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Candida bloodstream infection among immunocompromised paediatric patients admitted to the University College Hospital, Ibadan, Nigeria

*[1]Anozie, C. E., [2]Okesola, A., [3]Makanjuola, O., [4]Ayanbekun, T., [5]Mohammed, A. R., and [6]Fasuyi, T.

[1]Department of Medical Microbiology, Federal Medical Centre, Umuahia, Nigeria
[2]Department of Medical Microbiology and Parasitology, University College Hospital Ibadan, Nigeria
[3]Department of Medical Microbiology and Parasitology, University College Hospital Ibadan, Nigeria
[4]Department of Medical Microbiology, Federal Medical Centre, Bida, Niger State, Nigeria
[5]Department of Medical Microbiology, Lead City University, Ibadan, Nigeria
[6]Department of Medical Microbiology, Babcock University, Ilishan Remo, Ogun State, Nigeria
*Correspondence to: anoziechikezie@gmail.com; 08035607642

Abstract:

Background: Invasive candidiasis is a major hospital acquired fungal infection in Nigeria. Despite advances in support of critically ill patients, candidaemia is still associated with high morbidity and mortality. Data on *Candida* bloodstream infection among paediatric patients is limited in Nigeria and this informed this study, which was undertaken to investigate the prevalence, species distribution, antifungal susceptibility pattern for blood stream infections due to *Candida* species in University College Hospital, Ibadan, Nigeria.
Methodology: This was a descriptive study which recruited 322 immunocompromised paediatric patients. All *Candida* isolates obtained from their blood samples through blood culture were identified to species level by germ tube test and PCR-Restriction Fragment Length Polymorphism (RFLP) analysis of 16S rRNA genes with M*sp*I. Antifungal susceptibility test was performed on the isolates using the Vitek 2 system
Results: Eighteen (5.6%) of the 322 patients had candidaemia, with *Candida albicans* accounting for 14 (77.0%), and *Candida glabrata* and *Candida tropicalis* accounting for 2 (11.0%) of the isolates each. Fourteen (77.0%) isolates were susceptible to fluconazole and voriconazole, 8 (44.0%) were susceptible to caspofungin and micafungin, 10 (55.0%) were susceptible to amphotericin B and 17 (94.0%) were susceptible to flucytosine.
Conclusion: This study highlights the reality of candidaemia in hospitalized immunocompromised children, mostly caused by *Candida albicans* and other *Candida* species, exhibiting resistance to echinocandins, azoles and amphotericin B. It is important to have a high index of suspicion and efforts should be made to rightly identify the concerned *Candida* species and perform susceptibility testing before initiating antifungal treatment. This will ensure better outcome for the patients.

Keywords: Candidaemia, prevalence, paediatric, immunocompromised, PCR-RFLP

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Infection sanguine à *Candida* chez des patients pédiatriques immunodéprimés admis à l'Hôpital Universitaire, d'Ibadan, au Nigeria

*[1]Anozie, C. E., [2]Okesola, A., [3]Makanjuola, O., [4]Ayanbekun, T., [5]Mohammed, A. R., et [6]Fasuyi, T.

[1]Département de Microbiologie Médicale, Centre Médical Fédéral, Umuahia, Nigéria
[2]Département de Microbiologie Médicale et de Parasitologie, Hôpital Universitaire d'Ibadan, Nigéria
[3]Département de Microbiologie Médicale et de Parasitologie, Hôpital Universitaire d'Ibadan, Nigéria
[4]Département de Microbiologie Médicale, Centre Médical Fédéral, Bida, État du Niger, Nigéria
[5]Département de Microbiologie Médicale, Université Principale de la Ville, Ibadan, Nigéria
[6]Département de Microbiologie Médicale, Université Babcock, Ilishan Remo, État d'Ogun, Nigéria
*Correspondant a: anoziechikezie@gmail.com; 08035607642

Résumé:

