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Editorial Board

All correspondence to Editor-in-Chief
Department of Medical Microbiology,
LAUTECH Teaching Hospital,
PMB 4007, Ogbomoso, Nigeria
Email: afrjcem@gmail.com and ajcem2019@gmail.com
Mobile: +234 (0) 8033436344
OR
Department of Medical Microbiology,
College of Health Sciences,
LAUTECH, PMB 4000, Ogbomoso, Nigeria
E-mail: sstaiwo@lautech.edu.ng

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Original Article **Open Access**

Prevalence and antibiotic susceptibility of acute bacterial meningitis isolates among children with fever and convulsion in Federal Medical Centre, Bida, Nigeria

[1]Oyeleye, A. E., [1]Afeez, B. O., [1]Ayanbeku, T. S., and *[2]Ubesie, A. C.

[1]Department of Paediatrics, Federal Medical Centre, Bida, Niger State, Nigeria
[2]Department of Paediatrics, College of Medicine, University of Nigeria, Enugu State, Nigeria
*Correspondence to: agozie.ubesie@unn.edu.ng

Abstract:

Background: Acute bacterial meningitis (ABM) is a life-threatening illness associated with high morbidity and mortality as well as debilitating consequences among survivors. The objective of this study is to determine the prevalence of bacterial isolates and antibiotic susceptibility pattern of ABM among children presenting with fever and convulsion at the Federal Medical Centre, Bida, Nigeria.
Methodology: This was a descriptive cross-sectional study of children who presented with fever and convulsion at the Federal Medical Centre, Bida, Nigeria. The study participants were consecutively enrolled between December 2022 and November 2023. Ethical approval was obtained from the Health Research Ethics Committee (HREC) of the hospital, and informed consent from caregivers of the children was obtained. Demographic and clinical data of the participants were collected using a proforma. Samples of cerebrospinal fluid (CSF) were obtained by lumbar puncture for chemistry, microscopy and culture, while blood samples were obtained by venipuncture for random blood glucose. Data were analysed using IBM SPSS version 23. Associations between variables were determined using Chi-square (χ^2) or Fisher Exact test and $p<0.05$ was considered significant.
Results: Of the total 110 children aged 1 month - 14 years recruited, 69 (62.7%) were males, giving a male to female ratio of 1:0.6, and 60 (54.5%) were between age 1-5 years. Seven (6.4%) participants had acute bacterial meningitis (ABM), 5 (71.4%) of which were caused by *Streptococcus pneumoniae*. Age and vaccination status were significantly associated with ABM, with significantly higher prevalence of ABM in children 1 month-1 year old (20.8%) compared to children 1-<5 years old (1.7%), children 5-<10 years old (5.0%) and children 10-14 years old (0%) (p=0.011), and significantly higher prevalence in unvaccinated children (24.9%) compared to vaccinated children (1.2%) (p=0.000). All the *S. pneumoniae* isolates were susceptible to cefotaxime and rifampicin, while 80.0% were susceptible to ceftriaxone, penicillin, meropenem, clindamycin and vancomycin.
Conclusion: The prevalent cause of post-neonatal ABM in Bida from this current study is *S. pneumoniae*, which are sensitive to most commonly available antibiotics in this environment, especially the third generation cephalosporins.

Keywords: Acute bacterial meningitis, children, prevalence, antibiotics, susceptibility

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Prévalence et sensibilité aux antibiotiques des isolats de méningite bactérienne aiguë chez les enfants présentant de la fièvre et des convulsions au Centre Médical Fédéral de Bida, au Nigéria

[1]Oyeleye, A. E., [1]Afeez, B. O., [1]Ayanbeku, T. S., et *[2]Ubesie, A. C.

[1]Département de Pédiatrie, Centre Médical Fédéral de Bida, État du Niger, Nigéria
[2]Département de Pédiatrie, Faculté de Médecine, Université du Nigéria, État d'Enugu, Nigéria
*Correspondance : agozie.ubesie@unn.edu.ng

Résumé:

Contexte: La méningite bactérienne aiguë (MBA) est une maladie potentiellement mortelle associée à une morbidité et une mortalité élevées, ainsi qu'à des conséquences invalidantes chez les survivants. L'objectif de cette étude est de déterminer la prévalence des isolats bactériens et le profil de sensibilité aux antibiotiques de la MBA chez les enfants présentant de la fièvre et des convulsions au Centre Médical Fédéral de Bida, au Nigéria.
Méthodologie: Il s'agissait d'une étude transversale descriptive portant sur des enfants présentant de la fièvre et des convulsions au Centre Médical Fédéral de Bida, au Nigéria. Les participants à l'étude ont été recrutés

consécutivement entre décembre 2022 et novembre 2023. L'approbation éthique a été obtenue auprès du Comité d'éthique de la recherche en santé (HREC) de l'hôpital, et le consentement éclairé des soignants des enfants a été obtenu. Les données démographiques et cliniques des participants ont été recueillies à l'aide d'un formulaire. Des échantillons de liquide céphalorachidien (LCR) ont été obtenus par ponction lombaire pour la chimie, la microscopie et la culture, tandis que des échantillons de sang ont été obtenus par ponction veineuse pour une glycémie aléatoire. Les données ont été analysées à l'aide d'IBM SPSS version 23. Les associations entre les variables ont été déterminées à l'aide du test du Chi carré (χ^2) ou du test exact de Fisher et une valeur de $p<0,05$ a été considérée comme significative.
Résultats: Sur un total de 110 enfants âgés de 1 mois - 14 ans recrutés, 69 (62,7%) étaient de sexe masculin, soit un ratio homme/femme de 1:0,6, et 60 (54,5%) étaient âgés de 1-5 ans. Sept (6,4%) participants souffraient de méningite bactérienne aiguë (MBA), dont 5 (71,4%) étaient causées par *Streptococcus pneumoniae*. L'âge et le statut vaccinal étaient significativement associés à la BMA, avec une prévalence significativement plus élevée de la BMA chez les enfants de 1 mois - 1 an (20,8%) par rapport aux enfants de 1 -< 5 ans (1,7%), aux enfants de 5 -< 10 ans (5,0%) et aux enfants de 10 - 14 ans (0%) (p=0,011), et une prévalence significativement plus élevée chez les enfants non vaccinés (24,9%) par rapport aux enfants vaccinés (1,2%) (p=0,000). Tous les isolats de *S. pneumoniae* étaient sensibles à la céfotaxime et à la rifampicine, tandis que 80.0% étaient sensibles à la ceftriaxone, à la pénicilline, au méropénème, à la clindamycine et à la vancomycine.
Conclusion: La cause prévalente d'ABM post-néonatale à Bida dans cette étude actuelle est *S. pneumoniae*, qui est sensible à la plupart des antibiotiques couramment disponibles dans cet environnement, en particulier les céphalosporines de troisième génération.

