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Investigation of Antifungal Susceptibility Profile in Fungi Isolated from Patients with Chronic Wounds: A Focus on Venous Ulcers

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: The complexity and challenges in managing patients with chronic wounds, combined with high morbidity rates, make this condition a significant public health issue. The polymicrobial presence of microorganisms, particularly fungi, in these wounds poses a major challenge for understanding the infectious process and defining therapeutic approaches.
Aims: This study investigated, over the course of one year, the presence of fungi in 30 clinical samples collected from 10 patients with chronic wounds caused by venous ulcers and characterized the susceptibility profile to the antifungal agents amphotericin B and fluconazole using the microdilution method following the EUCAST protocol.
Results: We observed that 30% of the patients had positive fungal cultures, with *Candida tropicalis* (67%) being the most frequently isolated species, followed by *Candida guilliermondii* (33%). The highest minimum inhibitory concentration (MIC) values for fluconazole were observed in *Candida guilliermondii* (4 mg/L), while for amphotericin B (0.5 mg/L), MIC values did not differ significantly between the isolated species.
Conclusion: Our findings indicate that fungi can be present in chronic venous ulcer wounds and may impair the healing process. We demonstrated the persistent presence of yeasts in 30% patients with chronic wounds caused by venous ulcers, suggesting that they may negatively impact the healing process. Performing microbiological laboratory tests and monitoring antifungal susceptibility profiles are important for implementing appropriate therapeutic strategies and aiding in the clinical management of these patients.

Keywords: Chronic wounds; venous ulcer; antifungal susceptibility; minimum inhibitory concentration (MIC).

1. INTRODUCTION

Chronic wounds affect millions of people worldwide, significantly impacting the health of patients and generating high treatment costs (Nussbaum et al. 2018, Sen 2021, Yang et al. 2024). A wound is characterized as chronic when the healing or anatomical repair process does not occur within three months (Mustoe et al. 2006). Some wounds may take decades to heal, contributing to the emergence of secondary conditions such as isolation, depression, and a significant emotional burden (Bowers and Franco 2020). Chronic wounds can be classified by their etiology as venous stasis ulcers, neuropathic ulcers, arterial ulcers, diabetic foot ulcers, and pressure ulcers (Dissemond et al. 2017).

Venous ulcers are the most common type of chronic wound, typically superficial and located on the medial supramalleolar aspect of the lower extremities (Bowers and Franco 2020). Several factors contribute to delayed healing processes, including metabolic diseases (e.g., diabetes), vascular insufficiency, advanced age, obesity, and infections (Kirsner 2016). Chronic wounds provide a favorable environment for the growth of microorganisms, primarily due to tissue injury and the compromised immune response of the patient, elements that facilitate the proliferation of a polymicrobial flora (Baron et al. 2020).

The presence of bacteria, fungi, and/or adapted microorganisms interacting with one another can lead to the formation of biofilms in these chronic wounds, significantly hindering the healing process (Metcalf and Bowler 2013, Wang et al. 2024). The presence of fungi, particularly yeasts, can contribute to biofilm formation in chronic wounds and further impair wound healing (Kalan et al. 2016).

Few studies address the presence, interaction, and impact of yeasts on the pathogenesis of chronic wounds. The present study investigated the presence of fungi in wound secretion cultures from patients with venous ulcers and analyzed the antifungal susceptibility profile to fluconazole and amphotericin B.

2. MATERIAL AND METHODS

2.1 Study Design

The study included patients with chronic wounds caused by venous ulcers who were treated at the wound care outpatient clinic of the Midwestern Paraná State University (UNICENTRO), Brazil, from March 2023 to March 2024. Three collections of wound secretion were performed on each patient during the 12-month period, the first in month 1, the second in month 7 and the last in month 12. All patients underwent wound

cleaning using the debridement method weekly.

2.2 Wound Culture

Before performing secretion cultures, the wounds were cleansed with warm saline solution through debridement to remove necrotic tissue from the wound bed. After cleaning, secretion collection was conducted using a swab on the upper margin of the wound following Levine's technique (Levine et al. 1976). This technique consists of passing a sterile swab over an area of 1 cm^2 of the wound with light pressure. The swab containing the wound secretion was inoculated onto plates with Sabouraud dextrose agar (SDA) and sent to the laboratory, where it was incubated under aerobic conditions at 37°C for up to 7 days to facilitate colony growth and purification.

