

Mycological Profile of Diabetic Foot Ulcers in Kano, Nigeria: A Multicenter Study

Idris¹, A. M., Kabuga^{1,2*}, A. I., Ramadan^{1,3}, T. H., Getso¹, M. I., Sharif¹, A. A., Umar¹, M. B., Idris¹, S. S., Ahmed¹, B., Doguwa², N. M., Aliyu², A. U., Idris⁴, K. M.

¹Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, College of Health Sciences, Bayero University, Kano, P.M.B. 3011-Kano Nigeria. ²Department of Medical Microbiology, Aminu Kano Teaching Hospital, Kano, P.M.B. 3452, Zaria road, Kano State, Nigeria. ³Department of Medical Microbiology and Immunology, Faculty of Medicine, Al-Azhar University, Cairo, Egypt. ⁴Department of Biological Science, Faculty of Science, University of Abuja, PMB 117, Abuja, FCT, Nigeria.

*Corresponding Author: aikabuga.mcp@buk.edu.ng

ABSTRACT

This study investigated fungal infection in foot ulcers among People Living with Diabetes Mellitus (PLWDM) in Kano, northwestern Nigeria. One thousand and five hundred (1,500) consented PLWDM were screened for superficial body lesions (hair, nail, skin, and ulcers) and 300 fulfilled the selection criteria, of which 19 had diabetic foot ulcers. Swabs from the ulcer sites were cultured on Sabouraud Dextrose Agar (SDA), while identification of yeasts and moulds isolates was done using Gram staining, germ tube test, Chrom agar, lacto-phenol cotton blue mount and slide cultures. Antifungal susceptibility testing was by agar well diffusion method using fluconazole, itraconazole, ketoconazole, and clotrimazole. Socio-demographic data, medical history and other risk factors were also documented. Fungi were recovered in foot ulcers with a prevalence of 78.95% (15/19), with *Candida albicans* (33.33%), *Candida tropicalis* (20%), *Aspergillus fumigatus* (13.33%), and *Aspergillus flavus* (13.33%) as predominant species. Others include *Candida glabrata*, *Aspergillus niger*, and *Fusarium species*, each constituting 6.67%. All the isolates show excellent susceptibility to itraconazole, ketoconazole, and clotrimazole, but there was high resistance to fluconazole. This study demonstrates a significantly high prevalence of fungal infections in diabetic foot ulcers and thus, recommend routine screening and prompt treatment of fungi to minimize or eliminate complications associated with diabetic foot ulcers.

Keywords: Diabetes, Foot Ulcer, Mycoses Candida, Itraconazole, Ketoconazole, Kano, Nigeria.

Introduction

Generally, fungal infections (otherwise known as mycoses) are divided according to the body parts affected, they include superficial, subcutaneous, and systemic (or deep) mycosis (Al-Nasrawi, 2021). As the name implies, superficial mycoses affect superficial parts of the body. They are limited to hair, nails, epidermis and mucosa, and are caused by a heterogeneous group of fungi. Moreover, in immunocompromised individuals such as People Living With Diabetes Mellitus (PLWDM), superficial and subcutaneous mycoses can spread by penetrating the skin, connective and bone tissues, thereby causing debilitating effects on a person's quality of life, or even become invasive (Nwafia *et al.*, 2020).

Because of weakened immunity, PLWDM frequently encounter recurrent fungal infections, with skin, mucous membranes, nails, hair and other keratinous substrates as the most susceptible areas (Al-Nasrawi, 2021). Their risk is further worsened by poor glycaemic control, prolonged duration of DM, comorbidities (e.g., obesity, renal disease, liver disease, hypertension, *e.t.c.*) and use of steroids (Carey *et al.*, 2018).

With persistent hyperglycaemia, PLWDM tend to have reduced T-lymphocytes and neutrophils activity, poor secretion of inflammatory cytokines, dysregulated antibody mediated immunity alongside with angiopathy, neuropathy, and increased apoptosis of polymorphonuclear leukocytes (Saud *et al.*, 2020).

They can equally develop poor circulation, as the circulation slow down, blood moves more slowly, which makes it more difficult for the body to deliver nutrients to wounds. As a result, the injuries heal slowly, or may not heal at all and sometimes an infection might develop (Carey *et al.*, 2018).

