeasts and moulds. Briefly, genomic DNA was extracted using PrepMan Ultra sample preparation reagent (Applied Biosystems, Foster City, CA), and then ITS1-5.8S-ITS2 region was amplified [14]. Molecular identification of *Aspergillus* spp. isolates was performed amplifying a portion of the β-tubulin gene [15]. Amplicons were sequenced using BigDye™ terminators (Applied Biosystems, Foster City, CA) in an ABI PRISM1 310 Genetic Analyzer (Applied Biosystems), and nucleotide sequences were analysed using Finch TV software v.1.4.0 (Geospiza Inc.; https://digitalworldbiology.com/FinchTV). The consensus sequence was obtained using EMBOSS

explorer (http://www.bioinformatics.nl/emboss-explorer) and compared to the sequences present in the GenBank database by basic local alignment search tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi) analysis.

Antifungal susceptibility testing was performed by broth microdilution assay according to European Committee on Antimicrobial Susceptibility Testing (EUCAST) [16, 17] in order to determine the minimum inhibitory concentration (MIC) [18] for *Candida parapsilosis* and *Aspergillus* section *Fumigati*, the species with the highest resistance rates in Italy. In particular, *C. parapsilosis* isolates were tested for in vitro susceptibility to fluconazole, itraconazole, voriconazole and posaconazole (Sigma-Aldrich, St. Louis USA), whereas on *Aspergillus* section *Fumigati* isolates were tested for itraconazole, voriconazole, posaconazole, isavuconazole and amphotericin B (Sigma-Aldrich, St. Louis USA). *C. parapsilosis* ATCC 22019 and *Candida krusei* ATCC 6258 were included as quality controls.

Detection of mutations implicated in antifungal resistance in *C. parapsilosis* isolates was performed by amplification and sequencing of the *ERG11* gene [19]. The sequences obtained were aligned with the *ERG11* sequence of the wild-type reference strain ATCC 22019.

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RESULTS

During the period November 2019 to January 2020 401 environmental samples were collected from 61 bed stations and 17 medical workstations (MW) in 12 ICUs in 8 hospitals (Table I). Positive fungal cultures were obtained from all but one ICU (H4). Overall 48/401 (12%) of samples were positive, with the rate of positivity varying widely between hospitals (from 4% in H2 to 24.2% in H6) and between ICUs (from 3.3% to 26.7%).

The surfaces sampled were: floors (46 samples), vital signs monitors (45 samples), medication trolleys (45 samples), infusion pumps (41 samples), patient's tables (33 samples), bed handles (30 samples), ventilators (23 samples), curtains (22 samples), computer keyboards (20 samples), desks

in MW (19 samples), mouses (17 samples), telephones (15 samples), printers/label printers (11 samples), computers (8 samples), incubators (8 samples), surgical lamps (4 samples), other surfaces (i.e. cough stimulator, warmer; 14 samples).

Of the 48 positive samples, moulds were isolated from 34 (70.8%), yeasts from 13 (27.1%), and both yeasts and moulds from one (2.1%). Filamentous fungi were mainly detected on infusion pumps (10/43 samples, 23.2%) and patient tables (7/33 samples, 21.2%) (Figure 1). *Aspergillus fumigatus* (9 isolates) and *A. niger* (8 isolates) were the most frequently isolated moulds. Of the yeasts, *Candida* spp. were mainly isolated from floors (5/46, 10.9%) and computers (2/8, 25%) samples. The most frequent species were *C. parapsilosis* (6/14, 42.9%) and *C. glabrata* (4/14, 28.6%); no *Candida auris* was detected. Overall, fungi were isolated from 8 of 88 (9.1%) MW samples, mainly from computer keyboards (3/13, 23.1%) and computers (2/8, 25%).

Neosartorya hiratsukae grew in 6 out of 35 samples (17.1%), all collected in the same hospital (H3; one general and one post-surgical ICU). A second sampling was conducted in July 2020, in order to confirm the presence of *N. hiratsukae* in two ICUs of hospital H3, and the absence of fungi in the general ICU of the hospital H4. During this second sampling, *N. hiratsukae* was no longer isolated (0/50 samples positives for fungi) in H3, and the number of samples containing any fungi fell from 18.2% to 6%. The absence of fungi in 30 further samples from H4 was also confirmed.