2.3 Identification of Yeasts - *Phenotypic Identification*

Mycological identification of yeasts started with conventional methods employing the germ tube test, chlamydospores formation, and biochemical tests, as mentioned in the protocol, “The Yeasts", 2011 (Kurtzman et al. 2011).

2.4 Antifungal Susceptibility Testing (AFST)

Yeasts isolates from wounds were tested for *in vitro* susceptibility to amphotericin B (AmB), fluconazole (FLU) Sigma-Aldrich®, (Madrid, Spain). The test was performed using the European Committee on Antimicrobial Susceptibility Testing (EUCAST-AFST) method described in document E.Def 7.4 using broth microdilution (Arendrup et al. 2020). RPMI 1640 medium with 2.0% glucose Sigma-Aldrich® (St. Louis, USA), with L–glutamine but without sodium bicarbonate (Sigma-Aldrich) buffered to pH 7.0 with 0.165 M MOPS (Sigma-Aldrich) was used as the test medium. The final test concentration ranges were 8 - 0.015 mg/L for AmB and 64-0.125 mg/L for FLU. A stock solution was prepared in dimethyl sulfoxide (DMSO; Sigma-Aldrich®) for all tested antifungals, including for FLU, the according to the manufacturer’s instructions. Microdilution plates were incubated at 35°C and MIC endpoints were read at 24 h (OD at 530 nm) on an automated microplate reader spectrophotometer (GmbH, Salzburg, Austria). In addition, MIC results were evaluated after 48 h to measure the exposure of the isolate to antifungal agents. The MIC endpoints for azoles were defined as the lowest drug concentration that reduced the growth to 50% in relation to controls. For AmB, MIC endpoint was the lowest concentration that resulted in ≥ 90% growth inhibition, compared to the drug-free control. The breakpoints used were those defined by EUCAST document v.10.0 (https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/AFST/Clinical_breakpoints/AFST_BP_v10.0_200204_updatd_links_200924.pdf). The experiment was performed in triplicate.

3. RESULTS AND DISCUSSION

Ten patients with chronic wounds caused by venous ulcers located on the lower limb participated in the study, with 50% being male and 50% female. The duration of the wounds ranged from 1 to 30 years, and the patients' ages varied from 47 to 75 years. Mitura (2021) also observed that patients with chronic wounds were elderly, with a mean age of 70 years (Mitura 2021). Regarding fungal culture tests, 30% of the patients had positive cultures, comprising two men (patient’s A and B) and one woman (patient C). Dowd et al. (2011) also reported that 23% of patients with chronic wounds harbored fungi (Dowd et al. 2011).

Patient A was 75 years old, had a wound duration of 2 years, was pre-diabetic, hypertensive, and a non-smoker. Patient B was 70 years old, pre-diabetic, hypertensive, and a non-smoker. Patient C was 65 years old, had a wound duration of 2 years, was diabetic, hypertensive, and a smoker.

In our study, *Candida tropicalis* was the most isolated species (67%), followed by *Candida guilliermondii* (33%), as shown in Table 1 and Fig. 1, we did not observe mold growth in secretion cultures. Regarding the antifungal susceptibility profile of the tested isolates, we observed no resistance in *Candida tropicalis* isolates to fluconazole or amphotericin B.

For *Candida guilliermondii*, the EUCAST document does not define breakpoint values; however, we observed that *Candida guilliermondii* isolates exhibited high MIC values for fluconazole, suggesting possible tolerance to this antifungal agent, whereas no increased MIC values were noted for amphotericin B.

Fig. 1. *C. guilliermondii* corn meal agar with Tween-80, original magnification 400x

Concerning the clinical evolution of the wounds, we found that for patient A, the MIC for both drugs decreased as the wound dimensions also reduced (Fig. 2). For patient B, the MIC remained unchanged, and the wound showed no significant clinical changes. For patient C, we observed an improvement in clinical appearance, but MIC values remained constant. At 48-hour readings, no significant increases in MIC values were noted. It is worth mentioning, that during the study period, patients did not receive antifungal therapy.

