Research Article

iMedPub Journals
www.imedpub.com

Medical mycology: open access
ISSN 2471-8521

2021
Vol.7 No.6:009

Evaluation of the Sporicidal Effect of Glutaraldehyde used at the Yalgado Ouédraogo University Hospital Center on three Species of the Genus *Aspergillus*

Patindoilba Marcel Sawadogo[1,2*], Kiswendsida Thierry Guiguemdé[2,3], Adama Zida[1,2], Sidiki Sanou[1] and Robert Tinga Guiguemde[2]

[1]Parasitology-Mycology Service, Yalgado Ouédraogo University Hospital Center, Ouaga, Burkina Faso
[2]Training and Research Unit in Health Sciences, University Joseph Ki-Zerbo (UJKZ), Ouagadougou, Burkina Faso
[3]Parasitology-Mycology Service, Charles de Gaulle University Hospital Center, Ouagadougou, Burkina Faso.

[*]**Corresponding author:** Patindoilba Marcel Sawadogo, Parasitology-Mycology Service, Yalgado Ouédraogo University Hospital Center, Ouaga, Burkina Faso, E-mail: sawadogopmarcel@yahoo.fr

Received date: November 17, 2021; **Accepted date:** December 02, 2021; **Published date:** December 09, 2021

Citation: Patindoilba MS, Kiswendsida TG, Adama Z, Sidiki S, Robert TG (2021) Evaluation of the sporicidal effect of glutaraldehyde used at the Yalgado Ouédraogo University Hospital Center on three species of the genus Aspergillus. Med Mycol Open Access Vol.7 No.6:009.

Abstract

Introduction: In Burkina Faso, the effectiveness of the various disinfectants against Aspergillus spores used on medical devices has not yet been demonstrated. Thus the effectiveness of glutaraldehyde, one of the high-level disinfectants, used most often in the disinfection of these medical devices, deserves to be proven through a scientific study. Therefore, our study aimed to determine the minimum inhibitory concentration as a function of contact time of glutaraldehyde on Aspergillus flavus, Aspergillus niger and Aspergillus fumigatus.

Materials and Methods: This was an experimental study to evaluate the sporicidal effects of glutaraldehyde on the three species of Aspergillus. The survival of the spores of these species of Aspergillus is estimated after contact with solutions at a concentration of 0.5% to 2% of glutaraldehyde, and on a contact time of 1 to 30 minutes.

Results: Different concentrations of glutaraldehyde were tested for their sporicidal effects on Aspergillus flavus, Aspergillus niger and Aspergillus fumigatus. The germination of A. flavus spores was 100% inhibited at a dose of 2% and for a contact time of 5 minutes. While the germination of A. niger and A. fumigatus spores, was 100% inhibited at a dose of 2% and for a contact time of 30 minutes.

Conclusion: Glutaraldehyde exhibits a strong dose-dependent and contact time-dependent inhibitory effect of the germination of spores of A. fumigatus, A. flavus and A. niger. At this dose and at a minimum contact time of 5 min, glutaraldehyde is recommended for the disinfection of hospital equipment, concerning certain sensitive microorganisms such as A. flavus.

Keywords: *Aspergillus*; Glutaraldehyde; Sporicidal effect

Introduction

Healthcare Associated Infections (HAIs) are a health problem. They occur during or during the care of a patient in hospital. The occurrence of these infections in a hospital environment is favored by many factors. These are among others factors linked to patients, exposure to invasive medical devices and inadequacies in the quality of care. The same is true of defects in cleaning, disinfection and sterilization, which are potential factors in the development of many microorganisms [1-4].

Among the microorganisms encountered during HAIs, in addition to bacterial and viral agents, we find fungi, in particular molds of the genus *Aspergillus*. *Aspergillus* are responsible for various opportunistic infections (Aspergillosis) in humans through the spores they produce in abundance [5,6].

In hospital, aspergillosis occurs mainly in patients with compromised immunity such as patients with HIV/AIDS, patients treated for hemopathy, long-term neutropenic patients, patients transplanted with hematopoietic stem cells and solid organs, patients on long-term corticosteroids [7,8].