A significant number (85%) of individuals with long-standing diabetes mellitus suffer from superficial fungal foot infections (SFFIs), with infected nails as reservoir, thereby predisposing them to lower limb cellulites and increasing their risk of developing diabetic foot syndrome (Wijesuriya *et al.*, 2014). Since PLWDM are already at an increased risk of foot ulceration, cellulitis, gangrene, and lower limb amputation, presence of tinea pedis and/or onychomycosis further increases their risk of developing complications (Doğan & Karakaş, 2020).

Evidences have also suggested that majority of diabetic ulcers started with superficial mycoses, paving way for other pathogens to enter and thrive (Abilash *at al.*, 2015). Although a good number of microbiological assessments of foot ulcers have been carried out, there is still paucity of data with regards to fungal infections. In this study, we investigated the role of fungi in aetio-pathology of foot ulcers, the species distribution and anti-fungal susceptibility pattern in a cohort of PLWDM, Kano, Nigeria. The findings will provide information on the common fungal agents associated with foot fungal infections among diabetic individuals, guide selection of appropriate antifungal agent, and ultimately prevent severe complications such as gangrene and limb amputation.

Materials and Methods

Study Area and Collection of Specimen

The study was a cross-sectional multi-centered hospital-based in design. The protocol of the study was reviewed and approved by the Research and Ethics Committee, Kano state Ministry of Health (NHREC/17/03/2018), and informed consent collected from each participant before enrollment. Participants were recruited from two major diabetes treatment centers, in Murtala Muhammad Specialist Hospital and Muhammad Abdullahi Wase Teaching Hospital, all located in the ancient city of Kano, Nigeria.

A total of one-thousand-five hundred (1,500) diabetic individuals were initially approached and screened for body lesions. Different clinical samples were collected from the months of February to July, 2022, from three hundred (300) participants who fulfilled the selection criteria. Samples collected include, scalp, hair, nail scrapping/clipping, skin scrapping and swabs from foot ulcers.

Out of the 300 people with the lesions, 19 had foot ulcers and specimens were collected from the ulcer sites after cleaning with 70% alcohol. Specifically, sterile cotton swab was used to collect sample from the ulcer site and then placed inside sterile swab container (two swab samples were collected from each lesion) (Zafar *et al.*, 2017). Collected samples were immediately transported to the laboratory of Medical Microbiology Department, Aminu Kano Teaching Hospital, Kano for processing and mycological analysis.

Isolation of Fungi from Specimen Samples

All specimen samples were processed immediately on arrival in the Lab. Briefly, for direct microscopy, a portion of the specimen was placed in 2 drops of 10% potassium hydroxide solution (KOH) on a clean grease free glass slide, allowed to stay for about 30 minutes and the preparations viewed under the microscope (Leica, Leica Microsystems (Schweiz, Switzerland) at 10x and 40x objectives for the presence of fungal elements such as septate or non-septate hyphae, chlamydoconidia, microconidia and macroconidia, and gram-positive spherical cells with or without bud or pseudohyphae (Carroll *et al.*, 2019).

Subsequently, samples were inoculated on to Sabouraud Dextrose Agar (SDA) supplemented with 0.05 g chloramphenicol. Briefly, swabbed samples were wetted using 2 drops of sterile physiological saline and then used directly to streaked on the media in circles (about 20 mm size). The plates were sealed to avoid contamination, and incubated at room temperature and at 37°C, respectively. Observation for fungal growth was done daily in first week and then twice a week in subsequent weeks, and cultures considered negative if no growth was observed after four weeks incubation. Pure isolates were obtained by sub-culturing on new SDA plates and incubated at room temperature and at 37° C for 3-7 or until sufficient growth has occurred.

Identification of each pure culture of fungus was based on colony morphology and reverse pigmentation on SDA, Lactophenol cotton blue stain for sporulating fungus (Gohar *et al.*, 2019), and Gram staining and germ tube test for yeasts (Agu & Chidozie, 2021). Identification to species level was by slide culture technique and Chrome agar specifically for *Candida*.

Susceptibility testing was accomplished on Mueller Hinton Agar (MHA) using Agar well diffusion method in accordance with Clinical Laboratory Standards Institute (CLSI, 2019) recommendations.

The antifungal drugs and concentrations used for the study were; Fluconazole (Diflucan) capsule (50mg), Itraconazole (Iicare) capsule (100mg), Ketoconazole tablet (50mg), and Clotrimazole tablet (100mg) obtained from pharmaceutical stores. The antifungal solutions were prepared at the drug manufacturing unit of Aminu Kano Teaching Hospital.

