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Mixed secondary bacterial infection is associated with severe lesions of chromoblastomycosis in a neglected population from Brazil

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ABSTRACT

Chromoblastomycosis (CBM) is a chronic subcutaneous infection caused by melanotic fungi, affecting mainly rural workers in tropical and subtropical regions. Secondary bacterial infections (SBIs) in CBM lesions bring complications to the disease, but little is known about the agents involved. Fungal and bacterial identification and epidemiological profile of 50 patients with CBM were analyzed in this study. Bacteria were tested for susceptibility to antibacterial drugs. *Fonseacea pedrosoi* and *Rhinocladiella aquaspersa* were the fungal agents isolated. 88% of the patients presented SBI. Gram-positive bacteria coinfected mainly upper limbs, and Gram-negative bacteria were more isolated from lower limbs. *Streptococcus pyogenes* and mixed bacterial microbiota were associated with severe lesions. *Staphylococcus aureus* was associated with mixed infections and consequently with the severity of the infection. Resistance to β-lactams and methicillin was detected. Our results emphasize the necessity of bacterial culture and susceptibility testing as part of routine monitoring CBM cases.

1. Introduction

Chromoblastomycosis (CBM) is a chronic subcutaneous infection caused by melanotic fungi and affects mainly rural workers. The disease occurs worldwide but is more prevalent in regions with tropical and subtropical climates (Queiroz-Telles, 2015). In Brazil, the infection has been observed in all states, but the higher number of cases occurs in an endemic area in states within the Amazon forest (Avelar-Pires et al., 2013; Gomes et al., 2016; Queiroz-Telles et al., 2017). Of the 9 Amazon states, Maranhão ranks first in chromoblastomycosis notifications (Gomes et al., 2016), and most cases affect native populations and are concentrated in the "Baixada Maranhense" area (Marques et al., 2006). The main etiological agents are members of *Fonsecaea*, *Cladophialophora, Phialophora*, and *Rhinocladiella* genera and belong to the order Chaetothyriales. Infection occurs by transcutaneous trauma with the implantation of fungi (Gomes et al., 2016). The disease hallmark is the presence of muriform cells, embedded in granulomatous and suppurative tissue that can slowly evolve to a polymorphic lesion (nodules, plaques, tumors, warts, and scar tissue) causing pseudoepitheliomatous hyperplasia with keratolytic microabscess formation in the epidermis. The primary lesion is usually solitary, presenting as a small, pink, smooth, papular skin lesion. The papules gradually increase in size, forming irregular plaques with a squamous or verrucous center (Queiroz-Telles et al., 2009).

In this scenario, as the severity increases along with time, bacterial coinfections may bring significant complications to the disease, leading to limitations of labor activities. The damaged skin provides an opportunistic environment for different bacterial species to succeed and cause secondary/sequential cutaneous infections (Bakaletz, 2004; Pasman, 2012). In the most severe cases, chronic lymphoedema and ankylosis develop, and noninvasive squamous cell carcinomas may arise from chronic lesions (Azevedo et al., 2015a, 2015b). Nevertheless, studies focused on the determination of bacterial infection in CBM lesions are essential in order to improve treatment and prognosis. This study aimed

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to analyze the clinical and epidemiological profile of patients with CBM, identify the microorganisms responsible for secondary bacterial infections (SBIs) associated with the site and severity of lesions, and assess the resistance profile for antibacterial drugs.

2. Methods

2.1. Patients and ethic statements

Fifty patients with a clinical diagnosis of CBM were recruited by spontaneous demand in the period from September 2006 to October 2009 at the Clinic of Infectious and Parasitic Diseases, Department of Pathology, Federal University of Maranhão (UFMA), state of Maranhão, Brazil. This work was approved by the Research Ethics Committee-CEP-HUUFMA (University Hospital of the Federal University of Maranhão), according to Brazilian Resolution–Protocol number 019/06. These lesions were classified as mild/light forms when they involve a solitary plaque or nodule measuring less than 5 cm in diameter. The moderate form consisted of solitary or multiple lesions, which may be nodular or verrucous plaques and present alone or in combination, covering 1 or 2 regions of underlying skin and measuring less than 15 cm in diameter. Finally, the severe form included some types of lesions presenting alone or in combination that covered a large area adjacent or not adjacent to the skin (Queiroz-Telles, 2015; Queiroz-Telles et al., 2009, 2017).

