Disinfection efficacy of chlorine and peracetic acid alone or in combination against *Aspergillus* spp. and *Candida albicans* in drinking water

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ABSTRACT

The aim of the present study was to evaluate the fungicidal activity of chlorine and peracetic acid in drinking water against various pathogenic *Aspergillus* spp. and *Candida albicans* strains. *A. nidulans* exhibited the greatest resistance, requiring 10 ppm of chlorine for 30 min contact time for a complete inactivation. Under the same experimental conditions, peracetic acid was even less fungicidal. In this case, *A. niger* proved to be the most resistant species (50 ppm for 60 min for complete inactivation). All *Aspergillus* spp. were insensitive to 10 ppm even with extended exposure (>5 h). The combination of chlorine and peracetic acid against *Aspergillus* spp. did not show synergistic effects except in the case of *A. flavus*. Complete growth inhibition of *C. albicans* was observed after about 3 h contact time with 0.2 ppm. *C. albicans* was less sensitive to peracetic acid. Hence the concentrations of chlorine that are usually present in drinking water distribution systems are ineffective against several *Aspergillus* spp. and peracetic acid cannot be considered an alternative to chlorine for disinfecting drinking water. The combination of the two biocides is not very effective in eliminating filamentous fungi at the concentrations permitted for drinking water disinfection.

Key words | *Aspergillus*, *Candida albicans*, chlorine, drinking water, peracetic acid

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INTRODUCTION

Drinking water disinfection practices are designed to control most pathogenic microorganisms responsible for waterborne diseases. With regard to fungi, their occurrence in drinking water has received increasing attention in the last few decades given the risk they may pose to human health, in particular immunocompromised patients, either through the drinking of water or through dispersion, i.e nebulization, of spores in the air. Indeed, more and more studies point to water as a potential source of opportunistic, allergenic and toxigenic filamentous fungi (Kanzler *et al.* 2008; Hageskal *et al.* 2009). Such fungi may also survive conventional disinfection treatments, particularly within biofilm (Rosenzweig *et al.* 1983; Doggett 2000; Exner *et al.* 2005). Chlorine-based compounds such as sodium hypochlorite (NaClO) are the most widely used microbicidal halogens for drinking water disinfection. However, such chlorination treatments were found to be insufficient to eliminate fungi, and therefore, water distribution systems might be reservoirs for fungi, particularly the *Fusarium* and *Aspergillus* species (Anaissie & Costa 2001; Anaissie *et al.* 2001, 2002a, c; Gangneux *et al.* 2002; Warris *et al.* 2002, 2003; Kanzler *et al.* 2008). Fungal contamination of potable water systems is even more dangerous in hospital settings due to the presence of immunocompromised consumers. Although hospitals have adopted preventive measures such as high-efficiency particulate air (HEPA) filters and laminar air flow (LAF), the incidence of aspergillosis continues to increase. This suggests that, in addition to airborne aspergillus, there may be other sources of *Aspergillus* infection in hospitals, including water systems (Anaissie *et al.* 2002a, b, c, 2003;

Warris *et al.* 2002). Some authors caution that there are still few findings to support alternative vehicles of aspergillus diffusion in hospitals (Hajjeh & Warnock 2001). However, genotypic findings showing a relation between clinical and environmental isolates suggest that patients with invasive aspergillosis can be infected by strains originating from water or from air (Warris *et al.* 2003). *Candida albicans* is the most frequently isolated fungal pathogen in humans with superficial and systemic infection caused by yeast (Tuomanen 1996). It is also well known for its ability to form biofilm in drinking water distribution networks, which show a particular resistance to disinfection treatments (Doggett 2000; Kumamoto & Vinces 2005).

