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FRONT MATTER

TITLE: Microbiological air quality in heating, ventilation and air conditioning systems of surgical and intensive care areas: application of a disinfection procedure for the dehumidification devices.

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Abstract

International literature data report that the increase of infectious risk may be due to heating, ventilation and air conditioning (HVAC) systems contaminated by airborne pathogens. Moreover, the presence of complex rotating dehumidification wheels (RDWs) may complicate the cleaning and disinfection procedures of the HVAC systems.

We evaluated the efficacy of a disinfection strategy applied to the RDW of two hospitals HVAC systems.

Hospitals have 4 RDW systems related to the surgical areas (SA1 and SA2) and to the intensive and sub-intensive cares (IC and sIC). Microbiological air and surfaces analysis were performed in HVAC systems, before and after the disinfection treatment. Hydrogen peroxide (12%) with silver ions (10 mg/L) was aerosolized in all the air sampling points, located close to the RDW device.

After the air disinfection procedure, reductions of total microbial counts at 22°C and fungi were achieved in SA2 and IC HVAC systems. An *Aspergillus fumigatus* contamination (6 CFU/500L), detected in one air sample collected in the IC HVAC system, was eradicated after the disinfection. Surface samples proved a good microbiological quality.

Results suggest the need of a disinfection procedure aimed to improve the microbiological quality of the complex HVAC systems, mostly in surgical and intensive care areas.

Keywords: HVAC, *Aspergillus* spp., hydrogen peroxide, air disinfection.

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Introduction

Several studies report a lack of air quality control in operating rooms as a key factor for the surgical site infections following the most common general surgery procedures [1,2]. Surgical site infection accounts for 13%-17% of the total amount of nosocomial infections. Air quality control is therefore routinely performed in surgical settings [3-5] according to procedures described by international standards [6,7]. To achieve ultraclean air circulation in operating rooms, Italian standards highlight the technical requirements for the Heating, Ventilation and Air Conditioning (HVAC) plants [8,9]. Moreover, ISO 14644-1 [7] defines indications for the planning of HVAC and for the management of air quality in surgical areas. Despite this standard is aimed to prevent the occurrences of contaminations and infections for patients hosted in hospital high risk areas, several researches suggest that some HVAC systems may be a source of pathogens, because humidifiers, dirty air ducts and filters could be perfect sites for the growth and dissemination of indoor bacteria and fungi [10,11]. On the other hand, new updated studies state that improvement of microbiological quality of air entered in surgical areas may be achieved by low-turbulence airflows [12], and by the introduction of new devices and construction materials [13]. In fact, ISO 16890 [14] describes an efficiency classification system of air filters for general HVAC plants. This standard provides an overview of test procedures, and specifies general requirements for assessing and marking HEPA filters, as well as for documenting test results. Moreover, the HVAC systems may include special devices to improve indoor air quality and energy saving [15]. In this study we describe a microbiological risk management scheme applied to the HVAC systems in two Italian hospitals. We evaluated a disinfection strategy efficacy in hospital HVAC systems with complex dehumidifier devices.

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Results

HVAC systems SA1

From SA1 of both hospitals, microbiological parameters values were always within the limits provided by the Italian guidelines [6]. In details, before the chemical disinfection, air samples' total microbial counts at 22 and 37°C ranged from 2 to 13 CFU/500L, while fungi counts ranged from 1 to 5 CFU/500L.

Considering the low contamination state, after the treatment we did not observed a significant reduction of the contamination in both hospitals ($p>0.05$) (Figure 1). In fact, 24 hours after the disinfection all values were always below 5 CFU/500 L. Moreover, no differences were detected between the various sampling points. Surface samples' total microbial counts and fungi levels were always below 1 CFU/dm^2. This result may be due to the high temperatures of the analyzed surfaces.

