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FUNGICIDAL ACTIVITY OF FIVE DISINFECTANTS ON ISOLATES OF *CANDIDA SPECIES*

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ABSTRACT: Determine the fungicidal activity of five disinfectants on *Candida auris* of clinical and environmental origin. Assess the fungicidal efficiency of each disinfectant on the microorganism. Calculate the percentage of efficiency (% E) of each disinfectants on Candida strain and determine the specific death rate (k) and decimal reduction time for microorganisms. Analytic and experimental research with quantitative methods. We realized a strain's workbench by culturing it in corresponding culture media. Analysis was a challenge essay, performed on four surfaces and at different exposure times; 1, 5, 15, and 30 minutes of contact between disinfectant and yeast. Once the disinfection process was carried out, what was expected was that after 15 minutes of contact between the yeasts and the disinfectants, a percentage of inhibition of the microorganism between 90 and 95% was presented, thus guaranteeing the duration of elimination of the infectious agent by disinfectants. Results show that after one minute of contact between Candida auris and disinfectant in a gloved hand, the yeast was removed when the clinical and environmental strains were analyzed. Data showing disinfectants efficiency percentages greater than 90% overstrains of Candida used in the study. Decimal reduction times in seconds of the strains of Candida against the disinfectants used in the study were between 9.3 and 14.04 s. It was possible to evaluate effectiveness using the inhibition percentage of each disinfectant on the strains of *Candida auris* of clinical and environmental origin. Similarly, specific death rate and decimal reduction times in seconds were determined for strains under study.

INTRODUCTION: *Candida* genus is a group of yeasts responsible for most fungal infections. In a multicenter study in the United States, these yeasts were identified as the seventh most common microorganism related to health-care-associated infections, with increasing mortality rates in hospitalized patients [1] in Brazil it was the sixth most common isolate in intensive care unit infections (ICU) [2] and in German ICU´s it was identified as a common mortality cause among bloodstream infections [3] *Candida albicans, C. glabrata, C. krusei,* among others are the main species causing infections, but *C. aurishas* emerged as a microorganism capable of causing outbreaks worldwide [4], exposing major resistance to multiple anti-fungals, such as fluconazole, voriconazole and amphotericin, giving as a result treatment difficulties in managing affected individuals. In order to contain these outbreaks, chlorine-based

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products were proposed to disinfect areas or colonized patients with *C. auris* [5]. This study aimed to analyze the effect of chlorhexidine and four disinfectants currently used in hospitals and clinics to assess their anti-fungal activity and determine if they effectively eliminate the *Candida* isolates, including *C. auris*.

MATERIALS AND METHODS: This research was analytic research with quantitative methods.

Microorganisms: The microorganisms analyzed were *Candida auris, Candida albicans,* and *Candida guillermondii*, preserved in Petri dishes with Saboraud medium at 5°C.

A microscopic identification was made using a Gram stain. Human Mycosis provided the microorganisms and Proteomics Laboratory at Pontificia Universidad Javeriana and the Laboratory of National Institute of Health located in Bogotá-Colombia. The *C. auris* isolate used identified during an outbreak in Valledupar (Colombia) [6].

Cell Bank: Sabouraud broth was used. The broth was distributed in 4 flasks of 250 mL. Each includes 50 mL of a suspension of *Candida*in question. The flasks were left in a shaker for 24 hours to ensure the growth of the yeasts. Once the time had elapsed, the bank was made using Eppendorf tubes with 500 uL of medium and *Candida* strain and 500 uL of 85% glycerol. Each strain of *Candida* species used had a bank of 50 Eppendorf tubes that were frozen at -20 °C.