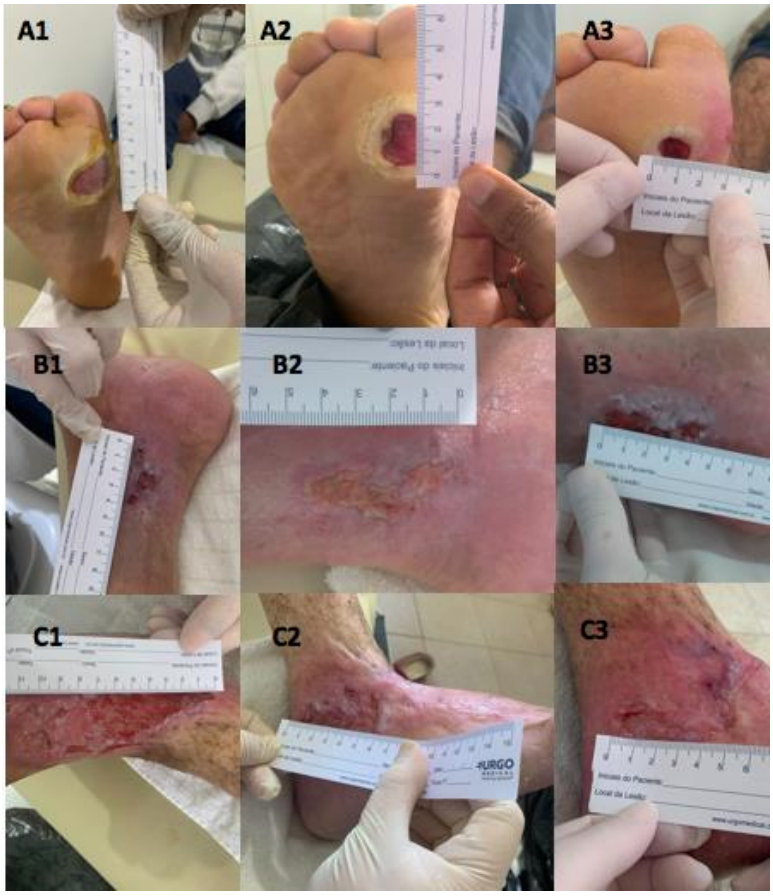

Fig. 2. Patient A, B and C: first collection of clinical material 1, second collection of clinical material 2 and third collection of clinical material 3

Table 1. Yeast species from patients with chronic wounds and MIC values for antifungal agents and wound dimensions

P	Dw (cm)	Cl	Species	Fluconazole			Amphotericin B		
				CIM 24H (mg/L)	CIM 48H (mg/L)	I	CIM 24H (mg/L)	CIM 48H (mg/L)	I
A	3.8 x 2.5	1ª	*C. guilliermondii*	4	8	IE	0,03	0,03	IE
	2.3 x 2.2	2ª	*C. guilliermondii*	2	8	IE	0,5	1	IE
	1.2 x 1.2	3ª	*C. guilliermondii*	0,125	0,25	IE	0,06	0,12	IE
B	2.0 x 4.0	1ª	*C. tropicalis*	0,12	0,12	S	0,6	0,12	S
	1.0 x 4.5	2ª	*C. tropicalis*	0,12	0,12	S	0,6	0,12	S
	1.0 x 4.5	3ª	*C. tropicalis*	0,12	0,12	S	0,6	0,12	S
C	12.5x 11.0	1ª	*C. tropicalis*	0,25	0,25	S	0,12	0,25	S
	6.0 x 3.0	2ª	*C. tropicalis*	0,12	0,25	S	0,015	0,03	S
	4.0 x 1.5	3ª	*C. tropicalis*	1	1	S	0,25	1	S

P:Patient A,B and C; Dw:wound dimensions; Cl:Clinical material collection stage; MIC:Minimum inhibitory concentration; IE:Insufficient evidence for therapy with the agent; S:Susceptible; I:Interpretation

Ge (2022) suggests that fungal prevalence rates in patients with chronic wounds are underestimated and indicates that fungal persistence in these wounds may promote biofilm formation, complicating the healing process (Ge and Wang 2022). It is worth noting that we observed the constant presence of yeasts throughout the study in three patients with chronic venous ulcers, even after wound cleaning using the debridement method. This observation suggests that fungi interact with the microbiome present in the wounds and benefit from this environment.

