



Review

Microbial contamination of air conditioning and humidifying systems in hospitals and in the built environment: a mapping review to assess potential microbial risks of adiabatic humidification

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SUMMARY

Adiabatic humidification is considered an energy-efficient alternative to steam humidification. However, it remains unclear whether these systems can act as reservoirs for pathogens that may subsequently be transmitted to patients. This mapping review assesses evidence on pathogen presence, biofilm formation and dissemination in air and aquatic environments of air conditioners and humidifiers analogous to adiabatic humidifiers. A search was conducted in PubMed, Web of Science and Embase until 6th July 2023. Data related to the 14 clinically most relevant bacteria based on expert assessment, and all fungi and protozoa were extracted. The search identified 468 studies, 22 were included, covering 184 air handling units (AHUs), none involving adiabatic humidification. In total, 216 bacterial and 149 fungal pathogens and 26 protozoa were classified. *Pseudomonas aeruginosa* and *Burkholderia cepacia* were occasionally found in system air. *Mycobacterium* spp. and *Acinetobacter* spp. were found on cooler surfaces and in drain pans. High-risk pathogenic fungi were reported in ambient air surrounding coolers. *Mucor* spp. and *Aspergillus fumigatus* were found in water and ambient air surrounding various components, whereas *Fusarium* spp. were more airborne. None of the studies examined the role of biofilms in AHUs. A variety of pathogenic bacteria and fungi were present in systems similar to adiabatic humidification,

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highlighting the need for risk-managed water safety, maintainable and hygienic design and routine microbiological surveillance, as deviations from recommended operation and maintenance may promote growth. The absence of adiabatic humidification studies necessitates further research to evaluate their microbiological safety in hospitals.

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Introduction

Relative humidity (RH) is the amount of water vapour present in the air at a specific temperature at any time, relative to the maximum water vapor capacity the air can hold at that temperature [1]. In hospitals, the RH is controlled between 40–60%. This range is considered optimal to prevent microbial growth and spread of pathogens that can cause healthcare-associated infections (HAIs) [2]. Additionally, controlling this RH range is important for (thermal) comfort and well-being of occupants and for patient safety because low humidity can lead to skin dryness and drying out of mucous membranes [1–7]. These effects might contribute to HAIs in hospitalized patients who are already vulnerable to infections [1,8–10].

Currently, RH in hospitals is most commonly regulated using steam humidification. This is an isothermal humidifier type that uses heat from an external source (e.g., electricity or natural gas) to convert water into steam in boilers and inject it directly into the airstream [6,11–13]. Steam humidification is considered to be a sterile method of humidification, which is extremely suitable for hospital environments [6,14]. However, steam humidification using natural gas or electricity is highly energy- and resource-intensive because generating steam requires substantial energy [12,14].

A promising alternative for steam humidification is adiabatic humidification, which is already used in some manufacturing sectors and hospitals worldwide [15,16]. This process involves directly introducing water into the air without prior heating of the water [5,6,12,14,17]. A previous study on cleanrooms in subtropical regions showed four-fold greater energy savings with high-pressure adiabatic humidification via evaporative cooling compared with steam humidification [18].

In general, there are two types of adiabatic humidification processes: evaporation and atomization. Figure 1 provides a schematic overview of a simplified example of these two types.

The atomizing system is aerosol-generating, wherein water is dispersed in small droplets into the air via mechanical vaporizers or nozzles (Figure 1). These droplets evaporate in the airstream, cooling down the air, and the excess droplets are subsequently removed by droplet separators [6,12]. For this method, the use of reverse osmosis is necessary in the water supply [6]. There are four main subtypes of adiabatic atomizing humidifiers: ultrasonic, centrifugal, pressurized-water humidifiers, and compressed air atomizers. In an evaporative adiabatic humidification system, water runs over evaporator mats or packages (wetted media), simultaneously exposing the air to be humidified by this wetted media by absorbing the moisture. Heat from a heater is required to facilitate this evaporative process imparting humidity into the air [12,15]. Wetted media humidifiers often make use of recirculated water. Therefore, this type poses a risk of microbial contamination (e.g., *Legionella*) and its use in critical settings should therefore be limited or carefully adapted [19,20].

Transitioning to non-steam alternative humidification systems to reduce CO₂ emissions presents challenges regarding stagnant water formation through condensation in drip trays or drain pans. Humid, nutrient-rich areas promote microbial growth and biofilms, from which micro-organisms may be released into the air [6,20]. Additionally, terminal filters that are moist can become reservoirs for micro-organisms, potentially leading to their dissemination into hospital's air and contributing to HAIs [21].

The extent of microbial colonization, biofilm formation in adiabatic humidification systems, and the risk of airborne dispersion remain unknown. Therefore, the aim of this study was to conduct a comprehensive review by mapping literature concerning the potential growth of micro-organisms in hospital adiabatic humidification systems. In the absence of scientific literature addressing adiabatic humidification systems, this has been translated in the formulation of two research questions: (1) which pathogenic micro-organisms are present in air and aquatic environments in different types of air conditioning (AC) systems and humidifiers may pose a threat to patients; and (2) could the presence of biofilm in these AC and humidification systems lead to the spread of micro-organisms into the air?

Methods

Review strategy

Given the absence of literature addressing adiabatic humidification and its relationship to microbiological safety, this study opted to explore alternative systems with similar components and functions (e.g., cooling, humidification, heating, dehumidification and air filtration). This mapping review therefore encompasses literature related to heating, ventilation, and air conditioning (HVAC) systems, and AC systems that are used in hospital settings and is conducted in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analyses extension for Scoping Reviews (PRISMA-ScR) guideline (Supplementary material A).