Contexte: La candidose invasive est une infection fongique nosocomiale majeure au Nigeria. Malgré les progrès réalisés dans la prise en charge des patients gravement malades, la candidémie reste associée à une morbidité et une mortalité élevées. Les données sur les infections sanguines à *Candida* chez les patients pédiatriques sont limitées au Nigéria et cela a éclairé cette étude, qui a été entreprise pour étudier la prévalence, la répartition des espèces et le profil de sensibilité aux antifongiques pour les infections sanguines dues aux espèces de *Candida* à l'Hôpital Universitaire d'Ibadan, au Nigeria.
Méthodologie: Il s'agissait d'une étude descriptive qui a recruté 322 patients pédiatriques immunodéprimés. Tous les isolats de *Candida* obtenus à partir de leurs échantillons de sang par hémoculture ont été identifiés au niveau de l'espèce par un test en tube germinal et une analyse PCR-Polymorphisme de Longueur des Fragments de Restriction (RFLP) des gènes de l'ARNr 16S avec M*sp*I. Un test de sensibilité aux antifongiques a été réalisé sur les isolats à l'aide du système Vitek 2.
Résultats: Dix-huit (5,6%) des 322 patients présentaient une candidémie, *Candida albicans* représentant 14 (77,0%) et *Candida glabrata* et *Candida tropicalis* représentant 2 (11,0%) des isolats chacun. Quatorze (77,0%) isolats étaient sensibles au fluconazole et au voriconazole, 8 (44,0%) étaient sensibles à la caspofungine et à la micafungine, 10 (55,0%) étaient sensibles à l'amphotéricine B et 17 (94,0%) étaient sensibles à la flucytosine.
Conclusion: Cette étude met en évidence la réalité des candidémies chez les enfants immunodéprimés hospitalisés, principalement causées par *Candida albicans* et d'autres espèces de *Candida*, présentant une résistance aux échinocandines, aux azoles et à l'amphotéricine B. Il est important d'avoir un indice de suspicion élevé et des efforts doivent être faits pour identifier correctement les espèces de Candida concernées et effectuer des tests de sensibilité avant de commencer un traitement antifongique. Cela garantira de meilleurs résultats pour les patients.

Mots clés: Candidémie, prévalence, pédiatrique, immunodéprimé, PCR-RFLP

Introduction:

Candidiasis refers to fungal infection caused by a yeast from the genus *Candida* (1). In the past 40-50 years, *Candida* species have changed from occasionally encountered pathogens that were initially regarded as mere contaminants to pathogens that cause myriad of superficial and invasive diseases (2). Superficial infections tend to be community-acquired, and cause considerable morbidity. On the other hand, invasive and systemic *Candida* infections tend to be acquired in hospitals or healthcare settings (1,2). Superficial candidiasis has different clinical manifestations, ranging from oropharyngeal and cutaneous, to vaginal candidiasis, while systemic candidiasis is made up of two syndromes; candidaemia, which refers to bloodstream infection by *Candida*, and disseminated candidiasis, which refers to organ infection by *Candida* species.

The genus *Candida* contains 150 to 200 species but only about 20 of them are known to cause disease in humans. From among these, the main pathogens that cause infections in man are *Candida albicans, Candida glabrata, Candida tropicalis, Candida parapsilosis, Candida krusei, Candida guilliermondii, Candida kefyr, Candida lusitaniae, Candida dubliniensis,* and *Candida pseudotropicalis,* with the first five *Candida* species causing the majority of systemic human infections (3,4).

Candida species are found everywhere in nature and are known to inhabit the human alimentary canal, the skin and the genital tract of females. Cases of candidiasis in debilitated patients have been known for decades but the emergence of *Candida* as an important cause of human infection started with the introduction and use of new drugs that inhibit normal human host defence mechanisms, especially the use of potent broad-spectrum antibiotics. These agents disrupt the normal microbiota of the human body and enable non-bacterial organisms to flourish (5,6).

Following the introduction and increasing use of antifungal drugs, the causes of *Candida* infections changed from primarily *C. albicans* to the other non-albicans species, and these are now responsible for about 50% of all cases of candidaemia and other forms of systemic disseminated candidiasis (7). This is a clinically important fact worth noting because the various *Candida* species differ in their susceptibility to antifungal agents, especially the newer agents. In the developed countries, where medical therapeutics are commonly used, *Candida* species are now among the most common nosocomial pathogens (8,9). *Candida* causes serious infection especially when it gets into the bloodstream. Globally, there are five species primarily involved in these bloodstream infections in both adult and paediatric patients; *C. albicans, C. glabrata, C. tropicalis, C. parapsilosis* and *C. krusei* but *C. albicans* is still the most common isolate in *Candida* bloodstream infection globally (10-12).

The diagnosis of *Candida* bloodstream infection is based on isolation of the offending organism from blood culture; however, the sensitivity remains low and it takes an average of 4-5 days to complete the process. This has led to the development of other complementary serological tests and even

molecular tests that detect fungal DNA directly in the blood sample, greatly speeding up the diagnostic process. There is still need to perform antifungal susceptibility testing of the isolate in selected cases to properly guide the therapy (13). Treatment is based on the local fungal epidemiology and the antifungal susceptibility pattern but generally, because of the changing landscape of fungal isolates, the recently updated guidelines from the Infectious Disease Society of America (IDSA) recommends echinocandin or fluconazole as the empirical treatment in most paediatric and adult patients (5).

