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PERSISTENCE OF CANDIDA AURIS ON ELEMENTS USED FOR PERSONAL PROTECTION (LATEX GLOVES AND DISPOSABLE GOWN)

Edith Sihomara Bohorquez [2], Janeth Arias Palacios [2] and Claudia Marcela Parra [*1]

Laboratory of Human and Proteomic Mycoses [1], Pontificia Universidad Javeriana, Faculty of Sciences, Department of Microbiology Bogota Colombia.
Bio-Technology Ambiental and Industrial Microbiology Group [2], Pontificia Universidad Javeriana, Faculty of Sciences, Department of Microbiology Bogotá Colombia.

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Correspondence to Author:
Mr. Claudia Marcela Parra

Laboratory of Human and Proteomic Mycoses. Pontificia Universidad Javeriana, Faculty of Sciences, Department of Microbiology Bogota Colombia.

E-mail: claudia.parra@javeriana.edu.com

ABSTRACT: Candida SP, is currently associated with nosocomial infection; in this study, we aimed to determine the persistence of *Candida auris* on inanimate surfaces. Two test surfaces (latex gloves and disposable gown) were inoculated with two strains of *Candida auris* (clinical Ca1 and environmental H005913). The initial concentration of the inoculum was 108 UFC / mL, sampling was performed by rubbing a sterile swab over the surface, being sampled every 48 h, the micro organisms recovered by means of MALDI-TOF mass spectrometry were identified. The persistence of *C. auris* on gloves surface was 624 h equivalent to 26 days and in disposable gown *C. auris* clinical 528 h 22 days, *C. auris* ambient 576 h 24 days; With respect to death kinetics, the values obtained on the glove surface are consistent with the death curve, but unlike disposable gowns, the values were not related to the death curves. According to the results obtained, it is important to manage and control Infections associated with health care since *C. auris* is a microorganism that persists for many days in fomites and can generate foci of new infections.

INTRODUCTION: The infections of nosocomial origin are known as infections that are acquired within the hospital or institutions that provide health services, which are not present in the patient at the time of admission [1]; some of these are the main cause of morbidity and Mortality with one in twenty hospitalized patients [2] is due to the increase in its incidence as well as due to the difficulty of its early diagnosis [3].

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Scientific evidence increasingly attributes infections associated with health care with surface contamination and environments including resistance to disinfectant agents [4, 5], micro organisms have been known for several years with the ability to develop such infections; Within this group are fungi, yeasts and bacteria; In the case of yeasts, a particular genus stands out causing a large number of cases, *Candida* sp. where the most common species reported are albicans, parapsilosis, tropicalis, glabrata, kruseiand lusitanie.

The genus Candida sp. several cases of infections associated with health care (IAAS) are attributed as a result of being more than a week in a health service in which candidiasis develops as a consequence due to colonization in different organs

or in some cases in torrent [6], early diagnosis in this type of candidiasis is a challenge, but it is key to diagnostic development and treatment [7]. Since 2009, several invasive candidiases attributed to *Candida auris* multi-drug fungus have been described worldwide, causing a wide range of infections associated with high mortality rates [8] in immunosuppressed and neonates. This microorganism has caused various outbreaks worldwide due to resistance to disinfectants; additionally, the rapid horizontal transfer has been described and one of the important factors involved with *C. auris* is its ability to adhere differentially to polymeric surfaces forming biofilms [9].

Candida auris is a novel and major fungal pathogen that has triggered several outbreaks in the last decade. The few drugs available to treat fungal diseases, the fact that this yeast has a high rate of multi-drug resistance and the occurrence of misleading identifications, and the ability to form biofilms (naturally more resistant to drugs) has made treatments of *C. auris* infections highly difficult [12]. *C. aurisin* America, was first reported in 2016 in immunosuppressed and neonatal patients who entered a medical office for reasons other than candidiasis; during their stay, they acquired the microorganism due to in-hospital infection [10], in Colombia, in the same year, 17 cases of isolated *C. auris* infection were reported in 6 institutions in the northern region of Colombia from February to July 20168. Considering the above, this project arises with the purpose of determining the persistence of *Candida auris* on different inanimate surfaces.