Micro-organisms of interest were chosen based on clinical relevance by clinical microbiologists (J.S., B.M.) and technical microbiology scientist expert opinion (J.V.) in accordance with the review team. Final consensus was reached during a discussion meeting (Supplementary material B: Search Strategy). Micro-organisms were selected due to their capacity to cause severe infections in patients, and because of the elevated risk they present when aerosolized and subsequently inhaled by patients.

Search and study selection

A literature search was conducted in electronic database EMBASE, Web of Science and PubMed on 6th July 2023. This search was not limited by date of publication, country of

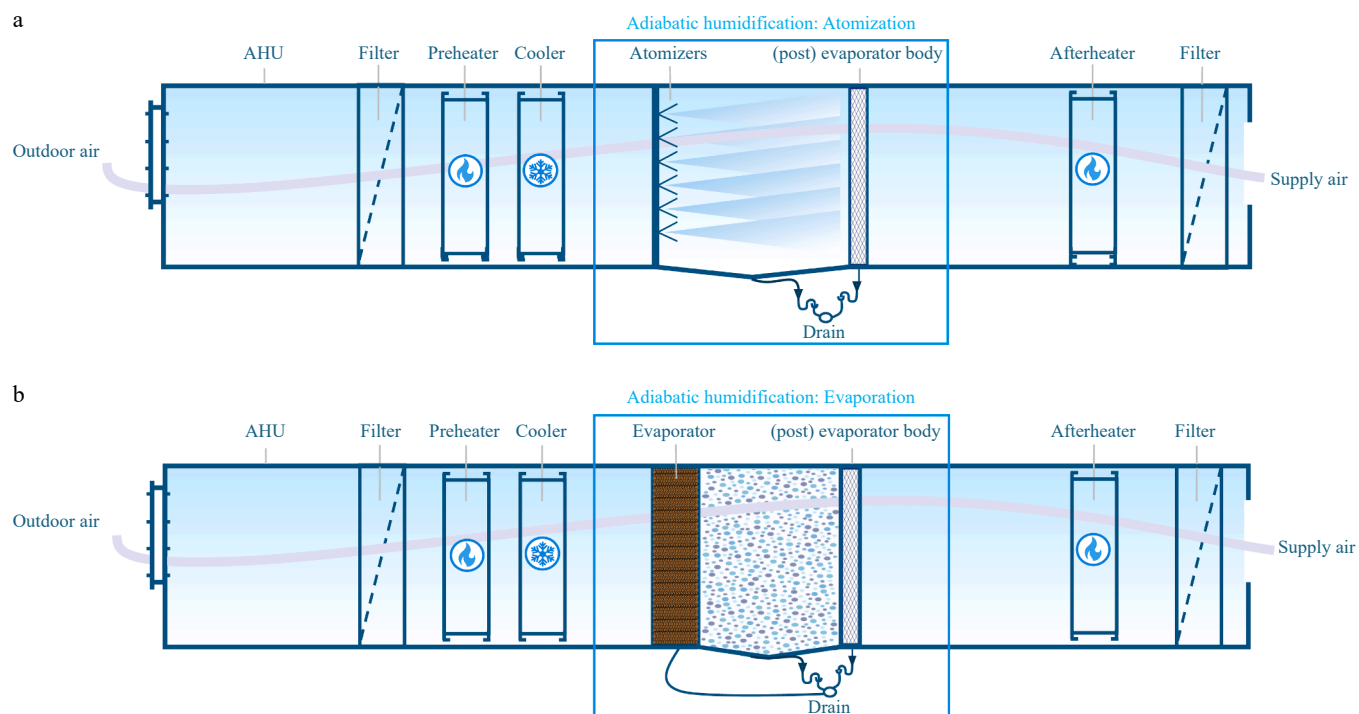


Figure 1. Schematic overview of a simplified air handling unit (AHU) with two types of adiabatic humidification process. (a) Atomization: an atomizing system uses pressurized water, which is dispersed into fine droplets or mist and introduced into the airstream. A post evaporator body or droplet separator is located behind the atomizers (e.g., nozzles) to collect droplets from the air, directing the excess water to a drain pan and sewer. (b) Evaporation: in this evaporative system, water runs over the mats or packages through which the airstream passes, cooling and humidifying the air. A droplet separator acts as a secondary evaporator to collect large droplets from the airstream, allowing them to evaporate. Any excess water is drained into a drain pan or directly into the sewer through a siphon.

publication, language, or study design. The complete search strategy with the study selection and the inclusion and exclusion criteria can be found in [Supplementary material B](#). In short, we excluded short notes, conference papers, letters and reviews, studies limited to indoor air quality; studies on home or portable humidifiers and small AC systems, studies on medical devices, and cooling towers. Finally, experimental, (computational) modelling and test set-up studies were excluded.

Data extraction

A data extraction file was created in Microsoft Excel (2016) to systematically extract data based on system properties, information on whether maintenance was performed, and data on whether there was microbial growth in these systems ([Supplementary material C](#)). Systems with similar conditions or principles were grouped: (1) coolers (evaporative and air coolers (units), cooling coils); (2) humidifiers (evaporative and water sources such as tubs, bowls, and reservoirs); (3) drain pan (condensate water (pan) from coolers and drain pans); (4) ductwork (e.g., ventilation ducts, ductwork, air vents and ducts); and (5) ventilators (e.g., fans, ventilators, and blower wheels).

Risk classification

A high-, high/medium-, medium-, medium/low- and low-risk classification was made for extracted microbial data

based on genera and species. This classification was based on expert opinion by two clinical microbiologists (J.S., B.M.). Furthermore, the list of micro-organisms is supported by World Health Organization (WHO) fungal priority pathogens list (2022) and the WHO bacterial priority pathogens list (2024) [22,23]. Genera and species classified as high-risk are known to cause severe infections in patients [22,23]. Medium-risk classified micro-organisms have the potential to cause infections, while low-risk classified micro-organisms are less likely to pose a threat to patients when they end up in air.