2.2. Mycological examination

A fragment of the lesion was examined by optical direct microscopy using potassium hydroxide (KOH) 20%. For macromorphological analysis, isolated clinical strains were grown on Sabouraud Dextrose Agar (SDA, Difco Laboratories, Detroit, MI) and mycobiotic agar (MA, Difco) and incubated at 28 °C, 37 °C, and 42 °C for 2–3 weeks. For micromorphological studies, slide cultures were prepared. To confirm the phenotypic identification of the fungal isolates, the genomic DNA was extracted with hexadecyl trimethyl ammonium bromide buffer, together with a 2:1 silica gel and celite mixture, following the method previously described by De Hoog et al. (2004). DNA amplification by PCR was performed with all samples with specific primers: Fon-F (forward; 5′-TAATGCGGGTGTTGCCTCTG-3′) and Fon-R (reverse; 5′-AGGGGTG GAAAGTGTGAACT-3′), as described by Abliz et al. (2003). Additionally, all samples were amplified with ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) primers (Abliz et al., 2003). PCR was performed in a 12.5-μL volume of reaction mixture containing 1× PCR buffer, 2.0 mM $MgCl_2$, 25 μM dNTPs, 0.5 μM of each forward and reverse primer, 1 U of BioTaq DNA polymerase, and 10 ng of genomic DNA. The homology evaluations were done on GenBank database using BLAST analysis, available at www.ncbi.nlm.nih.gov. All sequences attained in this study were submitted to GenBank database.

2.3. Bacterial culture and susceptibility testing to antibacterial drugs

Tissue fragments were also plated on MacConkey medium (Difco), blood agar, and brain heart infusion (BHI, Difco) and incubated at 35.5 °C for 24–48 h under aerobic, microaerophilic, and anaerobic conditions. The bacterial identification was performed through an automated method using the Vitek Compact system (bio-Merieux, Marcy l'Etoile, France). The antimicrobial susceptibility testing was conducted with the disk diffusion method (Kirby–Bauer) according to the interpretation criteria of the Clinical and Laboratory Standards Institute document M-100 (CLSI, 2008) Strains obtained from American Type Culture Collection (ATCC) were used as quality control for the culture media and reagents: *Pseudomonas aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 700603, *Staphylococcus aureus* ATCC 25923 and 29213, *Enterococcus faecalis* ATCC 29212, and *Proteus mirabilis* ATCC 7002.

2.4. Statistical analysis

Qualitative variables are presented by frequencies and percentages, and quantitative variables, as the mean and standard deviation (mean ± SD). To assess the association between qualitative variables (severity of the lesions, microorganisms isolated, absent microbiota, single microbiota, mixed microbiota, and injury location), we used the χ^2 test. The level of significance was set at 5%. The data were analyzed using the Stata data analysis software version 14.0.

3. Results

3.1. Demographics and clinical features of patients

The patients were predominantly male (98%), with a mean age of 59 ± 10.6 years. The percentage of subjects who could not read or write was 18%, and 82% had some difficulty reading and writing. Most (92.7%) were from a rural area of Maranhão state, where the main occupation was rural worker (88%; farmer, potter, and fisherman) (Table 1). Regarding the localization of the lesions, 80% were on the lower limbs, 14% on the upper limbs, and 4% in other anatomical sites. The patients involved in this study had lesional polymorphisms (60%), with the most common being plaques, with or without verrucous or nodular lesions. Concerning the extent of the lesions, 44% of patients had severe lesions; 32% were moderate; and in 24%, the lesions were mild. The duration of the disease ranged from 1 to 30 years, with a mean of 8.8 ± 7.8 years. Of the patients studied, 58% used medication at the time of collection; 52% took itraconazole alone or in combination with amphotericin B (6%).

3.2. Mycological identification of fungal isolates from patients who presented secondary bacterial infection

The morphological examination and molecular diagnosis based on ITS sequence analysis of the isolates studied revealed that 96% of the cases were caused by *F. pedrosoi* and 4% by *R. aquaspersa.*

3.3. Bacterial agents, susceptibility testing, and association with clinical aspects of the lesion

In the present study, all patients with SBI presented clinical signals of secondary single or mixed bacterial infection, as purulent discharge in lesions. Laboratory tests confirmed that 44 patients had SBI, and 22 (44%, considering all the patients included) of the cases were single SBI and 22 (44%) were mixed (Table 3). Sixty-eight strains of bacteria

Table 1
Chromoblastomycosis: demographic data.

