

Assessment of Gut Fungi and Haematological Parameters of Patients with Different Blood Glucose Levels in Federal Medical Centre, Yenagoa

Isaiah O. G.¹ Etebu E.² and Etebu E.^{1*}

1. Department of Microbiology, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

2. Haematology and blood transfusion Department, Medical Laboratory Services, Federal Medical Centre, Yenagoa, Bayelsa State, Nigeria.

* Corresponding author: eetebu@ndu.edu.ng

Abstract: This study examined gut fungi and haematological parameters in 20 patients with varying blood glucose levels separated into diabetic (≥ 7.0 mmol/L, n=9), pre-diabetic (5.6-6.9 mmol/L, n=8), and normal (3.9-5.5 mmol/L, n=3) groups. Fungal species and haematological parameters were analyzed using molecular and standard methods. Total fungal populations were comparable across all three groups; mean Log spore forming unit (sfu) per gram of stool of patients with varying glucose levels were 5.08 ± 0.21 (diabetic), 5.00 ± 0.37 (pre-diabetic) and 4.96 ± 0.14 (normal). Three fungal species, *Chaetomium* spp., *Fusarium equistei* and *Fusarium oxysporum* were identified in all patients. Fungal species populations were separately comparable across all three groups except *Fusarium oxysporum* which was significantly ($P \leq 0.05$) higher in patients with normal glucose levels than other groups. In contrast, haemoglobin (g/dL), mean corpuscular volume (fL), and mean corpuscular haemoglobin (pg) were separately lower in persons with normal glucose levels than diabetic and pre-diabetic patients. *Fusarium oxysporum* was positively correlated to lymphocytes ($r^2 = 0.62$) and negatively correlated with haematocrit ($r^2 = -0.52$) and neutrophils ($r^2 = -0.51$). *Fusarium equistei* was inversely correlated with glucose ($r^2 = -0.59$) and monocytes ($r^2 = -0.46$) while being positively related to mean corpuscular haemoglobin concentration ($r^2 = 0.47$). *Chaetomium* spp. was negatively related to red blood cell ($r^2 = -0.91$). The observed significant ($P \leq 0.05$) correlations between fungal populations, glucose, and haematological parameters suggest that gut fungi are involved in human health.

Key word: Blood glucose, diabetics, gut fungi, haematological parameters

INTRODUCTION

The human body hosts a diverse array of microorganisms, including bacteria, fungi, and viruses, within the gastrointestinal tract (GI) serving as the primary site of colonization. The gut is considered one of the most densely populated microbial communities on earth, containing over 100 trillion microorganisms (D'Argenio and Salvatore, 2015; Matijasic *et al.*, 2020), surpassing human cells by a ratio of 10:1 (Walter and Ley, 2011). Numerous studies have shown the astonishing diversity of microorganisms within the gut (Iatcu *et al.*, 2022). This has been a focal point of research in recent decades due to its significant implications for health and disease, including diabetes (Iatcu *et al.*, 2022; Bataineh *et al.*, 2023; Fliegerova *et al.*, 2025).

The gut microbiome has long been a focus of research, with significant emphasis on bacterial populations (Matijasic *et al.*, 2020), leaving the fungal population largely

overlooked despite its critical role in intestinal homeostasis and systemic immunity. Whilst gut bacterial populations and their role in health have garnered substantial research attention, Scientists have noted that fungi are also integral components of the gut microbiota, and as such deserve closer examination (Fliegerová *et al.*, 2025). Gut fungi, though constituting less than 0.1% of the gut microbiome, are pivotal to maintaining the microbiota's structural and functional integrity. However, some may be pathogenic species (Huffnagle ditto Hoverr, 2013), others may positively contribute to human immune response to disease, thus positively impact on human health (van Tilburg Bernardes *et al.*, 2020). Studies using next-generation sequencing (NGS) revealed the gut mycobiota's diversity, comprising fungal phyla such as Ascomycota, Basidiomycota, and Zygomycota, with genera like *Saccharomyces* and *Candida* frequently detected (Berman & Sudbey, 2002; Bliss *et*

al., 2008). However, significant variability exists in the reported composition of gut mycobiomes across studies, emphasizing the need for further investigation (Bik *et al.*, 2006).

Fungal species have been linked to various health conditions (Cai *et al.*, 2024) with genera such as *Cryptococcus*, *Candida*, and *Aspergillus* implicated in diseases. Given the variability in gut fungal populations and their influence on host health, understanding gut mycobiota's role is crucial, particularly in the context of conditions like diabetes, where microbial dysbiosis may play a role (Al Bataineh *et al.*, 2023; Cai *et al.*, 2024). This study, therefore, sought to assess gut fungi and haematological parameters in diabetic and non-diabetic patients to provide insights into the potential interplay between gut fungi and health.