Mots-clés: méningite bactérienne aiguë, enfants, prévalence, antibiotiques, sensibilité

Introduction:

Acute bacterial meningitis (ABM) is a life-threatening infection associated with high mortality and morbidity globally, especially among neonates and children under 5 years of age (1). The disease can occur sporadically or in epidemics. Africa bears a large portion of the burden with large part of sub-Saharan Africa (SSA) being referred to as the meningitis belt (1). Nigeria has her 19 northern States and the Federal Capital Territory (FCT) situated within the meningitis belt (2). The prevalence rates in the worst hit areas of Nigeria have ranged between 11.2% and 20.4% with mortalities as high as 7.6% to 11% (1,3–6). Meningitis epidemics in Nigeria, usually occur during the dry, hot and dusty season, from January to March and disappears when the rainy season begins (7). While many cases end fatally, survivors have suffered severe sequelae such as hearing loss, visual impairment, limb loss, and cognitive impairment, with lasting effects on individuals and their families (8, 9).

Globally*, Haemophilus influenzae* type b, *Neisseria meningitidis* and *Streptococcus pneumoniae* are the predominant agents of ABM in post-neonatal children under 5 years of age (10,11,12). Unlike the developed countries where group B streptococcus (GBS), *Listeria monocytogenes* and *Escherichia coli* are implicated in neonatal bacterial meningitis (13,14), agents reported in Nigerian neonates include *E. coli*, *Staphylococcus aureus*, other Gram negatives and *H. influenzae* (15). Odedina and Emumwen (16) in Bida, Nigeria evaluated the CSF samples of children aged 0 to 12 years for causative organisms of acute bacterial meningitis as well as clinical indicators of the disease over a year period (January to December 2001). The authors reported a prevalence of 16.7%. The major isolates in that study were *N. meningiditis* (50.0%)*, E. coli* (26.9%), and *S. pneumoniae* (15.4%). All the isolates were susceptible to chloramphenicol and ofloxacin.

Over the years, the epidemiology and aetiology of bacterial meningitis have been changing globally especially with the introduction of vaccines such as *H. influenzae* type B (Hib), meningococcal A (MEN A) and pneumococcal vaccines (PCV) (12,17). The study by Odedina and Emumwen (16) was done before the introduction of these vaccines to Bida which makes the current study compelling. Furthermore, Iregbu and Abdullahi (18) in a study at Abuja in 2015, identified *S. aureus* (32.2%) and *Klebsiella pneumoniae* (21.5%) as the most common causes of post-neonatal ABM with *S. pneumoniae* (17.6%) a distant third.

If the World Health Organisation (WHO) 2030 global goal of defeating meningitis will be attained, it is imperative to understand the current burden of ABM in Bida, the predominant aetiological agents and their antibiotic sensitivity pattern (7,10) This study thus sought to determine the prevalence and the antibiotic susceptibility of ABM isolates among post-neonatal children presenting with fever and convulsion in Bida, northcentral Nigeria.

Materials and method:

Study setting and design:

This was a descriptive cross-sectional study carried out between December 2022 and November 2023 at the Emergency Paediatric Unit of the Federal Medical Centre Bida, (FMCB), Niger State in the northcentral region of Nigeria.

Ethical approval was obtained from the Health Research Ethics Committee of Federal Medical Centre, Bida (FMCB/HCS/HR EC/APPR/15/2020).

Sample size, sampling and data collection:

The sample size formula for population proportion was used for the sample size calculation. A minimum sample size of 110 was calculated using a prevalence rate of 6.9% from a previous study in Zaria (6) and adjusting for non-response (20–22). Sampling was consecutive with infants and children aged 1 month to 14 years, who presented with fever and convulsion; whose parent/guardian gave informed consent and children above 7 years who gave assent, were recruited consecutively throughout the study.

Children with bleeding diathesis, infection/ulcer around lumbar spine, spinal defects and evidence of raised intracranial pressure (ICP) other than bulging fontanelle were excluded from the study. Information on participants' age, gender ethnic background and use of antibiotics prior to presentation were collected using a study proforma.

CSF sample collection and microscopy

Lumbar puncture was performed following strict aseptic procedures on each participant to obtain 1 ml each of cerebrospinal fluid (CSF) into sterile universal bottle and a fluoride oxalate bottle. The CSF samples were processed and analysed within an hour after collection, to ensure speed, accuracy and appropriate yield (15,23).

CSF samples were centrifuged at 1000 x g for 10 to 15 minutes after which a drop of the sediment was smeared on a glass slide. Each stained smear was examined under a compound light microscope (100x magnification) for bacteria and reported as Gram positive or Gram-negative cocci, diplococci or bacilli accordingly. The immediate CSF reports were used for treatment of participants while CSF culture results were awaited.

For CSF cell count, a drop of sediments from the centrifuged CSF sample was mixed with a drop of toluidine blue diluting fluid and charged into the improved Neubauer counting chamber. The charged Neubauer chamber was viewed under the 40x objective lens microscope. The white blood cells were counted and noted.

CSF culture for bacteria isolation:

For CSF culture, the sediments of the centrifuged CSF were inoculated on Chocolate agar, Blood agar and MacConkey agar aseptically. The inoculated Chocolate and Blood agar plates were incubated in CO_2 enriched atmosphere (using a candle Jar) at 35–37°C. After 48 hours of incubation, plates were inspected. Culture plates with growth had the bacteria colonies identified according to conventional microbiological techniques (24-26). In this study, confirmed bacterial meningitis was defined as presence of bacteria in CSF identified by Gram stain and/or by culture growth (24,27).

Antibiotic sensitivity testing:

The antibiotic susceptibility testing was done on positive cultures. The modified Kirby-Bauer disc diffusion technique was performed for *S. aureus* and *L. monocytogenes* isolates while the E-test was performed for *S. pneumoniae*. Antibiotic discs (Oxoid Ltd, Hampshire, UK) used were cefoxitin, ciprofloxacin, chloramphenicol, penicillin, clindamycin, gentamicin, vancomycin, and rifampicin for *S. aureus,* and penicillin, meropenem, ampicillin and sulphamethoxazole/trimethoprim for *L. monocytogenes.* E-test antibiotics strips (Bio-Mérieux) used were ceftriaxone, cefotaxime, chloramphenicol, penicillin, meropenem, clindamycin, vancomycin, and ciprofloxacin for *S. pneumoniae*.

Standardized inoculum (equivalent of 0.5 McFarland turbidity standard) prepared from colonies of *S. aureus* and *L. monocytegenes* were inoculated on Mueller Hinton (MH) agar containing 5% sheep blood using a sterile cotton swab. The antibiotic discs were firmly applied on the inoculated agar with a sterile forcep and incubated at 35±2°C in an atmosphere of 5% CO_2 for 24 hours. Zones of inhibition were measured with a calibrated ruler and interpreted as sensitive or resistant in line with the Clinical and Laboratory Standards Institute (CLSI) guideline (26).

For *S. pneumoniae,* standardized inoculum prepared from colonies were similarly inoculated uniformly on MH agar plate using sterile cotton swab. The E-test strips were applied on the inoculated agar using sterile forceps and the plates were incubated at 35±2°C in an atmosphere of 5% CO_2 for 24 hours. The ellipse of inhibition for each isolate against the antibiotic strips was read to determine the minimum inhibitory concentration (MICs), and the results interpreted as sensitive or resistant in line with the CLSI guideline (26).

