Species of *Candida* isolated from anatomically distinct sites in military personnel in Cuiabá, Mato Grosso, Brazil *

Leveduras do gênero *Candida* isoladas de sítios anatomicamente distintos de profissionais militares em Cuiabá (MT), Brasil

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Abstract: BACKGROUNDS: Some fungi are natural inhabitants of the human body but may result in disease when conditions are conducive to their development. Yeast infections are common and often occur in the skin and mucous membranes; however emerging species have changed this epidemiological profile. The ability to colonize different anatomical sites has been associated with the pathogenicity of Candida when environmental conditions are particularly favorable. In the case of hot, humid climates, the attrition suffered by the skin and weakened immune defenses may result in yeasts becoming pathogenic rather than commensal organisms.
OBJECTIVE: The objective of this study was to diagnose yeast infections in military personnel and to evaluate the frequency of these infections in the individuals evaluated.
METHODS: The clinical material analyzed was seeded in duplicate in Sabouraud dextrose agar (Difco™) and Mycosel medium (Difco™). The etiological agents were identified by observing the germ tubes, microculture and physiological characteristics, assimilation of carbon sources (auxanogram) and fermentation of carbon sources (zymogram).
RESULTS: Of a total of 197 patients evaluated, 91 (46.2%) had episodes of candidiasis. The genitocrural region was the most commonly affected area (47.7%) followed by the interdigital regions (between the toes or fingers) (27.8%). Candida albicans, *Candida parapsilosis*, *Candida tropicalis*, Candida glabrata and emergent species such as *Candida krusei* and *Candida guilliermondii* were found.
CONCLUSIONS: In the work environment, having to use shoes and uniforms for extended periods of time, in addition to stress and perspiration, were considered predisposing factors for the development of fungal infections.
Keywords: Candidiasis; Military personnel; Mycoses.

Resumo: FUNDAMENTOS: Alguns fungos são habitantes do organismo humano e podem vir a causar alguma doença, quando há condições propícias para seu desenvolvimento. Infecções por leveduras são comuns e frequentes em pele e mucosas; contudo, espécies emergentes têm alterado o perfil epidemiológico. A habilidade de colonizar diversos sítios anatômicos tem sido associada à patogenicidade do gênero *Candida*, quando as condições ambientais são particularmente favoráveis. No caso de climas quentes e úmidos, os atritos sofridos pela pele ou as defesas imunitárias debilitadas podem fazer com que as leveduras deixem de ser comensais para se tornarem organismos patógenos.
OBJETIVO: Diagnosticar candidíases em profissionais militares e avaliar a frequência dessas infecções nesses indivíduos.
MÉTODOS: Os materiais clínicos analisados foram semeados em duplicata nos meios Sabouraud Dextrose-ágar (Difco) e Mycosel (Difco). Identificaram-se os agentes etiológicos por meio da observação de tubo germinativo, microcultivo e caracteres fisiológicos, assimilação de fontes de carbono (auxanograma) e fermentação de fontes de carbono (zimograma).
RESULTADOS: De um total de 197 pacientes estudados, 91 (46,2%) apresentaram quadros clínicos de candidíases. A região genitocrural foi considerada a mais acometida (47,7%), seguida pelas regiões interdactilares (mãos e pés, 27,8%). *C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata* e espécies emergentes, como *C. krusei* e *C. guilliermondii*, foram identificadas.
CONCLUSÃO: No ambiente de trabalho, o uso de calçados e de uniformes por longos períodos de tempo, associado ao estresse e à sudorese, foi considerado fator predisponente para o desenvolvimento das infecções fúngicas.
Palavras-chave: Candidíase; Micoses; Militares

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INTRODUCTION

Fungi are free-living saprobes and are extremely common in nature. They are found as transient environmental colonizers on body surfaces; however, they obtain no obvious benefits. They have numerous effects on human beings; however, their role in infection is difficult to determine. [1]