Epidemiological studies have highlighted the association between the mutagenic and/or carcinogenic effects of disinfection by-products (DBP) and the chlorination of drinking water (Richardson *et al.* 2007). Hence attempts have been made to partially or totally replace chlorine with alternative disinfectants. Chlorine dioxide and ozone have been reported as the most effective water treatments against fungal spores (Warris *et al.* 2002; Hageskal *et al.* 2009); however, their use is limited due to the cost and complexity of their application. The fungicidal efficacy of many commercially available disinfectants has been well documented (Terleckyj & Axler 1993; Bundgaard-Nielsen & Nielsen 1995; Tortorano *et al.* 2005), but there is less information regarding whether these agents might be used for the disinfection of drinking water (Hageskal *et al.* 2009). Peracetic acid (PAA) belongs to this group of biocides, though our knowledge of its efficacy as a drinking water disinfectant is still limited (Ragazzo *et al.* 1997; Monarca *et al.* 2002; Schiavano *et al.* 2006). PAA has been proposed as a disinfectant with a wide spectrum of antimicrobial applications due to its effectiveness as a bactericidal, virucidal, and sporicidal agent shown in various food industries (Alvaro *et al.* 2009), as well as its use in the disinfection of a hospital water system (Ditommaso *et al.* 2005), medical devices, flexible scopes, and hemodialyzers (McDonnel & Denver Russel 1999). It has also been proposed for disinfecting wastewater (Briancesco *et al.* 2005; Koivunen & Heinonen-Tanski 2005) or water surface, for the eradication of *Legionella* and biofilm control (McDonnel 2007), as well as for disinfecting microfiltered water dispensers (Zanetti *et al.* 2009). With regard to the possible use of PAA in drinking water, studies

have been conducted using doses ranging from 0.5 to 5 ppm and for various contact times. These studies showed a high reduction of bacterial vitality already at doses of 1.5 to 2 ppm (Ragazzo *et al.* 1997; Goveas *et al.* 2010). Another study showed that in order to completely eliminate the total and fecal coliform present in the water supply a dose of 2 ppm PAA was required (Trujillo *et al.* 2008). The major advantages of PAA are the ease of its application, its broad spectrum of activity, even in the presence of heterogeneous organic matter, and the absence of persistent toxic or mutagenic residuals or DBP (Briancesco *et al.* 2005; McDonnel 2007).

The aim of the present study was to test the fungicidal activity of chlorine and PAA in drinking water against a panel of pathogenic *Aspergillus* spp. and *C. albicans* strains and to evaluate possible synergistic effects in order to reduce the amount of chlorine used in drinking water.

METHODS

Isolates

Twenty-eight clinical and environmental isolates belonging to two different genera were tested in this study. These included: *A. flavipes* (n. 3), *A. flavus* (n. 4), *A. fumigatus* (n. 4), *A. nidulans* (n. 3), *A. niger*, (n. 4), *A. terreus* (n. 2), *A. ustus* (n. 2), *A. versicolor* (n. 2) and four strains of *C. albicans* (three clinical isolates and one ATCC 14053). *Aspergillus* spp. were previously identified for their macroscopic and microscopic characteristics. Microscopic analyses were performed by slide culture and lactophenol blue staining methods (Lactophenol Blue Solution, Sigma-Aldrich). *C. albicans* isolates were identified by conventional methods (germ tube formation, microscopic morphology in corn meal-Tween 80 agar, and carbohydrate fermentation tests) and using a commercial kit API 20 °C (bioMerieux, Marcy I'Etoile-France).

Preparation of conidial and yeast suspensions

Filamentous fungi and yeasts were grown respectively on Potato dextrose agar (PDA) and Sabouraud dextrose agar (SDA) at 35 °C and subcultured at least twice to ensure purity and viability. Conidial suspensions were prepared

by flooding each slant with 1 ml of sterile 0.85% NaCl solution containing 0.05% Tween 80 and gently probed with a pipette tip. The resulting mixture was withdrawn and the heavy particles were allowed to settle for 3 to 5 min. The upper homogeneous suspensions containing the mixture of conidia and hyphal fragments were vortexed for 15 s. The transmittances of the mixture suspensions were adjusted according to the CLSI M38-A protocol (NCCLS 2002) using a spectrophotometer set at a wavelength of 530 nm to provide a final test inoculum of about 10^6 CFU/ml. Inoculum suspensions of *C. albicans* were prepared by selecting five colonies, each at least 1 mm in diameter, from 24-h-old culture and suspending the material in 5 ml of sterile 0.85% NaCl solution. The turbidity of the cell suspension was adjusted according to the CLSI M27-A protocol (NCCLS 1997) using a spectrophotometer set at a wavelength of 530 nm to provide a final test inoculum of 10^6 CFU/ml. The inoculum titres of *Aspergillus* and *C. albicans* samples were confirmed by plating serial dilutions of the suspensions on SDA plates.