The management of invasive candidiasis is negatively affected by the delays in prompt diagnosis and the dearth of reliable diagnostic methods that enable detection of both *Candida* bloodstream infection and tissue invasion by *Candida* species (1,7). This study was undertaken to investigate the prevalence, species distribution, antifungal susceptibility pattern of bloodstream infections caused by *Candida* species at the University College Hospital, Ibadan, Nigeria.

Materials and method:

Study setting:

This study was conducted in the department of paediatrics of the University College Hospital (UCH), a tertiary health facility situated in Ibadan, the second largest city in southwestern Nigeria. The hospital is an 820-bed facility and attends to patients from all over the country, especially southwest Nigeria.

The department of paediatrics is one of the core foundation departments of the hospital whose primary activity is the provision of specialized and patient-friendly services for children. It has 7 wards and an outpatients clinic which runs every weekday. Paediatric patients, when indicated, are admitted to the general intensive care unit (ICU) and burns unit.

Study design:

This was a cross-sectional study in which immunocompromised children at risk of invasive *Candida* infection in UCH were recruited for the study. The entire study was done between February 2019 and February 2020

Sampling technique/selection of study participants:

A purposive sampling method was adopted. Every week, the first 10 at-risk patients in the wards whose caregivers consented were recruited into the study. The hospital has a total of 7 wards involved in this study and these were grouped into two. Patients were recruited from alternate groups on alternate weeks which allowed for proportionate selection of patients.

Study population:

This comprised of immunocompromised children admitted into any of the paediatric wards of the department of paediatrics, ICU and burn unit of the hospital. An immunocompromised host is a patient who has a weakened immune system, and such patients have reduced ability to fight infections and other diseases. This may be due to certain diseases such as AIDS, cancers, diabetes, chronic renal diseases, severe malnutrition, prematurity, extremes of age and certain genetic disorders. Other causes include certain medicines or treatments such as anticancer drugs, radiation therapy, and stem cell or organ transplant.

Inclusion criteria:

Immunocompromised paediatric patients (0-18 years of age), whose caregivers consented to the study and who were admitted to UCH Ibadan with signs of bloodstream infection and sepsis with at least two of the following; fever (core temperature >38^{o}C) or hypothermia (core temperature <36^{o}C), abnormal leucocyte profile with counts either elevated or depressed for age or > 10% immature neutrophils, tachypnoea (RR>2SD above normal for age, and tachycardia (HR>2SD above normal for age) or bradycardia (HR< 10th percentile for age).

Ethical consideration:

Ethical approval was obtained from the Joint Ethics Committee of the University of Ibadan and University College Hospital, Ibadan prior to commencement of the study. Informed consent of parents or guardians of participants and informed assent of the older participants were obtained before recruitment into this study.

Specimen collection and handling:

About 2ml of venous blood was collected from septic neonates, infants and children under 5 years of age into to BD BACTEC –BD Peds Plus-Blood culture vials, while 8-10 mls of venous blood was collected from older children into BD BACTEC-Mycosis IC/F Medium culture vials, which was used for selective culture and recovery of fungi from blood. The sample vials were transported to the laboratory for culture.

Laboratory culture of samples:

The vials were incubated at 37^{o}C in the automated BACTEC FX40 blood culture system (Becton Dickinson, Inc., Sparks, MD, USA). The vials flagged positive for growth were removed from the incubator and inoculated onto SDA, Blood, Chocolate, and MacConkey agar plates. These were incubated

overnight at 37ºC under aerobic condition, after which the colonies were Gram stained.

Phenotypic identification of *Candida* from cultures:

Isolates identified from culture plate as *Candida* on Gram stain was tested by germ tube test (GTT) to presumptively identify *C. albicans* and non-albicans *Candida*.

Molecular identification of *Candida* from cultures by PCR and RFLP analysis:

Candida isolates were identified to species level by conventional PCR and Restriction Fragment Length Polymorphism (RFLP) analysis. The DNA was extracted from each *Candida* isolate using Yeast DNA preparation kit (Jena Bioscience) in accordance with the manufacturer's instructions, and amplified by PCR using primers that targets the internal transcribed spacer regions of the ribosomal DNA (ITS1 5'-TCC GTA GGT GAA CCT GCG G-3' and ITS4 5'-TCC TCC GCT TAT TGA TAT GC-3') (14).

The PCR reaction was performed in a thermal cycler (Applied Biosystems, ABS 7000) programmed as follows; initial denaturation of 95ºC for 5 minutes followed by 35 cycles of 95ºC for 30 seconds, 55ºC for 30 seconds and 72ºC and then a final elongation of 72ºC for 7 minutes. PCR grade water as the negative controls were included in each test. The amplified PCR products were electrophoresed in 2% agarose gel and photographed by a camera.