Data analysis

We qualitatively synthesized micro-organisms detected in air, surface, and water samples across different air-handling unit components. Reported genera and species were analysed separately for bacteria, fungi, and protozoa. Medium- to high-risk micro-organisms within each sample type were compared to identify whether the same pathogens consistently occurred under similar environmental conditions. We then compared these micro-organisms across sample types to assess their potential to proliferate in different environments and their suitability to conditions analogous to adiabatic humidification. Missing data were reported.

Data from upstream and downstream sampling of components were compared with each other. When only polymerase chain reaction (PCR) and sequencing methods were used, the results of micro-organisms may have indicated the presence of genetic material rather than viable micro-organisms. When the

numbers of systems were not described, the lowest countable number of systems was used. Additionally, when results of individual micro-organisms were not presented per system sampled, the findings of that study were combined and analysed as one result for all systems sampled.

Results

Description of included studies

The search identified 468 non-duplicate records (Supplementary material D: Figure S1). After abstract selection, 74 records were potentially relevant. The full-text screening resulted in including 22 studies, of which the extracted data was sent to the corresponding authors. Two corresponding authors provided additional and/or missing data. No studies were excluded due to non-response.

Study characteristics

The 22 included studies were published between 1980 and 2021. Twenty-one studies (95.5%) were single-country studies. One study was carried out across two different countries on separate continents. Thirteen studies took place in the built environment (59.1%), six in hospitals (27.3%), and five in school settings (22.7%). Two studies were conducted in buildings where the systems facilitate laboratories (9.1%).

A total number of 406 air-handling units (AHUs) were included across the 22 studies. We analysed the data from 184 systems wherein the components were sampled for microbial growth or the presence of genetic material of micro-organisms. Components of the systems sampled included filters ($N = 12$), coolers ($N = 7$), drain pans ($N = 4$), ductwork ($N = 4$), humidifiers ($N = 4$), indoor air inside the system ($N = 2$), insulation ($N = 2$), ventilators ($N = 2$), rotating dehumidification wheels (RDWs) ($N = 1$), and outdoor air ($N = 1$). Four studies (18.2%) specified the timing and frequency of maintenance on the sampled systems. Eight studies (36.4%) indicated that maintenance was performed but did not specify the timing or regularity (Supplementary material D: Table SI).

Nine studies (40.9%) did not report microbial findings per system. Twelve studies (54.5%) reported the presence of bacteria. Seventeen studies (77.3%) reported the presence of fungi, whereas nine studies (40.9%) studied the presence of both fungi and bacteria. Two studies (9.1%) investigated the presence of protozoa, limited to free-living amoeba (FLA).

From the 184 systems included, the majority reported micro-organisms on the components sampled. However, not all were identified to genus and species level as shown in Supplementary material E: Table SI and Figure S1. A total of 873 micro-organisms were identified (bacteria $N = 356$, fungi $N = 491$, protozoa $N = 26$). In total 142 genera were identified (bacteria $N = 55$, fungi $N = 85$, protozoa $N = 2$). Data lacking genus and/or species-level identification were excluded, except for Enterobacterales and Mucorales, due to their clinical relevance.

Sampling and identification of micro-organisms

Sampling methods varied across different sample types and culture techniques. Malt extract agar was commonly used for fungal identification, while diverse culture methods were used

for bacterial identification. Three of 22 studies conducted direct DNA-based identification methods (13.6%), bypassing microbial culturing, leaving uncertainty regarding the viability of the detected micro-organisms (Supplementary material D: Table SII). Although components were sampled this did not necessarily result in the identification of micro-organisms. Bacteria and fungi were most identified in systems where cooler surfaces and water from the drain pan was sampled. Surface sampling was the most preferred method for the systems. A full overview is shown in Supplementary material E: Figure S1 and Table SII.

Bacteria reported in the systems

Pathogenic bacteria

High-to-medium risk bacterial genera are listed in Table I for each component and sample type. A total of 216 of 356 bacteria from 20 identified genera and one family that were reported are a significant risk for patient health. *Staphylococcus* spp. ($N = 58$) and *Bacillus* spp. ($N = 51$) were identified the most, irrespective of the sample site. The majority of the pathogenic bacteria were observed in ambient air surrounding coolers ($N = 90$), followed by drain pan water ($N = 51$). The whole overview including low and medium-risk bacterial pathogens is shown in Supplementary material F: Figure S1A-B and Table SI.

Bacteria in air

With air sampling of the ambient air surrounding filters, coolers and the indoor air of the systems itself, a total of 130 bacteria (coolers $N = 90$, filters $N = 21$ and indoor air $N = 19$) of 10 genera (filters $N = 9$, coolers $N = 4$ and indoor air ($N = 7$) were documented. Most reported genera were *Staphylococcus* spp. ($N = 47$), *Bacillus* spp. ($N = 31$), *Micrococcus* spp. ($N = 22$) and *Sphingomonas* spp. ($N = 20$). Some noteworthy high-risk bacterial species that were found in the air include *Burkholderia cepacia* (filters, $N = 1$), *Pseudomonas aeruginosa* (filters $N = 1$ and indoor air $N = 1$) and *Staphylococcus aureus* (indoor air $N = 1$).

Surface-associated bacteria

On the surfaces a total of 31 bacteria were reported (coolers $N = 12$, filters $N = 10$ and ductwork $N = 9$) of 15 genera (coolers $N = 11$, filters $N = 6$, ductwork $N = 4$) and one family (coolers $N = 1$). *Bacillus* spp. ($N = 9$) was most documented genus, predominantly reported on filter surfaces ($N = 4$) and ductwork ($N = 4$). High-risk genera found on the surfaces include *Staphylococcus* spp. (filter $N = 1$, ductwork $N = 1$), *Legionella* spp., *Acinetobacter* spp., *Mycobacterium* spp., and *Stenotrophomonas* spp. which were all observed once on cooling coil surfaces of systems.