Biocides and neutralizing medium

All reagents used were of analytical grade. Stock solutions of active chlorine (added to cell suspensions as NaClO) and PAA were prepared in sterile and chlorine demand free distilled water from NaClO (4% chlorine) (Sigma) and PAA (32% WT of PAA) (Aldrich) respectively. The concentrations of the biocides were determined daily before starting tests. The DPD (N,N-diethyl-p-phenylenediamine) colorimetric method was used to measure levels of free chlorine and peroxiacid in NaClO and PAA respectively (*Standard Methods for Examination of Water and Wastewater*, APHA 2005). The interference caused by H_2O_2 and acetic acid in the determination of the titre of PAA was eliminated by adding catalase and NaOH. Biocides were inactivated at the end of treatments using sodium thiosulfate ($Na_2S_2O_3$), which was shown to be non-toxic to the cells in preliminary tests.

Preparation of drinking water

Samples of drinking water were taken from a municipal water distribution system and analyzed to exclude the presence of filamentous fungi and yeasts. Free chlorine was measured and then stoichiometrically neutralized with $Na_2S_2O_3$ 0.1 N before each experiment. The pH values were then measured. All treatments with biocides were performed at room temperature (20 °C) under light agitation (50 rpm).

Susceptibility testing for filamentous fungi

One ml of each conidial suspension was added to 9 ml of drinking water containing 0, 1, 2, 3, 5, 10, 20 and 50 ppm of active chlorine or 0, 10, 20, 50 and 100 ppm of PAA. After 0, 5, 10, 30, 60, 120, 240 and 1,440 min of exposure, an aliquot of 1 ml of the test mixture was drawn and biocides were immediately inactivated with 10 µl $Na_2S_2O_3$ 1 N. 100 µl of the mixture were then transferred to the microdilution well containing 100 µl 2× concentration of the standard RPMI 1640 medium (Sigma) and incubated at 35 °C for 46–50 h. The growth in each microdilution well was evaluated using a stereomicroscope. The minimal fungicidal concentration (MFC) was determined by plating 100 µl from each negative microdilution well on a PDA plate. MFC was defined as the lowest concentration of the biocides that allowed the growth of fewer than two colonies, representing a kill rate of 99.9%.

Susceptibility testing for yeasts

One ml of *C. albicans* suspension was added to 9 ml of drinking water containing 0, 0.1, 0.15, 0.2 and 0.4 ppm of active chlorine or 0, 1, 2, 3, 5 and 10 ppm of PAA. After time exposure of 0, 0.5, 1, 3 and 24 h, 1 ml of the test mixture was drawn and biocides were immediately inactivated with 10 µl $Na_2S_2O_3$ 1 N. Ten fold serial dilutions were performed in saline phosphate buffer (PBS) and 100 µl of each dilution suspension were spread plated on SDA media to enumerate CFU/ml.

Susceptibility testing with a combination of chlorine and PAA

The combination effect against filamentous fungi was assessed using the microdilution broth checkerboard

method on the basis of the fractional inhibitory concentration (FIC) index (Meletiadis *et al.* 2010). For the combination test 0.3, 0.6, 1.25, 2.5, 5 ppm of chlorine and 5, 10, 40, 80 ppm of PAA were selected, with a contact time of 120 min. The sum of the FICs (ΣFIC) of the first non-turbid (clear) well (corresponding to a minimum inhibitory concentration, MIC) found in each row and column along the turbidity/non-turbidity interface was calculated using the equation $\Sigma FIC = FIC_A + FIC_B = (C_A/MIC_A) + (C_B/MIC_B)$, where MIC_A and MIC_B are the MICs of drugs *A* and *B* alone, respectively, and C_A and C_B are the concentrations of the drugs in combination, respectively, in all of the wells corresponding to an MIC. The median of the ΣFICs index together with the minimum ΣFIC and the maximum ΣFIC calculated for each checkerboard test were averaged and reported. The values of the FIC index are considered indicative of synergy for FIC < 0.5, additivity for $0.5 \leq FIC \leq 2$ and antagonism for FIC > 2. For *C. albicans*, the concentrations of biocides selected for the combinations were 0.1, 0.2, 0.4 ppm for chlorine and 1, 3, 5 ppm for PAA. After 1, 3 and 24 h of

