Archives of Gerontology and Geriatrics 65 (2016) 204–210

Contents lists available at ScienceDirect

Archives of Gerontology and Geriatrics

journal homepage: www.elsevier.com/locate/archger

Oral *Candida* isolates and fluconazole susceptibility patterns in older Mexican women

Beatriz Benito-Cruz[a], Saray Aranda-Romo[b], Francisco Javier López-Esqueda[c], Estela de la Rosa-García[d], Rebeca Rosas-Hernández[e], Luis Octavio Sánchez-Vargas[b,*]

[a] Master in Biomedical Science, Faculty of Medicine, University Autonomous of San Luis Potosí, Mexico
[b] Oral Microbiology, Pathology and Biochemical laboratory, Faculty of Stomatology, University Autonomous of San Luis Potosí, Mexico
[c] Geriatric Unit. Central Hospital "Ignacio Morones Prieto", University Autonomous of San Luis Potosí, Mexico
[d] Department of Attention to Health, Metropolitan Autonomous University, Xochimilco, México City, Mexico
[e] Molecular Biophysics laboratory, Physics Institute "Manuel Sandoval Vallarta", University Autonomous of San Luis Potosí, Mexico

ARTICLE INFO

Article history:
Received 8 July 2015
Received in revised form 9 March 2016
Accepted 3 April 2016
Available online 9 April 2016

Keywords:
Candida
Diabetes
Elderly
Infection
Epidemiology

ABSTRACT

Objectives: To assess the epidemiologic and microbiologic profile and *in vitro* fluconazole susceptibility of yeasts isolated from the oral mucosa colonization/infection of elderly patients.
Background: It has been reported that in older adults increases the oral colonization by *Candida* particularly *C.* non-*albicans*, showing a decreased response to fluconazole, which increases the risk of recalcitrant local and disseminated candidiasis.
Materials and methods: This was a prospective cross-sectional study conducted in 120 elderly patients. Oral samples were obtained of mucosal *Candida* colonization or infection by swabbing. Each sample was plated on CHROMagar® *Candida* and incubated ($36 \pm 1.5\,^{\circ}C$) for two days. The yeast species were identified using the API®ID32-C-AUX. Fluconazole susceptibility was tested using a broth microdilution assay according to the CLSI methods.
Results: The yeast colonization/infection frequency in the total population was 65.8%. The frequency of the highest *Candida* carriers was 67.4% in the 70–79-year-old-group. Oral candidiasis was present in 20%, with a tendency to increase with age (33.3% of adults aged > 80 years), it was determined that the use of prosthesis is associated with a higher colonization rate (Chi2, $p = 0.011$). The frequency of colonization/infection cases with more than one species showed a tendency to increase with age; 18.9% in the 60–69 year-old-group, 20.9% in the 70–79-year-old-group and 29.2% in the ≥80 year-old-group. About fluconazole susceptibility: for *C. albicans,* 20.3%, about *Candida* non-*albicans* species 15.3% were dose dependently susceptible (DDS) and 17.9% were resistant.
Conclusions: After 80 years of age, there is a considerable increase in *Candida* non-*albicans* species and a reduced susceptibility to fluconazole.

1. Introduction

Healthcare services will face the challenge of meeting the needs of an increasingly aging population over the next decades. The world population of over 65-year-old individuals is predicted to reach 400 million by the year 2050 (DESIS, 2012). In Mexico, this population is 9% of the total population (INEG, 2013a) and is highly affected by chronic degenerative diseases including diabetes and hypertension (Gutiérrez et al., 2012).

An aging population is at risk of developing diseases due to a number of factors, including systemic diseases and oral lesions. There are inherent factors, such as tissular senescence, mucosal fragility, alteration of function due to an impaired immune response, and reduction of the protective effect of the saliva because of low salivary flow. Second, there are extrinsic factors, such as poly-pathologies, including chronic degenerative diseases and malignancies, poly-medications and malnutrition (Bodineau, Folliguet, & Séguier, 2009). These factors, which contribute to changes during aging, may also disturb the balance in the oral microbial ecosystem. A disturbance in oral homeostasis between bacteria and fungi may cause oral infectious diseases. *Candida*

* Corresponding author at: Laboratory of Biochemistry, Microbiology and Pathology, Facultad de Estomatología, Universidad Autónoma de San Luis Potosí, Av. Dr. Manuel Nava No. 2. Zona Universitaria. C.P. 78290. San Luis Potosí, S.L.P., Mexico.
E-mail addresses: lo.sanchezvargas@gmail.com, octavio.sanchez@uaslp.mx (L.O. Sánchez-Vargas).

http://dx.doi.org/10.1016/j.archger.2016.04.001
0167-4943/

species are a part of the commensal oral microbiota of healthy individuals at all ages with a reported cultivable prevalence between 15 and 75% (Ghannoum et al., 2010; Ten Cate, Klis, Pereira-Cenci, Crielaard, & de Groot, 2009) and up to 80% in elders, especially denture wearers (Gusmão et al., 2011; Vanden Abbeele et al., 2008). The use of antibiotics and corticosteroids (Ben Dhaou et al., 2012), chemotherapy, malnutrition, premature birth and old age are among the most common predisposing factors for opportunistic mycoses (Pfaller & Diekema, 2010).