Chlorhexidine and Disinfectants: The commercial products used were those available in the Colombian market that comply with local regulations and included: 1. 4% chlorhexidine gluconate with cetrimide 1% (v/v) (CHG/CTR, Quirucidal®, Quirumédica, Colombia); 2. quaternary ammonium combinations: a commercial combination of 50% glutaraldehyde, 40% formic aldehyde and 40% glyoxal and didecyldimethyl-ammonium chloride (GLUT 1, New Fagetriald ®, Fagesa Colombia) and a commercial combination with 50% glutaraldehyde and didecyl-dimethylammonium chloride (GLUT 2, New Desinfloor ®, Fagesa Colombia); 3. microbicidal surfactants in aqueous solution and alkalize alkanes with halogenated carboxylic acids (SURF, Tego ®, Quiminet Colombia) and 4. a chlorine-based surface disinfectant with 5.25% of sodium hypochlorite (Blanqueador Brilla King Natural® BNS. SA, Colombia).

Contamination Process:

Analysis of CHG / CTR Versus the 4 Strains of *Candida*: To analyze the action of CHG/CTR against the strains of *Candida*, a gloved hand was immersed into a solution with a known count of each yeast in question(*Candida albicans* SC5314, *Candida guillermondii* H025, *Candida auris* of clinical origin and *Candida auris* of environmental origin). After 30 seconds, once the solution dried, the glove was exposed to the disinfectants. Counts were made in triplicate at 1,5, 15 and 30 minutes, to analyze the percentage of inhibition of the microorganism against the disinfectant.

Analysis of GLUT1 and Chlorine against the 4 Strains of *Candida*: To analyze the action of GLUT 1 and the chlorine solution against the strains of *Candida*, different sections of a wall located in a laboratory at the Pontificia Universidad Javeriana in the Faculty of Basic Sciences, were exposed with a known count of each yeast in question (*Candida albicans* SC5314, *Candida guillermondii* H025, *Candida auris* of clinical origin and *Candida auris* of environmental origin). Cleaning was performed according to the manufacturer´s instructions. After the cleaning was executed, counts were made in triplicate at 1,5, 15, and 30 minutes to analyze the percentage of inhibition of the microorganism against GLUT 1 and the chlorine solution.

Analysis of GLUT 2 against the 4 strains of *Candida*: To analyze the action of GLUT 2 against the strains of *Candida*, a surface of a workbench located in a Laboratory of the Pontificia Universidad Javeriana in the Faculty of Basic Sciences, was exposed with a known count of each yeast in question.

(*Candida albicans*SC5314, *Candida guillermondii* H025, *Candida auris* of clinical origin and *Candida auris* of environmental origin). Cleaning was performed according to the manufacturer´s instructions. Counts were made in triplicate at 1, 5, 15 and 30 min, to analyze the percentage of inhibition of the microorganism against the disinfectant.

Analysis of SURF against the 4 Strains of *Candida*: To analyze the action of SURF against the strains of *Candida*, a surface of a metalwork bench located in a Laboratory of the Pontificia Universidad Javeriana in the Faculty of Basic Sciences, was exposed with a known count of each yeast in question. (*Candida albicans* SC5314, *Candida guillermondii* H025, *Candida auris* of clinical origin and *Candida auris* of environmental origin). Cleaning was performed according to the manufacturer´s instructions. Counts were made in triplicate at 1, 5, 15 and 30 min to analyze the percentage of inhibition of the microorganism against the disinfectant.

Calculation of Death Kinetics, Decimal Reduction time, and Inhibition Percentage: Death kinetics of microorganisms was established according to the following expression; where k is the specific death rate, corresponding to the slope of the line resulting from the graph ln (Nt) vs. time. A first-order kinetic equation is used, $N = N_0 \times e^{-kt}$ where N is the number of cells in colony-forming units per gram (CFU/g). No is the initial number of cells, t time, and k is the velocity constant. The following expression is obtained:

$$\ln (Nt / No) = -kt$$

Where, **No** is the number of initial microorganisms, **Nt** is the number of surviving microorganisms at time t, **t** is the contact time between the disinfectant and the microorganism, and **k** is the specific death rate, calculated as stated before. The line obtained in a graph ln Nt vs. time can present some deviations from the linearity.