The different strains of the *Candida* species were detected by RFLP analysis with M*sp*I and H*ae*II enzymes (Jena Biosciences, Jena Germany) following the manufacturers instruction. Briefly, 10µl of the PCR amplicon was added to 2µl of universal buffer, with 2µl of either of the restriction enzymes, and incubated at 38^{0}C for 30 minutes. The products were then run on agarose gel electrophoresis and the species determined based on the cutting pattern of the M*sp*I enzyme (15).

Antifungal susceptibility test:

This was done with Vitek 2 antifungal susceptibility system (BioMérieux) against the following antifungals; voriconazole, fluconazole, amphotericin B, flucytosine, micafungin and caspofungin**.** Quality control strain used was *C. parapsilosis* ATCC 22019. The test was done in accordance with CLSI document M27-A3, Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts, Approved Standard, 3rd edition.

Data analysis:

Data were analysed using the SPSS version 25.0 software and presented as descriptive and inferential statistics. Means (± SD) were derived for quantitative variables, while qualitative variables were summarized as proportions. Chi-square test was used to determine the association between variables. The multivariate analysis was performed by binary logistic regression to ascertain the association between the risk variables and prevalence of candidaemia. P values ≤ 0.05 was considered significant.

Results:

Demographic and clinical characteristics of the study participants:

A total of 322 participants were recruited into this study. The mean age was 2.4 years, with 129 (40.1%) patients being less than 1 month old and 3 (0.9%) patients more than 15 years of age. The male to female ratio was 1.28:1. All recruited patients were admitted into the different wards and the majority 222 (70.5%) were on admission for between 1-10 days, 26 (8.1%) patients were admitted for between 21-30 days while 3 patients (0.9%) were on admission for more than 30 days. About 80% of the patients had sepsis as the underlying pathology, 18 (5.6%) had malignancies, 45 (14.0%) had very low birth weight, 8 (2.5%) had HIV and 23 (7.1%) had sickle cell disease.

Majority of the patients received intravenous broad-spectrum antibiotics while on admission, and the average number of days of antibiotics administration was 7.3 days (Table 1). One hundred and eleven (34.0%) patients had antibiotics for a number of days ranging between 8-14 days, while 3 (0.9%) received antibiotics for more than 21 days. All (100.0%) the patients had intravenous lines, 16 (5.0%) had surgery, 8 (2.4%) had urethral catheter, 88 (27.3%) had total parenteral nutrition, 5 (1.6%) had haemo/peritoneal dialysis while 3 (0.9%) had mechanical ventilation in the course of hospital admission (Table 1).

Table 1: Demographic and clinical characteristics of the study participants

Variable	Frequency	Percent
Age group (days)		
0-28 days	129	40.1
> 28 days	193	59.9
Gender		
Male	181	56.2
Female	141	43.8
Days on admission		
1 -10	227	70.5
11- 20	66	20.5
21- 30	26	8.1
30 +	3	0.9
Underlying disease		
Sepsis	189	58.7
Systemic infection	31	9.6
Solid tumours	10	3.1
Haematological malignancy	8	2.5
Very low birth weight with sepsis	45	14.0
Human immunodeficiency virus infection	8	2.5
Renal disease	3	0.9
Severe burns	5	1.6
Sickle cell disease with sepsis	23	7.1

Prevalence of blood stream infection and distribution of *Candida* species:

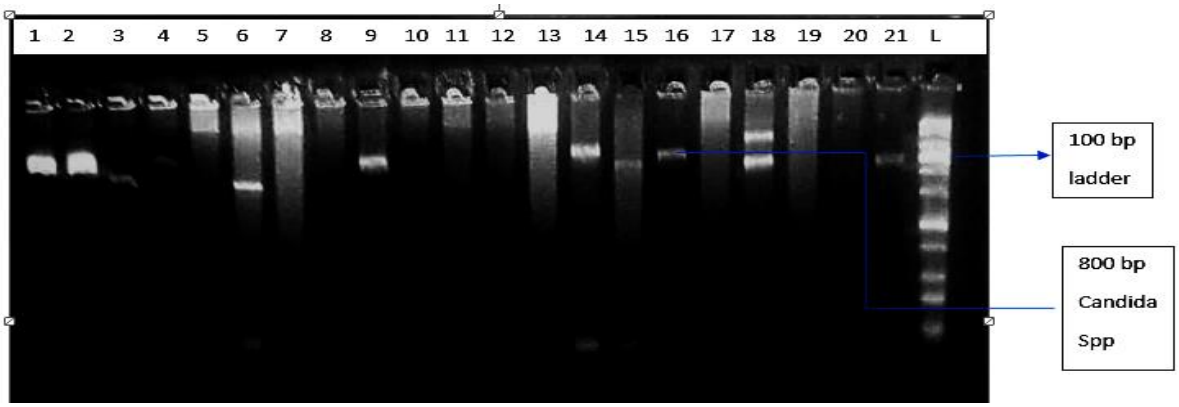
A total of 72 (22.4%) patient's blood samples yielded growth of one or more organisms while 252 (77.6%) samples yielded no growth. This gave the prevalence of microbiologically documented bloodstream infection of 22.4% in this study. Of those with positive blood cultures, 18 (24.3 %) had *Candida* bloodstream infection while 54 (75.7%) had bacterial bloodstream infection.