Candida in the oral cavity serves as a reservoir for inoculation and infections elsewhere in the body (Jobst & Kraft, 2006). When *Candida* penetrates the epithelium and invades the host tissues, septicemia and systemic infections may result (Mohandas & Ballal, 2011). These infections are difficult to treat with antifungals, particularly in older adults, and therefore have a high reported mortality (40%) (Pfaller & Diekema, 2007). The most common *Candida albicans* systemic infections, in addition to septicemia, are catheter-related, intra-abdominal and urinary tract infections; additionally, some groups have reported other *Candida* non-*albicans* species, which are particularly resistant to azoles such as fluconazole and other antifungals (Pfaller & Diekema, 2007). Candidemia is a leading cause of morbidity and mortality in both immunocompetent and immunocompromised critically ill patients (Pemán et al., 2011; Viale, 2009). The incidence of nosocomial yeast infections has increased markedly in recent decades, especially among older people. On the other hand, older adults are especially predisposed to oral infectious diseases such as candidiasis, which is associated with diabetes and prosthetic dentures. Wearing complete dentures is also a risk factor because they can promote *Candida* colonization, *Candidal* biofilm formation and oral candidiasis (Lotfi-Kamran, Jafari, Falah-Tafti, Tavakoli, & Falahzadeh, 2009).

Furthermore, the cases of invasive mycosis have been increasing; these are generated both in the intensive care unit and the outpatient setting, where most types of mycosis represent endogenous infection in which the normally commensal host microbiota take advantage of the "opportunity" to cause infection (Pfaller & Diekema, 2010). Given the reports of therapeutic failure (Pfaller & Diekema, 2004; Lattif, Mukhopadhyay, Banerjee, Goswami, & Prasad, 2011), it is very important to determine the profile of the antifungal susceptibility of oral *Candida* isolates and whether they are infectious or commensal. The aim of this study was to assess the epidemiologic and microbiologic profile and the *in vitro* susceptibility to fluconazole of yeasts isolated from the colonization or infection of the oral mucosa of elderly patients.

2. Materials and methods

2.1. Study design and clinical isolates

Following approval from the Ethical Committees of the participant institutions, we conducted a prospective cross-sectional study, conducted over a 6 month period in 120 older people participants (over the age of 65 during the period of study); half of these were sampled during a stay not exceeding three days in a second level hospital wing from the San Luis Potosí Central Hospital "Ignacio Morones Prieto". The second half of the population was sampled in recreation centers for older people [CEPITE—Centro Potosino de la Tercera Edad/Potosino Senior Center] under strict systemic, pharmacological and metabolic disease control. Characteristics considered to be risk factors for *Candida* infection were recorded. We analyzed the following factors: age, the presence of a complete and/or a partial dental prosthesis, natural dentition and oral hygiene. Underlying diseases including diabetes and arterial hypertension were noted. Other factors were also recorded, including systemic antibiotic therapy and all medications used. Older patients in intensive care and pre and post-surgical patients were excluded.

The participants were informed of the methods for sample collection and signed an informed consent form. Samples were taken following the same order in each patient. Briefly each sample was collected by passing a sterile cotton swab 10 times counter-clockwise across the dorsal surface of the tongue, soft and hard palate from incisive papila to uvula and buccal mucosa beginning from posterior right check and ending with the left. Each swab was placed in its sterile container with Stuart transportation medium (Copan, Italy) and taken to the laboratory within two hours to maintain the viability of *Candida*. Oral specimens were coded according to the clinical group and patient. Infection (candidiasis) was confirmed via cytology of the lesion: observing gemmation and *Candida* pseudohyphae and hyphae formation, using smears stained with periodic acid Schiff stain (Hycel, México).

2.2. Microbiological assessment and identification of isolates

Yeasts were identified to the species level using standard methods, such as the germ tube test, filamentation strains on Corn meal agar (BD, France)—Tween 80 (Hycel, México) plates (blastoconidia, pseudohyphae and true hyphae formation, as well as chlamydoconidia production) were identified by observing the plates at 10X magnification using a stereoscopic microscope (Leica® EZ4HD Microsystems, Singapore) also each colony smear was stained with Trypan blue (Sigma, USA) and observed by optical microscopy (100X) and carbohydrate assimilation patterns using the API ID 32C system (BioMerieux, France)

Each sample of oral mucosa collected was plated on differential and selective CHROMagar® *Candida* medium (CHROMAgar, París, France) and incubated aerobically at 36 °C for two days for *Candida* yeast growth; if fungi did not grow, they remained incubated for an additional period of seven days at a temperature of 30 ± 1 °C (ideal temperature for growth of fungi other than *Candida*) prior to evaluation as fungi-negative. With this chromogenic culture, a presumptive identification of *Candida* species was made. According to the colorimetric characteristics given by the manufacturer for each species, the use of this medium allowed us to separate two or more strains in overgrowth of different species from the same sample. Cultures that were positive for one or more species and purified cultures were reseeded and purified in plates of Sabouraud glucose agar (BD, USA) by incubating them at 36 °C (± 1 °C) for two days; the identifications were confirmed using an optical microscope (100X). Subsequently, the yeast species were identified through the API ID32C-AUX (BioMerieux, France) carbohydrate assimilation system and Apiweb database (BioMerieux, France). In our tests, reference *Candida* strains (*C. albicans* ATCC 90028, *C. krusei* ATCC 6258, *C. glabrata* ATCC 2001 and *C. tropicalis* ATCC 0750) were used as controls in each experiment. Previously phenotyped isolates were preserved in glycerol/YPD (Yeast extract, Peptone, Dextrose) broth 50:50 vol by freezing (−70 °C) for later use.

2.3. Fluconazole susceptibility testing

Prior to susceptibility testing, each isolate was sub-cultured on Sabouraud dextrose agar and CHROMagar *Candida* (CHROMagar, Paris, France) to ensure purity and viability. Susceptibility to fluconazole was tested using a broth microdilution assay, according to the methods (document M27-A3) of the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute, 2008) using fluconazole (Pfizer, Inc., New York, NY, USA).

For susceptibility test, a RPMI-1640 medium without bicarbonate was prepared with L-glutamine (SIGMA, USA) and buffered at