Sex	*n* (%)	Age, y	*n* (%)	Occupation	*n* (%)	Education	*n* (%)	HDI	*n* (%)
Male	49 (98)	40–50	12 (24)	Farmer	44 (88)	Illiterate	9 (18)	Low	25 (50)
Female	1 (2)	50–60	11 (22)	Potter	1 (2)	Low literate	41 (82)	Moderate	20 (40)
		60–70	17 (34)	Fisherman	1 (2)			High	5 (10)
		≥70	10 (20)	Carpenter	1 (2)				
				Greengrocer	1 (s2)				

y = years; HDI = human development index.

were isolated, and 54 (79.39%) were Gram-positive cocci and 14 (20.61%) were Gram-negative bacilli. Among the Gram-positive cocci, *S. aureus* was the most prevalent (30; 44.11%), followed by coagulase-negative *Staphylococci* (CoNS) (7; 10.29%), *Streptococcus agalactiae* (5; 7.35%), and *S. pyogenes* (5; 7.35%). Among the enterobacteria, *Proteus mirabilis* accounted for 3 (4.41%) of the infections, followed by *Klebsiella pneumoniae* (2; 2.94%) and *Morganella morganii* (2; 2.94%). Among the Gram-negative nonfermenting glucose, 2 (2.94%) of the isolates were *P. aeruginosa*, 2 (2.94%) were *Burkholderia cepacia*, and 1 (1.47%) was *Alcaligenes xylosoxidans* (Table 4).

When we analyzed the association between the bacterial agents and the clinical aspects of the lesions, we observed a statistically significant association between *S. pyogenes* and severe lesions ($P = 0.038$) (Table 3). There was also a statistically significant association between mixed bacterial microbiota and the severity of lesions ($P = 0.023$) (Table 3). In addition, *S. aureus* is associated with a mixed infection ($P = 0.001$) (Table 5).

The isolates were also tested for antibiotic susceptibility. A hundred percent of the Gram-positive cocci were susceptible to vancomycin, and the isolates of *S. aureus* and CoNS showed a high level of susceptibility to ampicillin/sulbactam, cefazolin, clindamycin, gentamicin, oxacillin, and rifampicin. These strains have shown a higher rate of resistance to erythromycin, penicillin, and sulfamethoxazole trimethoprim. The species of *Streptococcus* showed susceptibility to antimicrobials but also exhibited a high level 4 (50%) of resistance to tetracycline (Table 6). The 7 CoNS strains showed susceptibility only to ampicillin/sulbactam, ceftazidime, minocycline, rifampicin, and streptomycin (Table 6).

A hundred percent of Enterobacteriaceae showed susceptibility to ciprofloxacin, aztreonam, cefepime, ceftazidime, imipenem, meropenem, and ertapenem. Although only a single sample of *Citrobacter diversus* was isolated, it was resistant to several antibiotics. Overall, the Enterobacteriaceae were resistant to sulfamethoxazole-trimethoprim and ampicillin. Among the nonfermenting bacilli, *P. aeruginosa* showed the highest rate of resistance in relation to antimicrobials (Table 7).

4. Discussion

The studied population has similar HDI to African countries, such as South Africa, Congo, and Uganda (United Nations Development Programme, 2015), countries assisted by international humanitarian organizations, unlike the municipalities of Maranhão. CBM is an endemic disease whose intervention in social aspects plays a crucial role in its occurrence, chronicity, and coinfection with bacteria.

Secondary infections emerge when other risk factors contribute to the colonization of a second pathogen. The predisposition of patients with primary skin infection to the development of sequential bacterial infections is associated with a defective epidermal barrier, raised adhesion activity of bacteria to skin cells, impaired elimination of these bacteria, and impaired innate and acquired immunity (Brook, 2002). These secondary skin infections are frequently caused by microbiota from endogenous oral and skin sites, or by environmental bacteria, leading to the lesion worsening and to treatment failure. CBM is able to alter skin microbiota, where commensal bacteria may become pathogenic. This is the first study to detail secondary infections in CBM lesions. Our study included 50 cases, making it the largest epidemiological study of CBM in Brazil.