MATERIALS AND METHODS:

Reactivation of the Strain From the Primary Bank: *Candida auris* clinical (Ca1) and *Candida auris* environmental (H005913) was reactivated, as controls Candida parapsilosis (positive) strain (1172) [11] Saccharomyces cerevisiae (negative) strain (1184) was used [12].

The strains mentioned above were supplied by the Laboratory of Human Mycosis and Proteomics (MICOH-P) of the Pontificia Universidad Javeriana-Bogotá, the vials of the primary cell bank were taken, once thawed they were seeded in Papa dextrose Agar (PDA) (Potato extract 4.0 g/L, Dextrose 2.0 g/L, Agar 15.0 g/L, pH 5.6 ± 0.2) being incubated at 30 °C for 48 h.

Preparation of the Cell Suspension in Saline Solution: From the reactivated strains, a sample of each of the strains was taken with a round handle, which will be transferred to 16 ×150 mm tubes with 5 mL of 0.85% (w/v) saline solution until reaching the approximate turbidity to the tube number 3 of the Macfarland scale (9.0 × 108 UFC / mL), said the solution was made base 10 serial dilutions from 10^{-1} to 10-8 to determine the concentration in colony-forming units to be inoculated on surfaces.

Inoculation Purity Test: The purity test was carried out by means of identification by mass spectrometry (MALDI-TOF) [8, 13] of the isolated colonies; from these same counts, the selected concentration was determined, the identification. It was done by the direct identification method.

Direct Identification: With a sterile stick, a minimum amount of the colony was taken and spread over the circle of the plate, allowing to dry for one min, then 0.5 uL of formic acid was added again, dried, and the previous step was repeated again, afterward added the matrix allowing to dry, then the plate was placed in the MALDI-TOF reader.

Inoculation on Surfaces: The suspension prepared above is taken and inoculated on the surfaces to be evaluated (latex gloves and disposable gown); The selection criteria for these were due to the ease of space to be managed within the MICOH-P laboratory and are already implements used with frequencies within the hospital facilities. The area of the surfaces evaluated was (5 cm × 5 cm × 5 cm × 5 cm) to which 1 mL of said suspensions were added to each of them, Candida parapsilosis strain (1172) was used as a positive control [11], as a negative control Saccharomyces cerevisiae strain (1184) 12 and as study microorganisms will use two strains, Candida clinical auris (Ca1) and Candida environmental auris (H005913), all surfaces and hours sampling will be evaluated in triplicate.

Sampling For H: The inoculated surfaces were shown in the first hours as follows: 0, 0, 5, 1, 2, 4, 6, 12, 24, 48 h; after sampling the 48 h, it was continued sampled every 48 h until ho 672, the sampling was done with a sterile swab which was

rubbed in the delimited area of (5 cm × 5 cm × 5 cm × 5 cm), said swab was immersed in 4.5 mL of saline solution at 0, 85% (w/v), subsequently, dilutions were made up to [10, 3].