This can be attributed to several factors such as the specific speed of death does not remain constant, the concentration of the disinfectant might decrease over time or differences in temperature during the process. The decimal reduction time DRT indicates the time necessary to decrease in a logarithmic cycle, the quantity of microorganisms present in a test sample; this is determined by the following expression:

$$DRT = 2.3 / k$$

Where, **k** is the specific death rate. The formula expresses the percentage of inhibition:

$$I = R1 – R2/ R1 \times 100$$

Where, **R1 is the i**nitial count, R**2 is the f**inal count.

RESULTS AND DISCUSSION: Natural logarithm Counts of CFU/ml at the exposure times evaluated are shown in **Table 1**. The disinfectants used in the study show a significant result regarding the elimination of the microorganism within one minute of exposure. Solution C showed elimination of the strain in question within five minutes of exposure. **Table 2** shows the percentages of efficiency of the disinfectants over the strains in the study. CHG/CTR, SURF, and the chlorine-based disinfectant showed 100 % efficiency against the *Candida* strains. GLUT 1 showed a lower percentage of efficiency (42.86%) within five minutes of exposure. Percentages of inhibition of microorganisms as a result of the action of the disinfectants are showed in **Table 3**.

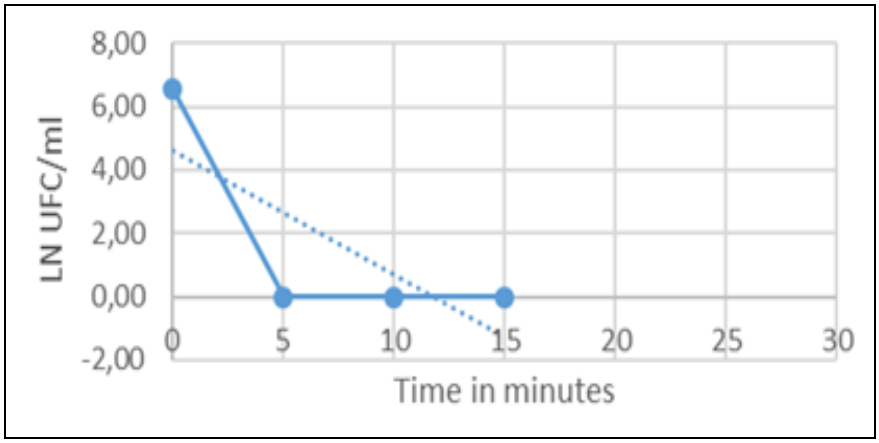
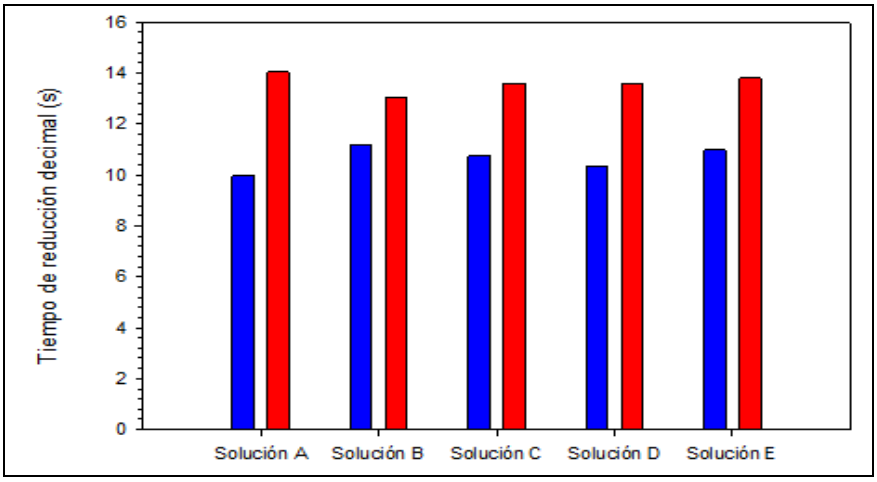


