In -Vitro Activities Of Some Common Disinfectants On *Aspergillus Fumigatus* Isolated From Selected Wards At The University College Hospital (Uch), Ibadan

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Abstract: *Aspergillus fumigatus* are considered important mycoses-causing agents in hospital environments and represents an infectious risk for patients. Incidence of invasive aspergillosis has been associated with fungal exposure and the most effective way of reducing its occurrence is to eliminate all reservoir and avoid direct patient exposure to environmental Aspergillus conidia using appropriate disinfectant. This study was therefore, designed to investigate the activities of some common disinfectants sold in Nigeria on *Aspergillus fumigatus* isolated from selected wards at the University College Hospital, Ibadan. Thirteen disinfectants commonly used in hospitals were randomly purchased and their effectiveness on *Aspergillus fumigatus* isolated from some wards were determined using time kill test, and Agar disc diffusion method to determine the MIC and MFC ratio. The result of the purity test showed that all the 13 disinfectants were sterile prior to use producing 100% reduction in growth of *Aspergillus fumigatus* in 30seconds. Analysis of anti-Aspergillus activities of the 13 diluted disinfectants by agar- disc diffusion method showed a significant difference ($P<0.05$). Formaldehyde was the most potent, followed by Dettol, Robert and Methylated spirit. The result of the quantitative test using the MBC/MIC ratio showed that the highest MIC of (1/2) was observed with Jik while the least MICs (1/64) were exhibited by Formaldehyde and Iodine. The presence of organic matter (plasma) in the undiluted and diluted disinfectants, significantly lowered the anti-*Aspergillus fumigatus* activities of the disinfectants ($t = 2.15$, $P<0.05$). The disinfectants tested possessed in-vitro anti-*Aspergillus fumigatus* activities.

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1.0 Introduction

Aspergillus is an opportunistic pathogenic mould that is widely spread in the human environment. The fungus produces spores known as conidia, which are abundantly disseminated into the air, and able to survive in a wide range of environmental conditions, especially those of Aspergillus fumigatus, which are considered to be the most pathogenic of the species (Ricardo *et. al.,* 2006). In addition to water and food, hospital fabrics and plastics represents reservoirs for transmission of medically important *Aspergillus spp.* However, the fungal species, as well as the characteristics of specific materials influences the length of survival of the fungi on different surfaces (Neely *et. al.,* 2001).

It is said that an estimate of several hundreds of *Aspergillus fumigatus* spores that are continually inhaled by human being have been implicated in the prevalence of diseases that range in severity from mild allegic rhinitis to life threatening invasive pulmonary and disseminated infection generally known as aspergillosis (Rhodes and Brakhage, 2006). However, major breakthrough has not been achieve in the treatment of the disease caused by *Aspergillus fumigatus* in man, which has been attributed to the seemingly unresponsiveness of aspergillosis to most antimicrobial agents including ketoconazole (Leonardelli *et. al.,* 2016). This therefore, suggests that the recent rise and persistence of highly lethal aspergillosis infection among both immuno-competent and immuno-compromised host (Weber *et. al.,* 2009), could be attributed to its unresponsiveness to most antimicrobial agents, coupled with the ubiquity nature of the mould (Saha *et. al.,* 2009).

Alternatively, preventive measure against inhalation of too numerous *Aspergillus fumigatus* spores, such as the disinfection of inanimate objects which humans are in contact with is highly recommended. Disinfectants are widely used in hospitals and homes to control the growth or eliminate potentially pathogenic microbes on inanimate objects. They are important aspect of infection control practices and in prevention of hospital acquired infections (Saha *et. al.,* 2009). However, there is paucity of knowledge about the activities of known disinfectants in the Nigerian markets, used against the spores of *Aspergillus fumigatus.* Furthermore, the selection of disinfectants that best mitigates the spread of Aspergillus spores has been a general problem, as response of pathogens to various disinfectants differs. This therefore suggests the need to test disinfectants like antibiotics, in order to determine their activity against a range of organisms prior to usage.