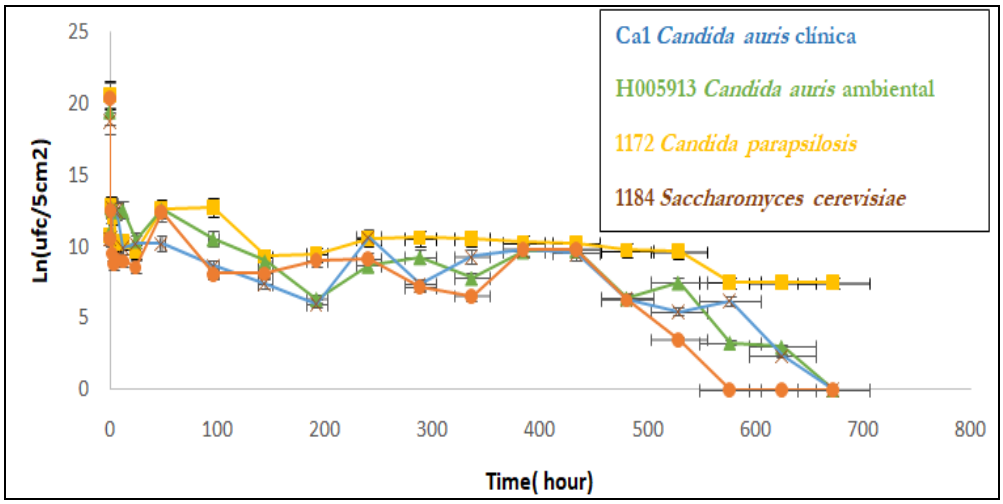

FIG. 1: DEATH CURVE OF CANDIDA AURIS, CANDIDA PARAPSILOSIS AND SACCHAROMYCES CEREVISIAE IN LATEX GLOVE

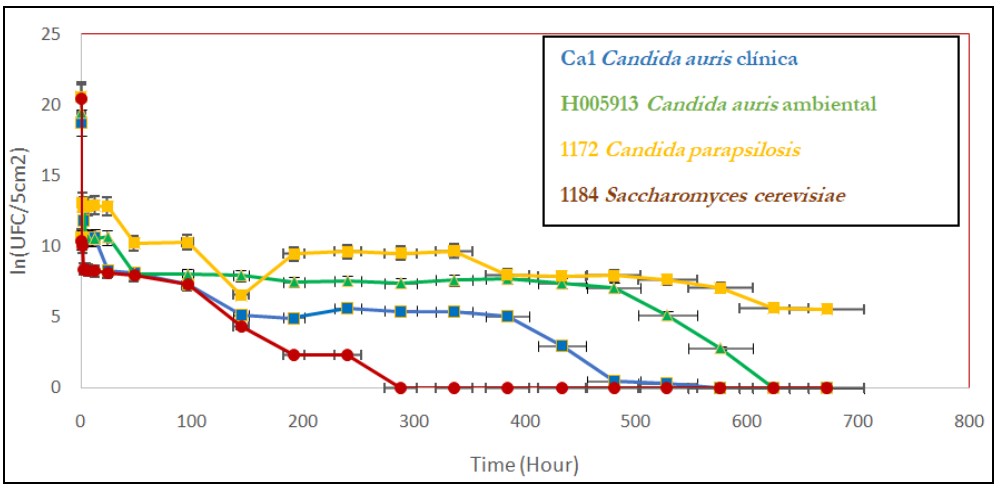

FIG. 2: DEATH CURVE OF *CANDIDA AURIS*, CANDIDA PARAPSILOSIS AND SACCHAROMYCES CEREVISIAE IN DISPOSABLE GOWN

Sowing by Surface: From each surface and sampling time, surface seeding was carried out in duplicate from dilution to 10^{-1} to 10^{-3} in Papa Dextrose Agar, incubated at 30 °C for 48 h, once growth is presented, counting will be performed in colonies plate.

Purification of Colonies: Of the colonies recovered at each h of sampling, isolation was performed in Papa Dextrose Agar, incubated at 30 °C for 24-48 h, in order to perform identification in MALDI-TOF.

Death Kinetics: Once the concentrations recovered from each surface were determined at different times, death kinetics analysis was performed using the formula where:

N: Concentration recovered/ no: initial concentration/ -kt: Specific death rate/ $N = N0e\ -kt$ (1)

Subsequently, by means of the straight equation, the specific speed of death is determined, which will be equal to the slope.