CBM is usually an occupationally related disease, affecting individuals worldwide, and is one of the most frequent implantation mycoses found among rural populations (Marques et al., 2015). Our results show that male farmers who are illiterate or possessing little education, aged 30–60 years and living in places of low HDI, are the most affected group by CBM (Table 1). The low degree of training associated with recurrent exposure to a fungal niche without suitable personal safety equipment classifies this population as neglected. In Maranhão, babaçu is the main source of income for rural workers, and (Marques et al., 2006; Nascimento et al., 2014) babaçu coconut breakers are often affected by CBM. Epidemiological data revealed that there are more than 300,000 coconut workers daily exposed to CBM agents since they use the babaçu coconut as a source of income in the states of Maranhão, Pará, Tocantins, and Piauí (Marques et al., 2006).

Several reports showed that *F. pedrosoi* is the main etiologic agent of this mycosis in various regions of the world, including Japan, India, Madagascar, Sri Lanka, Brazil, Mexico, China, and Cuba (Coelho et al., 2018; Marques et al., 2006). But other agents, although less frequent, may be involved, such as *F. monophora*, *F. nubica*, *F. pugnacious*, and *Rhinocladiella aquaspersa* (Azevedo et al., 2015a, 2015b). The ability to distinguish the species of agents of the disease is clinically significant because of differences in prognosis of the infection. But the diagnoses, which require tissue biopsy and culture, are rarely performed or do not exist in endemic areas (Queiroz-Telles et al., 2009) since health care services are mainly restricted to urban areas. This fact may explain the chronicity degree associated with the disease in patients from this study. According to Queiroz-Telles et al. (2009), CBM lesions are polymorphic and should be differentiated from those associated with various clinical conditions. In advanced cases of CBM, more than 1 type of lesion can be observed in the same patient. As noted in our results, patients with severe lesions were predominant compared with those with moderate lesions, and we found a lower rate of mild lesions. Many factors may have influenced this: absence of rapid diagnosis and treatment (which are directly influence by the social and economic status of the population studied), refractory therapy, and complications such as SBIs.

A large proportion of patients (88%) with CBM had SBI (Table 2). Our results revealed a large number of bacterial species isolated from these cases, and 79.39% were Gram-positive bacteria, with *S. aureus* being the pathogen most frequently isolated from all

Table 2
Chromoblastomycosis: clinical aspects.

Clinical topography	*n* (%)	Clinical lesion	*n* (%)	Disease duration/y	*n* (%)	Treatment	*n* (%)	Injury severity	*n* (%)	Etiology	*n* (%)
Lower limbs	40 (80)	N	1 (2)	1–10	34 (68	NT	21 (42)	Mild	12 (24)	*F. pedrosoi*	48 (96)
Upper limbs	7 (14)	V	1 (2)	10–20	10 (20)	ITRZ	26 (52)	Moderate	16 (32)	*R. aquaspersa*	2 (4)
Lower + upper limbs	1 (2)	P	18 (36)	20–30	6 (12)	ITRZ+AMB	3 (6)	Severe	22 (44)		
Cervical	1 (2)	P + V	10 (20								
Chest	1 (2)	P + N	6 (12)							**Bacterial infection**	*n* (%)
		P + T	1 (2)							Absent	6 (12)
		V + C	1 (2)							Present	44 (88)
		V + N	2 (4)								
		P + N + V	6 (12)								
		P + N + C	1 (2)								
		P + N + T	1 (2)								
		P + C + V	2 (4)								

y = years; N = nodular; V = verrucous; C = cicatricial; P = plaque; T = tumorous; ITRZ = itraconazole; AMB = amphotericin; NT = no treatment.

sites (lower and upper limbs, and chest) (Table 4) and associated with mixed infection ($P = 0.001$) (Table 5). Although *S. pyogenes* was associated with severe lesions ($P = 0.038$), in all cases of *S. pyogenes* coinfection, there was mixed *S. aureus* infection (Table 5). In this sense, *S. aureus* is an important secondary pathogen in CBM lesions, being prevalent in all types of lesions (mild, moderate and severe). Other studies investigating a variety of skin diseases, such as scabies, psoriasis, atopic dermatitis, eczema herpeticum, kerion, and leishmaniosis, have also shown a high prevalence of this microorganism (Amissah et al., 2015; Serra et al., 2015; Sonesson et al., 2017). In this context, it is important to discuss the composition of normal microbiota, which depends on the colonized area of the human host. Generally, *S. aureus* and negative-coagulase *Staphylococci*, mainly *S. epidermidis*, *S. haemolyticus,* and *S. hominis*, are present on the surface and in skin folds or hair follicles, as well as in nose mucosa. Smaller populations of other species have also been identified as *Micrococcus* sp., *Aerobacter* sp., and *Proteus* sp. (Findley and Grice, 2014). The opportunistic pathogen *S. aureus* may be a component of the normal microbiota of 5% to 100% of healthy individuals, and it turns into the main agent of SBI (Brook, 2002). However, a vast majority of people are either permanent or transient carriers of this species in the nasal vestibule, which can explain the occurrence in upper limbs (van Belkum et al., 2009).