MATERIALS AND METHODS

Study Area: The study was conducted in Yenagoa metropolis, Bayelsa State located at 4°58'6.132" N, 6° 5' 58.92" E in Nigeria; having being granted ethical approval from the ethical committee of the Centre for Medical Consultancy, Niger Delta University, Wilberforce Island, Amassoma. Owing to laid down clinical ethical standards, the name, age, sex and demographic data of participants were not be published as means of identifying the participants. Also, no name, file, or part of the samples were stored for future research.

Collection of Samples: Stool and blood samples were randomly collected from twenty volunteers (10 confirmed diabetic and 10 apparently non-diabetic patients) hospitalized in Medical Out-Patient Department (MOPD) of the Federal Medical Centre, Yenagoa, Bayelsa State, Nigeria. Samples were collected from volunteers between the ages of 18-70 who had not been on any antifungal drug within the last three months prior to the date of collection. Stool samples were assessed for fungal population and identification, whilst blood samples were used to assess glucose levels and other haematological parameters of patients.

Patients were re-categorized into three groups, diabetic, pre-diabetic and non-diabetic after evaluation of the glucose levels of the patients (World Health Organization, 2006; Clinical and Laboratory Standards Institute, 2018; American Diabetes Association, 2022).

Fungal Population Assessment of Stool

Samples: One gram (1 g) of each stool sample was aseptically homogenized in 9 mL of sterile distilled water to obtain a 10 mL stool suspension. The suspension was serially diluted, and 0.1 mL of appropriate dilutions was inoculated in triplicate onto potato dextrose agar (PDA) plates prepared according to the manufacturer's instructions. The PDA medium was supplemented with chloramphenicol at a final concentration of 50 mg/L to inhibit bacterial growth. The inoculated plates were incubated at a controlled temperature of 28 ± 2 °C for five (5) days. Fungal colonies were counted on the fifth day and recorded as spore-forming units (Sfu). Distinct fungal colonies observed on all three replicates for each sample were counted, and the mean colony count was calculated and expressed as gut fungal population per gram of stool.

Representative fungal colonies were observed for all stool samples and sub-cultured to obtain pure cultures for identification through fresh PDA plates using the streak plate technique to obtain pure cultures. Purified isolates were subsequently preserved and prepared for molecular identification using standard fungal DNA extraction, polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS) region, and sequencing, following established molecular mycological methods (Gardes & Bruns, 1993; White *et al.*, 1990; Sambrook & Russell, 2001).

Molecular Identification of Fungal Species

- DNA Extraction: Total DNA of fungal species was extracted and purified using ZR Fungal/Bacterial DNA MiniPrep™50 Preps. Model D6005 (Zymo Research, California, USA) according to the Manufacturer's protocol. Briefly, 0.5 g of fungal mycelia

was first suspended in 200 µl of sterile deionized water in a ZR Bashing Bead™ Lysis Tube, after which 750 µl of lysis solution was also dispensed into same tube. The fungal cell walls and membranes were then disrupted by bead beating for 5 min at maximum speed (Sambrook and Russel, 2001). Following the bead beating process, the ZR Bashing Bead Lysis tubes were subjected to centrifugation at a force of 10,000 x g for 1 minute, and 400µl of supernatant containing the lysate was carefully transferred into a Zymo-Spin™ IIC column in a collection tube and centrifuged at 10,000 x g for 1 minute to allow only DNA flow through the column into the Collection tube (Zymo, 2022). The filtrate was thereafter harvested and mixed with 1.2 ml of DNA binding buffer and 800 µl of the resultant mixture was dispensed into a Zymo-Spin™ IIC column in a new collection tube and centrifuge at 10,000 × g for 1 min, to ensure DNA bound to the column matrix while the flow-through, which contained unbound contaminants were discarded. The remaining volume was subsequently transferred to the same Zymo-Spin IIC column and spun again to ensure maximum recovery of the DNA (Sambrook and Russel, 2001). In the next phase, 200 microliters of DNA Pre-Wash buffer were added to the Zymo-Spin IIC column situated in a fresh collection tube and centrifuged at 10,000 x g for 1 minute. This pre-wash step was designed to remove any residual contaminants and inhibitors that could affect the subsequent steps of the extraction process. Following this, 500 microliters of fungal/bacterial DNA Wash Buffer were added to the column, and centrifuged once more at 10,000 x g for 1 minute. This wash step further purifies the DNA by removing additional impurities. To complete the DNA extraction process, the Zymo-Spin IIC column was transferred to a clean 1.5 microliter centrifuge tube. Into this tube, 100 microliters of DNA Elution Buffer were added to the column matrix. The column was then centrifuged at 10,000 x g for 30 seconds to release purified DNA from the

column matrix into the collection tube (Sambrook and Russel, 2001). The purified DNA samples were thereafter, sent to Inqaba Biotechnology Pretoria South Africa for Polymerase chain reaction (PCR) and sequencing of PCR amplicons.