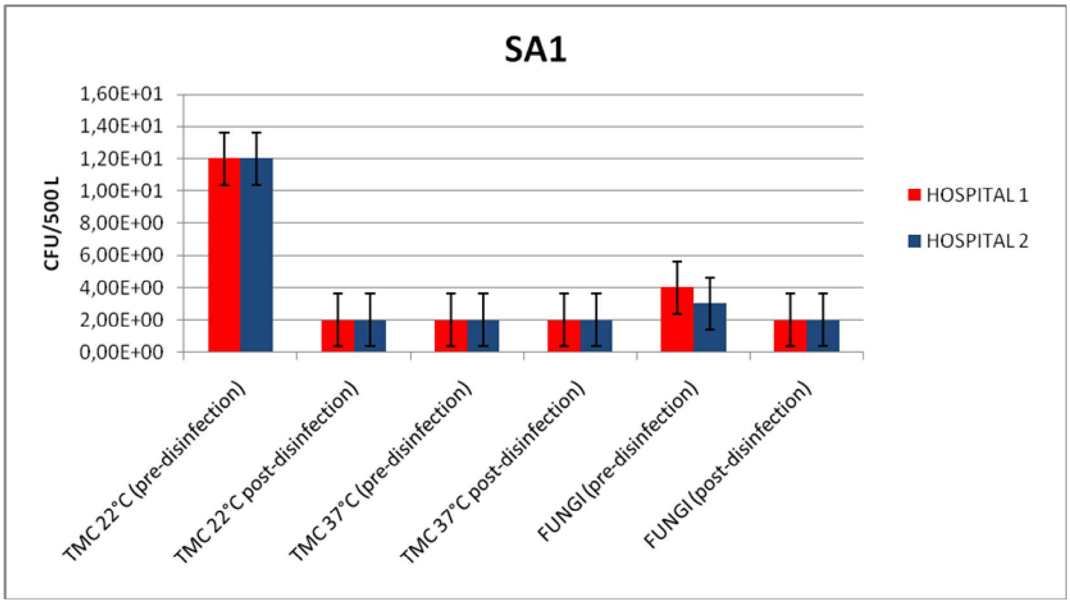

Figure 1: Mean values of total microbial counts (TMC) at 22/37°C and fungi counts detected in air samples (before and after disinfection) in HVAC system of surgical area 1 (SA1) in Hospital 1 and 2.

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HVAC systems SA2

In both hospitals, all microbiological data resulted within the limits provided by the Italian guidelines. On the analyzed surfaces a low contamination level was detected, with counts below 1 CFU/dm^2.

Statistically significant reductions of total microbial counts at 22°C and fungi were detected in both hospitals. In fact, disinfection treatment reduced the air bacterial counts from 39±6 and 65±13 CFU/500 L to 7±5 and 11±7 CFU/500L in hospital 1 and 2, respectively ($p=0.011$; $p=0.009$) (Figure 2). Fungi reduction was obtained in hospital 1 (from 18±8 to 7±4 CFU/500L) and in hospital 2 (from 15±6 to 9±7 CFU/500L) ($p=0.046$; $p=0.048$).

No air contamination differences were observed between the various sampling points.