The main locations are in the lung, brain, kidney, liver, heart and spleen. Among these infections, invasive pulmonary aspergillosis is the most common and serious. It is a more or less distal bronchial *Aspergillus* invasion, an invasion of the pulmonary and/or vascular parenchyma causing visceral dissemination [9-11]. Its mortality rate reaches 90% [12,13]. Treatment is expensive and the side effects of antifungals can be severe. No chemoprophylaxis protocol has yet demonstrated significant efficacy [14-17].

The most pathogenic and frequently encountered *Aspergillus* species in the hospital environment of the Hospital Center University-Yalgado Ouédraogo (CHU-YO) are: *A. fumigatus, A. flavus, A. niger*. These species are also the most isolated in the different forms of invasive aspergillosis [17].

The prevention of nosocomial invasive infections linked to *Aspergillus* spores in high-risk patients is therefore based on controlling the environmental risk. This involves the installation of an air treatment system, medical equipment by means of disinfection [14,16,18].

At CHU-YO, the effectiveness of the various disinfectants used has not yet been demonstrated. Thus, the effectiveness of glutaraldehyde, a high-level disinfectant used at CHU-YO, most often in the disinfection of these medical devices, deserves to be proven through an experimental study in the laboratory.

It is in this perspective that we evaluated the sporicidal effect of glutaraldehyde on the three main *Aspergillus* species most frequently encountered at CHU-YO.

Materials and Methods

Type of study

This was an experimental study to evaluate the sporicidal effects of glutaraldehyde on *A. fumigatus, A. flavus, and A. niger.* It took place over a two-month period from October to November 2020.

Disinfectant

The disinfectant tested is glutaraldehyde distributed by the hygiene department to certain departments of the CHU-YO. It has a wide spectrum of activity. It is an unstable product above 50°C and at pH above 8.5. It is used in the disinfection by soaking of heat-sensitive medical devices. It can also be used for the disinfection of floors and surfaces. It is used when the level of risk is high [19-21].

Culture medium for *Aspergillus* strains

We used as medium, Potato Dextrose Agar (PDA), at acidic pH of 5.6 with as reference: Liofilchem/Ref: 610102; Lot: 060619501. This is a medium used for the cultivation and enumeration of yeasts and molds. This medium contains 15.0 g of agar, 20.0 g of dextrose, and 4.0 g of potato infusion [22].

Fungal material

The antifungal activity of different concentrations of glutaraldehyde was evaluated on three fungal species of the genus *Aspergillus*, which are: *Aspergillus fumigatus*, *Aspergillus flavus and Aspergillus niger*. Our choice fell mainly on these species since they are often involved in various *Aspergillus* infections, and they are the most frequently encountered in the hospital environment of the CHU-YO.

Collection of the three fungal species

The strains of each species were obtained from the parasitology-mycology laboratory of the CHU-YO. These molds come from different samples from patients received in this laboratory. The colonies which had been isolated were sub cultured.

To do this, a colony fragment was removed using a sterilized pipette; this fragment was then placed in the center of a new Petri dish carefully labelled and containing the PDA medium. Subsequently the inoculated medium was incubated at 25°C for two weeks in the Parasitology laboratory of the UFR/SDS. Thus aged spores were obtained according to the protocol.

Preparation of *Aspergillus* spore suspensions

Spore suspensions of the selected *Aspergillus* species were prepared from the two-week-old PDA cultures at 25°C. For this the spores were collected with a sterile bacteriological loop to be diluted in 5 ml of sterile distilled water, and transferred to 100 ml of sterile distilled water in a 250 ml conical flask. The resulting suspensions were stirred for 10 mins at 25°C and filtered through two layers of sterile compresses to remove the fungal mycelium. The spore concentration was determined with a Malassez cell and was adjusted to 105 spores/ml.

Preparation of solutions with different concentrations of glutaraldehyde

Solutions with different concentrations of glutaraldehyde namely, 0%; 0.5%; 1% and 1.5% were obtained after diluting the 2% solution.