Fungi are more resistant to biocides than nonsporulating bacteria. However, the activity of biocides against fungal microorganisms is not as well documented as their activity against bacteria, evident by the availability of few studies for lethal concentrations of antiseptics and disinfectants towards yeasts and moulds (Vijayakumar *et al*., 2011). Therefore, research to determine activity of disinfectants against *Aspergillus fumigatus* in hospital environments and adequate disinfection program in these areas is of high recommendation. Hence, this study aimed to compare the efficacy of different disinfectants on *Aspergillus fumigatus* isolated from different wards at the University College Hospital.

2.0 Materials and Methods

2.1 Study Design

This was a hospital based study. A sample of thirteen (13) widely use disinfectants namely; Methylated spirit, Dettol, Lysol, Izal, Iodine, Hydrogen peroxide, Jik, Morigard, Nixoderm, Robert, Formaldehyde, Ethanol, and Tiscol, were purchased within Ibadan, Ile-Ife and Sagamu. However, the ethanol used for this study was prepared in two different concentrations of 50% and 70% respectively to determine the potency of ethanol as disinfectant at different concentration, and a table detailing the class/group of disinfectants, composition, and cost price of the different disinfectants was compiled (Table 1).

2.2 Selection of Wards and Isolation of *Aspergillus fumigatus*

Ten selected wards representing various patients' categories at the University College Hospital, Ibadan, were screened for surface fungi using moistened sterile swab sticks to swab inanimate objects such as the floors, baths, windows and sink. Moisten sterile swab sticks collected from swabbing of various surfaces in the selected wards were aseptically inoculated unto Sabouraud dextrose agar plates according to McClenny, (2005). The inoculated Sabouraud dextrose agar plates were incubated at room temperatures of 25^0C for 7 days. Reading of SDA plates were done daily and subsequently until 7 days. Cultures were not reported negative until after four weeks of incubations. Thermotolerance study was carried out by incubating the SDA plates at elevated temperature of 48^0C and this was used as confirmatory test for identification of *Aspergillus fumigatus* isolates. Suspected colonies were picked for further subculture on Sabouraud dextrose agar to obtain a pure isolate, and subsequently sub-cultured on SDA agar slant and maintained at room temperature of 25^0C for further use.

2.3 Purity test

The stock or undiluted disinfectants were opened aseptically and the liquids were screened for sterility by aseptically streaking a loopful on Blood agar plates, MacConkey agar plates and Sabouraud dextrose agar plates (SDA). The first two plates were incubated at 37^0C for 48 hours while the SDA plates were incubated at room temperature of 25^0C for 7 days with daily inspection of plates. Some milliliter of the disinfectants solution were further diluted according to manufacturer's recommendation for use in cleansing inanimate objects in the environment.

2.4 Preparation of *Aspergillus fumigatus* suspension

Aspergillus fumigatus inoculum was prepared according to the method adopted by Ogunledun *et al*., (2008). The suspension was compared against 1.0 McFarland standard turbidity, which corresponds to 3.0 x 10^8 organisms per milliliters (Finegold and Martins, 1982).

2.5 Determination of invitro activities

2.5.1 Time kill test

The time kill test was used for the quantitative determination of the effect of the disinfectant by evaluating the microbial reduction by disinfectants as described by Ogunledun *et al*., (2008). The colonies formed by the tests and controls were counted for each contact time using colony counter. The microbial cell reduction rate was determined in percentage (%) using the formula:

$$\text{Microbial cell reduction rate (\%)} = \frac{\text{CFU count at t second (control} - \text{test)}}{\text{CFU count at t second count of control}} \times 100$$

2.5.2 Agar-disc diffusion method

A sterile swab stick was dipped into the *Aspergillus fumigatus* prepared suspension and was used to inoculate the surface of well dried Sabouraud dextrose agar plate. Sterile paper discs soaked with different disinfectants diluted according to manufacturer's instruction were placed on the plate and incubated at 25ºC for 18 – 24 hours. Standard *Aspergillus fumigatus* ATCC 27853 was set up as control; all tests were performed in duplicates. After incubation, the plates were observed and the zones of inhibition were determined by diameter in millimeters of zone to which the disc inhibited the growth of the *Aspergillus fumigatus*. The zones of inhibition produced by the disinfectants were scored as describe by Adebayo, (1999) and CLSI (2008).

2.6 Minimum inhibition concentration

Minimum inhibition concentration method used was adapted from the combination of methods used by

CLSI, (2008); and Jennifer, (2006). The ratio of fungicidal and fungistatic concentration was determine. An agent is considered fungicidal in activities if the MFC/MIC ratio is less than or equal to four (4) and regarded as fungistatic if the ratio is greater than 4.