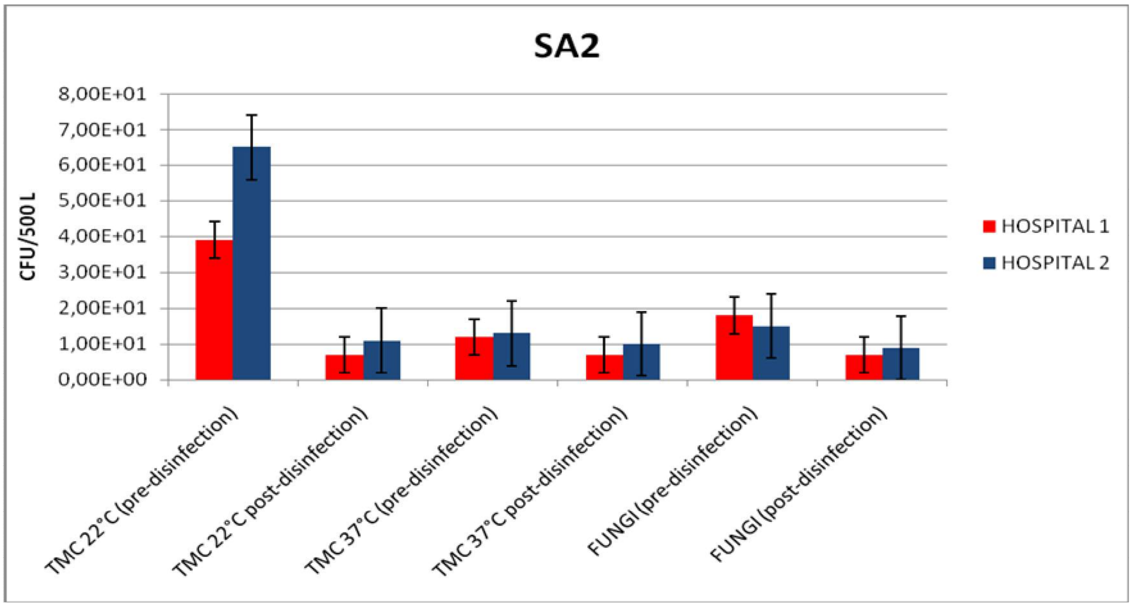

Figure 2: Mean values of total microbial counts (TMC) at 22/37°C and fungi counts detected in air samples (before and after disinfection) in HVAC system of surgical area 2 (SA2) in Hospital 1 and 2.

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HVAC systems IC

All microbiological data resulted within the Italian guidelines limits. Once again, from surface samples a low contamination level was observed, with counts below 1 CFU/dm^2.

From the intensive care wards of both hospitals, a statistically significant reduction of air total microbial counts at 22°C and fungi was detected. In details, the disinfection with hydrogen peroxide reduced the total microbial counts at 22°C from 17±4 and 11±3 CFU/500 L to 4±3 and 4±2 CFU/500L in hospital 1 and 2, respectively ($p=0.013$; $p=0.021$) (Figure 3).

Fungi reduction was obtained in hospital 1 (from 16±4 to 0 CFU/500L) and in hospital 2 (from 5±1 to 0 CFU/500L) ($p<0.001$).

Before the disinfection treatment, in hospital 1 an *Aspergillus fumigatus* contamination (6 UFC/500L) was detected and isolated from the point D (room vent). After the hydrogen peroxide aerosolization all fungi were eradicated from the HVAC system.