Inoculation of the different concentrations containing the strain on the PDA medium

One milliliter of the spore suspension of *A. fumigatus* or *A. flavus* or *A. niger* was added to a tube containing 9 ml of one of the solutions of the different concentrations v/v of glutaraldehyde (0%; 0.5%; 1%; 1.5% and 2.0%). Then each mixture was incubated at 25°C for 1, 5, 10, 15, 25, and 30 min. Then 0.1 ml of the suspension at each incubation period as well as the concentrated solution of glutaraldehyde used were inoculated on PDA medium and followed by incubation for 6 days at 25°C.

Calculation of percentage of inhibition

Survival of the fungus was expressed as the mean number of Colony Forming Units (CFU) per ml. The percentages of inhibition of spore germination for the three fungi studied were calculated according to the formula: P=(X-Xa/X)x100.

P: Spore germination inhibition rate

X: Estimation of germination in control medium.

Xa: Estimation of germination in the medium with glutaraldehyde.

Data analysis

The data collected was recorded on a collection sheet, and the analysis was carried out using excel version 2013 software.

Results

Determination of the Minimum Inhibitory Concentration (MIC) of glutaraldehyde on *A. flavus*

No colony was observed on PDA agar with the strain of *A. flavus*, 5 min after contact with 2% glutaraldehyde. This is shown in **Table 1.**

Contact time	Concentration (%)	Number of colonies	Percentage of inhibition (%)
1 minute	0	200	0
	0,5	144	28
	1	13	93,5
	1,5	4	98
	2	2	99
5 minutes	0	200	0
	0,5	17	91,5
	1	3	98,5
	1,5	2	99
	2	0	100
10 minutes	0	200	0
	0,5	16	92
	1	1	99,5
	1,5	0	100
	2	0	100
15 minutes	0	200	0
	0,5	6	97
	1	1	99,5
	1,5	0	100
	2	0	100
25 minutes	0	200	0
	0,5	6	97
	1	1	99,5
	1,5	0	100
	2	0	100
30 minutes	0	200	0
	0,5	5	97,5
	1	1	99,5
	1,5	0	100
	2	0	100

Table 1: Percentage of inhibition of the germination of spores of *A. flavus* in response to different concentrations of glutaraldehyde as a function of time.

At a concentration of 2% after one minute of contact time glutaraldehyde inhibits 80% of *A. flavus*, while within 5 min at the same concentration 100% of the spores are inhibited. The data presented in Table 1 are translated more explicitly in **(Figure 1).**

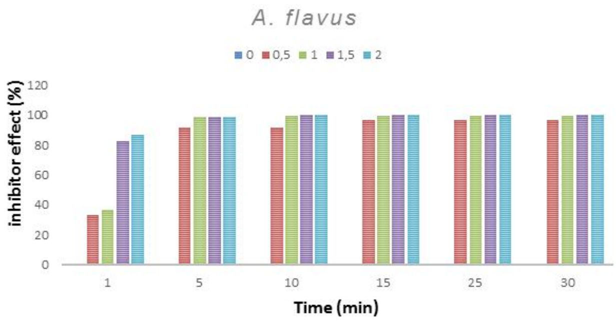

Figure 1: Percentage of inhibition of germination of *A. flavus* spores with lutaraldehyde.

Determination of the Minimum Inhibitory Concentration (MIC) of glutaraldehyde on *A. niger*

Regarding the strain of *A. niger*, it was only after 30 minutes of contact with 2% glutaraldehyde that no colony was observed on the PDA agar. This is shown in **Table 2.**

Contact time	Concentration (%)	Number of colonies	Percent of inhibition (%)
1 minute	0	120	0
	0,5	80	33,33
	1	76	36,66
	1,5	21	82,5
	2	16	86,66
5 minutes	0	120	0
	0,5	68	43,33
	1	48	60
	1,5	19	84,16
	2	5	95,83
10 minutes	0	120	0
	0,5	52	56,66
	1	44	63,33
	1,5	13	89,16
	2	5	95,83
15 minutes	0	120	0
	0,5	34	71,66
	1	20	83,33
	1,5	8	93,33
	2	5	95,83
25 minutes	0	120	0

	0,5	30	75
	1	20	83,33
	1,5	6	95
	2	2	98,33
30 minutes	0	120	0
	0,5	20	83,33
	1	15	87,5
	1,5	6	95
	2	0	100

Table 2: Percentage of inhibition of spore germination of *A. niger* in response to different concentrations of glutaraldehyde as a function of time.