The drugs were dissolved in 10 mL and 20 mL of their respective solvents to arrive at the desired concentrations required for agar well diffusion method. From the sub-cultured plates, tiny portion of the fungal colonies were emulsified in 5 mL sterile physiological saline each in a separate plain bottle and mixed with a vortex mixer. Sterile cork borer was used to punch/cut four wells out of the agar, each 6mm in diameter. A sterile swab dipped into the standardized inoculum suspension was used to inoculate the plates by making a lawn culture on the surface.

The plates were allowed to dry and 20 μ L of the individual antifungal agent were placed into separate wells which were labelled as A, B, C and D. The isolates were tested against Ketoconazole 10 μ g (A), Fluconazole 25 μ g (B), Itraconazole 10 μ g (C) and Clotrimazole 10 μ g (D) and incubated at room temperature 3–7 days.

After the duration of incubation, the diameter of zones of inhibition (clearance) were measured using a transparent foot rule and the results were recorded in millimeter (mm) (Table 1).

Statistical analysis

Data generated was prepared in Excel spreadsheet and analyzed using IBM Statistical Package for Social Sciences (SPSS) version 22. Demographic information, clinical data and other factors were analyzed with descriptive statistics and associations between variables assessed using Chi-square test or Fisher's Exact test as appropriate. A *P*-value < 0.05 was considered significant.

Results

Three hundred (300) PLWDM were initially selected, of which 250 (83.3%) presented with *Tinea pedis*, 30 (10%) *Tinea unguium*, 19 (6.3%) foot ulcers and one person (0.3%) having *Tinea cruris*. Of the 19 individuals with foot ulcers, 15 (78.95%) were mycologically confirmed as having FFIs. Their mean age was 63.10 ± 7.24 years, the minimum was 40 years and the maximum was 65 years. Most of them (73.7%) fall within the age range 45-64 years and females constituted the majority (68.4%). All were diagnosed as having Type II DM.

The socio-demographic characteristics, clinical profile and other potential risks associated with foot fungal infections are shown in Table 1.

The spectrum of fungi isolated from foot ulcers in PLWDM, Kano, Nigeria is presented in Figure 1. Among the mycologically proven cultures, yeasts were predominantly recovered with a prevalence of 9/15 (64.3%), and the dominant species include *Candida albicans* and *Candida tropicalis*. Non-dermatophyte moulds; *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, and *Fusarium* species were also recovered (Figure 1).

Result of the antifungal susceptibility testing of the fungal isolates is presented in Table 2. The isolates were 100% susceptible to itraconazole, followed by ketoconazole and clotrimazole in decreasing order of susceptibility. However, there was high resistance to fluconazole (Table 2).

Table 1 Clinical Characteristics of People Living With Diabetes Mellitus (PLWDM) in Kano, Nigeria

Variables	No of positive FFI (%)	No of negative FFI (%)	Total (%)	P-value
Age (years)				
<45	0 (0)	1 (100)	1 (5.3)	0.119
45-64	10 (71.4)	4 (28.6)	14 (73.7)	
≥65	4 (100)	0 (0)	4 (21.1)	
Gender				
Female	10 (76.9)	3 (23.1)	13 (68.4)	0.637
Male	4 (66.7)	2 (33.3)	6 (31.6)	
Level of education				
Quranic only	11 (78.6)	3 (21.4)	14 (73.7)	0.226
Primary	0 (0)	1 (100)	1 (5.3)	
Secondary	3 (75)	1 (25.0)	4 (21.1)	
Duration of DM (years)				
≤10	12 (70.6)	5 (29.4)	17 (89.5)	0.372
>10	2 (100)	0 (0)	2 (10.5)	
Diabetic medication used				
Oral	14 (77.8)	4 (22.2)	18 (94.7)	0.086
Insulin	0 (0)	0 (0)	0 (0)	
Both	0 (0)	1 (100)	1 (5.3)	
FBS (mmol/L)				
≤10.0	8 (80)	2 (20)	10 (52.6)	0.510
>10.0	6 (66.7)	3 (33.3)	13 (47.4)	
Comorbidities				
Hypertension	6 (60)	4 (40)	10 (52.6)	0.476
Hypertension & Obesity	3 (100)	0 (0)	3 (15.8)	
Obesity	1 (100)	0 (0)	1 (5.3)	
None	4 (80)	1 (20)	5 (26.3)	
History of Superficial mycoses				
No	12 (85.7)	2 (14.3)	14 (73.7)	*0.046
Yes	2 (40)	3 (60)	5 (26.3)	

Key: FBS, fasting blood glucose, No, number; FFI, foot fungal infection.