2.7 Effect of organic matter on the antifungal efficacy of disinfectants

Effect of organic matter on the antifungal efficacy of disinfectants was determined by adding 1ml of human plasma to 9mls of undiluted and diluted disinfectants according to manufacturer's instruction for use. Sterile discs were soaked in the mixture drained to remove excess; these were placed on SDA agar plates seeded with 0.5 MacFarland suspension of *Aspergillus fumigatus*. Plates were incubated at 25ºC for 7 days and standard *Aspergillus fumigatus* ATCC 27853 was used as control. The zones of inhibition were measured using a millimeter rule after 24hrs and 48hrs.

3.0 Results

The fourteen different disinfectants obtained from market survey with their chemical composition, class, and cost is as shown in Table 1. The disinfectants belong to the dichlolphenolic, cresol, Dichloroxylenol, alcoholic, peroxygen and halogen releasing agent groups. All the concentrated disinfectants subjected to purity test by time kill method produced 100% reduction in growth of *Aspergillus fumigatus* in 30seconds with formaldehyde having the highest zone of inhibition followed by Dettol. However, at manufacturers' specified dilutions, 12 out of the 14 disinfectants gave 100% cell reduction of *Aspergillus fumigatus* in 30seconds contact time while Tisco and Morigard produced 76.4% and 84.7% cell reduction in 120seconds and 150seconds respectively (Table2). The analysis of anti-Aspergillus activities of the 14 diluted disinfectants by agar –disc diffusion method showed a significant difference ($P<0.05$) in the comparative assessment of the zones of inhibition of the disinfectants, with formaldehyde having the highest zone of inhibition of 64mm, followed by Dettol which had 13mm, while Robert and methylated spirit had 12mm and 9mm respectively ($F = 167.13$, $P<0.05$) (Table3). Further analysis by least significant difference (LSD) revealed 6 (42.9%) of the disinfectants were observed to be least efficient. These disinfectants include Tisco, Jik, Lysol, 70% Ethanol, Izal and Morigard.

The result of the quantitative test using the MFC/MIC ratio showed that the highest MIC (1/2) result was observed with Jik while the least MICs (1/64) were exhibited by Formaldehyde and Iodine (Table 4). Also, the highest MFC (1/2) result was recorded with Robert, Izal, and 50% ethanol, representing 21.4% of the total disinfectants, while Formaldehyde and Iodine indicated the least MFC (1/64) as shown in Table 5. However, out of the 14 disinfectants used in the study, only Robert was observed to be fungistatic in action while the remaining 13 disinfectants were fungicidal (Table 6). The presence of organic matter (plasma) in the undiluted and diluted disinfectants, significantly lowered the anti-*Aspergillus fumigatus* activities of the disinfectants ($t = 2.15$, $P<0.05$), while the disinfectants indicated reduced zones of inhibition when compared with those without plasma (Table 7).

The disinfectants tested possessed in-vitro anti-*Aspergillus fumigatus* activities, their efficacy were however, reduced in the presence of organic matters. Less than 15% of the disinfectants had their least effective concentration higher than the dilution recommended by the manufacturers, while more than half of the disinfectants had their MICs higher than the dilutions recommended by the manufacturers.

Table 1. Disinfectants obtained from the market surveys

S/N	Trade Name	Active Ingredient	Class	Volume (ml)	Cost In (#)
1	Tisco	Dichlorophenol and Pine Oil	Phenolics	4000	1,500.00
2	Methylated Spirit (Moko Spirit)	Idoptophy Alcohol 95% v/v/	Alcohol	200	250.00
3	JIK	Sodium Hypochlorite 3.5% m/v	Halogen	1000	180.00
4	Dettol	Chloroxylenol B.P.C. 4.8% w/v, Oleum pini Aromaticum 9.9% v/v. Denatured spiries 11.3% w/v. Sapovegetalis 5.8% w/v. Saccharum ustum 9.5, agua ad 100 vols	Phenolics	500	750.00
5	Lysol	Saponated cresol, 50% cresol v/v	Phenolics	60	300.00
6	Nixoderm	Dichlorometaxylenol 205% w/v. Terpinceol 10% w/v	Phonolics	125	250.00
7	Izal	Saponated cresol	Phenolics	150	380.00
8	Morigard	32% phenol w/v. (Dichloroxtlenol + Chlorophenol)	Phenolics	4000	1,800.00
9	Foraldehyde	Formalin	Aldehyde	450	2,000.00
10	50% Ethanol	Ethyl alcohol	Alcohol	500	1,500.00
11	70% Ethanol	Ethyl alcohol	Alcohol	15	1,500.00
12	Iodine	Iodine - 0.025, Potassium iodine- 0.025g, Water - 0.025ml	Halogen	15	60.00
13	Hydrogen Peroxide	6% w/v approximately of H_2O_2 with stabilizer	Oxidizing agent	100	70.00
14	Robert	Dichloroxylenol 2%	Phenolics	125	250.00