Data analysis:

Data analysis was done using the IBM SPSS for Windows, Version 23.0 (Armonk, NY: IBM Corp) (28). Categorical variables (gender, and ethnicity) were expressed as frequencies and proportions. Age was expressed as mean. Prevalence of meningitis was expressed as a percentage of total number of participants.

The Chi-square test (χ^2) was used to determine significant associations between the prevalence of ABM and categorical variables of the participants. Fisher's Exact probability test was used where values were too small for Chi-squared test. Statistical significance was set at $p<0.05$ at 95% confidence interval.

Results:

Socio-demographics of the study participants:

The mean age of the study participants was 2.74±3.03 years. Fig 1 shows the distribution of participants by age group, with 60 (54.5%) aged 1-5 years. Sixty-nine study participants (62.7%) were males, giving a male to female ratio of 1:0.6. Ninety-five participants (86.4%) were of Nupe ethnic background as shown in Table 1.

Prevalence and bacterial agents of ABM:

Of the 110 participants, 7 had acute bacterial meningitis, giving a prevalence of 6.4% as shown in Fig 2. Six (85.7%) of the 7 participants with acute bacterial meningitis had CSF white cell counts of greater than 100 x 10^6 cells/litre. The CSF samples of all the 7 participants with acute bacterial meningitis stained positive on Gram staining (Table 2).

Of the bacterial isolates, *S. pneumoniae* accounted for 5 (71.4%) of the 7 isolates while *S. aureus* and *L. monocytogenes* accounted for 2 isolates (14.3% each) (Table 2).

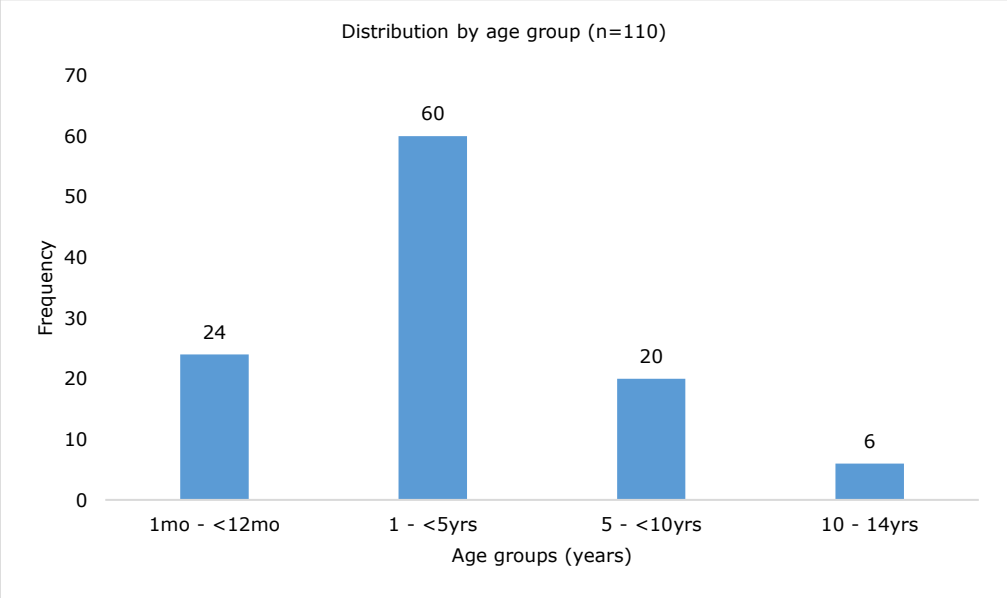

Fig 1: Distribution of the study participants by age groups

Table 1: Distribution of the study participants by gender, ethnicity and vaccination status

Variables	Frequency	Percentages (%)
Gender		
Male	69	62.7
Female	41	37.3
Total	110	100.0
Ethnicity		
Nupe	95	86.4
Hausa	5	4.5
Yoruba	4	3.6
Gbagyi	2	1.8
Fulani	2	1.8
Others	2	1.8
Total	110	100.0
Vaccination status		
Fully vaccinated	64	58.2
Incompletely vaccinated	21	19.1
Not vaccinated	25	22.7
Total	110	100.0

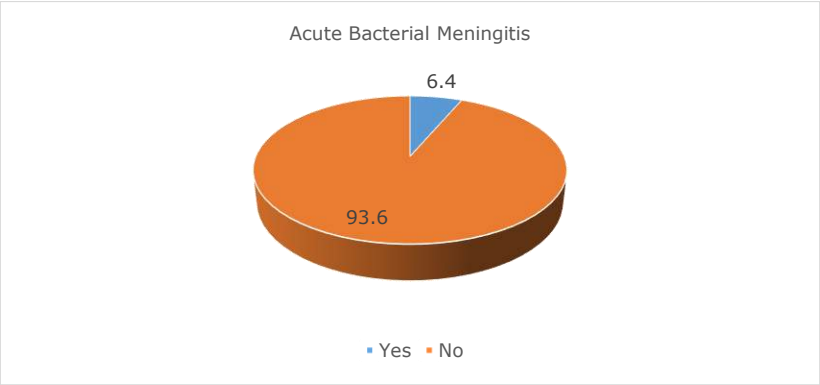

Fig 2: Prevalence of acute bacterial meningitis among the study participants

Table 2: Distribution of study participants by cerebrospinal fluid analysis findings

Variables	Bacterial Meningitis (%)	No Meningitis (%)	Total (%)	Fishers' Exact	*p*-value
White blood cells (cells/L)					
$<5x10^6$	0	103 (100.0)	103 (93.6)	110.000	0.000
5-100 $x10^6$	1 (14.3)	0	1 (0.9)		
101-500 $x10^6$	6 (85.7)	0	6 (5.5)		
Gram stain reaction					
Gram positive	7 (100.0)	0	7 (6.4)	110.000	0.000
None	0	103 (100.0)	103 (93.6)		
Growth on culture					
Growth	7 (100.0)	0	7 (6.4)	110.000	0.000
No growth	0	103 (100.0)	103 (93.6)		

Antibiotic susceptibility of isolates:

All the *S. pneumoniae* isolates were sensitive to cefotaxime and rifampicin while 4 (80%) were sensitive to ceftriaxone, penicillin, meropenem, clindamycin and vancomycin. The only *S. aureus* isolate was sensitive to cefoxitin, ciprofloxacin, penicillin, clindamycin and vancomycin but resistant to chloramphenicol, gentamicin and rifampicin. The only *L. monocytogenes* isolate was sensitive to penicillin, meropenem, ampicillin and sulphamethoxazole/trimethoprim as shown in Table 3.