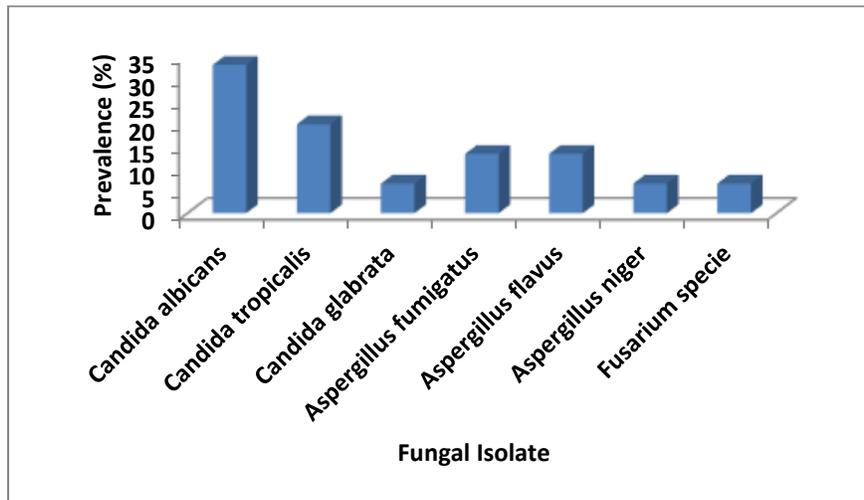


Figure 1: Spectrum of fungi isolated from foot ulcers in people living with diabetes mellitus (PLWDM)

Table 2 *In-vitro* Antifungal Susceptibility Pattern of the Fungal Isolates

Isolate	S. Pattern	Antifungal drug			
		Ketoconazole (%)	Fluconazole (%)	Itraconazole (%)	Clotrimazole (%)
<i>C. albicans</i> (n=5)	S	4 (100)	1 (20)	5 (100)	4 (100)
	I	1 (0)	1 (20)	0 (0)	1 (0)
	R	0 (0)	3 (0)	0 (0)	0 (0)
<i>C. tropicalis</i> (n=3)	S	3 (100)	1 (33.3)	3 (100)	2 (66.7)
	I	0 (0)	1 (0)	0 (0)	1 (0)
	R	0 (0)	1 (0)	0 (0)	0 (0)
<i>C. glabrata</i> (n=1)	S	1 (100)	0 (0)	1 (100)	1 (100)
	I	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	1 (100)	0 (0)	0 (0)
<i>A. fumigatus</i> (n=2)	S	2 (100)	0 (0)	2 (100)	2 (100)
	I	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	2 (100)	0 (0)	0 (0)
<i>A. niger</i> (n=1)	S	1 (100)	0 (0)	1 (100)	1 (100)
	I	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	1 (100)	0 (0)	0 (0)
<i>A. flavus</i> (n=2)	S	2 (100)	0 (0)	2 (100)	2 (100)
	I	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	2 (100)	0 (0)	0 (0)
<i>Fusarium</i> sp.(n=1)	S	1 (100)	0 (0)	1 (100)	1 (100)
	I	0 (0)	0 (0)	0 (0)	0 (0)
	R	0 (0)	1 (100)	0 (0)	0 (0)
Total (n=)	S	14 (93.33)	2 (13.33)	15 (100)	13 (86.67)
	I	1 (6.67)	2 (13.33)	0 (0)	2 (13.33)
	R	0 (0)	11 (73.33)	0 (0)	0 (0)

Key: S. Pattern = Sensitivity Pattern; S = susceptible; I = intermediate; R = resistant.

Discussion

This present study investigated the role of fungi in aetio-pathology of foot ulcers, the species distribution and anti-fungal susceptibility pattern in a cohort of People Living with Diabetes Mellitus (PLWDM), Kano, Nigeria.

Although superficial fungal infections (SFIs) are common and often neglected, they can cause debilitating effects over time if left untreated. People Living with Diabetes Mellitus (PLWDM) represent a unique group of individuals more prone to fungal infections because of uncontrolled high blood sugar level and weaken immune system. They are at risk of having recurrent/chronic fungal infections (Gupta *et al.*, 2018; Kareliya *et al.*, 2019; Richardson & Moyes, 2015), which can in turn result to increase frequency of diabetic foot syndromes such as foot ulcers (Doğan & Karakaş, 2020).