FIG. 1: DEATH KINETICS OF THE MICROORGANISM *VS.* THE ACTION OF THE DISINFECTANT IN THE EXPOSURE TIMES EVALUATED. A SINGLE FIGURE IS SHOWN BECAUSE THE SAME BEHAVIOR WAS SEEN THROUGHOUT THE STUDY. *Candida albicans* SC5314 *vs.* Solution B, y= -0.3931x + 4, 5858, R2 = 0.6.



CANDIDA AURIS* AND CANDIDA *AURIS

FIG. 2: DECIMAL REDUCTION TIMES IN SECONDS OF THE *CANDIDA AURIS* STRAINS USED IN THE STUDY AGAINST DISINFECTANTS ANALYZED

TABLE 1: DATA OF THE NATURAL LOGARITHM OF THE COUNTS IN CFU / ML AT THE EXPOSURE TIMES EVALUATED

	B CGH/CTR Gloved hand					**A Chlorine-based Solution Wall**					**C GLUT 1 Workbench**					**E GLUT 2 Workbench**					**D SURF Wall**				
	Natural logarithm in CFU /mL																								
Exposure time (min)	**0**	**1**	**5**	**15**	**30**	**0**	**1**	**5**	**15**	**30**	**0**	**1**	**5**	**15**	**30**	**0**	**1**	**5**	**15**	**30**	**0**	**1**	**5**	**15**	**30**
Candida auris 1	14,26	0	0	0	0	11,29	0	0	0	0	13,24	0	0	0	0	13,79	0	0	0	0	12,95	0	0	0	0
Candida auris 2	10,13	0	0	0	0	10,82	0	0	0	0	10,46	0	1.39	0	0	10,46	0	0	0	0	10,31	0	0	0	0
Candida albicans	14.62	0	0	0	0	12,96	0	0	0	0	13,20	0	0	0	0	13,45	0	0	0	0	12,78	0	0	0	0
Candida guillermondii	15,21	0	0	0	0	14,00	0	0	0	0	14,07	0	0	0	0	13,49	0	0	0	0	14,13	0	0	0	0

TABLE 2: DATA SHOWING PERCENTAGES OF EFFICIENCY OF THE DISINFECTANTS OVER THE STRAINS OF CANDIDA USED IN THE STUDY

	CHG/CTR												**GLUT 2**				**SURF**			
Exposure time (min)	**1**	**5**	**15**	**30**	**1**	**5**	**15**	**30**	**1**	**5**	**15**	**30**	**1**	**5**	**15**	**30**	**1**	**5**	**15**	**30**
	Microorganisms																			
Candida albicans	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Candida guillermon-dii	100	100	100	100	100	100	100	100	99.6	100	100	100	100	100	100	100	100	100	100	100
Candida auris 1	100	100	100	100	100	100	100	100	100	100	100	100	99,5	100	100	100	100	100	100	100
Candida auris 2	100	100	100	100	100	100	100	100	100	42.86	100	100	100	100	100	100	100	100	100	100

Our germicidal study demonstrated that all the disinfectants used in the study had an efficacy of 100% against *Candida auris* clinical origin, at four times of exposure.

For *Candida auris* environmental origin, GLUT 1 exhibits the lower percentage of inhibition (42%) within 5 min. For *Candida auris* 2 solution C exhibits 42% of inhibition in 5 min. *Candida auris, Candida albicans* and *Candida Guillermondii* was susceptible in the first minute for the action of disinfectant solutions. Death kinetics of the microorganism *vs.* the action of the disinfectant in the exposure times evaluated is shown in **Fig. 1**.