Table 3: Antibiotic sensitivity pattern of bacterial isolates from the study participants

Antibiotics	***Streptococcus pneumoniae*** **n=5 (%)**	***Staphylococcus aureus*** **n=1 (%)**	***Listeria monocytogenes*** **n=1 (%)**
Ceftriaxone	4 (80.0)	NT	NT
Cefotaxime	5 (100.0)	NT	NT
Cefoxitin	NT	1 (100.0)	NT
Ciprofloxacin	NT	1 (100.0)	NT
Chloramphenicol	2 (40.0)	0	NT
Penicillin	4 (80.0)	1 (100.0)	1 (100.0)
Meropenem	4 (80.0)	NT	1 (100.0)
Clindamycin	4 (80.0)	1 (100.0)	NT
Gentamicin	NT	0	NT
Vancomycin	4 (80.0)	1 (100.0)	NT
Rifampicin	5 (100.0)	0	NT
Ampicillin	NT	NT	1 (100.0)
Sulphamethoxazole/ Trimethoprim	NT	NT	1 (100.0)

NT- not tested

Table 4: Association of acute bacterial meningitis with age, gender, and prior exposure to antibiotics

Variables	Bacterial Meningitis (n=7, 6.4%)	No Meningitis (n=103, 93.6%)	Total (n=110)	Fisher's Exact	*p*-value
Age group (years)					
1 mo-<1	5 (20.8)	19 (79.2)	24	11.125	0.011
1 - < 5	1 (1.7)	59 (98.3)	60		
5 - <10	1 (5.0)	19 (95.0)	20		
10 – 14	0	6 (100.0)	6		
Gender					
Male	3 (4.3)	66 (95.7)	69	1.262	0.261
Female	4 (9.8)	37 (90.2)	41		
Use of antibiotics					
Yes	3 (5.9)	48 (94.1)	51	0.281	0.869
No	4 (7.1)	52 (92.9)	56		
Unknown	0	3 (100.0)	3		
Vaccination status					
Vaccinated (full/partial)	1 (1.2)	84 (98.8)	85	17.490	0.000
Not vaccinated	6 (24.9)	19 (76.0)	25		

Factors associated with ABM:

Five of the 7 participants with ABM were infants while 3 of the 7 had exposure to antibiotics prior presentation. Six of the 7 with ABM had received no vaccination. Age and vaccination status were significantly associated with ABM as shown in Table 4, with significantly higher prevalence of ABM in children 1 month-1 year old (20.8%) compared to children 1-<5 years old (1.7%), children 5-<10 years old (5.0%) and children 10-14 years old (0%) (p=0.011), and also significantly higher prevalence in unvaccinated children (24.9%) compared to vaccinated children (1.2%) (p= 0.000). No significant association of gender (p=0.261) and antibiotic use prior presentation (p=0.869) with prevalence of ABM.

Discussion:

The prevalence of acute bacterial meningitis among children presenting with fever and convulsions in this study was 6.4%. This compared favorably with a prevalence of 6.9% reported by Mado et al*.,* (6) in Zaria in 2012 and 4.2% reported by Akpede et al*.,* (29) in Benin in 1992. It also compared favorably with the prevalence of 5.2% reported by Sargazi et al*.,* (30) in Iran in 2022 and 5.9% in Multan, Pakistan by Urooj et al*.,* (31) in 2021. It was however significantly lower than the prevalence of 15.2% reported by Akpede and Sykes (32) in Benin in 1993, 10.2% reported by Owusu-Ofori et al., (33) in Kumasi, Ghana in 2004, and 16.7% reported by Odedina and Emumwen in Bida (16) in 2008. The lower prevalence in this study compared to the previous Bida study by Odedina and Emumwen (16) over 20 years ago, may be explained by the introduction of vaccines against some common bacterial agents causing ABM.

Furthermore, the prevalence of ABM by age group was highest among the younger age group of 1–11 months. This finding is similar to the reports by Mado et al., (6) and Akpede et al., (29). Owusu-Ofori et al., (33) in Ghana also had majority of ABM diagnosed among those under 12 months of age with decreasing prevalence of ABM as age increased. The higher prevalence of ABM with younger age group and lower prevalence with older age group recorded in this study may affirm the role of increasing maturity of the immune system with age (34).

Streptococcus pneumoniae was the predominant cause of ABM in this study, and agreed with findings by Mado et al*.,* (6) and Johnson et al*.,* (35). *Streptococcus pneumoniae* was also the predominant isolate in a multi-centre paediatric bacterial meningitis surveillance study conducted between 2010 and 2016, involving five Nigerian (Lagos, Enugu, Bauchi, Kwara and Edo) States (10). Contrary to established knowledge of agents causing ABM in the post-neonatal age group, *N. meningitidis* and *H. influenzae* type b were not isolated in this study. Over the years, previous studies conducted in Nigeria (3,5,6, 16,29,32,35) and sub-Saharan Africa (33,36, 37) reported *S. pneumoniae , N. meningitidis* and *H. influenzae* type B as the most common causes of ABM after the neonatal age.

The introduction of robust vaccination programs against these organisms have been proffered as the reason for the declining trend. It is therefore not surprising that the current study recorded neither *H. influenzae* type B nor *N. meningitidis* as causes of ABM in Bida. The earlier study by Odedina and Emumwen (16) in Bida in 2008 that reported *N. meningitidis* and *H. influenzae* type B, was done before the introduction of these vaccines. Seventy-seven percent of the participants (n=85) in the current study received vaccines either fully or partially, through the National program on Immunization (NPI) further buttressing the significant role vaccine may be

playing in reducing the prevalence of the usual isolates.

Other bacterial agents identified as causing ABM in this study were *S. aureus* and *L. monocytogenes*, although the latter is not usually found to cause ABM in children outside the neonatal age group. Reports of *L. monocytogenes* causing meningitis among Nigerian children have been few. Alao et al., (38) in Ogbomoso reported *L. meningitidis* meningitis in a 3-year old boy. *Listeria monocytogenes* is usually associated with immunodeficiency states and it is basically food-borne (39).

In our study, *S. pneumoniae* isolates were almost entirely (80-100%) sensitive to third generation cephalosporins, ciprofloxacin, penicillin, meropenem, clindamycin, rifampicin and vancomycin but 60% of the isolates were resistant to chloramphenicol. Previously in Bida, Odedina and Emumwen (16) in 2008, reported sensitivity of *S. pneumoniae* to chloramphenicol to be 87.5%, but totally resistant to penicillin. This current study utilized the epsilon test (E-test) for antibiotic sensitivity of *S. pneumoniae* which is recommended by CLSI (26), rather than the disc diffusion method used by Odedina and Emumwen (16). The antibiotic sensitivity pattern in our study is similar to that reported by Ogeneh et al., (40) in 2015 in Enugu, where *S. pneumoniae* were totally sensitive to cephalosporins and penicillin. A possible explanation could be that cephalosporins have not been as abused due to high cost compared with the cheaper chloramphenicol.

Our study was limited by the use of only phenotypic method for bacterial detection. The use of newer molecular techniques such as polymerase chain reaction (PCR) or antigen detection test such as the latex agglutination test would have been very helpful to improve yield from cerebrospinal fluid samples especially with the use of antibiotics among some participants prior to hospital presentation.

Conclusion:

There is a shift in the aetiologic pattern of ABM among children in the post-neonatal age groups presenting with fever and convulsions in Bida, Nigeria. *Streptococcus pneumoniae* is the major causative bacterial agent. Most of the bacterial agents causing meningitis in Bida are susceptible to the third generation cephalosporins.