Nine *A. fumigatus*, 2 *N. hiratsukae* and 6 *C. parapsilosis* isolates underwent antifungal susceptibility testing. All the *A. fumigatus sensu stricto* isolates were within the breakpoints (BPs) of susceptibility to amphotericin B (MIC range from <0.03 to 0.12 mg/L) and to azoles (MIC values ranging from 0.25 to 1 mg/L for itraconazole, from 0.06 to 0.12 mg/L for voriconazole, from <0.03 to 0.06 mg/L for posaconazole). *N. hiratsukae* isolates showed low amphotericin B (<0.03 mg/L) and azole (itraconazole 0.12 mg/L, voriconazole 0.03 mg/L, posaconazole 0.06 mg/L, isavuconazole 0.12 mg/L) MIC values; BPs are not available for this species. All *C. parapsilosis* isolates were susceptible to itraconazole (MIC value 0.06 mg/L), voriconazole (MIC value 0.06 mg/L), and posaconazole (MIC value ≤0.03 mg/L); 5 out of 6 were also susceptible to fluconazole

(MIC range from <0.25 to 2 mg/L); the other isolate had an MIC value of 32 mg/L. The molecular analysis of the sequence of the *ERG11* gene of this resistant isolate, evidenced a point mutation that led to an Y132F and R398I amino acid substitutions, and to an I197I synonymous substitution, compared to wild-type reference strain ATCC 22019.

DISCUSSION

HAIs represent a relevant public health problem worldwide; moreover, fungal infections, mainly candidaemia and aspergillosis, are associated with high morbidity and mortality. There is growing concern about multidrug-resistant fungal species, such as *C. auris, C. parapsilosis* and *C. glabrata,* which also show a high ability to persist on surfaces in the hospital environment.

According to the national guidelines [20], operating rooms are subjected to regular environmental control, while in other hospital departments, including ICUs, environmental sampling is usually performed only in the event of a suspected outbreak. The present study evaluated the presence and diversity of medically-relevant fungi in the environmental samples from surfaces of 12 ICUs located in 8 hospitals. Overall, fungi were isolated from 12% of the samples, and filamentous fungi were identified in the 70.8% of the positive samples. Among the moulds, *A. fumigatus* was the most frequently isolated, while the isolation in two ICUs of the same hospital of the extremely rare species *N. hiratsukae* aroused particular interest, being the first isolation reported in Italy [21]. Isolation of *N. hiratsukae* from both surfaces at close contact with the patients and at MWs, suggests spread by HCWs. The hands/gloves of HCWs could become contaminated by fungal spores, and then spread to a patient's nasal mucosa, increasing the risk of inhaling the spores [22].

In this study, yeasts were isolated from 27% of the positive samples, mainly *C. parapsilosis* (42.8%) and *C. glabrata* (28.6%). Yeast contaminated samples were both close to the patient's bed and surfaces of medical workstations (i.e. computers or keyboards), suggesting different potential transmissions routes of the pathogens: environment-to-patient, patient-to-patient, HCW-to-patient [23, 24].

The second sampling at H3 and H4 was conducted just after the first wave of the COVID-19 epidemic, when enhanced cleaning and disinfection had been implemented. This may explain the absence of *N. hiratsukae*, and the reduction in the overall positivity rate in H3, as well as the continuing absence of fungi in H4. It has previously been reported that adequate routine cleaning, strict hygiene measures and educational campaign about correct behaviors of healthcare workers can reduce the contamination of hospital surfaces [25, 26].

The major strength of our study is that environmental sampling of several different types of ICU in several hospitals has allowed us to build a picture of extent of fungal contamination. Our study also has one important weakness in that we incubated initial culture plates at 37 °C only. This may have limited our ability to detect even medically important moulds; nevertheless, we were able to show that such fungi are widely found in most ICUs.

CONCLUSION

Preventing and reducing HAIs is a public health goal. Among microorganisms, fungi deserve special attention due to their ability to survive in adverse conditions.

To prevent fungal and non-fungal infections, both in patients and hospital staff, surveillance in hospital settings through environmental sampling of air and surfaces may represents a valid tool that might be performed systematically. In particular, repeated sampling in ICUs would be useful and should be planned mainly during construction activities, when the contamination by fungi may increase [22, 27].

Information on species distribution and their sources could help to develop guidelines for preventive strategies against nosocomial fungal infections. Furthermore, molecular biology techniques can help in the identification of both rare and resistant fungal species, potentially dangerous for high-risk patients such as those admitted to the ICU. In addition, the continuous education of HCWs about the use of personal protection devices is crucial for the prevention of nosocomial fungal infection.