Bacteria in aquatic environments of the systems

In water samples of the drain pan and the humidifier, 55 bacteria of nine genera were identified that pose a threat to patients. *Sphingomonas* spp. ($N = 19$), *Bacillus* spp. ($N = 11$), *Staphylococcus* spp. ($N = 9$), and *Micrococcus* spp. ($N = 7$) were frequently documented, particularly in the drain pan consistent with findings of the other sample types. High-risk genera were found in the drain pan including, *Acinetobacter* spp. ($N = 5$), *Pseudomonas* spp. ($N = 1$) and *Mycobacterium* spp. ($N = 1$).

Table I
Classification of bacterial pathogenic genera and their corresponding species

Sample type	Components sampled (no. of systems where bacteria were identified)	Bacterial genera (no. of genera/total no. of bacteria)	Risk score	Bacterial species (no. of bacteria/total no. of bacteria)	Risk score
Air	Filter (6) ^a	<i>Burkholderia</i> spp. (1/21)	High/medium	<i>Burkholderia cepacia</i> (1/21)	High
		<i>Pseudomonas</i> spp. (2/21)	High/medium	<i>Pseudomonas aeruginosa</i> (1/21)	High
		<i>Staphylococcus</i> spp. (4/21)	High/medium	<i>Micrococcus luteus</i> (1/21)	Medium
		<i>Brevibacterium</i> spp. (1/21)	Medium	<i>Staphylococcus cohnii</i> (1/21)	Medium
		<i>Cellulomonas</i> spp. (1/21)	Medium	<i>Staphylococcus haemolyticus</i> (1/21)	Medium
		<i>Corynebacterium</i> spp. (1/21)	Medium	<i>Staphylococcus sciuri</i> (1/21)	Medium
		<i>Sphingomonas</i> spp. (1/21)	Medium	<i>Bacillus cereus</i> (1/21)	Medium
		<i>Bacillus</i> spp. (8/21)	Medium/low	<i>Bacillus licheniformis</i> (1/21)	Medium
		<i>Micrococcus</i> spp. (2/21)	Medium/low	<i>Sphingomonas paucimobilis</i> (1/21)	Medium
				<i>Pseudomonas oryzihabitans</i> (1/21)	Medium
				Unknown species (11/21)	
	Cooler (9) ^c	<i>Staphylococcus</i> spp. (36/90)	High/Medium	<i>Bacillus thuringiensis</i> (18/90)	Medium
		<i>Sphingomonas</i> spp. (18/90)	Medium	<i>Micrococcus luteus</i> (18/90)	Medium
		<i>Bacillus</i> spp. (18/90)	Medium/low	<i>Sphingomonas paucimobilis</i> (18/90)	Medium
		<i>Micrococcus</i> spp. (18/90)	Medium/low	<i>Staphylococcus haemolyticus</i> (36/90)	Medium
	Indoor air (8) ^a	<i>Pseudomonas</i> spp. (1/19)	High/medium	<i>Staphylococcus aureus</i> (1/19)	High
		<i>Staphylococcus</i> spp. (7/19)	High/medium	<i>Pseudomonas aeruginosa</i> (1/19)	High
		<i>Chryseobacterium</i> spp. (1/19)	Medium	<i>Micrococcus luteus</i> (1/19)	Medium
		<i>Corynebacterium</i> spp. (2/19)	Medium	<i>Staphylococcus cohnii</i> (1/19)	Medium
		<i>Sphingomonas</i> spp. (1/19)	Medium	<i>Staphylococcus haemolyticus</i> (1/19)	Medium
		<i>Bacillus</i> spp. (5/19)	Medium/low	<i>Staphylococcus sciuri</i> (1/19)	Medium
		<i>Micrococcus</i> spp. (2/19)	Medium/low	<i>Staphylococcus xylosus</i> (1/19)	Medium
				<i>Bacillus cereus</i> (1/19)	Medium
				<i>Bacillus licheniformis</i> (1/19)	Medium
				<i>Chryseobacterium meningosepticum</i> (1/19)	Medium
				<i>Sphingomonas paucimobilis</i> (1/19)	Medium
				Unknown species (8/19)	
Surface	Filter (7)	<i>Staphylococcus</i> spp. (1/10) ^a	High/medium	<i>Staphylococcus xylosus</i> (1/10) ^a	Medium
		<i>Corynebacterium</i> spp. (1/10) ^a	Medium	<i>Bacillus licheniformis</i> (1/10) ^a	Medium
		<i>Nocardia</i> spp. (1/10) ^a	Medium	<i>Sphingomonas frigidaeris</i> sp. nov. (1/10)	Medium
		<i>Sphingomonas</i> spp. (2/10)	Medium	Unknown species (7/10)	
		<i>Bacillus</i> spp. (4/10) ^a	Medium/low		
		<i>Micrococcus</i> spp. (1/10) ^a	Medium/low		
	Cooling coils (40) ^{a,b}	<i>Legionella</i> spp. (1/12)	High		
		<i>Enterobacterales</i> (1/12) ^d	High/medium	Unknown species (12/12)	
		<i>Acinetobacter</i> spp. (1/12)	High/medium		
		<i>Mycobacterium</i> spp. (1/12)	High/medium		
		<i>Stenotrophomonas</i> spp. (1/12)	High/medium		
		<i>Aerococcus</i> spp. (1/12)	Medium		
		<i>Corynebacterium</i> spp. (1/12)	Medium		
		<i>Enterococcus</i> spp. (1/12)	Medium		

(continued on next page)

Table I (continued)