There should be a high index of suspicion for ABM in infants presenting with fever and convulsion. Furthermore, third generation cephalosporins, should be considered for empirical treatment in suspected cases of ABM.

Contributions of authors:

AEO, ACU, BOA and TSA conceptualized and designed the study. AEO, ACU, BOA and TSA were involved in data collection/ acquisition and statistical analysis. AEO, ACU and BOA were involved in the writing and revising the manuscript for intellectual content. All authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

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Conflict of interest:

Authors declare no conflict of interest.

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Original Article **Open Access**

Molecular epidemiology of genital *Chlamydia trachomatis* infections among women of reproductive age living with HIV/AIDS in a Nigerian tertiary health institution

*[1,2,3]Abayomi, S. A., [3]Akanbi, A. A., [2]Taiwo, S. S., [4]Omoare, A. A., and [5]Adegboro, B.

[1]Cancer Research UK, Swansea, Wales, United Kingdom
[2]Department of Medical Microbiology and Parasitology, LAUTECH Teaching Hospital, Ogbomoso, Nigeria
[3]Department of Medical Microbiology and Parasitology, University of Ilorin Teaching Hospital, Ilorin, Nigeria
[4]Public Health Laboratory Services, Nigeria Centre for Disease Control and Prevention (NCDC), Abuja, Nigeria
[5]Department of Medical Microbiology and Immunology, Nile University of Nigeria, Abuja, Nigeria
*Correspondence to: subslaabayomi@gmail.com

Abstract:

Background: *Chlamydia trachomatis* is the most prevalent bacterial pathogen causing sexually transmitted infections (STIs) globally. This study investigates the genovars of genital *C. trachomatis* infection among women of reproductive age living with HIV/AIDS in Ilorin, Nigeria.
Methodology: Endocervical samples were collected from a total of 402 consecutively recruited HIV-infected women attending the highly active anti-retroviral therapy (HAART) clinic of the University of Ilorin Teaching Hospital. Socio-demographic and clinical data of participants were collected through interviewer-administered structured questionnaire. *Chlamydia trachomatis* was detected in the samples by conventional polymerase chain reaction (PCR) amplification of *C. trachomatis* cryptic plasmid, and genotyping was performed using multiplex allele-specific (MAS) PCR to detect serovars D-K and L1-L3. Data were analyzed using SPSS version 20.0 Associations between variables were determined using Chi-square test, with significance set at $p<0.05$.
Results: The overall prevalence of *C. trachomatis* was 18.0%, and highest in women aged 18–25 years (33.3%). Genovars D–K were identified in 43.0% of cases, L1–L3 in 23.6%, and 33.3% could not be genotyped by the method used. Significant risk factors associated with *C. trachomatis* infection included previous STI history ($p=0.000$), non-use of barrier contraceptives ($p=0.009$), and prior STI treatment ($p=0.000$). There was a significant association of *C. trachomatis* infection and HIV viral load ($p=0.041$).
Conclusion: This study provides information on the epidemiological distribution of *C. trachomatis* genovars among women with HIV/AIDS in Ilorin, Nigeria. The genovars D-K and L1-L3 identified are good guide for appropriate management of *C. trachomatis* infections, including complications, contact tracing and prevention.

Keywords: *Chlamydia trachomatis*, HIV/AIDS, genovars, STI, Viral load

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Épidémiologie moléculaire des infections génitales à *Chlamydia trachomatis* chez les femmes en âge de procréer vivant avec le VIH/sida dans un établissement de santé tertiaire Nigérian

*[1,2,3]Abayomi, S. A., [3]Akanbi, A. A., [2]Taiwo, S. S., [4]Omoare, A. A., et [5]Adegboro, B.

[1]Recherche sur le Cancer au Royaume-Uni, Swansea, Pays de Galles, Royaume-Uni
[2]Département de Microbiologie Médicale et Parasitologie, Hôpital Universitaire LAUTECH, Ogbomoso, Nigéria
[3]Département de Microbiologie Médicale et Parasitologie, Hôpital Universitaire d'Ilorin, Ilorin, Nigéria
[4]Services Laboratoire Santé Publique, Centre Nigérian Contrôle et Prévention des Maladies (NCDC), Abuja, Nigéria
[5]Département de Microbiologie Médicale et d'Immunologie, Université du Nil du Nigéria, Abuja, Nigéria
*Correspondance à: subslaabayomi@gmail.com

Résumé:

Contexte: *Chlamydia trachomatis* est l'agent pathogène bactérien responsable d'infections sexuellement transmissibles (IST) le plus répandu dans le monde. Cette étude examine les génovars d'infection génitale à *C. trachomatis* chez des femmes en âge de procréer vivant avec le VIH/sida à Ilorin, au Nigéria.
Méthodologie: Des échantillons endocervicaux ont été prélevés auprès de 402 femmes infectées par le VIH,

recrutées consécutivement et fréquentant la clinique de traitement antirétroviral hautement actif (HAART) du CHU d'Ilorin. Les données sociodémographiques et cliniques des participantes ont été recueillies au moyen d'un questionnaire structuré administré par un intervieweur. *Chlamydia trachomatis* a été détectée dans les échantillons par amplification par réaction en chaîne par polymérase (PCR) conventionnelle du plasmide cryptique de *C. trachomatis*, et le génotypage a été réalisé par PCR multiplex spécifique d'allèle (MAS) pour détecter les sérovars D à K et L1 à L3. Français Les données ont été analysées à l'aide de SPSS version 20.0. Les associations entre les variables ont été déterminées à l'aide du test du Chi carré, avec une signification fixée à $p<0,05$.
Résultats: La prévalence globale de *C. trachomatis* était de 18,0%, et la plus élevée chez les femmes âgées de 18 à 25 ans (33,3 %). Les génovars D à K ont été identifiés dans 43,0% des cas, L1 à L3 dans 23,6%, et 33,3% n'ont pas pu être génotypés par la méthode utilisée. Les facteurs de risque significatifs associés à l'infection à C. trachomatis comprenaient les antécédents d'IST ($p=0,000$), la non-utilisation de contraceptifs barrières ($p=0,009$) et un traitement antérieur contre les IST ($p=0,000$). Il y avait une association significative entre l'infection à *C. trachomatis* et la charge virale du VIH ($p=0,041$).
Conclusion: Cette étude fournit des informations sur la répartition épidémiologique des génovars de *C. trachomatis* chez les femmes vivant avec le VIH/sida à Ilorin, au Nigéria. Les génovars D à K et L1 à L3 identifiés constituent une bonne indication pour la prise en charge appropriée des infections à *C. trachomatis*, notamment en ce qui concerne les complications, la recherche des contacts et la prévention.