Internal Transcribed Spacer (ITS)

Amplification: The ITS region of the rRNA genes of the fungal isolates were amplified targeting the ITS region using Fungal Primers ITS1F (CTT GGT CAT TTA GAG GAA GTA A) described by Gardes and Bruns (1993) and ITS4 (TCC TCC GCT TAT TGA TAT GC) described by White *et al.* (1990). Amplification was carried out on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 50 µl for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (*Taq* polymerase, DNTPs, MgCl₂), the primers at a concentration of 0.4M and the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95°C for 5 minutes; final denaturation, 95°C for 30 seconds; annealing, 53°C for 30 seconds; initial extension, 72°C for 30 seconds for 35 cycles and final extension, 72°C for 5 minutes. The product was resolved on a 1% agarose gel at 200V for 15 minutes and visualized on a blue light transilluminator for a 550bp product.

DNA Sequencing: Purified, post PCR DNA samples were sequenced using the BigDye terminator V1.1 cycle sequencing kit (Applied Biosystems, California, USA) and electrophoresis on an ABI 3510 automated DNA capillary sequencer (Applied Biosystems) at Inqaba Biotechnological, Pretoria South Africa. Sequences generated were manually edited using Chromas (www.technelysium.com.au), and thereafter compared to sequences in Gen Bank National deposited at the Center for Biotechnology Information (NCBI) to obtain the topmost hit of fungal species with closest related sequence (Williamson, 2011).

Assessment of Haematological Parameters and Blood Glucose of Patients: Five milliliters (ml) of blood were collected

through venipuncture with a syringe and needle from patients. Of this volume, 3ml was mixed with ethylene diaminetetraacetic acid (EDTA) for assessment of haematological parameters, while the other 2ml was mixed with fluoride oxalate anticoagulants prior to assessment for glucose. Each sample bottle was labeled with name of patient and serial number. Haematological parameters including haemoglobin concentration (Hb), red blood cells count (RBC), white blood cells count (WBC), haematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC), platelets, and white blood cell differentials (Monocytes, neutrophils and leucocytes) were assessed using standard hematological method as described by Shamila *et al.* (2019), while blood glucose levels were assessed using enzymatic procedure in a Semi-Automatic Biochemistry Analyzer, BS-3000M Model.

RESULTS AND DISCUSSION

Patients had varying glucose levels (Table 1). Eight of the 20 patients representing 40% had their glucose levels ranging between 7.00-15.30 mmol/L (Table 1; Figure 1). These patients were categorized as diabetic patients in line with the World Health Organization (2025) classification. The findings also showed that glucose levels of nine out of the 20 patients representing 45% ranged 5.80-6.20 mmol/L. These were also categorized as pre-diabetic patients in line with (World Health Organization, (2025). The remaining three of the 20 patients representing 15% were categorized as normal (non-diabetic) because their glucose levels ranged 4.20-5.50 mmol/L. The World Health Organization (2025) reported that a fasting blood glucose level of 3.9-5.5 mmol/L is normal. These findings showed that some of the patients sampled as normal and non-diabetic had their glucose levels within the pre-diabetic range. In particular, 7 out of the 10 patients that were presumed to be normal and non-diabetic had glucose

levels within the pre-diabetic (5.6-6.9 mmol/L) range, while the remaining 3 had diabetic (≥ 7.0 mmol/L) blood glucose levels (Table 1).

Similarly, findings showed that only 5 out of the 10 confirmed diabetic patients had glucose levels commensurate with diabetic classification when assessments were carried out. The remaining 5 already had their glucose levels considerably controlled at the time samples were taken; glucose levels of 3 of them had dropped to either normal level, while glucose levels of the remaining 2 patients had dropped to pre-diabetic levels. These findings informed the decision to regroup patients in this work based on their respective glucose levels evaluated at the time of sampling. Findings of this work showed that glucose levels of some people yet to be confirmed diabetic and considered as normal may indeed be within the diabetic or pre-diabetic ranges. It is therefore, important for people to regularly assess their glucose levels to enable them seek audience with their doctors on time when necessary.