At a concentration of 1.5% and at one minute of contact time glutaraldehyde inhibits 80% of the spores of *A. niger*. This same percentage of inhibition is achieved at the concentration of 1% from 15 minutes of contact time and complete inhibition is achieved at the concentration of 2% in 30 minutes of contact time. This is illustrated in **(Figure 2)**.

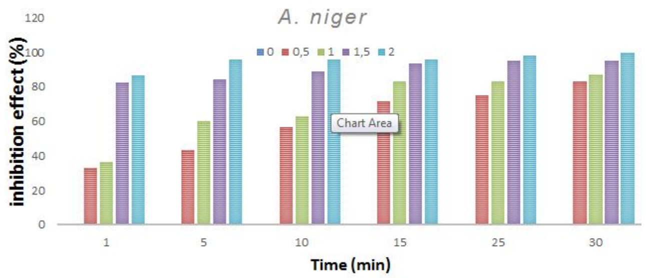

Figure 2: Percentage of inhibition o f germination of *A. niger* spores with glutaraldehyde.

Determination of the Minimum Inhibitory Concentration (MIC) of glutaraldehyde on *A. fumigatus*

As for A. fumigatus, it was also after 30 minutes of contact with 2% glutaraldehyde that no colony was observed on the PDA agar. This is shown in **Table 3** below.

Contact time	Concentration (%)	Number of colonies	Percentage of inhibition (%)
1 minute	0	200	0
	0,5	192	4
	1	110	45
	1,5	96	52
	2	52	74
5 minutes	0	200	0
	0,5	104	48
	1	66	67
	1,5	33	83,5
	2	11	94,5
10 minutes	0	200	0
	0,5	90	55
	1	28	86
	1,5	9	95,5
	2	7	96,5
15 minutes	0	200	0
	0,5	80	60
	1	18	91
	1,5	6	97
	2	2	99
25 minutes	0	200	0
	0,5	54	73
	1	12	94
	1,5	5	97,5
	2	1	99,5
30 minutes	0	200	0
	0,5	48	76
	1	7	96,5
	1,5	4	98
	2	0	100

Table 3: Percentage of inhibition of the germination of spores of *A. fumigatus* in response to different concentrations of glutaraldehyde as a function of time.

At a concentration of 1.5%, and from 5 minutes of contact time glutaraldehyde inhibits 80% of the spores. This same percentage of inhibition is achieved at the concentration of 1% from 10 minutes of contact time and complete inhibition is achieved at the concentration of 2% in 30 minutes of contact time. This is illustrated in **Figure 3** below.

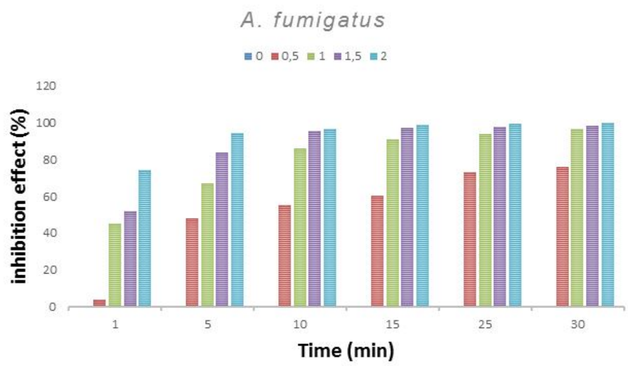

Figure 3: Percentage of inhibition o f germination of *A. fumigatus* spores with glutaraldehyde.

Summary of MICs according to fungal species and contact time

The summary of MICs by fungal species and contact time is presented in **Table 4**.