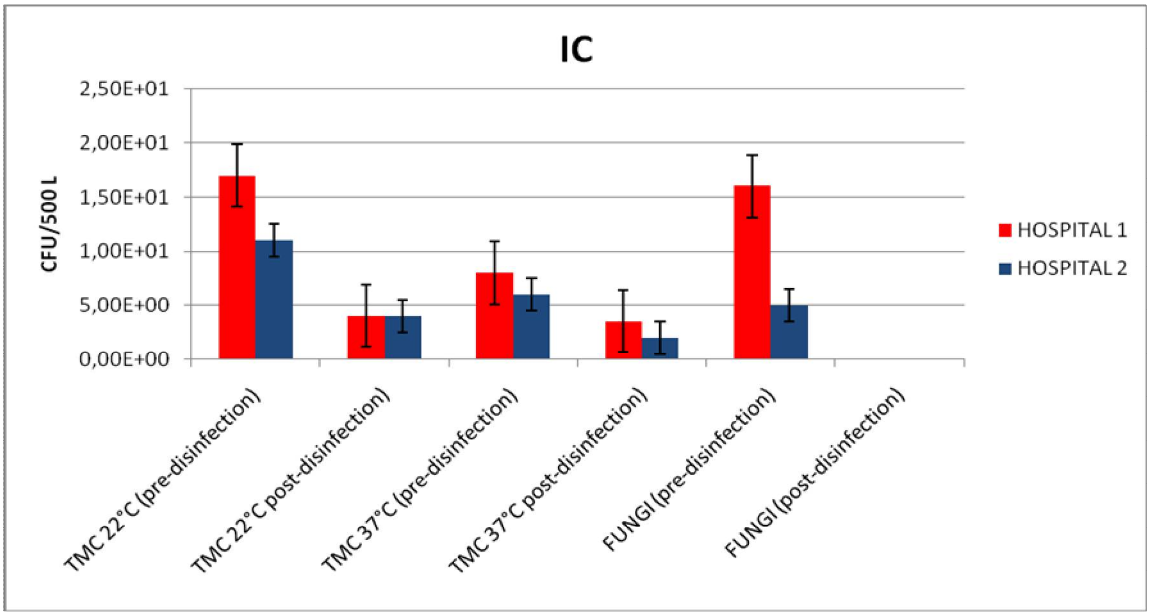

Figure 3: Mean values of total microbial counts (TMC) at 22/37°C and fungi counts detected in air samples (before and after disinfection) in HVAC system of intensive care (IC) in Hospital 1 and 2.

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HVAC systems sIC

In sub-intensive care wards, microbiological results obtained from air and surfaces were within the Italian guidelines limits. Surface samples total microbial and fungi counts resulted below 1 CFU/dm^2.

The disinfection treatment reduced the total air microbial counts at 22°C from 21±5 and 6±5 CFU/500 L to 5±3 and 3±2 CFU/500L in hospital 1 and 2, respectively (p=0.032; p=0.046) (Figure 4).

Fungi reductions (from 5±4 to 0 CFU/500L and from 2±1 to 0 CFU/500L) were not statistically significant (p>0.05).

No air contamination differences were observed between the various sampling points.

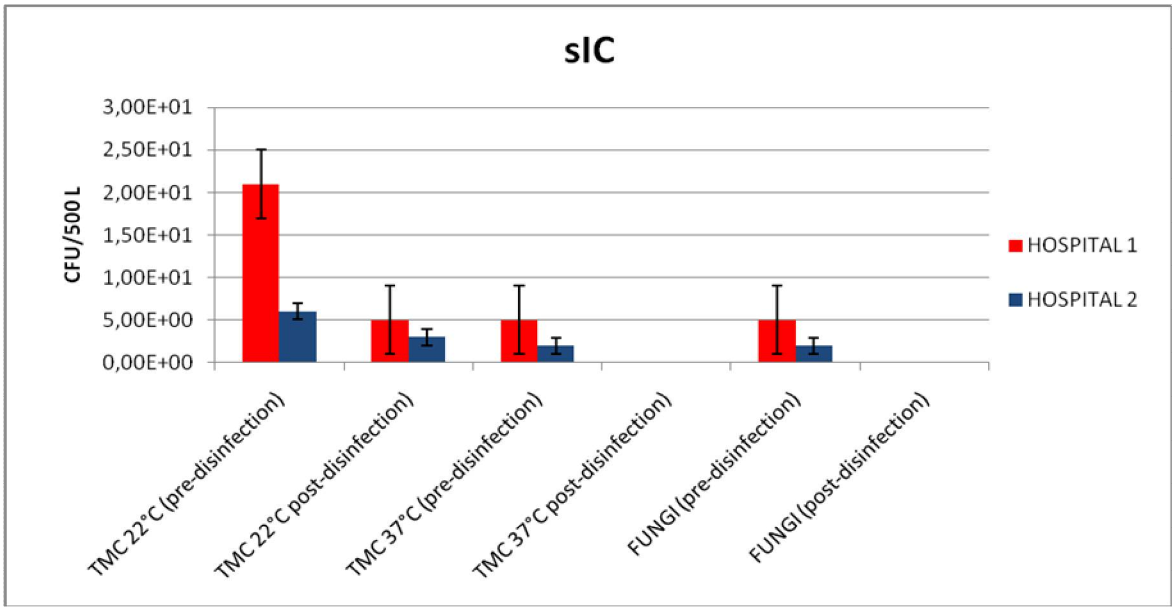

Figure 4: Mean values of total microbial counts (TMC) at 22/37°C and fungi counts detected in air samples (before and after disinfection) in HVAC system of sub-intensive care (sIC) in Hospital 1 and 2.