Mean glucose levels of diabetic, pre-diabetic and non-diabetic patients were 10.45 ± 3.69 mmol/L, 6.07 ± 0.17 mmol/L and 4.85 ± 0.65 mmol/L, respectively (Table 1). Whilst mean glucose levels were significantly ($P \leq 0.05$) higher among the diabetic group of patients compared to pre-diabetic and non-diabetic patients. The difference in mean glucose levels between the latter two groups were not significant at the 5% probability level. This probably explains why people with pre-diabetic glucose levels who had never been confirmed diabetic are not placed on same medications recommended for diabetics. Pre-diabetic patients are rather advised to adopt healthy lifestyles that would keep their glucose levels in check (World Health Organization, 2025).

Molecular profile of ITS sequences of the five fungi (coded F1-F5) isolated from all patients revealed three different species (Table 2 and Fig. 1). Isolate F1 had ITS sequence closely related to a fungal sequence *Chaetomium* species with the

ascension number MN559961.1 deposited in the NCBI genetic data bank. The partial ITS sequences of three isolates (F2, F3 and F4) were separately closely related to *Fusarium equiseti* fungal sequences deposited in the NCBI genetic data bank with ascension numbers MH260596.1, KX463025.1 and OP900553.1, respectively. The ITS sequence of the fifth isolate (F5) was closely related to *Fusarium oxysporum* fungal sequence deposited in the NCBI genetic data bank with ascension number PP565979.1.

Cumulative mean fungal population counts were comparable across all three blood glucose levels (diabetic, pre-diabetic and non-diabetic) investigated in this work (Table 3). Mean log Sfu of patients with normal (non-diabetic), pre-diabetic and diabetic glucose levels were observed to be 4.96 ± 0.14 , 5.00 ± 0.37 , and 5.08 ± 0.2 respectively (Table 3). Mean de-transformed data of fungal populations were observed to be 1.20×10^5 , 1.00×10^5 and 9.12×10^4 Sfu/g⁻¹ faeces for people with diabetic, pre-diabetic and normal (non-diabetic) glucose levels respectively (Table 3).

Similar to cumulative fungal populations, mean spore forming units of *Chaetomium* sp and *F. equiseti* were comparable in all three groups of varying glucose levels. In contrast, however, the population of *F. oxysporum* was observed to be significantly ($P \leq 0.05$) higher among individuals with the normal (non-diabetic) glucose levels than people who either had pre-diabetic or diabetic glucose levels as shown in Table 3. Difference in *F. oxysporum* population between individuals with pre-diabetic and diabetic glucose levels was not significant at ($P \leq 0.05$) (Table 3).

These findings provide insight that the presence of *Chaetomium* and *F. oxysporum* does not appear to be strongly associated with diabetes as these fungi separately did not show any significant relationship with glucose levels. The consistent log fungal population values across the three groups suggest that the overall population fungal size may not be significantly affected by an individual's glucose level. However, mean

log population of *Fusarium equiseti* was found to be negatively correlated with glucose levels, meaning the fungus may potentially lead to lower levels of glucose. Also, previous researchers had noted that diabetes can lead to dysbiosis, characterized by an imbalance in microbial populations, including fungi. In particular, Cai et al. (2024) had reported that diabetic patients exhibited lower diversity in their gut mycobiota. This seemed to align with the significant lower population of *F. oxysporum* observed amongst persons with diabetic and pre-diabetic glucose levels compared to persons with normal glucose levels in this study. Decrease in fungal diversity has been linked to alterations in metabolic pathways and immune responses in diabetic individuals (Al Bataineh et al., 2023).

Similarly, Li et al. (2019) emphasized that the gut fungal microbiome's composition could be influenced by glucose levels, which are often dysregulated in diabetes. This relationship might explain the observed negative correlation between *F. equiseti* populations and glucose levels. Elevated glucose levels would provide a more favourable environment for certain fungal species over others. Although, the populations of *Fusarium oxysporum* was observed to be low across all three diabetes status compared to the other two fungi isolated in this study, its population was nonetheless significantly higher among patients with normal glucose levels than those with pre-diabetic and diabetic glucose levels (Table 3). This suggests that this *Fusarium oxysporum* may be influenced by diabetes-related metabolic changes such as glucose levels compared to other fungal species. As noted by Mosca et al. (2016), the composition of gut microbiota, including fungi, can fluctuate due to various environmental and medical conditions. Diet is a significant factor influencing gut fungal populations. High-fiber diets, for example, have been associated with greater gut microbial diversity, including fungi (Mosca et al., 2016). Dietary changes could alter the

gut environment, thereby impacting fungal populations. Our study did not assess dietary intake, but future research could explore how diet interacts with diabetes status to influence gut fungal diversity.