Candida yeasts infect the skin and mucous membranes such as the lining of the mouth and vagina. They may invade deeper tissues such as the blood, resulting in systemic candidiasis. This much more severe infection is more common in immunodepressed individuals such as those infected by the human immunodeficiency virus (HIV), patients undergoing chemotherapy and those hospitalized for long periods of time in intensive care units. [2]

The capacity of yeasts to change from a commensal to a pathogenic condition when host conditions are favorable depends on several factors that are propitious to the fungus, allowing it to live off patients who are debilitated and susceptible to the opportunistic microorganism. [3,4]

Candida yeasts live in the mucous membranes, skin, gastrointestinal tract and vaginal canal, and are normally harmless. When environmental conditions are particularly favorable, especially in hot, humid climates or when the host immune defenses are debilitated, yeasts manage to infect the skin, becoming pathogenic rather than commensal and resulting in a skin infection or even a generalized and potentially fatal infection. [2,5]

Candida yeast infections are known as candidiasis or candidosis. These yeasts form part of the endogenous microbiota present in the human body. Candida albicans and other pathogenic species are rarely found on the hairless skin of healthy individuals, with the majority of yeast infections of this type tending to develop in moist intertriginous areas, caused by maceration or occlusion. [6]

The objective of the present study was to evaluate the frequency of candidiasis in the skin, nails and mucous membranes of military personnel and to identify the etiological agents involved in infections found in this sample population. It should be emphasized that this type of occupational activity may increase the rates of colonization in certain regions of the body, particularly those involving Candida yeast infections. This is the first study of this type to be conducted in the state of Mato Grosso, Brazil.

MATERIAL AND METHODS

Patients: A total of 197 male military personnel with symptomatology and/or lesions compatible or suggestive of yeast infections were evaluated between January and October 2009. The most common clinical findings were: foot intertrigo, Candida nail infection, genitocrural intertrigo and localized lesions.

All the military personnel included in this study filled out a questionnaire regarding their type of work, the number of hours they worked per day, hygiene and personal care, predisposing factors and any previous use of antifungal medication. Biological samples were collected in accordance with the anatomical site and the characteristics of the lesions reported by the individuals. The study was approved by the Internal Review Board of the Júlio Muller Teaching Hospital (HUJM) under approval number 495/CEP-HUJM/08. The samples were processed at the Mycology Laboratory of the General Teaching Hospital, using standard methodology (direct examination and culture for fungi) in accordance with the techniques described by Lacaz et al. [6] The direct examination of specimens was performed by preparing fresh slides clarified with 20% or 40% potassium hydroxide (KOH) aqueous solution in accordance with the specificity of the clinical material. Next, the material for analysis was seeded in duplicate in Sabouraud dextrose agar (Difco™) and Mycosel medium (Difco™). The tubes were kept at 25oC and 30oC for 7-10 days after which the species were identified according to the criteria established by De Hoog. [7]

Clinical isolates: Following appearance of the colonies in culture medium, the micromorphological features of the yeast were analyzed using the Gram staining technique. [8] The etiological agents were identified in accordance with the classic methodology based on germ tube formation - Reynolds & Braude effect, micromorphological features (presence of pseudomycelium, true mycelium and the production of chlamydospores) and physiological characteristics - assimilation of carbon sources (auxanogram) and fermentation of carbon sources (zymogram). [6,7,9]

RESULTS

A total of 91 individuals (46.2%) tested positive for candidiasis in accordance with the aforementioned methodology. Overall, 210 Candida yeast isolates were obtained from various anatomical sites: the skin, mucous membranes and nails. This difference is due to the fact that, in many cases, more than one species of yeast was found in the same sample of material collected, thus resulting in a greater number of isolates (210) compared to the number of patients analyzed (n=197). In 94 of these isolates (44.7%), numerous sprouting yeast cells with pseudomycelia formation had already been detected by direct microscopy, while in 104 (49.6%), yeast identification followed isolation in culture (Figure 1).

The age of the individuals evaluated ranged from 18 to 50 years, with a mean of 26.6 ± 9.1 years (mean ± standard deviation [SD]); 95% confidence interval (95%CI) ± 1.3. C. albicans was the most commonly found species in all the age groups evaluated, while C. parapsilosis was more common in individuals of 18-20 years of age (Table 1).