exposure, the samples were processed as reported for the single biocide and synergistic effects were assessed.

Statistical analysis

Data of susceptibility testing for filamentous fungi are representative of three separate experiments; variable results are expressed as ± values. Data of susceptibility testing for yeast and FICs values from checkerboard test are expressed as means ± SEM of at least three separate experiments.

RESULTS

In all the water samples analyzed before artificial contamination, both yeasts and filamentous fungi were always absent, while indigenous microflora was often absent or found in rather low quantities (<10 cfu/ml).

As shown in Table 1, the susceptibility of filamentous fungi to oxidants is time and dose dependent, in particular in the response to chlorine. However, all the *Aspergillus* spp.

Table 1 │ Susceptibility testing of *Aspergillus* spp to chlorine and peracetic acid

Species (*n*)	Time of contact (min)	Chlorine concentration (ppm)						Peracetic acid (concentration ppm)			
		1	2	3	5	10	20	10	20	50	100
A. flavipes (3)	5	+	+	+	+	+	−	+	+	+	+
	10	+	+	+	+	+	−	+	+	±	−
	30	+	+	+	±	−	−	+	−	−	−
	60	+	+	+	−	−	−	+	−	−	−
	120	+	+	+	−	−	−	+	−	−	−
	240	+	+	+	−	−	−	+	−	−	−
	1,440	+	+	−	−	−	−	+	−	−	−
A. flavus (4)	5	+	+	+	+	+	−	+	+	+	−
	10	+	+	+	+	−	−	+	+	±	−
	30	+	+	+	±	−	−	+	−	−	−
	60	+	+	+	−	−	−	+	−	−	−
	120	+	+	−	−	−	−	+	−	−	−
	240	+	+	−	−	−	−	+	−	−	−
	1,440	+	−	−	−	−	−	+	−	−	−
A. fumigatus (4)	5	+	+	+	+	+	−	+	+	+	+
	10	+	+	+	+	+	−	+	+	+	−
	30	+	+	+	+	±	−	+	+	+	−
	60	+	+	+	−	−	−	+	+	±	−
	120	+	+	−	−	−	−	+	+	±	−
	240	+	+	−	−	−	−	+	+	−	−
	1,440	+	−	−	−	−	−	+	±	−	−

(*continued*)

Table 1 | continued

Species (n)	Time of contact (min)	Chlorine concentration (ppm)						Peracetic acid (concentration ppm)			
		1	2	3	5	10	20	10	20	50	100
A. nidulans (3)	5	+	+	+	+	+	−	+	+	+	+
	10	+	+	+	+	+	−	+	+	+	±
	30	+	+	+	+	+	−	+	+	−	−
	60	+	+	+	−	−	−	+	+	−	−
	120	+	+	+	−	−	−	+	+	−	−
	240	+	+	+	−	−	−	+	−	−	−
	1,440	+	+	±	−	−	−	+	−	−	−
A. niger (4)	5	+	+	+	+	+	−	+	+	+	+
	10	+	+	+	+	±	−	+	+	+	+
	30	+	+	+	+	−	−	+	+	+	−
	60	+	+	+	−	−	−	+	+	+	−
	120	+	+	+	−	−	−	+	+	±	−
	240	+	+	−	−	−	−	+	±	−	−
	1,440	+	−	−	−	−	−	+	−	−	−
A. terreus (2)	5	+	+	+	+	+	−	+	+	+	+
	10	+	+	+	+	+	−	+	+	−	−
	30	+	+	+	±	−	−	+	+	−	−
	60	+	+	+	−	−	−	+	+	−	−
	120	+	+	+	−	−	−	+	+	−	−
	240	+	+	−	−	−	−	+	−	−	−
	1,440	+	−	−	−	−	−	+	−	−	−
A. ustus (2)	5	+	+	+	+	+	−	+	+	+	+
	10	+	+	+	+	+	−	+	+	−	−
	30	+	+	±	−	−	−	+	±	−	−
	60	+	+	−	−	−	−	+	−	−	−
	120	+	+	−	−	−	−	+	−	−	−
	240	+	+	−	−	−	−	+	−	−	−
	1,440	+	+	−	−	−	−	+	−	−	−
A. versicolor (2)	5	+	+	+	+	+	−	+	+	+	+
	10	+	+	+	+	−	−	+	+	−	−
	30	+	+	+	±	−	−	+	+	−	−
	60	+	+	±	−	−	−	+	−	−	−
	120	+	+	−	−	−	−	+	−	−	−
	240	+	+	−	−	−	−	+	−	−	−
	1,440	+	−	−	−	−	−	+	−	−	−