In this study, the mean glucose levels were significantly higher among participants with diabetic glucose levels (10.45 ± 3.69 mmol/L) compared to individuals with pre-diabetic (6.07 ± 0.17 mmol/L) and normal (4.85 ± 0.65 mmol/L) glucose levels (Tables 1 and 3). The white blood cell (WBC) counts across all clinical groups were comparable, and no significant differences were observed between any of the three groups (Table 4), suggesting that glucose level may not significantly influence total white blood cell counts in humans. WBC counts are expressed as $\times 10^9/L$. Unlike WBC, haemoglobin (Hb) appeared to be influenced by glucose levels. The mean Hb concentration of individuals with normal glucose levels was 10.0 ± 1.85 g/dL, which was significantly lower than that observed in pre-diabetic (12.4 ± 1.25 g/dL) and diabetic (12.0 ± 1.42 g/dL) participants. Mean haematocrit (HCT) values, expressed as percentages (%), were higher in pre-diabetic (37.3 ± 3.77 %) and diabetic (36.4 ± 4.31 %) groups compared to individuals with normal glucose levels (30.3 ± 5.68 %).

Mean red blood cell (RBC) counts were significantly higher in the diabetic group ($5.97 \pm 4.70 \times 10^{12}/L$) compared to the pre-diabetic ($4.21 \pm 0.13 \times 10^{12}/L$) and normal ($4.20 \pm 0.25 \times 10^{12}/L$) groups. Mean corpuscular volume (MCV) values were statistically higher in pre-diabetic (88.62 ± 8.07 fL) and diabetic (85.13 ± 9.07 fL) participants compared to those with normal glucose levels (43.25 ± 36.75 fL). There were no significant differences in mean corpuscular haemoglobin (MCH), expressed in picograms (pg), across the three groups. Similarly, mean corpuscular haemoglobin concentration (MCHC), expressed in g/dL, did not differ significantly among the groups. Mean platelet counts ($\times 10^9/L$), as well as differential counts for monocytes (MO), lymphocytes, and neutrophils (all

expressed as percentages, %), were statistically comparable across all three groups at the 5% probability level (Table 4). The present study demonstrates clear alterations in selected haematological parameters across normal, pre-diabetic, and diabetic participants, findings that are largely consistent with previous scholarly reports. The significantly higher glucose levels observed in the diabetic group compared with pre-diabetic and normoglycaemic individuals align with established diagnostic patterns reported in earlier epidemiological and clinical studies (Arkew *et al.*, 2021; Mansoori *et al.*, 2023). The absence of significant differences in total white blood cell (WBC) counts among the three groups in this study suggests that hyperglycaemia may not invariably influence total leukocyte count. This agrees with reports indicating that WBC changes in diabetes are often inconsistent and may depend on disease duration, inflammatory status, and glycaemic control rather than glucose level alone (Kachekouche *et al.*, 2018; Essawi *et al.*, 2023). However, other studies have reported elevated WBC counts in poorly controlled diabetes, attributing this to chronic low-grade inflammation (Narjis *et al.*, 2021; Arkew *et al.*, 2021), highlighting that inflammatory responses may vary between populations.

In contrast, haemoglobin (Hb), haematocrit (HCT), and red blood cell (RBC) counts showed significant associations with glycaemic status in this study. The higher Hb and HCT values observed in pre-diabetic and diabetic participants compared with normoglycaemic individuals are consistent with findings that elevated Hb or HCT may predict or accompany dysglycaemia (Kachekouche *et al.*, 2018; Milosevic *et al.*, 2019). Similarly, the significantly higher RBC count in diabetic participants agrees with reports that hyperglycaemia can stimulate erythropoiesis or alter RBC survival, thereby increasing circulating RBC numbers (Alamri, 2019; Arkew *et al.*, 2022). The observed increases in mean corpuscular volume (MCV) among pre-diabetic and