The distribution of the *Candida* species by PCR and RFLP analyses is as follows; *C. albicans* 14 (77.8%), *C. glabrata* 2 (11.1%) and *C. tropicalis* 2 (11.1%), while the germ tube test (GTT) identification of the *Candida* isolates is as follows; *C. albicans* 10 (55.5%) and non-albicans *Candida* 8 (44.4%).

Table 2: Distribution of *Candida* species by PCR-RFLP analysis

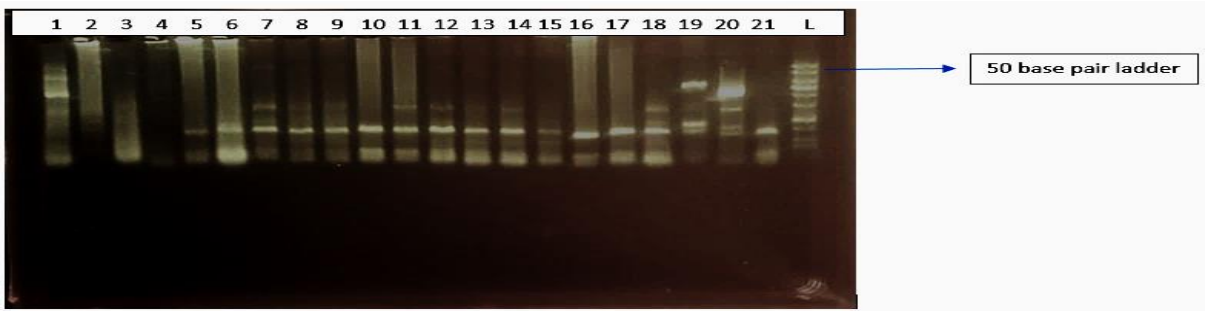
Isolates	Frequency	Percentage
Candida albicans	14	77 .77
Candida glabrata	2	11.11
Candida tropicalis	2	11.11
Total	**18**	**100**

Table 2 shows the species distribution of the *Candida* isolates identified using PCR while Fig 1 shows the image of agarose gel electrophoresis after PCR. Fig 2 shows the agarose gel electrophoresis after RFLP with restriction enzyme M*sp*I.



Samples in lanes 1,2,3,4,6,9, 14,15,16, 18 and 21 had the expected band sizes of 700 to 800bp.

Fig 1: Agarose gel electrophoresis picture of PCR amplicons for detection of *Candida* species using primers ITS1 and ITS4



Lanes 7-18 = *C. albicans,* Lanes 1 and 19 = *C. glabrata;* Lanes 5 and 21 = *C. tropicalis*

Fig 2: Agarose gel electrophoresis picture of PCR-RFLP with restriction enzyme M*sp*I

Antifungal susceptibility of *Candida* species: Fourteen (77%) *Candida* isolates were susceptible to fluconazole and voriconazole, 3 (16.6%) were resistant while 1 (5.5%) was of intermediate susceptibility to the antifungals respectively. Eight (44.4%) of the isolates were susceptible to caspofungin and micafungin respectively while 10 (55.5%) were resistant to both antifungals. Ten (55.5%) of the isolates were susceptible to amphotericin B, 5 (27.7%) were resistant while 3 (16.6%) had intermediate susceptibility to the antifungal. All but one isolate was susceptible to the antifungal, flucytosine (Table 3).