Aspergillus species	Contact time (min)					
	1	5	10	15	25	30
A. flavus	>2%	2%	1,5%	1,5%	1,5%	1,5%
A. niger	>2%	>2%	>2%	>2%	>2%	2%
A. fumigat us	>2%	>2%	>2%	>2%	>2%	2%

Table 4: Summary of MICs according to fungal species and contact time.

Table 4 shows us that from 5 min of contact time 2% glutaraldehyde inhibits 100% of the spores of the species *A. flavus*, while for the other two species it takes up to 30 minutes of contact time at the same concentration to obtain the same result.

Discussion

MIC analysis revealed that glutaraldehyde exhibited an inhibitory effect on the germination of *Aspergillus* spores tested with varying sensitivity depending on the concentration and the contact time from one species to another. It is well known that contact time is an important parameter in the effectiveness of disinfectants. This efficiency is equal to the product of the contact time and the concentration. Failure to observe the recommended contact time if it is shorter does not allow the active ingredient to fully inhibit germs. It should also be noted that a very prolonged contact time could deteriorate the plastic surfaces [23,24]. Our choice of the three species of *Aspergillus* for the study is explained by their pathogenicity and their frequency in the hospital environment of the Yalgado Ouédraogo University Hospital [2,17].

The spores of *A. flavus* were inhibited after 5 min of exposure to 2% glutaraldehyde and from 10 min of contact time at the 1.5% concentration all of the spores were inhibited. This is explained by the very high sensitivity of *A. flavus* to glutaraldehyde.

With regard to *A. niger*, from 1 to 25 minutes of contact time and whatever the concentration of glutaraldehyde the spores were always present. It was not until 30 minutes of contact and at the concentration of 2% that the inhibition was complete. The same is true for the strain of *A. fumigatus.* This could be explained by the fact that *A. niger* and *A. fumigatus* would show a dose-dependent sensitivity to glutaraldehyde. These results corroborate those of Lensing HH et al. who found 2% glutaraldehyde to be an inhibitor of *A. niger* and *A. fumigatus* but only after a long exposure period [25].

The choice of a disinfectant must be made according to nine basic parameters to consider which are:

- Sought objective: all-purpose product or specialized product
- Degree of disinfection: regular maintenance, terminal disinfection
- Action time: a contact time of 15 minutes or less
- Wide spectrum of activity: effect on as many microbes as possible
- Nature of the surfaces to be treated
- Compatibility with materials: product that damages materials as little as possible
- Health and safety for employees and beneficiaries: follow standards or best practices
- Environment: use products that have reduced effects on the environment
- Quality/price ratio [24,25].

Of these nine parameters cited our study concerned the contact time. For this we noticed that only *A. flavus* meets this criterion because at 5 min already the inhibition rate is 100%. However, for *A. niger* and *A. fumigatus* this inhibition rate is reached after 15 minutes. Hence with regard to glutaraldehyde while taking into account these nine parameters concerning its sporicidal effect we must also take into account the species of *Aspergillus* targeted in the disinfection.

Conclusion

Glutaraldehyde exhibits a strong dose-dependent and contact time-dependent inhibitory effect of the germination of spores of *A. fumigatus, A. flavus* and *A. niger*. Due to its significant disinfection potential it allows the eradication of the fungi in question on reusable medical devices in service rooms and this from a dose of 2%. At this dose and at a minimum contact time of 5 min glutaraldehyde is recommended for the disinfection of hospital equipment concerning certain sensitive microorganisms such as *A. flavus.*