According to the individual interviews, the duration of the disease ranged from 1 to over 12 months.

Overall, C. parapsilosis was the second most commonly found species, accounting for 31 cases (20.5%), followed by C. tropicalis (n=21; 13.9%) (Table 2).

The anatomical site most commonly affected by the fungal infections was the genitocrural region (n=72 cases; 47.6%) followed by the feet (n=30 cases; 19.9%) (Table 2).

The isolates referring to yeast infections of the inguinocrural region and genital mucosa of this male population were identified as: Candida albicans (n=25; 16.5%), Candida parapsilosis (n=14; 9.2%) and Candida tropicalis (n=12; 7.9%). Other species such as Candida krusei and Candida guilliermondii, considered emerging pathogens, were also isolated (Table 2).

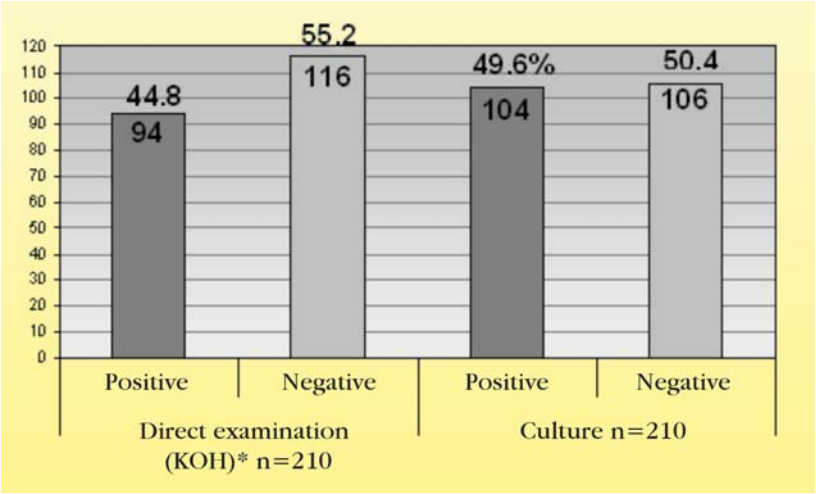
DISCUSSION

Different profiles of Candida species in different geographical locations have been reported in the literature in numerous samples evaluated in Brazil and worldwide. [10-14]

Some studies that analyzed a variety of anatomical sites reported C. albicans as being the most commonly isolated yeast. One study conducted in Brazil to evaluate yeast infections in 100 patients affected by superficial candidiasis confirmed this finding, reporting the presence of C. albicans in 76.0% of superficial lesions in various parts of the body. 10 According to King, this fact is explained by the greater capacity of C. albicans to adhere to the mucosal surface. [15] However, Law et al. commented that adherence is a prerequisite for the colonization and multiplication of yeasts. [16] In this study, C. albicans was the most commonly isolated agent (n=52; 34.4%). On the other hand, some authors evaluating fungal skin infections have reported C. parapsilosis as being the most commonly found microorganism. [11] Others, however, failed to find C. albicans in skin scrapings from healthy individuals, with C. tropicalis, present in 3.87% of cases, being the most commonly found species. [17]

The conditions in the work environment and the continuous use of closed footwear, the stress associated with military service and the body heat provoked by the use of uniforms leading to intense perspiration, all constitute factors possibly related to the yeast infections in the anatomical sites investigated.

The most common relevant habits in the population evaluated included the friction caused by the use of often tightly-fitting military boots and the impact caused by the weight of these boots, together with the perspiration and heat caused by socks and also the contact of feet with the floors of communal bathrooms. Military personnel commonly experience trauma to the feet, which may be caused by continuous walking and marching, activities that are inherent to the profession. In general, the feet of these professionals are exposed to the risk of developing blisters, calluses, various types of lesions, and broken nails. These hypotheses are in agreement with reports published by Purim et al. affirming that the incidence of Candida yeast infections in Brazilian football players was attributed to alterations in skin pH, injury and perspiration, in addition to the emo-