Table 3: Antifungal susceptibility test result of the *Candida* species to selected antifungal agents

Antifungal/Isolates		***Candida albicans*** **(n = 14)**	***Candida glabrata*** **(n = 2)**	***Candida tropicalis*** **(n = 2)**
Fluconazole	S (%)	10 (71.0)	2 (100.0)	1 (50.0)
	I (%)	1 (7.0)	-	-
	R (%)	3 (21.0)	-	1 (50.0)
Voriconazole	S (%)	10 (71.0)	2 (100.0)	2 (100.0)
	I (%)	1 (7.0)	-	-
	R (%)	3 (21.0)	-	-
Caspofungin	S (%)	7 (50.0)	-	1 (50.0)
	I (%)	-	-	-
	R (%)	7 (50.0)	2 (100.0)	1 (50.0)
Micafungin	S (%)	6 (43.0)	1 (50.0)	1 (50.0)
	I (%)	-	-	-
	R (%)	8 (57.0)	1 (50.0)	1 (50.0)
Amphotericin B	S (%)	9 (64.0)	1 (50.0)	1 (50.0)
	I (%)	3 (22.0)	-	-
	R (%)	2 (14.0)	1 (50.0)	1 (50.0)
Flucytosine	S (%)	14 (100.0)	2 (100.0)	1 (50.0)
	I (%)	-	-	-
	R (%)	-	-	1 (50.0)

S = Susceptible, I = Intermediate, R = Resistant, N = Number of isolates

Discussion:

Candidaemia is a type of common hospital acquired bloodstream infection that occurs primarily in the immunocompromised. In this study, *Candida* spp as a group was found to be the most common aetiological agent of bloodstream infection among immunocompromised paediatric patients admitted into the hospital during the period of this study. A study conducted among immunosuppressed patients in Turkey put *Candida* species as the third commonest cause of bloodstream infection while another study in South Africa among oncology paediatric patients reported *Candida* species as the fifth commonest cause of bloodstream infections. Studies from the United States and Europe however showed that *Candida* species are the third most common pathogen isolated in paediatric bloodstream infection (12,16-19). The difference could be because in our study, all the participants were immunocompromised and had signs and symptoms of bloodstream infection, together with the fact that a selective blood culture media was used. In addition, the developed world, unlike our environment, has long established protocols and guidelines which are applied consistently in diagnosis, management and prevention of invasive fungal infections.

The prevalence of candidaemia in our study was 5.6% (18/322). This is lower than 7.4% reported in a study in India (19) but higher than 3.5% reported in a study in the United States (20). Although this rate is still high, it could reflect changes made in the management of invasive candidiasis in our environment in the last couple of years, which include high index of suspicion of such infections, better and more sensitive diagnostic techniques employed in the evaluation of patients, stricter adherence to infection control measures in the management of at-risk patients and local availability of more groups of antifungal drugs employed in the management of such patients when a diagnosis is made.

The male to female ratio of the participants in our study was 1.28:1 while the ratio of patients with candidaemia was slightly less at 1.25:1. Eleven of the patients with candidaemia were neonates, buttressing the observation made by Saiman et al., (21) who reported that candidaemia is more common in neonates than in other children because of their immature immune system, prematurity, low birth weight, and more frequent level of intubation. Two of the patients with candidaemia had malignancies, one was HIV infected, and these also corroborates other studies that show a higher incidence of candidaemia in patients with immunosuppression caused by these conditions (18).

Candida albicans accounted for 77% of the *Candida* isolates. This is similar to the results of the studies by Ezenwa et al., (22) and Santolaya et al., (23) which show that *C. albicans* is still the predominant *Candida* species that cause invasive *Candida* infection, but differs from the result of the studies by Awasthi et al., (20) and Kaur et al., (24) which showed *C. tropicalis* and *C. parapsilosis* respectively to be the primary *Candida* species implicated in candidaemia. In this study, resistance rate to fluconazole was 22.2% for *Candida* spp, which is higher than the rate reported by Oladele et al., (25) in a previous study conducted in UCH Ibadan over a decade ago where no resistance to fluconazole was reported. It is however lower than the rate reported in a study by Kaur et al., (24) who reported rate of 37.8%.

Candida albicans exhibited resistance rate of 21.0% to fluconazole, *C. tropicalis* exhibited resistance rate of 50.0% while *C. glabrata* did not exhibit any resistance to fluconazole. It is important to note the increasing level of resistance to fluconazole in Ibadan and take measures aimed at countering it since fluconazole is still the primary antifungal drug used for most invasive fungal infections in Nigerian hospitals. Another study by Adhikary et al., (26) in Southern India reported higher level of resistance (47.9%) for all *Candida* isolates to fluconazole, however, these isolates were susceptible to polyenes, flucytosine and echinocandins. Fortunately, the level of fluconazole resistance is still relatively low in our study despite the fact that it is the main antifungal drug used in the treatment of candidaemia in our environment.