The occurrence of Gram-positive bacteria (46; 67.63%) is predominant in lower limbs. This finding can be explained by the type of activity that these patients undertake in agriculture, without proper protection and in contact with soil, plants, and decaying organic matter, which are also the natural habitat of fungi. Furthermore, as the lesions were in constant contact with

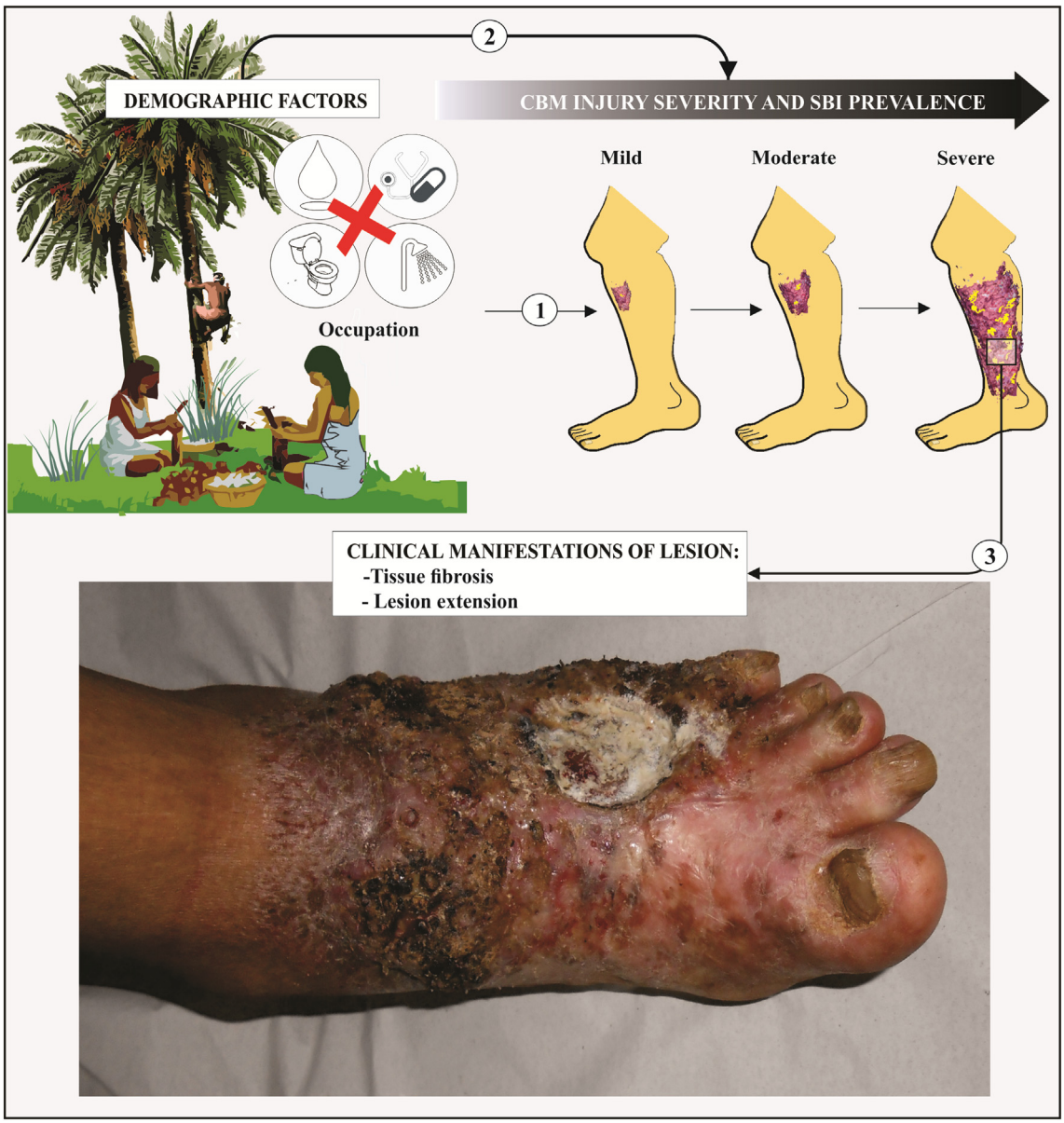

Fig. 1. CBM infection: predisposing factors, disease progression, and SBI prevalence. (1) Demographic factors, such as lack of basic sanitation (wastewater treatment, basic hygiene practices) and occupation (rural workers), contribute to exposure and infection by CBM agents. (2) After CBM infection, the limited access to diagnostic services and treatment and demographic factors contribute to disease progression and higher prevalence of SBI. (3) Clinical manifestations of severe lesions, such as large extension and tissue fibrosis, are associated to a higher prevalence of SBI.

Table 3
Association between injury severity and the microorganisms isolated from the lesions of the patients with chromoblastomycosis and the type of bacterial infection.

Bacteria	Injury severity *n* (%)			Total isolates	*P* value
	Mild	Moderate	Severe		
Staphylococcus aureus	6 (8.83)	11 (16.17)	13 (19.11)	30 (44.11)	0.683
Staphylococcus spp. (CoNS)	0	2 (2.94)	5 (7.35)	7 (10.29)	0.227
Streptococcus agalactiae	1 (1.47)	1 (1.47)	3 (4.41)	5 (7.35)	0.780
Streptococcus pyogenes	0	0	5 (7.35)	5 (7.35)	0.038