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Discussion

Literature reports that almost $10 billions are spent annually on hospital acquired infections, with surgical site accounting for 34% of the overall cost. Besides, air quality in operating rooms is an important factor that may contribute to surgical site infections [16,17]. HVAC systems in healthcare buildings are important to ensure clean air and prevent hospital infections, but at the same time, they require significant amounts of energy to operate [18].

Considering that the HVAC systems use for operating rooms and intensive care areas has increased over the years in worldwide hospitals, literature data suggest the need of new devices associated with HVAC systems, aimed to save energy costs [15]. New technical devices included in the HVAC systems are often large and complex and their cleaning and disinfection is difficult to achieve. In our study the installation of RDWs in 4 out of 90 HVAC systems has been included in the energy saving plan of the hospitals management. Despite the RDW devices allow the air dehumidification and the high temperatures prevent occurrences as the air contaminations, our experience reports the presence of *Aspergillus fumigatus* in the room vent of one hospital intensive care area. *Aspergillus* spp. are ubiquitous thermotolerant fungi which may disperse on air currents and deposit into human alveoli causing syndromes such as the allergic bronchopulmonary aspergillosis, mostly in immunocompromised patients hosted in hospital high risk areas [19]. Outbreak of invasive *Aspergillus fumigatus* infection in several surgical and intensive care settings, associated with a contaminated HVAC systems, are reported in many recent and old studies [20-23], which assert that mold contaminations were due to the lack of plant maintenances, and the structural complexity of the HVAC system.

Moreover, our research shows that mold contamination was eradicated after the introduction of the disinfection procedure with hydrogen peroxide applied to the RDWs devices. This result is frequently associated with statistically significant reductions of the total microbial counts at 22°C. It is known that aerosolized hydrogen peroxide is often used for the hospital indoor air disinfection, preventing outbreaks of multidrug-resistant bacteria and fungi infections [24,25]. Hydrogen

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peroxide is a strong oxidizer, bactericidal at 3% solution, a sterilant at 6% with six hours of exposure, more powerful than chlorine dioxide, and more stable at high temperatures and pH compared to chlorine-based disinfectants. It is non-toxic to humans and it cannot damage several type of technical materials. The lack of toxicity of HP to people and animals and its lack of environmental impact have been confirmed by the U.S. Food and Drug Administration (FDA) and the U.S. Environmental Protection Agency [26-27].

Materials and Methods

Hospital settings

The healthcare settings (Hospital 1 and Hospital 2) are two general hospitals in North-Western Tuscany local health unit (Italy), a 386 and a 360 beds hospitals with catchment areas of about 165.000 and 140.000 inhabitants, respectively. Hospital 1 has been active since 2014, while Hospital 2 has been active since 2016. Both architectural structures are monoblocks with a central plate on 5 levels. The warehouses and the car parks are in the basement. The ground floor houses the emergency department. Medical and clinical areas are located on the first and second floor, while surgery areas are on the third floor.

HVAC systems

Each hospital presents 90 HVAC systems located on the hospital rooftop. They have the following devices (Figure 5): outdoor air intake damper; pre-filtration and filtration systems, air recirculation device, heating battery, humidifier and dehumidifier-cooling devices, and air transport channels inside the wards (with terminal HEPA filters).