Mots-clés: *Chlamydia trachomatis*, VIH/sida, génovars, IST, charge virale

Introduction:

Sexually transmitted infections (STIs) represent not only a public health challenge but also a personal crisis for millions, silently crippling reproductive health worldwide (1,2). Among these, *C. trachomatis* stands out as a bacterial infection with far-reaching consequences, capable of remaining asymptomatic while causing severe reproductive damage (3). The WHO reports over one million new STI cases daily, underscoring the global scale of this issue (1). Among these, *C. trachomatis* has emerged as the most prevalent bacterial STI, with profound implications for reproductive health and fertility. Often asymptomatic, *C. trachomatis* infections can silently progress to severe complications, including pelvic inflammatory disease (PID), ectopic pregnancy, and infertility. These risks are compounded in HIV-positive populations, where co-infections can influence HIV disease progression and complicate clinical management.

Globally, *C. trachomatis* continues to represent a significant burden in various regions. In the United States, *C. trachomatis* is the most reported STI, with over two million cases annually and a higher prevalence in young adults aged 15–24 years (2). In Europe, routine screening programs report a prevalence of 3%–6% among sexually active women, underscoring regional differences in diagnostic and preventive strategies (4). Similarly, in Asia, studies have revealed substantial challenges in diagnosing and managing *C. trachomatis*, with prevalence rates ranging from 5-10% in countries like China and India, particularly among high-risk groups (5).

In sub-Saharan Africa, including Nigeria, the prevalence of *C. trachomatis* infection among reproductive-age women varies widely, ranging from 6% to 40%, with significant socioeconomic and healthcare disparities contributing to this variation (6). This aligns with studies in similar Nigerian settings, where *C. trachomatis* infection has been linked to poor reproductive outcomes and an increased risk of HIV transmission. Despite these alarming statistics, limited research has explored the molecular epidemiology of *C. trachomatis* in resource-limited settings like Nigeria. Addressing this gap is critical for improving screening strategies and ensuring tailored interventions. This study aims to fill this critical gap by investigating the molecular epidemiology of *C. trachomatis* among HIV-positive women in Ilorin, Nigeria.

Materials and method:

Study area, design and ethical approval:
This was a hospital-based descriptive cross-sectional study of HIV-infected women aged 18 to 49 years attending the highly active antiretroviral therapy (HAART) clinic of the University of Ilorin Teaching Hospital (UITH), Ilorin, Nigeria. UITH is a healthcare institution with a capacity of 600 beds, which additionally operates as a referral center for residents of Kwara and the adjacent States of Niger, Ekiti, Osun, Oyo, and Ondo. Geographically, it is situated at a latitude of 8.3ºN and a longitude of 4.3ºE in the northcentral region of Nigeria.

The mean frequency of patient visits for HAART treatment is approximately 6000 monthly consultations (7). The Institutional Review Board (IRB) of UITH approved the conduct of the study with approval number UITH/CAT/189/19B/195. At recruitment, each participant provided informed consent to participate in the study.

Sample size and participant selection:
The sample size of 402 patients was calculated using the modified Fisher's formula for proportions with population less than 10000 (8), with an estimated prevalence rate set at 50% and adjusting for 10% attrition. The patients who satisfied the inclusion criteria were recruited consecutively using the simple random sampling technique until the sample size of 402 was attained over a period of 3 months.

Data, sample collection and transportation:

A structured questionnaire was designed and interviewer-administered to collect relevant demographic and clinical information including age, marital status, educational level, relevant symptoms of sexually transmitted infections such as history of vaginal discharge and lower abdominal pain.

Endocervical swab was collected aseptically from all participants at the HAART clinic by one of the researchers (ASA). A Cusco's speculum was inserted into the vagina to expose the cervix while the patient was lying supine with the legs supported with a stirrup. Endocervical swab was taken by inserting a cytobrush into the endocervical canal, followed by a gentle 360º rotation of the cytobrush 5 times. The cytobrush was withdrawn carefully to avoid contact with the vaginal mucosa and then placed inside a tube containing a liquid-based cytology (Eziprep™) preservative, stored at 2-8°C, and transported to the laboratory for genomic DNA extraction.

Five millilitres (5 ml) of venous blood was also collected from each participant into ethylene diamine tetra acetic acid (EDTA) bottle by aseptic venepuncture, for HIV testing.

Detection of HIV and viral load estimation:

Presumptive detection of HIV was done using Determine HIV 1/2 Ag/Ab (Abbot) kit. A Pasteur pipette was used to place a drop of blood onto the absorbent pad on the strip of the kit. A drop of chase buffer was added to the specimen pad and the result was interpreted after 15 minutes and recorded. A reactive result was interpreted by two lines appearing in both control and test areas of the strip.

Confirmatory HIV detection was performed using UniGold™ (Trinity Biotech) strip, a rapid immunoassay for qualitative detection of antibodies to HIV-1 and HIV-2 in the blood. Two drops of blood were applied to the same port, followed by two drops of wash solution and allowed to react for 15 minutes. A reactive result is interpreted by a line at the test and another one at the control region of the test device.

The results of the viral load of each HIV-infected participant performed by real-time (rt) PCR at Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Nigeria, in the 3 months preceding enrolment were retrieved from the laboratory records of each participant.

DNA extraction:

Chlamydia trachomatis DNA was extracted from each endocervical swab sample using a commercially available DNA extraction kit (FavorPrep™Ping Tong, Taiwan) according to the manufacturer's instructions. Eluted total DNA was subsequently stored at -20℃ for PCR analysis.

Polymerase chain reaction amplification detection of *C. trachomatis* cryptic plasmid:

Conventional PCR as described by Cufinni and co-workers (9) was used to amplify the cryptic plasmid DNA (usually present in all *C. trachomatis* strains) in all the endocervical samples from which DNA has been extracted. The appropriate volumes of Master Mix (made up of thermostable Taq DNA polymerase, $MgCl_2$, nucleotide triphosphates, reaction buffer), forward (5'-AGTATTTCCGGAGCAGGC-3') and reverse (5'-TTACCTCGAAAAACTGTTCC-3') primer, template genomic DNA and distilled water were dispensed into each PCR tube and the mixture vortexed.

Amplification was done in a thermal cycler (Eppendorf) programmed with 35 cycles of denaturation at 94°C for 45 seconds, annealing at 55°C for 60 seconds, extension at 72°C for 90 seconds, and final extension at 72°C for 5 minutes. Positive control (*C. trachomatis* reference serovar-L2 strain obtained from National Microbiology Laboratory, Winnipeg, Canada), and negative controls (distilled water) were also processed using the same protocol.

The tubes were removed from the thermal cycler after the reaction has been completed and the amplicons were resolved on electrophoresis in 1.5% agarose gel containing a gel stain dye (10µl SYBR) safe to produce bands that were visualized in a gel documentation system with an ultraviolet trans-illuminator and a documentation computer system. The 100 base pair molecular weight standard DNA marker was used to determine the size of the amplified PCR product. A positive reaction for *C. trachomatis* was confirmed by the presence of a band corresponding to 201 amplicon size.

Multiplex allele specific PCR (MAS-PCR) for *C. trachomatis* serovar identification:

The multiplex allele-specific PCR (MAS-PCR) for *C. trachomatis* designed by Cai and colleagues (10), was used to identify the serovars of *C. trachomatis* in all the samples that amplified with the conventional PCR for cryptic plasmid, and categorized them into serovars D-K, L1-L3 or untyped strain.