4. CONCLUSION

Understanding the interaction between yeasts and chronic wounds presents a significant challenge. Our study demonstrated the persistent presence of yeasts in three patients with chronic wounds caused by venous ulcers, suggesting that they may negatively impact the healing process. Although we did not observe resistance in *Candida tropicalis* isolates, we identified high MIC values in *Candida guilliermondii* isolates for fluconazole. This finding underscores the ongoing need for vigilance regarding antifungal susceptibility profiles and the monitoring of yeast presence in these wounds.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT

All participants signed the Informed Consent Form (ICF)

ETHICAL APPROVAL

The study was approved by the UNICENTRO Research Ethics Committee (CAAE No. 33589020.5.0000.0106).

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

Arendrup, M.C., et al., 2020. EUCAST definitive document E.DEF 7.3.2. Method for the determination of broth dilution minimum inhibitory concentrations of antifungal agents for yeasts.

Baron, J.M., M. Glatz, and E. Proksch, 2020. Optimal Support of Wound Healing: New Insights. Dermatology, 236(6): p. 593-600.

Bowers, S. and E. Franco, 2020. Chronic Wounds: Evaluation and Management. Am Fam Physician, 101(3): p. 159-166.

Dissemond, J., et al., 2017. Diagnosis and treatment of chronic wounds: current standards of Germany's Initiative for

Chronic Wounds e. V. J Wound Care, 26(12): p. 727-732.
Dowd, S.E., et al., 2011. Survey of fungi and yeast in polymicrobial infections in chronic wounds. J Wound Care, 20(1): p. 40-7.
Ge, Y. and Q. Wang, 2022. Current research on fungi in chronic wounds. Front Mol Biosci, 9: p. 1057766.
Kalan, L., et al., 2016. Redefining the Chronic-Wound Microbiome: Fungal Communities Are Prevalent, Dynamic, and Associated with Delayed Healing. mBio, 7(5).
Kirsner, R.S., 2016. The Wound Healing Society chronic wound ulcer healing guidelines update of the 2006 guidelines--blending old with new. Wound Repair Regen, 24(1): p. 110-1.
Kurtzman, C.P., J.W. Fell, and T. Boekhout, 2011. The Yeasts: A Taxonomic Study. Elsevier Science, (5th edn).
Levine, N.S., et al., 1976. The quantitative swab culture and smear: A quick, simple method for determining the number of viable aerobic bacteria on open wounds. J Trauma, 16(2): p. 89-94.
Metcalf, D.G. and P.G. Bowler, 2013. Biofilm delays wound healing: A review of the evidence. Burns Trauma, 1(1): p. 5-12.
Mitura, K., 2021. An Innovative National Programme for Comprehensive Chronic Wound Therapy and Its Impact on Treatment Outcomes. World J Surg, 45(4): p. 1071-1079.
Mustoe, T.A., K. O'Shaughnessy, and O. Kloeters, 2006. Chronic wound pathogenesis and current treatment strategies: a unifying hypothesis. Plast Reconstr Surg, 117(7 Suppl): p. 35S-41S.
Nussbaum, S.R., et al., 2018. An Economic Evaluation of the Impact, Cost, and Medicare Policy Implications of Chronic Nonhealing Wounds. Value Health, 21(1): p. 27-32.
Sen, C.K., 2021. Human Wound and Its Burden: Updated 2020 Compendium of Estimates. Adv Wound Care (New Rochelle), 10(5): p. 281-292.
Wang, Q., et al., 2024. Deciphering microbiome and fungi-bacteria interactions in chronic wound infections using metagenomic sequencing. Eur J Clin Microbiol Infect Dis, 43(12): p. 2383-2396.
Yang, H., X. Zhang, and B. Xue, 2024. New insights into the role of cellular senescence and chronic wounds. Front Endocrinol (Lausanne), 15: p. 1400462.

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