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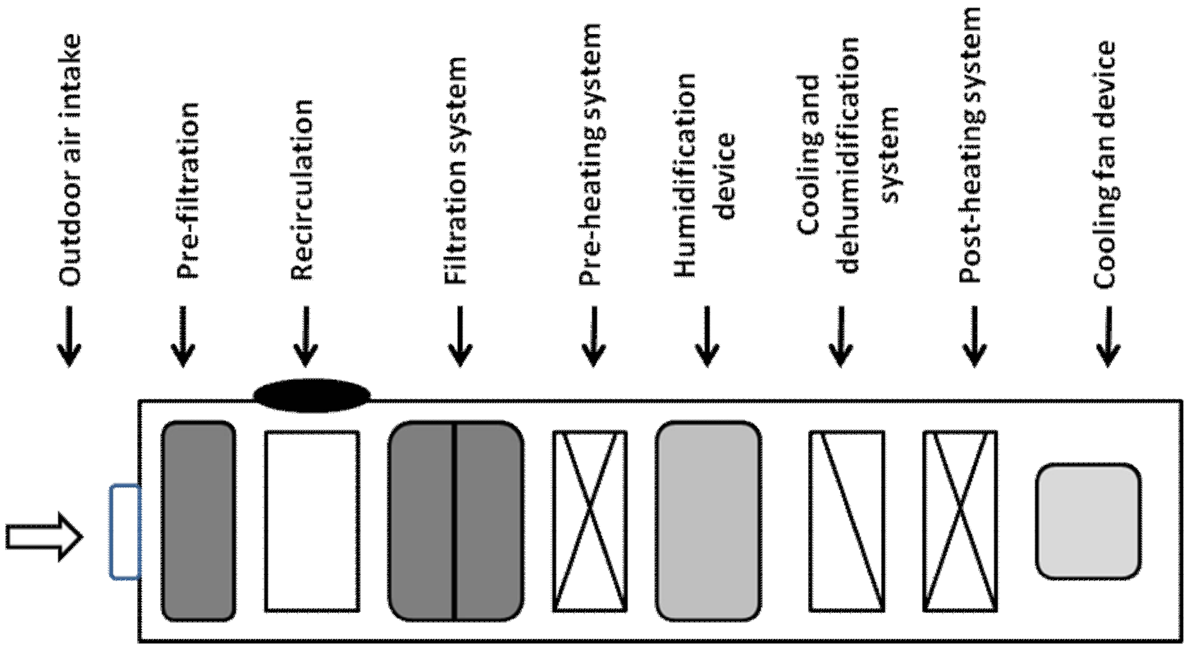

Figure 5: Schematic diagram of a typical HVAC plant.

Only 4 out of the 90 HVAC systems have a rotating dehumidification wheel (RDW), whose aim is removing the water vapor from the air and saving energy during the air recirculation. RDW temperature range, while operating, is from 70 to 100°C.

RDW devices are made of silicon gel (82%), glass fiber (16%) and acrylic coating (2%). As showed in Figure 6, surfaces present a honeycomb shape, allowing the flow air impact. The device allows the air input (98% of relative humidity (RH); 10°C), which passes through it. Dehumidified air (0% of RH; 25°C) enters into the channels and is subsequently recirculated (50% of RH; 20°C) entering into the RDW.

RDW are present in the HVAC systems related to the two surgery areas (SA1 and SA2), the intensive care areas (IC) and the sub-intensive care (sIC).

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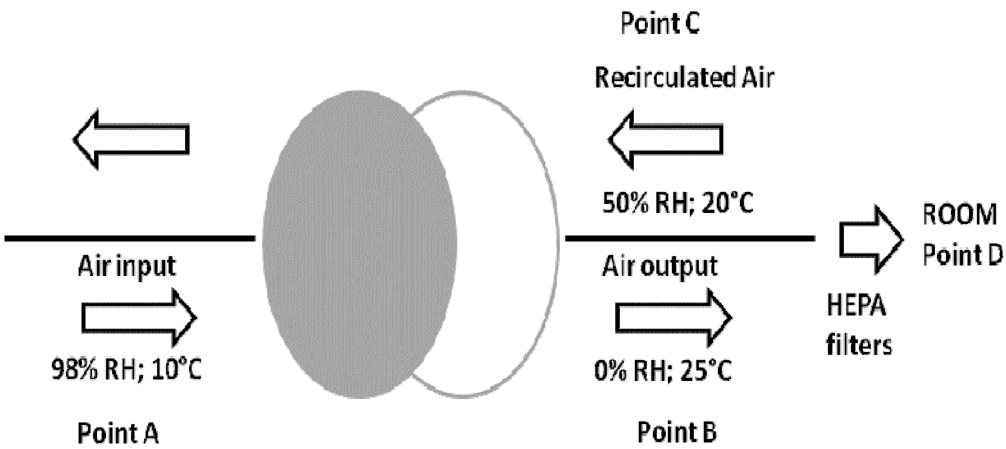

Figure 6: Process of dehumidification and recirculation air obtained from the rotating dehumidification wheel (RDW). (RH= Relative Humidity).