The MAS-PCR is based on the variation in the sequence of the polymorphic membrane protein H (*pmpH*) gene of *C. trachomatis* (which has a unique 36-nucleotide base deletion compared with the other *C. trachomatis* serovars) for which primers that can amplify the unique specific sequences in a multiplex PCR system to distinguish serovars A-C, D-K and L1-L3 have been developed.

The reagents used for MAS-PCR includes ready to use Master Mix (5µl), 0.5µl of outer primers (*pmph*-F and *pmph*-R), 0.5µl of inner primers (*pmph*-R2-LGV and *pmph*-R3-CT) (Macrogen, Europe), 17.5µl of distilled

water and 1.5µl of template DNA. Two sets of MAS-PCR were done with two primers in combination of each set; set one with *pmpH*-F (forward outer primer), *pmph*-R (reverse outer primer) and *pmpH*-R2-LGV (inner primer) for *C. trachomatis* serovars L1, L2 or L3; and set two with *pmpH*-F (forward outer primer), *pmpH*-R (reverse outer primer) and *pmpH*-R3-CT (inner primer) for *C. trachomatis* serovars D-K (Table 1).

After an initial denaturation step at 95ºC for 15 minutes, the following amplification conditions were utilized; denaturation at 94ºC for 30 seconds, annealing at 55ºC for 30 seconds and extension at 72ºC for 30 seconds, followed by 25 cycles at 94ºC for 30 seconds, 53ºC for 40 seconds and 72ºC for 40 seconds, with a final extension at 72ºC for 2 minutes. A non-template control containing sterile distilled water was included in each experimental run. Amplified products were electrophoresed on 2% agarose gel and visualised under ultraviolet light and photographed with a gel documentation system.

The set of *pmph*-F, *pmph*-R and *pmph*-R3-CT that positively amplified is expected to produce two bands of 224 bp and 122 bp sizes to indicate *C. trachomatis* serovars D-K while the set of *pmph*-F, *pmph*-R and *pmph*-R2-LGV that positively amplified is expected to produce two bands of 224 bp and 106 bp sizes to indicate *C. trachomatis* serovars L1, L2 or L3.

Course of treatment:

Participants who tested positive for *C. trachomatis* were treated with doxycycline or azithromycin based on the National STI guidelines. Counselling was offered to promote adherence, educate on safe sexual practices, and address stigma associated with STIs and HIV. Follow-up appointments were scheduled to monitor treatment outcomes and reinforce behavioral changes aimed at reducing reinfection risk.

Statistical analysis:

Data were analyzed using SPSS version 20.0. Associations between variables were assessed using Chi-square test, with significance set at $p<0.05$.

Results:

Prevalence of *Chlamydia trachomatis* infection*:*

The overall prevalence of *C. trachomatis* infection was 18.0% (n=72/402). Women aged 18–25 years had the highest prevalence (33.3%, n=1/3), followed by age group 46–49 years (22.0%, n=22/100), and 31–40 years (17.8%, n=24/135) (Table 1), but there was no significant association of *C. trachomatis* infection with age ($p=0.5656$).

However, there was significant association of *C. trachomatis* infection with marital status ($p=0.0014$), with higher prevalence in divorced (50.0%, n=1/2) and married (23.0%, n=63/274) women compared to other women, but no significant association with educational status of the participants ($p=0.2156$) (Table 2).

Symptoms associated with *C. trachomatis* infection:

Among 72 women positive for *C. trachomatis*, 72.2% (n=52) reported vaginal discharge, compared to 43.0% (n=142) among 330 women negative for *C. trachomatis* infection ($p=0.000$). Other symptoms significantly more frequent in women with *C. trachomatis* infections are pain during sexual intercourse ($p=0.048$), bleeding after sexual intercourse ($p=0.004$), bleeding between periods ($p=0.019$) and history of STIs ($p=0.000$) (Table 3).

Table 1: Primers and conditions of the multiplex allele-specific PCR for *Chlamydia trachomatis* genotyping

Target gene	Primer sequence	Amplicon size	Melting point	Specificity	Reference
pmpH	Outer primers- *pmph*-F-AGTATTTCCGGAGCAGGC *pmph*-R-TTACCTCGAAAAACTGTTCC	224 bp or 260 bp	55ºC	*C. trachomatis*	10
pmpH	Inner primers- *pmph*-R2-LGV–TAACTGTTGGAGCAGGCG *pmph*-R3-CT- CTTGAAGCAGCAGGAGCT	106 bp 122 bp	52ºC 52ºC	Serovar L1-L3 Serovar D-K	10

Table 2: Socio-demographic characteristics of HIV-infected women with *Chlamydia trachomatis* infections in Ilorin, Nigeria

Characteristic	No positive for *C. trachomatis* (n=72, 18.0%)	No negative for *C. trachomatis* (n=330, 82.0%)	Chi square (x^2)	*p* value
Age group (years)				
18-25	1 (33.3)	2 (66.7)	2.954	0.5656
26-30	2 (9.5)	19 (90.5)		
31-40	24 (17.8)	111 (82.2)		
41-45	23 (16.1)	120 (83.9)		
46-49	22 (22.0)	78 (78.0)		
Marital status				
Single	2 (7.7)	24 (92.3)		
Married	63 (23.0)	211 (77.0)		
Widowed	4 (6.3)	59 (93.7)		
Separated	2 (5.4)	35 (94.6)		
Divorced	1 (50.0)	1 (50.0)	17.723	0.0014*
Educational status				
None	7 (16.3)	36 (83.7)		
Primary	15 (19.0)	64 (81.0)		
Secondary	17 (12.9)	115 (87.1)		
Tertiary	33 (22.4)	114 (77.6)	4.463	0.2156

* = statistically significant

Table 3: Comparative analysis of clinical symptoms between HIV-infected women with and without *Chlamydia trachomatis* infections in Ilorin, Nigeria

Clinical symptom	No positive for *C. trachomatis* (%) (n=72)	No negative for *C. trachomatis* (%) (n=330)	Chi square (x^2)	*p* value
History of vaginal discharge	52 (72.2)	142 (43.0)	20.171	0.000*
History of blisters and spots	2 (2.8)	2 (0.6)	2.803	0.150
History of lower abdominal pain	16 (22.2)	48 (14.5)	2.602	0.079
Pain during sexual intercourse	8 (11.1)	19 (5.8)	4.033	0.048*
Bleeding after sexual intercourse	4 (5.6)	1 (0.3)	13.275	0.004*
Bleeding between periods	3 (4.2)	1 (0.3)	8.956	0.019*
History of pain on passing urine	4 (5.6)	12 (3.6)	0.570	0.317
Previous STI	53 (73.6)	140 (42.4)	23.031	0.000*

* = statistically significant; STI=Sexually transmitted infection

Risk factors associated with *C. trachomatis* infection:

Risk factors associated with *C. trachomatis* infection included age at first intercourse (p=0.000), history of previous STI (p=0.000), use of protection against STIs (p=0.009), types of protection against STIs (p=0.000), and prior STD treatment (p=0.000) (Table 3).