This study has revealed that, the prevalence of fungal infections in diabetic foot ulcers is relatively high 78.95% (15/19). The observed prevalence is consistent with regional patterns seen in African settings and close to the figure reported in Sri Lanka (Wijesuriya *et al.*, 2014), however, it was much higher than what was previously reported from other regions (EL-Nagar *et al.*, 2018; Papini *et al.*, 2013; Sanniyasi *et al.*, 2015).

Although, the frequency of positive cultures was highest in the age group 45–64 years, the association was not statistically significant, possibly reflecting increased physical activity as suggested by Gupta and colleagues (Gupta *et al.*, 2018). The obvious female preponderance in the infection status can be explained by having more female participants in the study rather than a true sex-based predisposition, a conclusion shared by Ngwogu and colleagues (Ngwogu *et al.*, 2014) and other researchers (Surja *et al.*, 2018).

The fact that we isolated *Candida albicans*, *Candida tropicalis*, *Aspergillus fumigatus*, and *Aspergillus flavus* as the predominant species concur with other previous studies among PLWDM (Abilash *et al.*, 2015; EL-Nagar *et al.*, 2018; Kareliya *et al.*, 2019; Wijesuriya *et al.*, 2014). The variation observed can be attributed to methodological differences including the diagnostic approaches, hygiene practices, and/or patient health education and awareness. The fungal agents were isolated in 78.9% (15/19) of the patients with diabetic foot ulcers (DFUs), indicating a remarkably high burden of fungal infections among diabetic patients with foot complications in Kano. Yeasts constituted the majority of the isolates with 64.3% (9/15), while moulds accounted for 35.7% (6/15). Among the yeasts, *Candida albicans* was the most frequently isolated organism (33.3%) followed by *Candida tropicalis* (20%) and *Candida glabrata* (6.7%). The high prevalence of *Candida albicans* agrees with several studies which highlights its dominance in diabetic fungal infections due to its adaptability and bio-film production in high glucose environment.

Among the non-dermatophyte moulds, *Aspergillus fumigatus* and *Aspergillus flavus* were isolated at equal rates (13.3%) each, followed by *Aspergillus niger* and *Fusarium species* (6.67% each). These filamentous fungi are increasingly being recognized as opportunistic pathogens in immunocompromised individuals, particularly in the presence of wounds. The predominance of yeasts over moulds in this study may be attributed to their ability to colonize mucousal and epithelial surfaces. However, the presence of filamentous fungi like *Aspergillus* and *Fusarium* should not be underestimated, as these agents are more likely to cause invasive disease if left undiagnosed or inadequately treated.

In evaluating the socio-demographic factors, level of education emerged as an important consideration. Although, the association of infection and education was not statistically significant ($P = 0.226$). In examining the relationship between the infection status and socio-demographic profile of the respondents, such as age, gender, and level of education, this study revealed that, women especially in the middle age group (45-64 years) and those with no formal education had the highest infection rates.

However, the variations were no statistically significant ($P > 0.05$). Similar findings were previously reported (Akkus *et al.*, 2016; Leelavathi *et al.*, 2013). This supports the fact that fungal infections in diabetic patients are less influenced by demographic factors and more by metabolic and environmental factors. On the other hand, significant associations were observed with use of oral diabetic medications, previous use of systemic antifungal agents, and exposure to domestic animals. However, these findings contrast that of Cheikhrouhou and Colleagues (Cheikhrouhou *et al.*, 2021), possibly reflecting regional differences in hygiene and animal husbandry practices, and species distribution. The antifungal susceptibility pattern discloses promising therapeutic insights, with itraconazole, ketoconazole, and clotrimazole showing high efficacy across the isolates. Unfortunately, fluconazole demonstrated poor activity likely reflecting its availability over the counter, abuse and misuse as echoed by Khan and other researchers (Khan *et al.*, 2022; Khatri *et al.*, 2017).

Conclusion

This study revealed a significantly high prevalence of fungal infections in diabetic foot ulcers in a cohort of People Living with Diabetes Mellitus (PLWDM) in Kano, northwestern Nigeria. Findings from this study showed that itraconazole, ketoconazole and clotrimazole are the most sensitive antifungal drugs against the fungi isolated in this study. We therefore recommend the use of the aforementioned antifungal agent(s) as complimentary therapy in the management of diabetic foot ulcers so as to enhance healing and minimize or eliminate complications and ultimately prevent severe complications such as gangrene and limb amputation.

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