Streptococcus β *haemolyticus*	0	0	3 (4.41)	3 (4.41)	0.154
Streptococcus constellatus	0	0	1 (1.47)	1 (1.47)	0.549
Enterococcus faecalis	0	1 (1.47)	2 (2.94)	3 (4.41)	0.606
Peptoestreptococcus spp.	0	0	1 (1.47)	1 (1.47)	0.549
Alcaligenes xylosoxidans	0	1 (1.47)	0	1 (1.47)	0.338
Burkholderia cepacia	1 (1.47)	1 (1.47)	0	2 (2.94)	0.384
Citrobacter diversus	0	1 (1.47)	0	1 (1.47)	0.338
Klebsiella pneumoniae	1 (1.47)	0	1 (1.47)	2 (2.94)	0.493
Morganella morganii	0	1 (1.47)	1 (1.47)	2 (2.94)	0.713
Proteus mirabilis	1 (1.47)	1 (1.47)	1 (1.47)	3 (4.41)	0.861
Pseudomonas aeruginosa	0	0	2 (2.94)	2 (2.94)	0.284
Enterobacter cloacae	0	0	1 (1.47)	1 (1,47)	0.549
Total isolates	10 (14.71)	19 (27.94)	39 (57.35)	68 (100)	
Bacterial infection					
Absent	3(6)	1 (2)	2 (4)	6 (12)	0.205
Single	6(12)	10 (8)	6 (12)	22 (44)	0.075
Mixed	2(4)	5 (10)	15 (26)	22 (44)	0.023

CoNS = coagulase-negative *Staphylococci*.

the environment, it is consequentially predictable that many individuals would experience combined infections with environmental saprophyte bacteria. In this study, besides being affected by Gram-positive cocci of skin microbiota, the lower limbs were the unique site affected by enteric (*M. morganii*, *K. pneumoniae*, *E. cloacae,* and *P. mirabilis*) and other Gram-negative bacilli from environmental sources (*A. baumanni*, *P. aeruginosa,* and *B. cepacia*), frequently

Table 4
Distribution of microorganisms isolated from secondary infections of patients with chromoblastomycosis according to the injury location.