Graph 1: Results of mycological examination in 210 clinical samples collected from 197 military personnel affected by suspected candidiasis. Cuibá, Mato Grosso, Brazil, 2009

**KOH: Potassium hydroxide*

Table 1: Distribution of yeasts of the Candida genus according to age-group, isolated from 210 clinical samples collected from 197 military personnel affected by suspected candidiasis lesions. Cuiabá, Mato Grosso, Brazil, 2009

	Age-group (years)			
Species	**18|20**	**21|30**	**31|40**	**41|50**
Candida albicans	21	13	11	7
Candida catenulata	1	-	-	-
Candida ciferrii	1	-	-	-
Candida glabrata	7	5	3	
Candida guilliermondii	3	5	3	2
Candida intermedia	1	-	-	-
Candida kefyr	1	-	1	-
Candida krusei	2	1	2	1
Candida lusitaniae	2	1	-	1
Candida novergensis	2	-	-	-
Candida parapsilosis	15	6	9	1
Candida pulcherrima	-	-	1	-
Candida tropicalis	11	7	1	2
Candida viswanathii	1	-	-	-
Total	68	38	31	14

tional stress that is present in all sporting activities, thus contributing towards reducing the body's defenses and facilitating fungal infections. [18] A similar study conducted to investigate the routine of military police officers evaluated the functional characteristics of the boots worn by these professionals on their daily beat and found that this type of footwear may contribute towards discomfort, physical tiredness and overheated feet, favoring the appearance of trauma and infection in this part of the body. [19]

Micromorphological structures such as pseudomycelium and true mycelium, as found at direct examination, reinforce the state of infection in these patients. Some authors consider that this differentiation facilitates adhesion, invasion and dissemination in the host tissue. [6]

In 2005, investigators in Turkey evaluated 155 male patients with various pathologies, who were receiving routine care at the Urology Department of a city hospital, and reported 10 cases of intertrigo caused by Candida yeast in the inguinocrural region (25.6%), Candida albicans being the most common agent isolated. [12] Nevertheless, other authors evaluated the enzymatic capacity of Candida yeast and identified virulence in strains of C. albicans isolated in the urogenital tract and skin of adult patients, these yeasts being associated with the incidence of infections. [13]

The results obtained in the present study may reflect the colonization of skin yeasts in the interdigital regions of the feet of military personnel using occlusive footwear. A study conducted in the Amazon region in which the authors investigated yeast colonization in skin scrapings from the hands and feet of 1,296 apparently healthy inhabitants of three riverbank communities in the Amazon region showed that 10.1% of the population evaluated tested positive for yeast infection. [17] Purim et al. investigated fungal microbiota from the feet of Chinese and Brazilian football players and found Candida yeast infections in 34.4% of a sample of 129 Brazilian users of occlusive footwear. [18] On the other hand, in 2006 and 2007, investigators evaluating the prevalence of etiological factors of superficial mycoses on the feet and nails of 1,300 and 650 Algerian military personnel, respectively, found a prevalence of 68% of Candida yeast infection on the feet of these individuals, C. parapsilosis being the principal causal agent both on the skin of the feet and in the toenails in 28.2% and 18.7% of individuals, respectively.[14,20] Other authors, however, have reported lower rates compared to the present study, in which a prevalence of 15.2% (n=23) of Candida yeast infections was found in the toenails. [17] Some investigators have shown C. parapsilosis to be the principal agent in infections in this part of the body. 20 A study conducted in Brazil showed C. tropicalis (30%) to be the most commonly found species in interdigital mycosis of the feet in military personnel in the southwestern part of the state of Paraná. [21] These percentages are in conflict with the results of the present study in which C. albicans was the most common etiological agent (Table 2).