The initial concentration of microorganisms decreases dramatically in the first five minutes of exposure to the disinfectant. **Table 4** and **Fig. 2** show the decimal reduction times (DRT) in seconds of the strains studied against the disinfectants.

TABLE 3: DATA SHOWING PERCENTAGES OF INHIBITION OF MICROORGANISMS BECAUSE OF THE ACTION OF THE DISINFECTANTS USED IN THE STUDY

	CHG/CTR												GLUT 2				SURF			
Exposure time (min)	**1**	**5**	**15**	**30**	**1**	**5**	**15**	**30**	**1**	**5**	**15**	**30**	**1**	**5**	**15**	**30**	**1**	**5**	**15**	**30**
Microorganisms																				
Candida albicans	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
Candida guillermon-dii	100	100	100	100	100	100	100	100	99.6	100	100	100	100	100	100	100	100	100	100	100
Candida auris 1	100	100	100	100	100	100	100	100	100	100	100	100	99,5	100	100	100	100	100	100	100
Candida auris 2	100	100	100	100	100	100	100	100	100	42.86	100	100	100	100	100	100	100	100	100	100

TABLE 4: DATA SHOWING DECIMAL REDUCTION TIMES IN SECONDS OF THE STRAINS OF CANDIDA AGAINST THE DISINFECTANTS USED IN THE STUDY

	CHG/CTR												GLUT 2				SURF			
Exposure time (min)	**1**	**5**	**15**	**30**	**1**	**5**	**15**	**30**	**1**	**5**	**15**	**30**	**1**	**5**	**15**	**30**	**1**	**5**	**15**	**30**
Microorganisms	**Decimal reduction times**																			
Candida auris 1	9,973					11.155				10,743				10,314				10,983		
Candida auris 2	14,041					13.142				13,601				13,601				13,797		
Candida albicans	9,729					10,973				10,778				10,574				11,127		
Candida guillermondii	9,353					10,159				10,109				10,546				10,065		

The highest DRT was between *Candida auris* environmental origin and CHG/CTR. *Candida auris* Clinical origin has the lowest decimal reduction time, so is a most sensitive strain in this study. Solution A has the best reduction time. In *Candida auris* 2 the average was around 13.6s. Using disinfection solutions, there was a complete elimination of the controls used in the study, *Candida albicans* SC5314 and *Candida guillermondii* H025 after 1 minute of action of the disinfectant in gloved hand, wall and workbench. Our results show an important effect of chlorhexidine in combination with cetrimide and some of the conventional disinfectants over clinical isolates of *Candida spp.*, including two isolates of *C. auris,* identified during an outbreak in Colombia [6].

This study used two controls of the genus, *C. albicans* SC5314 and *Candida guillermondii* H025, due to the ability to form biofilms[7] and the specie in question, *Candida auris*, multi-resistant emergent yeast that represents a major risk to the public health [8]. These two isolates were chosen due to the uncertainty related to the effect of this kind of product over such specie. The current recommendation is the use of an Environmental Protection Agency (EPA)-registered hospital-grade disinfectant effective against *Clostridium difficile* spores, the majority of which correspond to chlorine solutions [9]. In order to guarantee a correct elimination and prevent dissemination throughout the hospital surfaces and areas, and to prevent patient colonization and infection with the infectious agent, it´s demanding to exercise correct antisepsis and disinfection. Analyzing disinfection and survival of *Candida* on surfaces, a study made by[10], determined how *Candida auris* and other *Candida* species are prone to develop on surfaces, especially when they are moist, creating a potential source of transmission of the microorganism. In their study, quaternary ammonium compounds, acetic acid, and ethyl alcohol 29% were unable to eliminate a considerable amount of *Candida* in the study. In another study conducted by [11], chlorhexidine gluconate was used at different concentrations to control outbreaks of *Candida auris* in hospitals and clinics.