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CONFLICT OF INTEREST STATEMENT

AP received speaker honorarium from Gilead. The other authors report no conflicts of interest in this work.

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FIGURE LEGEND

Figure I. Number of fungal species isolated from each sampled surface. MW=Medical workstation

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Table I. Description of study area locations, positive surface and distribution of fungal species

Hospital	ICU type	N. beds + N. of MW sampled	N. positives/ N. samples (%)	Positive surface type	Species
H1	Paediatric	3 + 2	3/25 (12%)	Cough stimulator, infusion pump	*Candida albicans* *Aspergillus nidulans* *Aspergillus niger*
	Neonatal	7 + 2	3/45 (6.7%)	Infusion pump, ventilator	*Aspergillus flavus* *Aspergillus niger*
	Total	10 + 4	6/70 (8.6%)		
H2	Paediatric	3 + 2	1/25 (4%)	Label printer in MW	*Trichosporon dermatis* *Trichosporon mucoides*
H3	General	2 + 1	2/15 (13.3%)	Floor, printer in MW	*Candida parapsilosis* *Neosartorya hiratsuke*
	Post-surgical	7 + 1	8/40 (20%)	Surgical lamp, vital signs monitor, bed handle, ventilator, computer keyboard in MW	*Candida glabrata* *Aspergillus fumigatus* *Aspergillus niger* *Neosartorya hiratsuke*
	Total	9 + 2	10/55 (18.2%)		
H4	General	5 + 1	0/30 (0%)		
H5	General	9 + 2	3/70 (4.3%)	Infusion pump vital signs monitor	*Aspergillus flavus* *Neurospora tetrasperma*
	Neuro-surgical	4 + 1	1/30 (3.3%)	Vital signs monitor	*Aspergillus fumigatus*
	Paediatric	5 + 1	8/30 (26.7%)	Infusion pump, floor, ventilator, keyboard, vital signs monitor, patient's table	*Candida guillermondi* *Candida parapsilosis* *Rhodotorula mucillaginosa* *Aspergillus fumigatus* *Aspergillus insuetus* *Aspergillus niger* *Penicillium rubens* *Rhizopus microsporus*
	Total	18 + 4	12/130 (9.2%)		
H6	General	7 + 1	8/33 (24.2%)	Patient's table, floor, medication trolley, computer keyboard in MW	*Candida albicans* *Candida glabrata* *Candida parapsilosis* *Aspergillus fumigatus* *Aspergillus insuetus*
H7	General	7 + 1	8/35 (22.8%)	Floor, patient's table, curtains, medication trolley, computer and telephone in MW	*Candida glabrata* *Candida parapsilosis* *Aspergillus fumigatus* *Aspergillus niger* *Penicillium pinophilum* *Rhizomucor miehei* *Thermoascus crustaceus*
H8	General	2 + 1	3/23 (13%)	Warmer, computer and keyboard in MW	*Candida parapsilosis* *Aspergillus niger*

MW= Medical workstation

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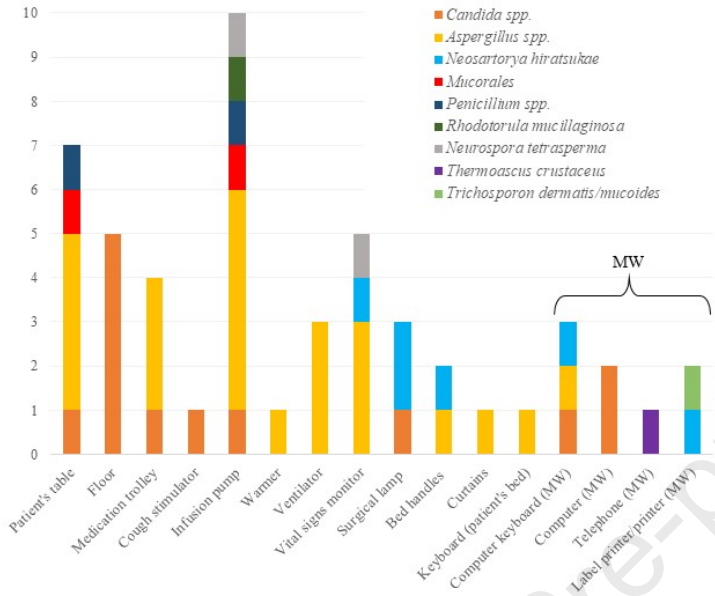

Figure 1. Number of fungal species isolated from each sampled surface. MW= Medical workstation

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