Sample type	Components sampled (no. of systems where bacteria were identified)	Bacterial genera (no. of genera/total no. of bacteria)	Risk score	Bacterial species (no. of bacteria/total no. of bacteria)	Risk score
Water	Ductwork (9)	<i>Finegoldia</i> spp. (1/12)	Medium		
		<i>Streptococcus</i> spp. (1/12)	Medium		
		<i>Neisseria</i> spp. (1/12)	Medium		
		<i>Bacillus</i> spp. (1/12)	Medium/low		
		<i>Staphylococcus</i> spp. (1/9) ^a	High/medium	<i>Micrococcus luteus</i> (1/9) ^a	Medium
		<i>Nocardia</i> spp. (1/9) ^a	Medium	<i>Staphylococcus xylosus</i> (1/9) ^a	Medium
		<i>Bacillus</i> spp. (4/9) ^a	Medium/low	<i>Bacillus cereus</i> (1/9) ^a	Medium
		<i>Micrococcus</i> spp. (3/9) ^a	Medium/low	<i>Bacillus licheniformis</i> (1/9) ^a	Medium
	Humidifier water reservoir (34)			Unknown species (5/9) ^a	
				Unknown species (4/4) ^a	
	Drain pan (19)	<i>Pseudomonas</i> spp. (1/4) ^a	High/medium		
		<i>Streptococcus</i> spp. (1/4) ^a	Medium		
		<i>Bacillus</i> spp. (2/4) ^a	Medium/low		
		<i>Staphylococcus</i> spp. (9/51)	High/medium	<i>Staphylococcus haemolyticus</i> (9/51)	Medium
		<i>Acinetobacter</i> spp. (5/51)	High/medium	<i>Bacillus thuringiensis</i> (9/51)	Medium
		<i>Mycobacterium</i> spp. (1/51) ^a	High/medium	<i>Sphingomonas paucimobilis</i> (9/51)	Medium
		<i>Sphingomonas</i> spp. (19/51)	Medium	<i>Micrococcus luteus</i> (7/51)	Medium
		<i>Chryseobacterium</i> spp. (1/51) ^a	Medium	Unknown species (17/51) ^a	
		<i>Bacillus</i> spp. (9/51) ^a	Medium/low		
		<i>Micrococcus</i> spp. (7/51)	Medium/low		

^a The authors of these studies reported the results of all systems together as one result and/or the microbial results specifically for every system sampled were not (fully) described.

^b The results of identification of the bacteria were based on DNA techniques.

^c Includes studies that had two separate sample moments and therefore these sample moments are used twice in the analysis.

^d Includes the members of the Enterobacterales family.

Fungi reported in the systems

Pathogenic fungi

High-to-medium risk fungi are listed in Table II. A total of 149 fungal pathogens were identified from 20 genera and one order (Mucorales). The most reported fungal pathogens were *Aspergillus* spp. ($N = 64$), *Mucor* spp. ($N = 30$), and *Fusarium* spp. ($N = 12$). The majority of pathogenic fungi were documented in the ambient air surrounding coolers ($N = 45$), surfaces of filters ($N = 23$) and coolers ($N = 20$). The most diverse composition of fungal pathogens was found on surfaces of coolers ($N = 13$) and filters ($N = 8$). The whole overview including low and medium-risk fungal pathogens is shown in Supplementary material F: Figure S2A-B and Table SII.

Fungi in air

In ambient air, 76 fungi were reported, particularly in areas surrounding components such as coolers ($N = 45$), filters ($N = 12$), indoor air ($N = 9$), outdoor air ($N = 9$), and humidifiers ($N = 1$). These fungi belonged to eight genera. *Aspergillus* spp. ($N = 33$) and *Mucor* spp. ($N = 22$) were predominantly identified, especially in the ambient air surrounding coolers (*Aspergillus fumigatus* and *Mucor ramosissimus*, $N = 18$, each). *Aspergillus* spp. were additionally commonly reported in the ambient air around filters ($N = 5$), and the indoor air ($N = 4$). In contrast, *Fusarium* spp. ($N = 10$) was most frequently detected in the outdoor air ($N = 3$) and indoor air ($N = 3$). Only one species was reported in the supply air.

Surface-associated fungi

On component surfaces, a total of 62 fungi were identified on filters ($N = 23$), coolers ($N = 20$), insulation ($N = 9$), ductwork ($N = 5$), ventilators ($N = 4$), and RDWs ($N = 1$). In total 15 fungal genera (coolers $N = 13$, filters $N = 8$, ductwork $N = 4$, ventilators $N = 2$, RDWs $N = 1$) and one order (ventilator $N = 1$ and ductwork $N = 1$) were found. *Aspergillus* spp. was again most frequently identified ($N = 28$), predominantly found on the surfaces of filters ($N = 16$), insulation ($N = 4$), ductwork ($N = 2$), ventilators ($N = 3$) and RDWs ($N = 1$). In contrast, *Candida* spp. ($N = 4$) was more commonly reported on the cooler surfaces ($N = 3$). *Fusarium solani* was detected on filter surfaces as well ($N = 1$).

Fungi in aquatic environments of systems

In the drain pan water samples, 11 fungi were identified, representing two distinct high-risk species. Among these, *Mucor ramosissimus* was the most frequent reported fungus ($N = 8$) followed by *Aspergillus fumigatus* ($N = 3$).

Protozoa reported in the systems

A total of 26 protozoa were reported in 26 systems of two studies together. These protozoa include FLA such as *Acanthamoeba* spp. ($N = 23$), and *Balamuthia mandrillaris* ($N = 3$) which were found on the filter surfaces. Other protozoa that could be a threat to patient health were not identified.