The authors analyzed that exposure times less than two minutes and concentrations below 2% of chlorhexidine gluconate were not enough to eliminate *Candida auris*. In our study, a commercial combination of chlorhexidine 4% plus ceramide 1% was successful to eliminate the isolates in the hand glove model with effectiveness of 100%. A commonly used strategy is to combine the action of chlorhexidine with isopropyl alcohol, to guarantee antisepsis of skin and hands. A study demonstrated that levels of *Candida auris* decrease compared to the initial population of the yeast when exposed to chlorhexidine in combination with

isopropyl alcohol, while the use of chlorhexidine alone at 2% was not able to eradicate *C. albicans*. This study and others also showed the effectiveness of 10% povidone-iodine in reducing the number of colonies of *Candida* strains. In our case, the tested product included chlorhexidine at 4% in combination with cetrimide, and the observed effect was a clear reduction in the number of colonies. Worth noting, that several studies have shown that *Candida auris* strains have the ability to form large aggregation of cells, protecting the yeast against the action of chemical disinfectants; the reason that can be attributed to the difficult elimination of the microorganism on surfaces.

A study conducted by [12], analyses the role of the extracellular matrix of Candida species in biofilm formation leading to drug resistance and difficult elimination of the microorganism on surfaces. A polysaccharide complex of mannan-glucan is found in most *Candida* species. This, combined with an abundance of efflux pump transcript and an interestingly high expression of glucan modifying genes, could be considered signature components in antifungal sequestration and drug resistance biofilm in *Candida auris.* To analyze the effectiveness of the disinfectants in the study correctly, several factors need to be taken into consideration. According to [13], wet contact time directly affects antimicrobial efficacy. When analyzing *Candida auris*, it is demanding to guarantee continuous wetness during microbial efficacy testing. The EPA requires this method when the analysis involves either *Candida auris* or *Clostridium difficile.*

The action of quaternary ammonium compounds has been questioned. In the study by [10] Candum *et al*, these products could not reach the same level of activity compared with others. Since, there are different combinations, it is difficult to ascertain if this is a class effect, a difference in the concentrations and products tested, or even technical differences among studies performed. In a study conducted by [14, 15] the action of quaternary ammonium with potential use in dental care was tested against the genus *Candida*. Minimal inhibitory concentrations (MIC) and minimal fungicidal concentrations were found in a range that is possible to be reached in clinical or environmental scenarios.

In another study conducted by [16], quaternary ammoniums were tested against *Candida albicans* in combinations with products used for the care of contact lenses. This study showed a limited effect on fungal cells, showing the need for doing different tests against these products in the different settings in which they are intended to be used. A study conducted by [17, 18] analyzed the action of glutaraldehyde in combination with quaternary ammonium against strains of *C. albicans*. Glutaraldehyde acts by distorting the membrane, affecting its integrity and function, and preventing the formation of biofilms, which are important in the process of colonization and surface infection, as mentioned before. Since, there are different combinations commercially available, before its use, at least for the disinfection of surfaces potentially containing *Candida* isolates, a formal test should be done to prove their effectiveness. Carboxylic acid is a chemical agent used for the elimination of Gram-positive and Gram-negative bacteria including *Salmonella sp, Campylobacter sp, Listeria sp* and yeasts. In a study carried out by Hai-Nam *et al* it was found that carboxylic acid affects the lipid matrix of the cells, facilitating the entry of the chemical agent into the microorganism [19, 20]. In our study, a combination of products including carboxilic acids showed complete elimination of the *Candida* isolates in the tests done. The main limitation for our work is that the individual agents were not tested against the *Candida* isolates, and no conclusion can be drawn from each individual agent.

In conclusion, the present work evaluates the effects of some disinfectant products and chlohexidine in *Candida* isolates over organic free surfaces, providing evidence of the effects of such combination products over isolates of from clinical and environmental samples of *C. auris.* Further experiments are required to establish firm recommendations, but our results suggest that other products different from chlorine derivate might be useful in environmental cleaning and clinical antisepsis.

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