In general, onychomycosis is reported as being the most common fungal infection in adults. Investigators claim that the increasing age of the population is an important cause of this increase in onychomycosis. [22] Cases of yeast infection of the nail were reported in the scientific literature by investigators evaluating onychomycoses in 504 patients in Tehran. [23] Onychomycoses were confirmed in 216 (42.8%) of the 504 cases analyzed. Those authors found yeast infections in 129/216 cases (59.7%), the most common causal agents being Candida albicans (n=42) and Candida spp (n=56). Almost half the cases with a clinical suspicion of a fungal infection of the nails were caused by yeast infections. Iranian authors evaluating the epidemiology of 137 cases of onychomycoses in the city of Kashan found yeast infections to be the principal cause of onychomycoses, with C. albicans being the most common (n=7; 26.9%), followed by the other species, which in 4 cases (15.4%) were unidentified. [24]

Differences between nail infections may be the result of peculiarities in the lifestyle of the different populations analyzed. On the other hand, the use of

Table 2: Distribution of the species of yeasts of the Candida genus, according to the anatomical sites of the infection, isolated from 210 clinical samples collected from 197 military personnel affected by suspected candidiasis lesions. Cuiabá, Mato Grosso, Brazil, 2009

	Folliculitis	Candidiasis Genitocrural			Candidíasis interdigital		Candidíasis Nails			
Candida spp.	**Beard**	**genital**	**crural**	**Mucous Membranes**	**Hands**	**Feet**	**Hands**	**Feet**	**N**	**%**
C. albicans	3	1	11	13	4	10	4	6	52	34,4
C. catenulata	-	-	-	-	-	-	-	1	1	0,7
C. ciferrii	-	-	-	-	-	1	-	-	1	0,7
C. glabrata	1	-	4	2	1	4	-	3	15	9,9
C. guilliermondii	-	1	5	1	-	2	1	3	13	8,6
C. intermedia	-	-	-	-	1	-	-	-	1	0,7
C. kefyr	-	-	1	-	1	-	-	-	2	1,3
C. krusei	-	-	1	1	2	-	-	2	6	4,0
C. lusitaniae	-	-	2	1	-	1	-	-	4	2,6
C. novergensis	-	-	1	1	-	-	-	-	2	1,3
C. parapsilosis	3	2	7	5	2	7	1	4	31	20,5
C. pulcherrima	-	-	-	-	-	1	-	-	1	0,7
C. tropicalis	1	-	7	5	1	3	-	4	21	13,9
C. viswanathii	-	-	-	-	-	1	-	-	1	0,7
Total	8	4	39	29	12	30	6	23	151	100
%	5,3	2,6	25,8	19,2	7,9	19,9	4,0	15,2	-	100

boots, often for periods exceeding 11 hours a day, may promote perspiration, friction and a propensity to develop fungal infections. Investigators in Spain reported that the use of enclosed footwear and socks contributes to fungal infection. [25] In a study conducted in Adana, Turkey, Ilkit reported that the toenails were more commonly involved in fungal infections than the fingernails. [26] The prevalence of onychomycoses of yeast origin found by this Turkish investigator in the toenails was 87.6%. Similar findings were also reported in Iran, Algiers, Canada and Italy, these investigators reporting the isolation of yeasts in fungal infections of the nail. [14,23,27,28] Investigators in Brazil conducted dermatophytic screening at the Araraquara Health Unit and found 15 cases of yeast infection (16.2%). [29] The results obtained in the present study, in which 23 cases of Candida yeast infection were detected in the toenails, a rate of 15.2%, are in agreement with the findings of the abovementioned investigators.

With respect to the species of Candida yeast identified, investigators in Iran evaluated patients with fungal infections of the skin and nails in Tehran and reported similar findings to those reported in the present study with respect to the species isolated: Candida albicans 44 (16.73%), C. parapsilosis 24 (9.12%) and C. tropicalis 23 (8.74%). 30 These percentages are close to those found in the present study: C. albicans 52 (33.4%), C. parapsilosis 31 (20.5%) and C. tropicalis 21 (13.9%) (Table 2).

This was a pioneering study in Brazil and highlights the idiosyncrasies associated with military activities as possible factors that may predispose towards yeast infection, particularly by species of Candida. It should be emphasized that the bioclimatic conditions in Cuiabá, Mato Grosso may provide an extremely favorable environment for these infections, a factor that is potentiated when associated with the type of clothing used during military duty. ❑

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