Differences upstream and downstream the system

In six of 22 studies, the authors either assumed or found significant evidence that microbial growth was propagated

downstream through the airstream within the systems, eventually contaminating room air [24–29]. In contrast, two other studies stated clearly that they did not find significant differences of microbial growth in terms of location [30] or significant differences upstream or downstream the humidifier [31]. Only two studies have assessed upstream–downstream microbial correlations in ventilation systems: one found no significant quantitative correlations but presence of similar species [32], while the other reported clear positive correlations, suggesting downstream spread of fungi through the airstream [33].

Biofilm formation in the systems

None of the included studies investigated biofilm formation in AC and humidification systems and dissemination into the air. Only one study investigated the ability of *Sphingomonas paucimobilis* and *Micrococcus luteus* to form biofilm *in vitro* on aluminium fin coupons at temperatures ranging from 10 to 30°C, and resistance to changing moisture conditions. They found that both were able to form biofilms on the coupons and maintain viability after multiple wet–dry–wet cycles [34].

Discussion

In absence of literature regarding adiabatic humidification, this review included studies on other AC systems and humidifiers. Our findings indicate that the majority of research focuses on fungal contamination, with less attention given to bacteria and protozoa. Fungi are ecologically dominant, stable in air and dust, and easier to culture and identify. Fungal spores are well adapted for airborne dispersal and readily proliferate in moist HVAC environments, contributing to impaired system performance [35,36]. In contrast, bacterial communities are more variable and may require appropriate culture methods due to viable but non culturable states [37,38].

A study by Hugenholtz and Fuerst, along with the included studies, collectively supports the hypothesis that high-risk pathogens can proliferate in systems and eventually can be spread through the air [39]. Therefore, special attention is required to prevent the growth of high-risk pathogens such as *Pseudomonas* spp., *Staphylococcus* spp., *Acinetobacter* spp., *Legionella* spp., *Aspergillus* spp., and *Fusarium* spp. These micro-organisms can cause severe infections, particularly in immunocompromised patients, with high mortality rates [40–48]. Some of these fungal pathogens can also infect immunocompetent individuals via airborne exposure [49–57]. Hugenholtz and Fuerst, found in addition to our findings that *Acinetobacter* spp. and *Flavobacterium* spp. were present in visually clean AHUs in the return-fresh mix air, evaporative condenser sump water, and supply air. *Flavobacterium* spp. was also found downstream the cooling coils and in the drain pan [39]. This is particularly concerning because Gram-negative bacteria are commonly found in hospital wet environments and pose a risk when ‘non-sterile’ water is introduced into hospitals’ air through adiabatic humidification processes [58]. Gram-negative bacteria such as *Enterobacter* spp., *Citrobacter* spp. and *Klebsiella* spp. that play a role in many water-related outbreaks in hospitals, were not reported by any of the included studies [59–66]. This absence may be attributable to the use of limited and potentially suboptimal selective media used in the studies included.

Table II
Classification of fungal pathogenic genera and their corresponding species

Sample type	Components sampled (no. of systems where pathogenic fungi are identified)	Fungal genera (no. of genera/total no. of pathogenic fungi)	Risk score	Fungal species (no. of species/total number of pathogenic fungi)	Risk score
Air	Filter (8)	<i>Fusarium</i> spp. (2/12) ^a	High	<i>Aspergillus fumigatus</i> (1/12) ^a	High
		<i>Mucor</i> spp. (2/12) ^a	High	<i>Fusarium oxysporum</i> (1/12) ^a	High
		<i>Rhizopus</i> spp. (3/12) ^{a,d}	High	<i>Fusarium solani</i> (1/12) ^a	High
		<i>Aspergillus</i> spp. (5/12) ^{a,d}	High/medium	<i>Mucor plumbeus</i> (1/12) ^a	High
				<i>Rhizopus stolonifer</i> (2/12) ^{a,d}	High
				<i>Rhizopus oryzae</i> (1/12) ^d	High
				<i>Aspergillus niger</i> (1/12) ^a	Medium
				Unknown species (4/12)	
	Cooler (19)	<i>Fusarium</i> spp. (2/45) ^{a,d}	High	<i>Fusarium acuminatum</i> (1/45) ^d	High
		<i>Mucor</i> spp. (18/45) ^e	High	<i>Mucor ramosissimus</i> (18/45) ^e	High
		<i>Aspergillus</i> spp. (21/45) ^{a,d,e}	High/medium	<i>Fusarium cerealis</i> (1/45) ^d	High
		<i>Sporothrix</i> spp. (1/45)	Medium	<i>Aspergillus fumigatus</i> (18/45) ^e	High
		<i>Acremonium</i> spp. (3/45)	Medium/low	Unknown species (7/45) ^d	
	Humidifier unspecified (4) ^{d, e} Outdoor air (2)	<i>Aspergillus</i> spp. (1/1) ^{a,d}	High/Medium	Unknown species (1/1) ^{a,d}	
		<i>Fusarium</i> spp. (3/9)	High	<i>Fusarium acuminatum</i> (2/9)	High
		<i>Mucor</i> spp. (1/9)	High	<i>Fusarium cerealis</i> (1/9)	High
		<i>Aspergillus</i> spp. (2/9)	High/medium	<i>Mucor racemosus</i> (1/9)	High
		<i>Nigrospora</i> spp. (1/9)	Medium	<i>Aspergillus purpurascens</i> (1/9)	Medium
		<i>Paecilomyces</i> spp. (1/9)	Medium	<i>Aspergillus niger</i> (1/9)	Medium
		<i>Acremonium</i> spp. (1/9)	Medium/low	<i>Acremonium strictum</i> (1/9)	Medium
				<i>Paecilomyces variotii</i> (1/9)	Medium
				Unknown species (1/9)	
		<i>Fusarium</i> spp. (3/9) ^a	High	<i>Aspergillus fumigatus</i> (1/9) ^a	High
		<i>Mucor</i> spp. (1/9) ^a	High	<i>Fusarium solani</i> (1/9) ^a	High
		<i>Rhizopus</i> spp. (1/9) ^a	High	<i>Rhizopus stolonifer</i> (1/9) ^a	High
	Indoor air (unspecified) (10)	<i>Aspergillus</i> spp. (4/9) ^a	High/medium	<i>Aspergillus niger</i> (1/9) ^a	Medium
				<i>Aspergillus ochraceus</i> (1/9) ^a	Medium
				Unknown species (4/9) ^a	
				<i>Fusarium acuminatum</i> (1/1)	High
Surface	Supply air (2)	<i>Fusarium</i> spp. (1/1)	High	<i>Fusarium acuminatum</i> (1/1)	High
	Return air (1)	<i>Fusarium</i> spp. (1/1)	High	<i>Aspergillus fumigatus</i> (2/23) ^{a, b}	High
	Filter (10)	<i>Fusarium</i> spp. (1/23) ^a	High	<i>Fusarium solani</i> (1/23) ^a	High
		<i>Aspergillus</i> spp. (16/23) ^{a,b}	High/medium	<i>Aspergillus flavus</i> (1/23) ^{a, b}	Medium
		<i>Candida</i> spp. (1/23) ^a	High/medium	<i>Aspergillus ochraceus</i> (2/23) ^{a,b}	Medium
		<i>Cryptococcus</i> spp. (1/23)	High/medium	<i>Aspergillus versicolor</i> (1/23)	Medium
		<i>Curvularia</i> spp. (1/23)	Medium	<i>Curvularia geniculata</i> (1/23)	Medium
		<i>Phialemonium</i> spp. (1/23)	Medium	<i>Phaeoacremonium parasiticum</i> (1/23)	Medium
		<i>Rhodotorula</i> spp. (1/23)	Medium	<i>Phialemonium curvatum</i> (1/23)	Medium
		<i>Acremonium</i> spp. (1/23) ^a	Medium/low	<i>Aspergillus restrictus</i> (1/23)	Medium
				<i>Aspergillus ustus</i> (1/23)	Medium
				<i>Aspergillus niger</i> (3/23) ^{a,b}	Medium