Table 2: Antifungal Activities of diluted disinfectants to *Aspergillus fumigatus* at manufacturers' specified dilutions using time kill test

S/N	Disinfectants (Trade Name)	Initial CFU/Ml 0s	CFU/Ml After Challenge With Diluted Disinfectant						Microbial Cell Reduction (%)					
			30s	60s	90s	120s	150s	180s	30s	60s	90s	120s	150s	180s
1	Tisco	3.0×10^8	3.0×10^2	2.0×10^2	1.0×10^2	1.0×10^2	0	0	70.8	72.9	76.4	76.4	0	0
2	Methylated spirit (Moko spirit)	3.0×10^8	0	0	0	0	0	0	100	100	100	100	100	100
3	JIK	3.0×10^8	0	0	0	0	0	0	100	100	100	100	100	100
4	Dettol	3.0×10^8	0	0	0	0	0	0	100	100	100	100	100	100
5	Lysol	3.0×10^8	0	0	0	0	0	0	100	100	100	100	100	100
6	Nixoderm	3.0×10^8	0	0	0	0	0	0	100	100	100	100	100	100
7	Izal	3.0×10^8	0	0	0	0	0	0	100	100	100	100	100	100
8	Morigard	3.0×10^8	2.5×10^2	2.0×10^2	0.5×10^2	0.3×10^2	0.2×10^2	0	71.7	72.9	80.0	82.3	84.7	0
9	Fomaldehyde	3.0×10^8	0	0	0	0	0	0	100	100	100	100	100	100
10	50% ethanol	3.0×10^8	0	0	0	0	0	0	100	100	100	100	100	100
11	70% ethanol	3.0×10^8	0	0	0	0	0	0	100	100	100	100	100	100
12	Tincture of iodine	3.0×10^8	0	0	0	0	0	0	100	100	100	100	100	100
13	Hydrogen peroxide	3.0×10^8	0	0	0	0	0	0	100	100	100	100	100	100
14	Robert	3.0×10^8	0	0	0	0	0	0	100	100	100	100	100	100

Table 3: Anti- aspergillus activities of selected disinfectants using agar-disc diffusion method

S/N	Disinfectants	N	Means zone of inhibition ± SD	F	p-value	Least efficient Disinfectant by LSD
1	Tiscol	5	0.00± 0.00			Tisco
2	Methylated	5	9.00 ± 0.71			JIK
3	JIK	5	0.00 ± 0.00			Lysol
4	Dettol	5	13.00 ± 1.58			70% ethanol
5	Lysol	5	0.00 ± 0.00			Izal
6	Nixoderm	5	6.00 ± 1.58			Morigard
7	Izal	5	2,00 ± 0.71			
8	Morigard	5	4.00 ± 1.58			
9	Formaladelyde	5	64.00 ± 9.17			
10	50% Ethanol	5	6.00 ± 2.12	167.13	<0.05	
11	70% Ethanol	5	1.00 ± 0.71			
12	Iodine	5	6.00 ± 2.24			
13	H_2O_2	5	7.00 ± 2.24			
14	Robert	5	12.00 ± 2.55			

Table 4: Minimum inhibitory concentration of disinfectants on *Aspergillus fumigatus*