diabetic groups further support previous evidence that hyperglycaemia affects RBC morphology and indices, possibly through glycation-induced membrane changes and altered RBC lifespan (Alamri, 2019; Meshesha *et al.*, 2024). Conversely, the lack of significant differences in MCH and MCHC across groups in this study is in line with reports suggesting that these indices are less consistently affected by glycaemic status, particularly in the absence of overt anaemia or advanced diabetic complications (Al-Shehri *et al.*, 2017; Essawi *et al.*, 2023). Haematological parameters, glucose and fungal populations correlated significantly ($P \leq 0.05$) amongst themselves (Table 5). All three fungal species encountered in this work was observed to be significantly correlated to one or more haematological parameters, indicating the potential roles of gut fungi on human health. In particular, *Chaetomium* was strongly, inversely correlated to RBC ($r^2 = -0.91$). Similarly, *Fusarium oxysporum* was also separately, inversely correlated to Hb ($r^2 = -0.59$), HCT ($r^2 = -0.60$), MCV ($r^2 = -0.64$) and MCH ($r^2 = -0.53$). In contrast, unlike *F. oxysporum*, *Fusarium equiseti* was positively correlated to MCHC ($r^2 = 0.47$; $P \leq 0.05$), whilst being significantly, negatively correlated to glucose ($r^2 = -0.59$). This suggests that *F. equiseti* plays a potential role in lowering glucose levels.

Interestingly, *F. equiseti* was also observed to be negatively, though weakly to monocytes ($r^2 = -0.46$). It would be interesting to further investigate how this fungus could possibly lower both glucose levels and monocytes. Whilst further

research would be recommended, the contrasting, but significant correlations between these fungal species and haematological parameters indicated the potential roles of gut fungi on human health. Recent studies have shown that gut fungi seemed to significantly influence diabetics in humans and animal studies (Bataineh *et al.*, 2020; Cai *et al.*, 2024).

Haematological parameters were also observed to be correlated with glucose levels and amongst themselves. Glucose level was observed to be negatively correlated with MCHC ($r^2 = -0.55$), indicating a relationship between glucose levels and mean corpuscular haemoglobin concentration. Also, Hb and HCT were separately positively correlated with MCV ($r^2 = 0.85$ in each case) and MCH ($r^2 \geq 0.92$), suggesting a potential direct relationship between these pairs of haematological parameters. Haematocrit had a perfect positive correlation with hemoglobin ($r^2 = 1$) which was expected. Other significant correlations included positive correlations between neutrophil and MCHC ($r^2 = 0.73$), MCV and Hb ($r^2 = 0.85$), MCV and HCT ($r^2 = 0.85$), MCH and Hb ($r^2 = 0.93$). Similarly, MCH correlated positively with HCT ($r^2 = 0.92$) and MCV ($r^2 = 0.86$). On the other hand, Lymphocytes had negative relationships with MCHC ($r^2 = -0.69$), monocytes ($r^2 = -0.52$), and neutrophils ($r^2 = -0.99$), respectively, indicating a strong inverse interaction between immune cells. *Fusarium equiseti* showed negative correlations with glucose ($r^2 = -0.53$) and monocytes ($r^2 = -0.58$).

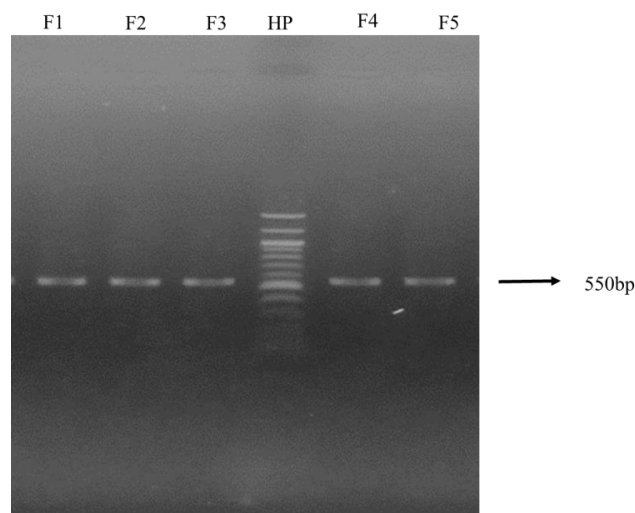
Table 1: Patient Diabetic Status and Their Corresponding Blood Glucose Levels

Clinical Status	No. of Patients	Mean Blood Glucose $\pm$ SD (mmol/L)	Range of Blood Glucose
Diabetic	8 (40%)	10.45 $\pm$ 3.69 ^b	7.0-15.3
Pre-diabetic	9 (45%)	6.07 $\pm$ 0.17 ^a	5.8-6.2
Normal	3 (15%)	4.85 $\pm$ 0.65 ^a	4.2-5.5

Keys: Blood glucose levels (WHO, 2025), Diabetic = ≥ 7.0 mmol/L, Pre-diabetic = 5.6-6.9 mmol/L, Normal = 3.9-5.5 mmol/L

Table 2: Molecular Identification of Fungi Isolates from Human Faeces Across People with Different Blood Glucose Levels