Values are average of three experiments.
+: growth fungal cells in microdilution wells; − : no growth fungal cells in microdilution wells or less than two colonies, representing a kill rate of >99.9%; ±: variable results such as growth only one of three microdilution test.

tested were rather resistant to the effects of both active chlorine and PAA. Of these species, A. *nidulans* showed the greatest resistance against active chlorine withstanding doses of up to 10 ppm for 30 min contact time, while *A. versicolor* was the least resistant (10 ppm for 5 min). The action of PAA varied against the different species of Aspergillus. The growth of *A. fumigatus* was completely inhibited by 50 ppm PAA after 2–3 h contact time, *A. niger* displayed the highest resistance (100 ppm PAA for 10 min), while

A. *flavus* was moderately susceptible. Since the efficacy of a disinfectant could depend on microbial concentration, we repeated the experiment using 10^3 CFU/ml *Aspergillus* spp. suspensions obtaining similar results of resistance to PAA (not shown).

Chlorine showed good efficacy in a dose-dependent manner against *C. albicans*. Indeed, exposure to 0.2 ppm of the oxidant for 3 h in tap water resulted in the complete elimination of viable cells, while with 0.4 ppm, 30–60 min

of treatment were necessary to obtain the same result (Figure 1(a)). Conversely, PAA was not very effective agaínt *C. albicans*. In fact, only the treatment with 10 ppm for 24 h resulted in the complete elimination of the yeast (Figure 1(b)), while with the same time exposure 3 ppm and 5 ppm of PAA reduced the viable cells of only 1 and 2.3 log, respectively.

In a set of experiments, combinations of PAA and chlorine were selected and the degree of synergy against filamentous fungi and *C. albicans* was evaluated. Table 2 shows the median of the ΣFIC index and the ΣFIC min and ΣFIC max for each strain used in the combinatory tests. Filamentous fungi were chosen for the checkerboard tests based on their high sensitivity (*A. nidulans*, *A. niger*), average sensitivity (*A. fumigatus*) and low sensitivity (*A. versicolor*, *A. flavus*) to two biocides. The combination tested by checkerboard methods was not found to be synergistic, except for *A. flavus* at 0.6 ppm of chlorine and 20 ppm of
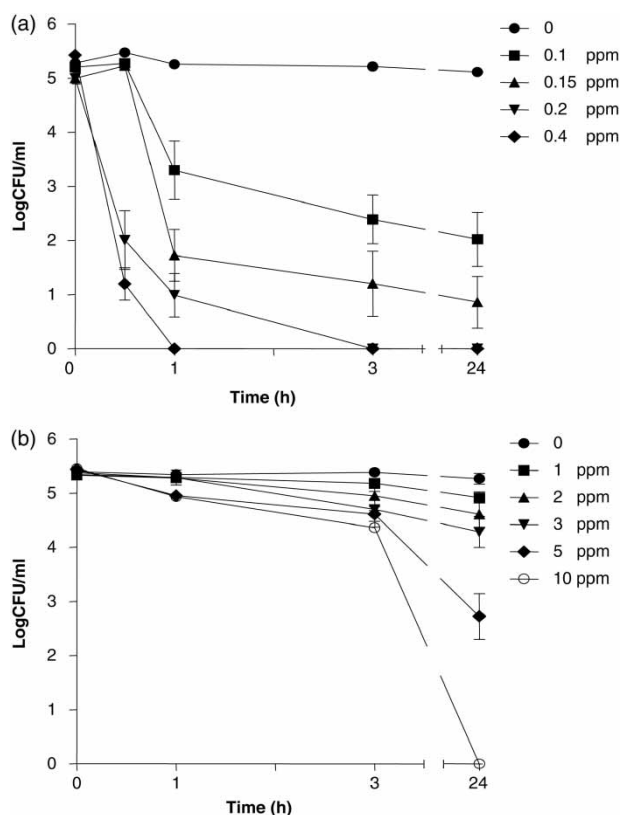
Table 2 | ΣFICs (fractional inhibitory concentration indices) for *Aspergillus* spp from checkerboard tests