S/N	Disinfectants	Concentrations											
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/512	1/256	1/1024	Dtd – O	DtO – d
1	Tiscol	-	-	-	+	+	+	+	+	+	+	-	+
2	Methylated spirit (Moko spirit)	-	-	-	-	+	+	+	+	+	+	-	+
3	JIK	-	+	+	+	+	+	+	+	+	+	-	+
4	Dettol	-	-	-	-	-	+	+	+	+	+	-	+
5	Lysol	-	-	-	-	+	+	+	+	+	+	-	+
6	Nixoderm	-	-	-	-	-	+	+	+	+	+	-	+
7	Izal	-	-	-	+	+	+	+	+	+	+	-	+
8	Morigard	-	-	-	-	+	+	+	+	+	+	-	+
9	Fomaldehyde	-	-	-	-	-	-	+	+	+	+	-	+
10	50% ethanol	-	-	+	+	+	+	+	+	+	+	-	+
11	70% ethanol	-	-	+	+	+	+	+	+	+	+	-	+
12	Ticture of iodine	-	-	-	-	-	-	+	+	+	+	-	+
13	Hydrogen peroxide	-	-	+	+	+	+	+	+	+	+	-	+
14	Robert	-	-	-	-	-	+	+	+	+	+	-	+

Key:

- = No growth

\+ = Growth

d + d – 0 = disinfectant + diluents minus organism (*Aspergillus fumigatus)*

d + 0 – d = diluents (SD broth) + Organism (*Aspergillus fumigatus)* minus disinfectant

Table 5: Minimum fungicidal concentrations of disinfectants on A*spergillus fumigatus*

S/N	Disinfectants	Concentrations									
		1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/512	1/256	1/1024
1	Tiscol	-	-	+	+	+	+	+	+	+	+
2	Methylated spirit (Moko spirit)	-	-	-	+	+	+	+	+	+	+
3	JIK	-	+	+	+	+	+	+	+	+	+
4	Dettol	-	-	-	-	+	+	+	+	+	+
5	Lysol	-	-	-	+	+	+	+	+	+	+
6	Nixoderm	-	-	-	+	+	+	+	+	+	+
7	Izal	-	-	-	+	+	+	+	+	+	+
8	Morigard	-	-	-	-	+	+	+	+	+	+
9	Formaldehyde	-	-	-	-	-	-	+	+	+	+
10	50% ethanol	-	+	+	+	+	+	+	+	+	+
11	70% ethanol	-	-	+	+	+	+	+	+	+	+
12	Ticture of iodine	-	-	-	-	-	-	+	+	+	+
13	Hydrogen peroxide	-	-	+	+	+	+	+	+	+	+
14	Robert	-	+	+	+	+	+	+	+	+	+

Key:
- = No growth
+ = Growth

Table 6: MFC/MIC Ratio of Disinfectants.

Disinfectants	MFC	MIC	MFC/MIC Ratio
Tiscol	1/4	1/8	2
Methylated spirit (Moko spirit)	1/8	1/16	2
JIK	1/2	½	1
Dettol	1/16	1/32	2
Lysol	1/8	1/16	2
Nixoderm	1/8	1/32	4
Izal	1/8	1/8	1
Morigard	1/16	1/16	1
Formaldehyde	1/64	1/64	1
50% ethanol	1/2	¼	2
70% ethanol	1/4	¼	1
Ticture of iodine	1/64	1/64	1
Hydrogen peroxide	1/4	¼	1
Robert	1/2	1/32	16

Table 7: Effect of organic matter on the activities of selected disinfectants

Organic Matter (Plasma)	N	Mean zone of Inhibition	T-test	p-value
In the presence of plasma	70	7.99		
In the absence of plasma	70	19.21	2.15	<0.05