S/No.	Fungal Isolate Code	Query length	Query cover	Fungus identified with closest related Species	Accension number	% identity	E-Value
1	F1	526	100%	<i>Chaetomium</i> species	MN559961.1	99%	0
2	F2	495	100%	<i>Fusarium equiseti</i>	MH260596.1	100%	0
3	F3	504	99%	<i>Fusarium equiseti</i>	KX463025.1	99%	0
4	F4	503	99%	<i>Fusarium equiseti</i>	OP900553.1	99%	0
5	F5	503	99%	<i>Fusarium oxysporum</i>	PP565979.1	100%	0

**Figure 1: Agarose Gel Electrophoresis of Fungal Isolates - Lane F1- F5 Represent Its Gene Bands (550bp). Lane HP Represents 1500bp Molecular Ladder (Hyper Ladder)****Table 3: Mean Log Fungal Populations Per Gram of Faeces Obtained from Patients with Varying Blood Glucose Levels**

Clinical Status	Spore forming units (sfu)	<i>Chaetomium</i> sp.	<i>Fusarium equiseti</i>	<i>Fusarium oxysporum</i>
Log Transformed				
Diabetic	5.08 ± 0.21 ^a	4.41 ± 1.78 ^a	1.79±2.40 ^a	0.37±0.93 ^a
Pre-Diabetic	5.00 ± 0.37 ^a	4.70 ± 0.43 ^a	3.34±0.55 ^a	0.17±0.39 ^a
Normal	4.96 ± 0.14 ^a	4.12±0.83 ^a	3.12±2.68 ^a	0.68±0.39 ^b
De-Transformed				
Diabetic	1.20E+05	2.57E+04	6.17E+01	2.34E+00
Pre-Diabetic	1.00E+05	5.01E+04	2.19E+03	1.48E+00
Normal	9.12E+04	1.32E+04	1.32E+03	4.79E+00

Keys: Blood glucose level categories, Diabetic = ≥ 7.0 mmol/L, Pre-diabetic = 5.6-6.9 mmol/L Non-diabetic = 3.9-5.5 mmol/L

Table 4: Haematological Profiles of Participants Blood Samples

Haematological Parameters	Normal	Pre-Diabetic	Diabetic
White Blood Cells ($\times 10^9/L$)	5.90±0.80 ^a	5.73±0.64 ^a	6.10±1.81 ^a
Haemoglobin (g/dL)	9.30±1.30 ^a	12.4±1.26 ^b	12.06±1.43 ^b
Haematocrit (%)	30.3±5.68 ^a	37.3±3.77 ^b	36.4±4.31 ^{ab}
Red Blood Cell ($\times 10^{12}/L$)	3.95±0.05 ^a	4.21±0.14 ^a	5.98±4.71 ^a
Mean Corpuscular Volume (fL)	43.25±36.75 ^a	88.62±8.07 ^b	85.13±9.07 ^b
Mean Corpuscular Haemoglobin (pg)	23.50±3.11 ^a	29.47±2.69 ^b	28.20±3.11 ^b
Mean Corpuscular Haemoglobin Concentration (g/dL)	33.20±0.10 ^a	33.2±0.07 ^a	33.05±0.32 ^a
Platelets ($\times 10^9/L$)	227.50±5.50 ^a	197.44±51.20 ^a	255.75±42.46 ^a
Monocytes (%)	9.50±0.50 ^a	8.89±1.27 ^a	9.88±2.10 ^a
Lymphocytes (%)	37.50±6.50 ^a	36.44±4.72 ^a	41.75±18.74 ^a
Neutrophils (%)	52.50±7.50 ^a	53.89±4.94 ^a	47.13±17.51 ^a

Rows with different superscript alphabets are significantly different at $P \leq 0.05$, while rows with same superscript alphabet are not significantly different at $P \leq 0.05$