Strain	ΣFICs[a] Median	Range Min	max
A. fumigatus	1.08 ± 0.04	0.78 ± 0.16	1.25 ± 0.00
A. nidulans	0.82 ± 0.11	0.58 ± 0.03	1.14 ± 0.11
A. niger	1.06 ± 0.04	0.69 ± 0.09	1.25 ± 0.00
A. versicolor	1.23 ± 0.04	1.04 ± 0.03	1.50 ± 0.00
A. flavus	0.59 ± 0.03	0.37 ± 0.00	1.06 ± 0.00

[a]The ΣFICs from checkerboard tests are the means ± SD of at least three separate experiments.
The ΣFIC indicates synergy for FIC < 0.5, additivity for 0.5 ≤ FIC ≤ 2 and antagonism for FIC > 2.

PAA (ΣFIC = 0.37). Figure 2 shows one representative checkerboard test against *A. flavus*.

The disinfectant actions of the two biocides against *C. albicans* were synergic only after 24 h of treatment with the combinations containing 0.4 ppm chlorine and 3 ppm or 5 ppm of PAA (not shown).

DISCUSSION

The data obtained in this study show that all *Aspergillus* spp. are able to withstand concentrations of chlorine or PAA many times higher than those usually found in drinking water for long periods. Hence the current water disinfection procedures, such as chlorination, do not ensure the
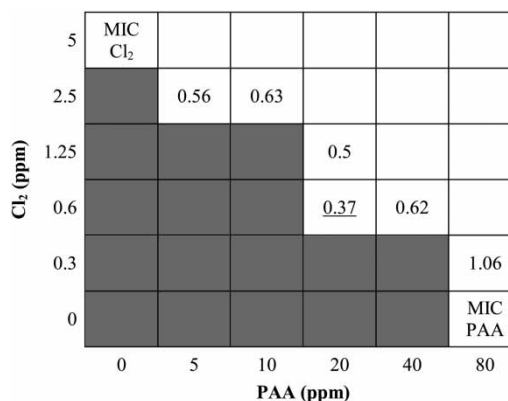

Figure 1 | Susceptibility of *C. albicans* to chlorine (a) or peracetic acid (b) in drinking water. Each point represents the mean value ± SEM of at least six experiments performed in four different strains.

Figure 2 | Checkerboard test obtained by combination treatments of chlorine and peracetic acid against *A. flavus*. The values of FICs (fractional inhibitory concentration indices) are considered indicative of synergy for FIC < 0.5, additivity for 0.5 ≤ FIC ≤ 2 and antagonism for FIC > 2. Checkerboard data are from one representative experiment. Grey area: visible growth.