RESULTS:

Persistence of *Candida Auris* in Latex Gloves: **Fig. 1**. shows the behavior on latex gloves with each of the strains evaluated individually, the initial concentration of inoculum for one of the strains was 108 UFC/mL; the persistence of the *C. auris* strains (Ca1 and H005913) was of similar behavior from the h 0 -0.5, time in which a drastic decrease of (CFU / over the analyzed area) is evidenced; subsequently at h 1 there was a slight increase (Ca1

3.7 × 104 and H005913 3.4 × 10 5UFC/ 5 Cm^{-2}); after hour 1 until h 433 the behavior for the two strains was variable, in some h there were minimal increases and then decreases in UFC; from 433 to 624 the trend was decreasing, and at 672 there was no growth in any of the experimental *C. auris* strains (Ca1 and H005913). With respect to the behavior of the control microorganisms, Saccharomyces cerevisiae (1184), a negative control was evidenced by a decrease of almost 50% (4.0 × 104 CFU / 5 cm^{-2}) at h 0.5, later from this h until h 433 the behavior it was variable maintaining an approximate concentration of 103UFC/ 5 cm^{-2}, after h 433 and until 528 showed a linear tendency of decrease, as of h 576 there was no growth; situation contrary to what happened with C. parapsilosis (1172) positive control, who was persistent until h 672, this microorganism in h 0.5 as well as the other strains evaluated had a drastic decrease and after this time maintained a behavior almost stable until the last hour of sampling.

Persistence of *Candida Auris* in Disposable Gown: Fig. 2 represents the death curve of each microorganism evaluated on a disposable surgical gown, just like the previous experiment, each of these strains had an initial concentration of 108UFC / mL, at h 0.5 all microorganisms decreased 50%. of inoculum; *C. auris*, in this case, presented different behaviors among the strains analyzed, the most persistent in this case was *C. auris* Ambiental (H005913), which remained stable at the time 48-480 at approximate concentrations of 103UFC/ 5cm^{-2}, after this The h had a rapid decline, being persistent until 576; on the contrary, *C. auris* clinica (Ca1) from h 0.5 to h 96 showed a decrease in the concentration of UFC/ 5 cm^{-2}, from hour 144 to 384 it was stable at concentrations of 102UFC/ 5 cm^{-2} and again the low count from 433 to 528 last hour in which this microorganism persisted.

The positive control C. parapsilosis (1172) reduced the concentration from the 0.5 hour to the 144 h; once this time was reached, the behavior was stable at concentrations of 103-102UFC/ 5 cm^{-2}, the microorganism was present until the last hour of sampling, inverse to the negative control S. cerevisiae (1184) strain which decreased from 0.5 h to 240 h, the h evaluated from 288 to 672 resulted in 0UFC / 5 cm^{-2}.

DISCUSSION: When evaluating the persistence of *Candida auris* on glovessurface´s, it was found that for the *Candida auris* strains studied (Ca1-H005913), the microorganism was present until 624 h in both cases; the initial behavior from the 0 h to the 0.5 h evidenced a drastic decrease this possibly due to the requirement of nutritional sources of the microorganism [14, 15] which, when not being present, could have an influence on the adaptation of the microorganism on the surface; After this time, as shown in **Fig. 1**. there were slight increases in the UFC, a possible explanation for these increases as reported by Welsh in his study "is the adhesion of living cells to the exposed surface"8, in this case, latex gloves where the yeast cells divide but these, since they do not have sufficient nutritional substrates, the increase is minimal and for this same reason consecutively descends.

In this study, it was possible to determine that the persistence of *C. auris* in latex gloves is 624 h equivalent to 26 days without control of physical parameters; This finding contrasts with other studies in which they report the persistence of up to 14 days of *C. auris* on plastic surfaces under conditions of temperature and controlled humidity8; It has also been shown that *C. auris* survives on a variety of surface types, including dry, wet and plastic surfaces, indicating the potential for environmental contamination that this microorganism can develop [16] Persistence is attributed to stress tolerance levels, high temperatures [17] and biofilm formation [18, 19] however this study cannot be attributed the ability to persist the formation of biofilms since they were not studied, but at levels of tolerance to stress due to nutrient shortages.