			<i>Aspergillus paradoxus</i> (1/23)	Medium
			<i>Aspergillus penicillioides</i> (1/23)	Medium
			<i>Aspergillus candidus</i> (1/23) ^a	Medium
			<i>Rhodotorula mucilaginosa</i> (1/23)	Medium
			Unknown species (4/23)	
			Unknown species (20/20) ^{a,c}	
Cooler (43) ^{a,c}	<i>Fusarium</i> spp. (1/20) ^{a,c}	High		
	<i>Aspergillus</i> spp. (2/20) ^{a,c}	High/medium		
	<i>Candida</i> spp. (3/20) ^{a,c}	High/medium		
	<i>Cryptococcus</i> spp. (2/20) ^{a,c}	High/medium		
	<i>Exophiala</i> spp. (1/20) ^{a,c}	Medium		
	<i>Malassezia</i> spp. (1/20) ^{a,c}	Medium		
	<i>Paecilomyces</i> spp. (1/20) ^{a,c}	Medium		
	<i>Paraconiothyrium</i> spp. (1/20) ^{a,c}	Medium		
	<i>Stachybotrys</i> spp. (1/20)	Medium		
	<i>Talaromyces</i> spp. (1/20) ^{a,c}	Medium		
	<i>Trichosporon</i> spp. (2/20) ^{a,c}	Medium		
	<i>Rhodotorula</i> spp. (2/20) ^{a,c}	Medium		
	<i>Acremonium</i> spp. (2/20)	Medium/Low		
Ventilator (3)	Mucorales (order) (1/4)	High	<i>Aspergillus niger</i> (1/4)	Medium
	<i>Aspergillus</i> spp. (3/4)	High/medium	Unknown species (3/4)	
Rotating Dehumidification Wheel (RDW) (1)	<i>Aspergillus</i> spp. (1/1)	High/medium	<i>Aspergillus fumigatus</i> (1/1)	High
Ductwork (9) ^a	Mucorales (order) (1/5) ^{a,f}	High	<i>Aspergillus candidus</i> (1/5) ^a	Medium
	<i>Aspergillus</i> spp. (2/5) ^a	High/medium	<i>Aspergillus niger</i> (1/5) ^a	Medium
	<i>Rhodotorula</i> spp. (1/5) ^a	Medium	<i>Rhodotorula mucilaginosa</i> (1/5) ^a	Medium
	<i>Acremonium</i> spp. (1/5) ^a	Medium/low	Unknown species (2/5) ^{a,f}	
Insulation (11)	<i>Aspergillus</i> spp. (4/9) ^{a,e}	High/medium	<i>Aspergillus versicolor</i> (3/9) ^{a,e}	Medium
	<i>Paecilomyces</i> spp. (1/9)	Medium	Unknown species (6/9) ^{a,e}	
	<i>Scedosporium</i> spp. (1/9)	Medium		
	<i>Acremonium</i> spp. (3/9) ^{a,e}	Medium/low		
Water	<i>Mucor</i> spp. (8/11)	High	<i>Mucor ramosissimus</i> (8/11)	High
Drain pan (8) ^a	<i>Aspergillus</i> spp. (3/11)	High/medium	<i>Aspergillus fumigatus</i> (3/11)	High

AC, air conditioning system.

^a The authors of these studies reported the results of all systems together as one result and/or the microbial results specifically for every system sampled were not (fully) described.

^b Includes a study that reported multiple AC systems that were tested for school classrooms and offices.

^c Identification was only partly done on basis of DNA techniques.

^d Result outcomes before and after a specific component were combined.