elimination of fungal spores and, consequently, water distribution systems might be vehicles for these fungi. Conversely, the concentration of chlorine generally present in potable water (e.g 0.2 ppm) seems to be sufficient for a marked or complete inactivation of *C. albicans* in only a few hours. However, the ability of *Candida* spp. to form biofilms, where they may survive conventional disinfection strategies (Walker *et al.* 2003), should be considered. On the other hand, PAA was shown to have limited efficacy and does not seem to be a good candidate for the inactivation of yeast in potable water. Our data are in agreement with those of other authors who have observed that the conidia of *A. fumigatus* and *A. niger* in drinking water can survive a chlorine concentration of 10 ppm for 10 min (Rosenzweig *et al.* 1983). This finding may be of particular relevance to hospital intensive care units. There appears to be support for the assumption that water distribution systems in hospitals can play an important role in the onset of fungal infections, especially when water contaminated by fungi is aerosolized, e.g. through taps and showers, into the air environments where immunocompromised patients are housed (Anaissie *et al.* 2002c). Indeed, although hospitals have adopted preventive measures such as HEPA filters and LAF, the incidence of aspergillosis continues to increase. This suggests that, in addition to airborne aspergillus, there may be other sources of *Aspergillus* infection in hospitals, including water systems (Anaissie *et al.* 2002a). Hence there appears to be a rationale for the so-called 'French approach', which lists strategies to reduce these risks such as the adoption of adequate air filtration systems, ongoing monitoring of their performance and in-depth studies concerning sources and transmission routes (Gangneux *et al.* 2002). In addition, this approach calls for high-risk patients to be provided with sterile (boiled) water for drinking and sterile sponges for bathing (to avoid the aerosolization associated with showering) (Anaissie *et al.* 2003).

Furthermore, it should be considered that the contamination level of water distributed in hospitals may depend on whether it is derived from groundwater or surface water. For example, *A. fumigatus* was found in tap water originating from surface water but not in drinking water which originated from groundwater (Warris *et al.* 2003, 2010).

It was also shown that of 2,657 samples of drinking water from different sources, 7.5% of them tested positive for the presence of fungal flora (Gottlich *et al.* 2002), and fungi were isolated in all 38 samples of drinking water and groundwater examined with a mean value of 9.1 CFU/100 ml and 5,400 CFU/100 ml respectively (Kanzler *et al.* 2008).

Hence there appears to be some evidence supporting several authors who have called for the use of tap filters and disposable shower heads in hospital settings, such as pediatric bone marrow transplant units, to limit the so-called 'wet route of transmission' (Warris *et al.* 2010) or mobile air treatment systems in high risk units lacking of HEPA filters or LAF (Sixt *et al.* 2007).

Since *Aspergillus* spp. was shown to be highly resistant to chlorine, an alternative biocide, PAA, was tested against these fungi in drinking water. However, the disinfectant activity of PAA was found to be poor and therefore it appears that it cannot be used as an alternative to chlorine.

The combination of chlorine and PAA evaluated by checkerboard methods, which are frequently used to determine the synergistic and antagonistic interactions between antifungal agents (Maesaki *et al.* 1994; Perea *et al* 2002; Meletiadis *et al.* 2003), showed synergistic effects only for *A. flavus*, whereas the same combination proved to be only additive when used against the other *Aspergillus* spp. It should be noted, however, that both synergistic and addictive effects were found with concentrations of the two biocides several times higher than those used in drinking water. Hence we can conclude that these combinations of PAA and chlorine can only be used for specific treatments, such as 'shock disinfection', and not for conventional disinfectant treatment of potable water. Finally, our data indicate that PAA, alone or in combination with chlorine, does not seem to be a good candidate even for disinfection of *C. albicans* in potable water.

The higher resistance of fungi to biocides compared to bacteria may be related to differences in cell wall composition, while the greater resistance of moulds compared to yeasts is probably related to the higher content of cell wall glycan in moulds (McDonnel & Denver Russel 1999).

CONCLUSION

The present study provides additional information on the high resistance of *Aspergillus* spp. to chlorination and

shows that PAA is not a viable alternative fungicide to chlorine. Furthermore, not even a combination of PAA and chlorine proved to be effective in eliminating filamentous fungi, at least at the concentrations permissible in drinking water. The persistence of potentially pathogenic fungi in potable water highlight the relevance of waterborne fungi to human health. Further studies are needed to test the effectiveness of other biocides or treatment strategies to inactivate *Aspergillus* spp. in potable water distribution systems.

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