Bacteria	Injury location *n* (%)			Cervical	Total isolates
	Lower limbs	Upper limbs	Chest		
Staphylococcus aureus	22 (32.34)	6 (8.82)	1 (1.47)	1 (1.47)	30 (44.11)
Staphylococcus spp. (CoNS)	6 (8.82)	1 (1.47)	0	0	7 (10.29)
Streptococcus agalactiae	5 (7.35)	0	0	0	5 (7.35)
Streptococcus pyogenes	5 (7.35)	0	0	0	5 (7.35)
Streptococcus β *haemolyticus*	3 (4.41)	0	0	0	3 (4.41)
Streptococcus constellatus	1 (1.47)	0	0	0	1 (1.47)
Enterococcus faecalis	3 (4.41)	0	0	0	3 (4.41)
Peptoestreptococcus spp.	1 (1.47)	0	0	0	1 (1.47)
Alcaligenes xylosoxidans	1 (1.47)	0	0	0	1 (1.47)
Burkholderia cepacia	2 (2.94)	0	0	0	2 (2.94)
Citrobacter diversus	1 (1.47)	0	0	0	1 (1.47)
Klebsiella pneumoniae	2 (2.94)	0	0	0	2 (2.94)
Morganella morganii	2 (2.94)	0	0	0	2 (2.94)
Proteus mirabilis	3 (4.41)	0	0	0	3 (4.41)
Pseudomonas aeruginosa	1 (1.47)	1 (1.47)	0	0	2 (2.94)
Enterobacter cloacae	1 (1.47)	0	0	0	1 (1.47)
Total Gram-positive	46 (67.63)	6 (8.82)	1 (1.47)	1(1.47)	55 (79.39)
Total Gram-negative	13 (19.11)	1 (1.47)	0	0	14 (20.61)
Total isolates	59 (86.74)	7 (10.29)	1 (1.47)	1 (1.47)	68 (100)

CoNS = coagulase-negative *Staphylococci*.

Table 5
Association between single and mixed microbiota and microorganisms isolated from secondary infections of patients with chromoblastomycosis according to the injury location.

Bacteria	Bacterial infection *n* (%)		
	Single	Mixed	*P* value
Staphylococcus aureus	12 (17.64)	18 (26.46)	0.001
Staphylococcus spp. (CoNS)	2 (2.94)	5 (7.35)	0.246
Streptococcus agalactiae	2 (2.94)	3 (4.41)	0.603
Streptococcus pyogenes	1 (1.47)	4 (5.88)	0.220
Streptococcus β *haemolyticus*	0	3 (4.41)	0.131
Streptococcus constellatus	0	1 (1.47)	0.522
Enterococcus faecalis	0	3 (4.41)	0.131
Peptoestreptococcus spp.	1 (1.47)	0	0.522
Alcaligenes xylosoxidans	1 (1.47)	0	0.522
Burkholderia cepacia	2 (2.94)	0	0.266
Citrobacter diversus	0	1 (1.47)	0. 522
Klebsiella pneumoniae	0	2 (2.94)	0.266
Morganella morganii	1 (1.47)	1 (1.47)	0.688
Proteus mirabilis	0	3 (4.41)	0.131
Pseudomonas aeruginosa	0	2 (2.94)	0.266
Enterobacter cloacae	0	1 (1.47)	0.522
Total isolates	22 (32.35)	46 (67.64)	68 (100)

CoNS = coagulase-negative *Staphylococci*.

isolated from SBIs of the leg during the occurrence of skin lesions (Brook, 2002). The presence of these microorganisms in lesions of CBM may reflect the low level of personal hygiene and socioeconomic and sanitary conditions found in the housing of these patients since patients were not previously hospitalized.

In the severe forms of CBM, fibrosis formation occurs in subcutaneous tissue (Fig. 1) associated with chronic inflammatory infiltrate. Previous authors have shown that this lesion profile was characterized by a TH2 type immune response, where there was an increase in IL10, resulting in a reduction in cell-mediated response and high fungal burden (D'Avila et al., 2003; Mazo Fávero Gimenes et al., 2005; Silva et al., 2014). This scenario may predispose patients to SBI. In this study, we found an association between severe CBM lesions and mixed bacterial infection ($P = 0.023$) (Table 3). However, we believe that the immune response in this type of lesion favors the occurrence of bacterial infections because it is a chronic and less resolute response. In addition, the extensive lesion area, in addition to inadequate sanitary conditions of the study population, may predispose them to infection by bacteria present in the environment (Fig. 1). In this context, combined antimicrobial therapy for mixed fungi and bacterial coinfection can improve treatment. Because *S. aureus* and CoNS infection predominates in most case series, including the present study, initiation of antibiotics with activity against *Staphylococci* is mandatory. Resistance to β-lactams was

Table 6
Antimicrobial susceptibility profile of Gram-positive cocci isolated from the lesions of chromoblastomycosis patients.