4.0 Discussion

The in-vitro antifungal activity of some commonly selected disinfectant liquids was determined in this study. The data obtained gave useful information on these disinfectants that were found to be potent against *Aspergillus fumigatus*. The observation made with Tiscol disinfectant was in agreement with the work of Ogunsola *et al*., (2000); where they found out that when Tisco was diluted as indicated by the manufacturers, it was not active against tested bacterial. Tisco was found not to inhibit gram negative organism even at 90 minutes contact time. Also, this finding agreed with the reports of Ogunledun *et al*., (2008); where Carex antiseptic liquids tested in it undiluted and diluted forms gave 100% reduction against some tested microbes except *Pseudomonas aeroginosa* which produced 100% and 62 % in its undiluted and diluted form respectively. The study carried out by these workers made use of *Trichophyton mentagrophyte*, which is a mould but a dermatophyte, despite the relative lack of data describing the use of time kill method for the study of fungi, the techniques have been found to be valuable in the examination of antifungal dynamics. Although factors such as starting innocular and sampling method can significantly influence time kill results and or the interpretation of results, however, the results generated are reproducible (CLSI, 2008). Lewis *et al*., (2002) reported that the time kill studies could help elucidate the pharmacodynamics of an antifungal combination by measuring the effects of the antifungal interaction on the rate of and extent of fungal killing. However, the drawback of time kill studies is that they are laborious to complete. Moreover, lacks of antifungal carry over were also observed with the time kill method (Lewis *et al*., 2002; Klepser, 2000).

Comparative assessment of the activities of the two methods showed that both method are equally good in testing the activities of disinfectants against *Aspergillus fumigatus* ($p > 0.05$). Although, time-kill test recorded higher activity against *Aspergillus fumigatus* than Agar- disc diffusion method in this study, slight discrepancy in the result may be attributed to agar diffusion difficulties that some of the disinfectants may encounter. Also, organisms are in direct contact with exposed disinfectant in time kill method, while lots of factors including substantial dependence on inoculum, temperature and duration of incubation could result in inefficacy of disinfectants when using the agar disc diffusion method (Adebayo, 1999). The estimation of the size of inhibitory zones can be difficult because of partial growth inhibition (Drouhet *et al*., 1986) or the presence of persistent colonies within otherwise clear zones of inhibition (Utz and Shadony, 1997). The wider zones of inhibition observed with formaldehyde in the Agar disc diffusion method concurred with the report of Adebayo (1999); that formaldehyde is fungicidal and sporicidal in its action.

The minimum inhibitory concentration (MIC) and minimum fungicidal concentration (MFC) of these selected disinfectants tested against *Aspergillus fumigatus* isolate showed that, Jik had the highest MIC of 1in 2 while formaldehyde and Iodine had the lowest MIC of 1 in 64. The minimum fungicidal concentration ratio to minimum inhibitory concentration (MFC/MIC ratio) showed that 13 out of the 14 which represents 92.9% of the disinfectants tested against *A. fumigatus* isolates were fungicidal, while only one (1), Robert was found to be fungistatic with MFC/MIC ratio equal to 16. According to Hazen 1998, MFC/MIC ratio $\geq$ 4 of any disinfectant is fungicidal in action but MFC/MIC ratio $\leq$ 4 is fungistatic in action.

Also, more than half of the studied disinfectants had their MICs higher than the manufacturers recommended dilution. This is in contrast with the reports of Jennifer (2006), who reported that, for the disinfectant to be effective the MIC must be well below the concentration of the manufacturer's recommended concentration. This may be due to some factors such as poor storage as well as volatility nature of some of the disinfectants. From a critical evaluation of the chemical composition of the disinfectants, it was observed that majority of them have multiple compositions. Hence, it was a bit difficult in categorising them based on their active ingredients. However, majority of them contain chlorine while only few are devoid of this halogen.

In this study, there was variation in the antifungal activities of the disinfectants against *Aspergillus fumigatus* where some of them were active while others were less active. In a bid to establish some of the factors that may be responsible for discrepancies in the studied disinfectants, their anti-Aspergillus activities was assessed with respect to organic matter (plasma). Presence of organic matter (plasma) was observed to inversely influence the disinfectants' action against *Aspergillus fumigatus*. This findings agrees with Adebayo (1999), who reported that, organic matter tends to reduce the activity of disinfectants to an extends which varies from one antimicrobial substances to another. Also, Ewart (2001) and, Shulaw and Bowman (2001) reported that organic matter provides physical barrier that protects microorganism from contact with disinfectants and can neutralize many disinfectants especially chlorine and iodine containing compounds. This also corroborated the findings of Olowe *et al.,* (2004) who stated that disinfectants take time to act and their activity is greatly affected by amount of organic matter present and concentration of the disinfectants. It is therefore recommended that materials should be free of plasma before treating them with disinfectants. In conclusion, formal aldehyde, Dettol are efficient against *Aspergillus fumigatus* isolated from UCH environments, whereas sodium hypochlorite showed lower efficiency.

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