About 71.0% of *C. albicans* in our study were susceptible to voriconazole but the other non-albicans *Candida* spp were 100.0% susceptible to this antifungal. This is the contrary to the study by Kaur et al., (24), who reported *C. albicans* to be 100.0% susceptible to voriconazole while *C. parapsilosis* was 62.5% susceptible to the drug. It is obvious that voriconazole was an effective azole among all the *Candida* isolates tested, and it is worth noting that in our study, almost all *Candida* isolates resistant to fluconazole were susceptible to voriconazole. This beyond doubt proves that voriconazole is a very effective azole. Such finding implies that voriconazole due to its vast species coverage, can be used in the treatment of candidemia caused by fluconazole resistant strains. These observations are in accordance with previous studies done by Madhavan et al., (27).

This study demonstrated that the *Candida* spp were 44.0% susceptible to caspofungin, 77.0% susceptible to amphotericin B and 94.0% susceptible to flucytosine. Althou-

gh these antifungals are not readily available in our environment, it would be a very useful measure to make them more available, especially the flucytosine because they will come in handy when an azole resistant *Candida* spp is implicated in cases of invasive candidiasis. Unfortunately, these drugs are however very expensive and not affordable by many of the patients who might need them in our resource limited environment.

Conclusion:

Candida bloodstream infection is a very present in our environment and is also increasing in prevalence even among paediatric patients on admission, especially those who are immunocompromised. It is necessary to have a high index of suspicion when managing these patients and endeavour to correctly identify the culpable organism and perform antifungal susceptibility test as regularly as possible. This will substantially improve patients' survival and reduce the mortality of this infection.

Contribution of authors:

ACE is the principal investigator, responsible for all aspects of the study: conceptualization and design, patients' recruitment, sample collection, laboratory analysis, statistical analysis, and final manuscript write up; AO was responsible for data collation; OM was responsible for proof reading the manuscript; TA was involved in sample collection; ARM was involved in the laboratory analysis; and TF was involved in the sample collection and proof reading. All authors approved the manuscript submitted for publication.

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No conflict of interest is declared.

Previous presentation:

Oral presentation of the findings of this study was made at 3rd Virtual Conference of CLIMIDSON that held on November 23-24, 2023 and the abstract was published in the conference brochure.

References:

1. McCarty, T. P., and Pappas, P. G. Invasive Candidiasis. Infect Dis Clin North Am. 2016; 30 (1):103-124. doi: 10.1016/j.idc.2015.10.013
2. Martins, N., Ferreira, I. C., Barros, L., et al. Candidiasis: predisposing factors, prevention, diagnosis and alternative treatment. Mycopathologia. 2014; 177 (5-6): 223-240. doi:10.1007/s11046-014-9749-1
3. Dutta, A., Zaoutis, T, E., and Palazzi, D. L. An Update on the Epidemiology of Candidemia in Children. Curr Fungal infect Rep. 2012; 6 (4): 296-302. doi:10.1007/s12281-012-0113-y
4. Green, L., and Dolen, W. K. Chronic Candidiasis in Children. Curr Allergy and Asthma Rep. 2017; 17 (5): 31. doi:10.1007/s11882-017-0699-9.
5. Pappas, P. G., Kauffman, C. A., Andes, D. R., et al. Clinical Practice Guideline for the Management of Candidiasis: 2016 Update by the Infectious Diseases Society of America. Clin Infect Dis. 2016; 62 (4): e1-50. doi:10.1093/cid/civ933.
6. Pfaller, M. A., and Diekema, D. J. Epidemiology of invasive candidiasis: a persistent public health problem. Clin Microbiol Rev. 2007; 20 (1): 133-163. doi:10.1128/CMR.00029-06.
7. Prigitano, A., Cavanna, C., Passera, M., et al. CAND-LO 2014-15 study: changing epidemiology of candidemia in Lombardy (Italy). Infection. 2016; 44 (6): 765-780. doi:10.1007/s15010-016-0951-6.
8. Al-Rawahi, G. N., and Roscoe, D. L. Ten-year review of candidemia in a Canadian tertiary care centre: Predominance of non-albicans *Candida* species. Canad J Infect Dis Med Microbiol. 2013; 24 (3): e65-e68. doi:10.1155/2013/929717.
9. Caggiano, G., Coretti, C., Bartolomeo, N., et al. *Candida* Bloodstream Infections in Italy: Changing Epidemiology during 16 Years of Surveillance. BioMed Res Int. 2015; 2015: 256580. doi:10.1155/2015/256580.
10. Guinea, J., Global trends in the distribution of *Candida* species causing candidemia. Clin Microbiol Int. 2014; 20 (Suppl 6): 5-10. doi: 10.1111/1469-0691.12539
11. Quindós, G. Epidemiology of candidaemia and invasive candidiasis. A changing face. Revista iberoamericana de micologia. 2014; 31 (1): 42-48. doi:10.1016/j.riam.2013.10.001
12. Wisplinghoff, H., Ebbers, J., Geurtz, L., et al. Nosocomial bloodstream infections due to *Candida* spp in the USA: species distribution, clinical features and antifungal susceptibilities. Int J Antimicrob Agents. 2014; 43 (1): 78-81. doi:10.1016/j.ijantimicag.2013.09.005.
13. Downey, L. C., Smith, P. B., and Benjamin, D. K, Jr., Recent Advances in the Detection of Neonatal Candidiasis. Curr Fungal Infect Rep. 2010; 4 (1): 17-22. doi:10.1007/s12281-010-0002-1.
14. White, T. J., Bruns, T., and Taylor, J. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M. A., Gelfand, D. H., Sninsky, J. J., and White, T. J (eds). PCR Protocols: A Guide to Methods and Applications. London, UK Academic Press. 1990: 315-322
15. Mirhendi, H., Makimura, K., Khoramizadeh, M., and Yamaguchi, H. A one-enzyme PCR-RFLP assay for identification of six medically important *Candida* species. Nihon Ishinkin Gakkai Zasshi. 2006; 47: 225–229.doi:10.3314/jjmm.47.225
16. Lovero, G., De Giglio, O., Montagna, O., et al. Epidemiology of candidemia in neonatal intensive care units: a persistent public health problem. Annali di Igiene: Medicina Preventivae di Comunita. 2016; 28 (4): 282-287. doi:10.7416/ai.2016.2107.
17. Mvalo, T., Eley, B., Bamford, C., et al. Bloodstream infections in oncology patients at Red Cross War Memorial Children's Hospital, Cape Town, from 2012 to 2014. 2018; 77: 40-47. doi:10.1016/j.ijid.2018.09.012
18. Tural Kara, T., Erat, T., Yahşi, A., et al. Bloodstream infections in paediatric haemato logy/oncology patients: Six years' experience of a single center in Turkey. Turkish J Med Sci. 2019; 49 (4): 1157-1164. doi:10.3906/sag-1812-101.