^e The results of studies with multiple sampling moments for a specific component include the number of times the sampling occurred.

^f Including the members of the Mucorales order.

FLA have been reported in the filters of included studies. The presence of moisture, dust, and improper maintenance can facilitate the proliferation of FLA and other micro-organisms inside these filters. Consequently, cysts, trophozoites, and other micro-organisms may become dislodged and re-aerosolized into the system's airstream [67–70].

None of the included studies investigated whether biofilms in these systems could lead to the spread of micro-organisms into the air. Hugenholtz and Fuerst suggested that *Blastobacter* spp. cells detached from the biofilm into condensate, potentially contaminating drain pan water, post-coil air, and ultimately the supply air delivered to occupied spaces [39]. This supports the notion that biofilms potentially can facilitate the spread of micro-organisms in these systems. Adiabatic humidification systems create moist environments in which suboptimal system performance, inadequate drainage design and insufficient maintenance (e.g., component inspection and filter replacement) can lead to moist accumulation and promote microbial growth. Therefore, preventing biofilm formation in adiabatic humidification systems may be of importance to avoid microbial proliferation and reservoirs for pathogenic micro-organisms [71–77].

Strengths and limitations

Our study's strength lies in its comprehensive analysis of microbial presence across components and sample types. Risk classification helped identify high-risk pathogens and their likely proliferation sites, providing insights useful for hospitals considering adiabatic humidification systems. This mapping review has certain limitations. First, relevant studies may have been missed possibly due to the omission of the term 'air handling units' in our search strategy. However, these studies were used to discuss our results and are consistent with our findings. The lack of literature on *Legionella* represents a limitation, however, this is addressed by established international guidelines for *Legionella* risk management, whereas equivalent guidance for other pathogens remains absent [19,78–81]. Other limitations include lack of the availability of detailed information on types of systems and maintenance practices. Some studies reported combined microbial outcomes across multiple systems, precluding system- and component-specific attribution, likely underestimating the number of systems harbouring specific pathogens and associated component-level risks. Upstream and downstream sampling of the components was often not well described, leaving a gap in understanding micro-organism dissemination from system components into the air [32,33]. Moreover, none of the studies assessed aerosolization from biofilms, leaving a critical gap in understanding their role in airborne transmission and potential impact on patient air quality.

Implications for infection prevention and control

Implications for hospital design and infection prevention and control could include: (1) treating adiabatic humidification as part of the facility's water safety framework; (2) a system that requires a hygienic and a maintainable design (e.g., layout, proper air duct and drainage and windows for visual inspection); (3) adequate technical performance (e.g., air

changes, pressure differentials, filtration, humidity and temperature control); (4) routine maintenance and inspection (e.g., cleaning protocols, filter replacement, duct inspection); and (5) surveillance (e.g., regular sampling and targeted cleaning and disinfection). These mitigation strategies should follow manufacturer's instructions and recommended guidelines for hospitals, which may altogether contribute to maintaining air quality. Deviations from optimal operation may comprise performance and promote microbial proliferation [82–86]. High-risk areas (e.g., operating rooms and intensive care units) and immunocompromised patients require particular attention, as even low-level contamination may have clinical consequences [83]. The suitability of adiabatic humidification in these settings should therefore be evaluated.

Future research

Future research should focus on sampling supply air and downstream air, and in water accumulation areas in installed adiabatic humidification systems. Test setups should also be used to introduce high-risk species to study biofilm formation and aerosolization across different humidifier types. Modelling is needed to estimate how many particles and bioaerosols could reach patient areas. Finally, improved design, performance, maintenance, and surveillance strategies are essential to prevent microbial growth and downstream dissemination, reducing patient infection risk.

Conclusion

This study shows that high-risk bacteria and fungi are present in wet and condensate-accumulating areas of AC and humidification systems similar to adiabatic humidifiers. Components such as filters, humidifiers, coolers, and drain pans pose particular risks for microbial growth, and surrounding air may also harbour pathogens that can contaminate supply air. Whether biofilms form in these systems and release micro-organisms into the airstream remains uncertain. Our findings suggest that pathogenic micro-organisms may proliferate in adiabatic systems, reflecting risks comparable to those in current AC and humidification setups. These systems should therefore be properly designed and include routine maintenance and surveillance to mitigate microbial growth. Further research is needed to clarify microbial colonization, proliferation, biofilm formation, and airborne spread in adiabatic humidification systems. This evidence will support the development of effective mitigation strategies and help ensure safe implementation of adiabatic humidification, thereby supporting more sustainable, low-energy healthcare environments.

CRediT authorship contribution statement

J.L. van Rheenen: Conceptualization, Methodology, Formal analysis, Data curation, Visualization, Writing – original draft, Writing – review & editing. K. Kompatscher: Conceptualization, Methodology, Writing – review & editing. J.M.B.M. van der Vossen: Conceptualization, Methodology, Writing – review & editing. B.C.G.C. Mason-Slingerland: Conceptualization, Methodology, Writing – review & editing. W. de Groot: Conceptualization, Writing – review & editing. I. Immerzeel: Conceptualization, Writing – review & editing. R. van

Mansfeld: Conceptualization, Writing – review & editing. **R.A.A.L. Traversari:** Conceptualization, Methodology, Writing – review & editing. **J.A. Severin:** Conceptualization, Methodology, Supervision, Writing – review & editing. **A.F. Voor in 't holt:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Ethics statement

Not required.

Declaration of generative AI and AI-assisted technologies in the writing process

During the preparation of this work the authors used Microsoft Co-pilot and OpenAI ChatGPT to assist in writing, improve readability and language of the work. After using these tools/services, the authors reviewed and edited the content as needed and take full responsibility for the content of the published article.

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Conflicts of interest statement

All authors report no conflicts of interest relevant to this article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jhin.2026.07.020>.

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