Bacteria	*S. aureus*	CoNS	*S. pyogenes*	*S.* β hemolítico	*E. faecalis*
Isolates *n* (%)	30 (100)	7 (100)	5 (100)	3 (100)	3 (100)
Antimicrobial	**Resistance profile *n* (%)**				
Ampicillin	21 (70)	3 (42.86)	0 (0)	0 (0)	0 (0)
Amp/Sulb	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Cefazolin	0 (0)	1 (14.29)	0 (0)	0 (0)	NT
Ceftazidime	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Clindamycin	7 (23.33)	1 (14.29)	0 (0)	0 (0)	NT
Erythromycin	9 (30)	1 (14.29)	0 (0)	0 (0)	NT
Gentamicin	0 (0)	1 (14.29)	NT	NT	1 (33.33)
Minocycline	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Oxacillin	0 (0)	1 (14.29)	NT	NT	NT
Penicillin	21 (70)	3 (42.86)	0 (0)	0 (0)	0 (0)
Rifampicin	1 (3.33)	0 (0)	NT	NT	NT
SFZ + TMP	10 (33.33)	1 (14.29)	NT	NT	NT
Streptomycin	0 (0)	0 (0)	0 (0)	0 (0)	1 (33.33)
Tetracycline	12 (40)	3 (42.86)	2 (40)	2 (66.67)	3 (100)

NT = not tested; CoNS = coagulase-negative *Staphylococci*.

Table 7
Antimicrobial susceptibility profile of Gram-negative bacilli isolated from the lesions of chromoblastomycosis patients.

Bacteria	*B. cepacia*	*C. diversus*	*K. pneumoniae*	*M. morganii*	*Proteus*	*P. aeruginosa*
Isolates *n* (%)	1 (100)	1 (100)	2 (100)	2 (100)	3 (100)	2 (100)
Antimicrobial	**Resistance profile *n* (%)**					
Ampicillin	NT	1 (100)	2 (100)	2 (100)	1 (33, 33)	2 (100)
Amp/Sulb	NT	1 (100)	0 (0)	1 (50)	0 (0)	1 (50)
Amikacin	NT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Aztreonam	NT	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Ceftazidime	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)
Cefepime	NT	1 (100)	0 (0)	0 (0)	0 (0)	1 (50)
Cefotaxime	NT	1 (100)	0 (0)	0 (0)	0 (0)	2 (100)
Cefoxitin	NT	1 (100)	0 (0)	2 (100)	0 (0)	2 (100)
Cephalothin	NT	1 (100)	0 (0)	2 (100)	0 (0)	2 (100)
Ciprofloxacin	NT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Clavulanate	NT	1 (100)	0 (0)	1 (50)	0 (0)	2 (100)
Ertapenem	NT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Gentamicin	NT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Imipenem	NT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Levofloxacin	NT	NT	NT	0 (0)	0 (0)	0 (0)
Minocycline	1 (100)	NT	NT	1 (50)	1 (33, 33)	2 (100)
Meropenem	NT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Piperacillin	NT	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
SFZ + TMP	0 (0)	1 (100)	1 (50)	0 (0)	1 (33, 33)	0 (0)

NT = not tested.

detected, but all isolated bacteria were susceptible to vancomycin and linezolid. Interestingly, bacteria with this susceptibility profile are commonly found in hospitalized patients (Hawser et al., 2011). Other pathogens, particularly *P. mirabilis* and *K. pneumoniae*, were the most common microorganisms isolated that showed high rates of antimicrobial susceptibility; however, *C. diversus* had a high profile of antimicrobial resistance (Table 7). These results emphasize the necessity of including bacterial culture and antibacterial agent susceptibility testing for routine monitoring of CBM cases, considering the high profile of refractory therapy and chronicity observed in these patients. Furthermore, our study suggests that antifungal therapy in association with antibacterials to fight Gram-positive bacteria may improve the prognosis.

This pioneering work has demonstrated the main bacterial groups either from environmental sources or from human microbiota that are involved in secondary bacterial infection in CBM lesions. We also provide additional evidence and support for including bacterial culture and antibacterial agents' susceptibility testing in routine monitoring of bacterial involved in secondary infection, in cases of CBM, to prevent possible therapeutic failures, thus guaranteeing the success of treatment.

Conflicts of interest

The authors declare no conflicts of interest.

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