Air and surface samplings

From September 2016 to July 2018, in both hospitals microbiological tests were performed on the four HVAC systems with RDW devices (Table 1).

HOSPITAL	MONTHS OF SAMPLINGS	HVAC SYSTEMS	SAMPLING CONDITIONS	MATRIX
HOSPITAL 1	September - December 2016	SA1 SA2 IC sIC	First samplings with RDW in operation	Air and surfaces
	May– July 2017		Second samplings with RDW in operation (24 hours after the disinfection)	
HOSPITAL 2	April– May 2018	SA1 SA2 IC sIC	First samplings with RDW in operation	Air and surfaces
	July 2018		Second samplings with RDW in operation (24 hours after the disinfection)	

Table 1: Air and surfaces samplings protocol applied to HVAC systems of Hospital 1 and 2 before and after the rotating dehumidification wheel (RDW) disinfection. (SA1 and SA2: surgery areas; IC and sIC: intensive and sub-intensive areas).

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The same analyses were applied before and after the disinfection procedures.

From each HVAC system, air microbiological samplings were carried out using Microflow 90 (Aquaria, Italy) during the HVAC activities. From the points shown in Figure 2 (air input - point A, air output - point B, recirculation - point C; room - point D), 500 L of air (flow rate of 120 L/min) were aspired for the research of fungi and total microbial counts at 22 and 37°C.

From all the investigated HVAC systems, the surface microbiological samplings were performed in both RDW frontages, and in the channels carrying the air into the rooms. Microbiological samplings on surfaces were performed using Contact Agar Plates (VWR, Italy) for the research of fungi and total microbial counts at 22 and 37°C.

Culture media used for fungi and total microbial counts detection were Sabouraud Dextrose Agar and Plate Count Agar (VWR, Italy), respectively. After the samplings, all plates were incubated (fungi at 22°C for 120 hours; total microbial counts at 22°C for 72 hours; total microbial counts at 37°C for 24 hours), as described elsewhere (Istituto superiore per la prevenzione e la sicurezza del lavoro 2009).

Species confirmation of suspect colonies of *Aspergillus* spp. was obtained by Vitek 2 (Biomeriéux, France).

HVAC systems disinfection

HVAC systems disinfection procedures were performed in both hospitals after the first air and surfaces samplings. A mixture of Hydrogen peroxide (12%) with silver ions (10 mg/L) was aerosolized in all the air sampling points. The disinfectant was applied for at least 90 minutes contact time, in order to cover the whole RDW surfaces and the channels. All treatments were applied in HVAC "at rest" conditions. Moreover, the room vents were temporarily sealed avoiding patients and healthcare personnel exposure to the chemical compound.

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Statistical analysis

The Kolmogorov–Smirnov test was performed to verify normality of distributions. For each HVAC system the Kruskall-Wallis test and the Dunn's test were used to evaluate the reduction of the total microbial counts at 22 and 37°C and the fungi after the disinfection. Power tests were carried out to estimate the sample sizes. The 1-beta values of the significant variables were >0.8, proving acceptable sample sizes. The statistical analysis was fulfilled using the IBM SPSS software package, version 17.0.1.

Conclusions

Our study is the first one highlighting the need of new procedures for the disinfection of complex rotating dehumidification wheels, planned and installed in the HVAC systems. Despite literature data does not report disinfection strategy for the internal complex devices of the HVAC systems, we recommend the application of a safety plan for the indoor air quality, preventing the infectious risk from airborne pathogens in HVAC systems of hospital surgical and intensive care areas.

Competing Interests

All authors have no conflict of interest to declare.

Author Contribution

A.B., G.P., B.C. and A.P. conceived and designed the experiments.

M.T, L.F. and P.V. performed the samplings performed the laboratory tests.

M.T., A.L.C., S.P. and A.G. performed the data processing and wrote the paper.

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