The positive control C. parapsilosis (1172) was persistent until the last hour of sampling (672h); Like the *C. auris* strains, the control showed a decrease in UFC in the first h, a decrease attributable to the initial adaptation. The behavior of C. parapsilos is stable, maintaining approximate concentrations of 104 UFC/ 5 cm^{-2} from h 6 to h 528, then decreased a logarithmic unit until hour 672 (28 days); The research carried out by Traore in 2002 showed the persistence capacity of C. parapsilos is for 14 days on different surfaces, steel, glass and tissues [20].

Meanwhile, Welsh in 2017, used C. parapsilos is as a control microorganism and found a 28-day persistence on plastic 8; these results are equivalent to those obtained in Our study on latex gloves. On the other hand, Saccharomyces cerevisiae (1184) used negative control; it was present until 528 h, being the least persistent micro organism evaluated, however for the surface of latex gloves, it was not a negative control according to the study since it remained on the surface with a few hours apart from *C. auris* Clinical and environmental, with respect to Saccharomyces cerevisiae it is known that the mechanisms of adhesion of this microorganism are due to the presence of adhesins that generate the propagation of the microorganism on surfaces [21] and in this way they favor their colonization of surfaces.

The persistence of microorganisms in a disposable gown was lower compared to the surface of latex gloves *C. auris*; except C. parapsilosis (1172) strain that was persistent until the time 672 fig. two; the strains of *C. auris* analyzed showed different behaviors in latex gloves, in this case *C. auris* Ambiental (H005913) remained for a longer time on the surface of the disposable gown (576h- 24 days) but in comparison with gloves it persisted 48 h less, according to the results of Welsh again the persistence of the microorganism in this study is superior compared in the study carried out by 8; the strain *C. auris* (Ca1) remained present until the hour (528h-22days) with the results obtained and with previous studies, it can be considered that the material to be evaluated may or may not favor the colonization of *C. auris*; In this case, the lower porosity surface kept the microorganism longer.

Saccharomyces cerevisiae on this surface only persisted for (240 h 10 days) the concentration recovered from the microorganism was lower compared to the experiment developed in gloves at the h evaluated. A study realized by Jabeen in 2020, shows that *Candida auris* had higher persistence on latex and nitrile gloves with a transmission on the agar surface. It also had higher transmission from contaminated latex gloves to catheter surfaces. After *C. auris*, C. albicans showed the highest transmission from contaminated latex gloves to catheter surfaces. Proper glove use can therefore decrease the risk of healthcare-associated Candida infections, as would hand hygiene before touching other patients and surfaces [22]. In this study, the persistence of *C. auris*, C. parapsilosis and S. cerevisiae on surfaces of latex gloves and disposable gown was evaluated; it was determined that the physical and chemical characteristics may or may not allow colonization by microorganisms; in the area of health, it is sought that most medical devices are for sterile use; however, the spread of microorganisms depends on the use and composition of the implement; in the case of latex gloves the microorganisms used were less persistent than in the disposable gown, one of the possibilities is the porosity of the two surfaces, the gloves have less porosity compared to the gown, at the time the inoculum is added (suspension), the surface of the glove does not absorb but keeps the inoculum on it, contrary to the gown who does absorb the added inoculum, this is due to the composition of the implement.

Latex gloves consist of elastomer, polyethylene, silicone, or viny l23 hydrophobic substances 24, which do not allow the penetration of the inoculum but the adhesion on the surface of the glove. The disposable gowns are made of multiple fibers of fabric and polyethylene that, when interwoven, allow spaces, so it is presumed that the inoculum is filtered. Its concentration is lost or decreased, although the concentrations recovered from C. auris, were different in gowns. Latex gloves in the h evaluated, it is found that it is a microorganism resistant to physical conditions and is even able to stay on surfaces without nutritional sources for prolonged periods of time, causing possible sources of infection in institutions providing services of health.

CONCLUSION: The persistence of both clinical and environmental strains of *C. auris* (Ca1-h005913) in latex gloves was 624 h, and in disposable gowns, *C. auris* clinical (Ca1) was 528h and *C. auris* environmental (H005913) 576 h.

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