References

1. Afle FCD, Kisito JMK, Quenum, Hessou S, Johnson RC (2018) Etat des lieux des infections associées aux soins dans deux hôpitaux publics du sud Benin, Centre Hospitalier Universitaire de Zone d'Abomey-Calavi/Sô-Ava et de Zone de Cotonou 5. J Appl Biosci 121: 12192-12201.
2. French Association of Parasitology and Mycology Teachers (2014). Parasitosis and Mycosis (3rd edn). Paris, Masson.
3. Halewyn MA, Leclerc JM (2002) The health risks associated with the presence of mold indoors. (1st edn). INSPQ,Québec.
4. Maslin J, Morand JJ, Menard G, Camparo P(2004) Aspergilloses. Med Trop 64: 11-17.
5. Botton B (1990) Useful and harmful molds. Industrial importance (2nd edn). Paris, Masson.
6. Botton B (1999) Useful and harmful molds. Industrial importance (3rd edn). Paris, Masson.
7. Herbrecht R, Bories P, Moulin JC, Ledoux MP, Letscher BV (2012) Risk stratification for invasive aspergillosis in immunocompromised patients. Ann N'Y Acad Sci 1272: 23-30.
8. Thiebaut A (2008) Prophylaxie des infections fongiques invasives : Nouvelles études cliniques. John Libbey Eurotext 14: 12-19.

9. Ader F, Nseir S, Guery B, Tillie LI (2006) Acute invasive pulmonary aspergillosis in chronic lung disease. Rev Mal Respir 23: 6S11-6S20.

10. Chabasse D, Guiguem C (1999) Mycologie médicale (1st edn). Paris, Masson.

11. Hope WW, Walsh TJ, Denning DW (2005) The invasive and saprophytic syndromes due to *Aspergillus Spp. Med Mycol 43: S207-S238.*

12. Dagenais T, Keller N (2009) Pathogenesis of Aspergillus fumigatus in Invasive Aspergillosis. Clin Microbiol Rev 22: 447-465.

13. Graf K, Khani SM, Ott E, Mattner F, Gastmeier P, et al. (2011) Five-years surveillance of invasive aspergillosis in a university hospital. BMC Infect Dis 11: 163

14. Cornet M, Levy V, Fleury L, Lortholary J, Barquins S, et al. (1999) Efficacy of prevention by high-efficiency particulate air filtration or laminar airflow against Aspergillus airborne contamination during hospital renovation. Infect Control Hosp Epidemiol 20: 508-513.

15. Cheikh EJ, Venton G, Crocchiolo R, Fürst S, Faucher C, et al. (2013) Efficacy and safety of micafungin for prophylaxis of invasive fungal infections in patients undergoing haplo-identical hematopoietic SCT. Bone Marrow Transplant 48: 1472-1477.

16. Gangneux JP, Poirot JL, Morin O, Derouin F, Bretagne S, et al. (2002) Mycologic surveillance of the environment for preventive invasive aspergillosis: Proposals for standardization of the methodologies and implementation. Presse Médicale Paris 31: 841-848.

17. TRAORE MS (2016) Study of *Aspergillus* biocontamination at the Yalgado Ouédraogo university hospital center. Thesis diploma of specialized studies in clinical biology. Ouaga University I Pr joseph KI-ZERBO BF.

18. Hahn T, Cummings KM, Michalek AM, Lipman BJ, Segal BH, et al. (2017) Efficacy of high-efficiency particulate air filtration in preventing aspergillosis in immunocompromised patients with hematologic malignancies. Infect Control Hosp Epidemiol 23: 525-531.

19. Pierre M (2002) Disinfectants, efficiency and requirement levels (1st edn). Paris: Bull Acad Vét de France.

20. Coordination center for the fight against nosocomial infections (2000). Antiseptics and disinfectants. Paris: C CLIN.

21. Christine D, Isabelle B Disinfection of surfaces in a biological laboratory (1st edn). Paris, INRS.

22. M Gélose Potato Dextrose (2016) Product Instructions.

23. Springthorpe S (2000) Disinfection of surfaces and equipment. J Can Dent Assoc 66: 558-560.

24. Taccone FS, Van den Abeele AM, Bulpa P, Misset B, Meersseman W, et al. (2015) Epidemiology of invasive aspergillosis in critically ill patients: Clinical presentation, underlying conditions and outcomes. Critical Care 19: 1-15.

25. Gorman SP, Eileen MS (1980) Antimicrobial activity, uses and mechanism of action of glutaraldéhyde, Journal of Applied Bacteriology 48: 161-190.