Table 5: Correlation Amongst Fungal and Haematological Parameters

	*FPop	*CSP	*FEQ	*FOX	GLU	WBC	Hb	HCT	RBC	MCV	MCH	MCHC	PLT	MO	LYMP	NEU
*FPop	1															
*CSP	0.55	1														
*FEQ	0.19	-0.34	1													
*FOX	-0.33	-0.09	0.33	1												
GLU	0.21	0.20	-0.59	-0.23	1											
WBC	0.22	0.35	-0.09	0.23	-0.02	1										
Hb	0.04	-0.02	0.04	-0.59	0.24	-0.05	1									
HCT	0.05	-0.02	-0.02	-0.60	0.26	-0.08	1.00	1								
RBC	-2.00	-0.91	0.19	-0.12	-0.02	-0.29	0.14	0.15	1							
MCV	0.24	0.16	-0.17	-0.64	0.33	0.15	0.85	0.85	0.05	1						
MCH	0.06	0.12	-0.06	-0.53	0.25	0.14	0.93	0.92	-0.04	0.86	1					
MCHC	-0.23	-0.19	0.47	0.03	-0.55	0.26	0.15	0.11	0.02	0.01	0.27	1				
B-P	0.16	-0.13	-0.06	0.10	0.40	0.25	-0.12	-0.11	0.22	-0.05	-0.12	-0.33	1			
MO	-0.09	-0.04	-0.46	-0.20	0.25	-0.07	0.21	0.21	0.07	0.15	0.25	0.17	-0.12	1		
LYMP	0.36	0.34	-0.11	0.32	0.31	0.14	-0.32	-0.31	-0.20	-0.05	-0.32	-0.69	0.28	-0.52	1	
NEU	-0.35	-0.34	0.19	-0.30	-0.39	-0.13	0.31	0.29	0.19	0.03	0.31	0.73	-0.3	0.42	-0.99	1

Keys: *Log Spore forming Units (SFU) were used in correlation analysis; CSP (*Chaetomium* species, SFU); FEQ (*Fusarium equiseti*, SFU); FOX (*Fusarium oxysporum*, SFU); GLU (Glucose, mmol/L); WBC (White Blood Cells, $\times 10^9$ cells/L); Hb (Haemoglobin, g/dL); HCT (Haematocrit, %); RBC (Red Blood Cell, $\times 10^{12}$ cells/L); MCV (Mean Corpuscular Volume, fL); MCH (Mean Corpuscular Haemoglobin, pg); MCHC (Mean Corpuscular Haemoglobin Concentration, g/dL); PLT (Platelets, $\times 10^9$ cells/L); MO (Monocytes, %); LYMP (Lymphocytes, %); NEU (Neutrophils, %); $\times$ = Regression coefficient (r^2) in bold letters denotes significance at ($P \leq 0.05$)

CONCLUSION

This study assessed the gut fungal populations and haematological parameters of patients with varying blood glucose levels attending the Federal Medical Centre, Yenagoa, Bayelsa State, Nigeria. Stool and blood samples were collected from adult participants and analyzed using standard microbiological, molecular, biochemical, and haematological methods. Patients were reclassified into diabetic, pre-diabetic, and normal groups based on their blood glucose levels at the time of sampling. Gut fungal populations were evaluated through culture-based enumeration and molecular identification using internal transcribed spacer (ITS) sequencing, while haematological parameters and blood glucose levels were determined using established laboratory procedures. The findings of the study demonstrated that several individuals initially presumed to be non-diabetic had glucose levels within pre-diabetic or diabetic ranges, emphasizing the importance of regular glucose screening for early detection and management of dysglycaemia. Molecular characterization of fungal isolates revealed the presence of *Chaetomium* species, *Fusarium equiseti*, and *Fusarium oxysporum* in stool samples across all glucose categories. Although cumulative gut fungal populations were comparable

among diabetic, pre-diabetic, and normal individuals, species-level differences were observed. Notably, *Fusarium oxysporum* was significantly more abundant in individuals with normal glucose levels, while *Fusarium equiseti* showed a negative correlation with blood glucose levels, suggesting a potential association between specific gut fungi and glucose regulation. Haematological analysis revealed that white blood cell counts, platelet counts, and differential leukocyte parameters were not significantly influenced by glucose status. However, haemoglobin concentration, haematocrit, red blood cell count, and mean corpuscular volume were significantly higher in pre-diabetic and diabetic individuals compared to those with normal glucose levels. These findings indicate that dysglycaemia is associated with alterations in erythrocytic parameters, which may reflect metabolic, inflammatory, or compensatory physiological changes in individuals with elevated blood glucose levels. The study provides evidence that while total gut fungal population size may not vary significantly with glucose status, diabetes and pre-diabetes are associated with shifts in specific fungal species and alterations in key haematological parameters. These observations highlight the complex interplay between gut mycobiota,

blood glucose regulation, and systemic haematological health, and underscore the importance of considering the fungal component of the gut microbiome in metabolic disorders such as diabetes.

Based on the findings of this study, the following recommendations are made:

1. Future studies should involve larger sample sizes and longitudinal designs to better clarify the causal relationships

between gut fungi, blood glucose levels, and haematological parameters.

2. Dietary habits, lifestyle factors, and medication use should be incorporated into future research to determine their influence on gut fungal diversity and metabolic health.

3. Routine monitoring of blood glucose levels should be encouraged, and further exploration of gut mycobiota as a potential adjunct marker in diabetes management is recommended.

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