19. Zaoutis, T. E., Argon, J., Chu, J., et al. The epidemiology and attributable outcomes of candidemia in adults and children hospitalized in the United States: a propensity analysis. Clin Infect Dis. 2005; 41 (9): 1232-1239. doi:10.1086/496922
20. Awasthi, A. K., Jain, A., Awasthi, S., Ambast, A., Singh, K., and Mishra, V. Epidemiology and microbiology of nosocomial pediatric candidemia at a northern Indian tertiary care hospital. Mycopathologia. 2011; 172 (4): 269-277. doi:10.1007/s11046-011-9431-9.
21. Saiman, L., Ludington, E., Pfaller, M., et al. Risk factors for candidemia in Neonatal Intensive Care Unit patients. The National Epidemiology of Mycosis Survey study group. Paediatr Infect Dis J. 2000; 19 (4): 319-324. doi:10.1097/00006454-200004000-00011
22. Ezenwa, B. N., Oladele, R. O., Akintan, P. E., et al. Invasive candidiasis in a neonatal intensive care unit in Lagos, Nigeria. Nig Postgrad Med J. 2017; 24 (3): 150-154. doi:10.4103/npmj.npmj_104_17.
23. Santolaya, M. E., Alvarado, T., Queiroz-Telles, F., et al. Active surveillance of candidemia in children from Latin America: a key requirement for improving disease outcome. Paediatr Infect Dis J. 2014; 33 (2): e40-e44. doi:10.1097/INF.0000000000000039.
24. Kaur, R., Jaggi, S., and Dhakad, M. S. An etiological and antifungal profile of candidemia in children. Int J Comm Med. 2019; 6 (9): 3899-3904.doi:10.18203/2394-6040.ijcmph20193990
25. Oladele, R. O., Bakare, R. A., Petrou, M. A., et al. Candidaemia in a tertiary hospital in Nigeria. Afr J Lab Med. 2014; 3 (1): 89-83. doi:10.4102/ajlm.v3i1.89.
26. Adhikary, R., and Joshi, S. Species distribution and anti-fungal susceptibility of Candidaemia at a multi super-specialty center in Southern India. Indian J Med Microbiol. 2011; 29 (3): 309-311. doi:10.4103/0255-0857.83920.
27. Madhavan, P., Jamal, F., Chong, P. P, and Ng, K. P. In vitro activity of fluconazole and voriconazole against clinical isolates of *Candida* spp. by E-test method. Trop Biomed. 2010; 27 (2): 200-207.