Frequency distribution of *C. trachomatis* genovars:

Of the 72 women with *C. trachomatis* infection, as confirmed by PCR amplification of the cryptic plasmid (Fig 1), 31 (43.0%) were genovars D-K (Fig 2), 17 (23.6%) were genovars L1-L3 (Fig 3) while 24 (33.3%) could not be genotyped by the method used.

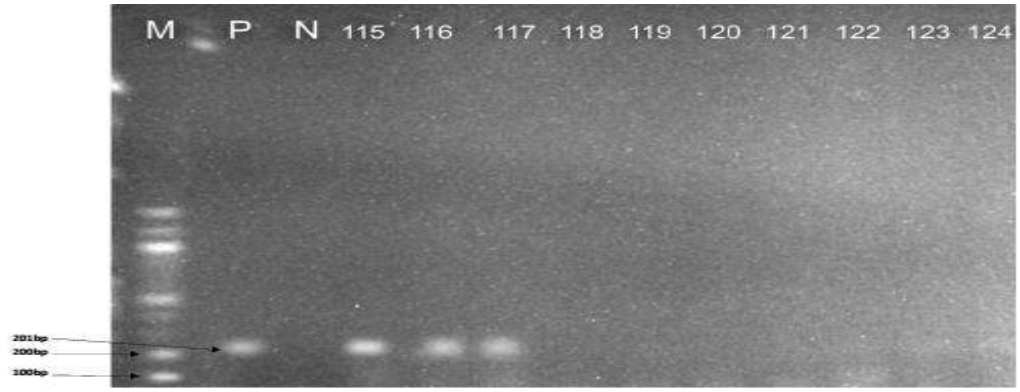
HIV viral load and *C. trachomatis* infection:

HIV load was significantly associated with *C. trachomatis* infection (p=0.0419). HIV loads of 101-1000 and >1000 copies/µl were significantly higher in women with *C. trachomatis* infections (11.3% and 16.1% respectively) compared to women without *C. trachomatis* infection (8.9% and 14.2% respectively) (Table 4).

Table 4: Risk factors of *Chlamydia trachomatis* in HIV infected women in Ilorin, Nigeria

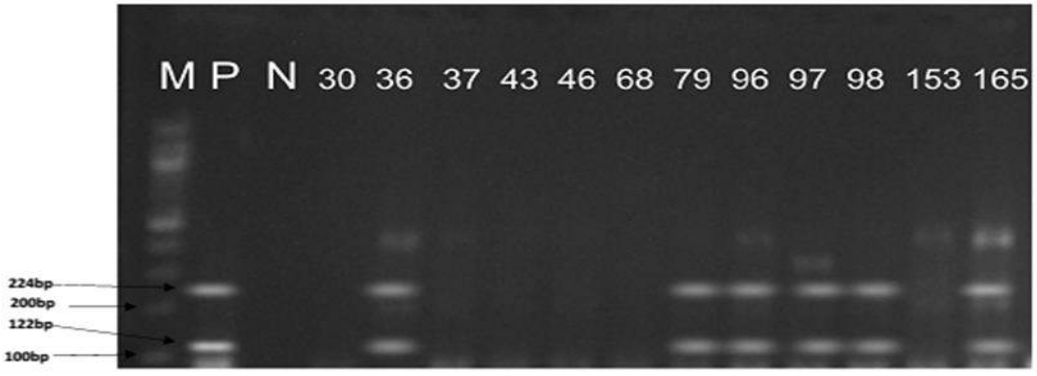
Risk factor	No positive for *C. trachomatis* (n=72, 18.0%)	No negative for *C. trachomatis* (n=330, 82.0%)	Chi square (x^2)	*p*-value
Age at first intercourse (years)				
18-25	20 (25.0)	60 (75.0)		
26-49	52 (16.6)	262 (83.4)	18.501	0.000*
Number of sexual partners				
None	4 (7.4)	50 (92.6)		
1	68 (19.7)	278 (80.3)		
>1	0	1 (100.0)	5.301	0.064
Use of protection against STI				
Yes	27 (13.2)	177 (86.8)		
No	45 (62.2)	152 (46.2)	6.279	0.009*
History of previous STI				
Yes	53 (26.9)	140 (73.1)		
No	19 (9.1)	190 (90.9)	23.031	0.000*
Type of protection against STI				
None	27 (13.3)	176 (86.7)		
Condom	40 (20.8)	152 (79.2)		
Others	5 (100.0)	0	31.804	0.000*
Treated for STI				
Yes	45 (23.4)	147 (76.6)		
No	3 (75.0)	1 (25.0)	32.94	0.000*

* = statistically significant; STI=Sexually transmitted infection



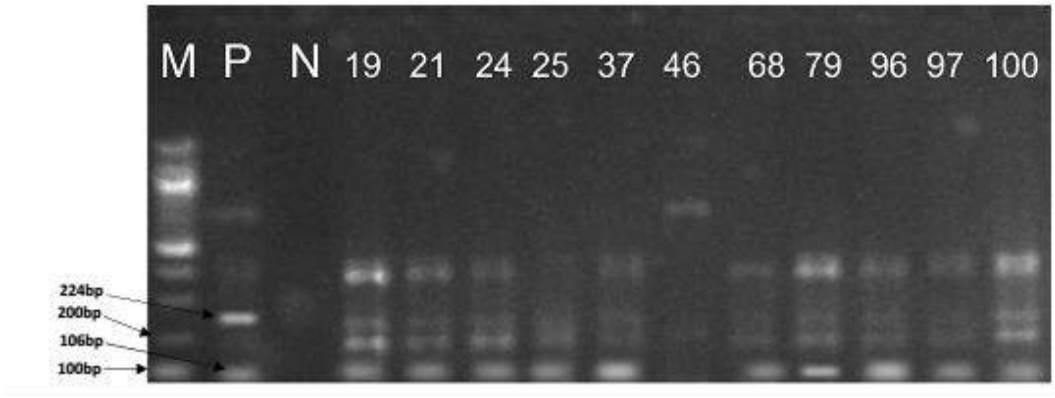
M= Molecular ladder; P = Positive Control; N = Negative Control; Positive samples 115, 116 and 117 amplified at 201bp for *C. trachomatis*; Negative samples 118-124 did not amplify

Fig 1: Gel electrophoresis of representative *Chlamydia trachomatis* showing amplicon size of 201 bp for cryptic plasmid



M= Molecular ladder; P = Positive Control; N = Negative Control; Positive samples 36, 79, 96, 97, 98, 165 amplified at 224bp for *C. trachomatis;* Negative samples 30, 37, 43, 46, 68 did not amplify

Fig 2: Gel electrophoresis of representative *Chlamydia trachomatis* showing amplicon size 224 bp (*pmph* gene with outer primer) and another amplicon size 122 bp ((*pmph* gene with inner primer) for genovars D-K



M= Molecular ladder; P = Positive Control; N = Negative Control; Positive samples 19, 21, 24, 25, 37, 68, 79, 96, 97, 100 amplified at 224bp for *C. trachomatis* and 106 bp for *C. trachomatis* genovars L1-L3

Fig 3: Gel electrophoresis of representative *Chlamydia trachomatis* showing amplicon size 224bp (*pmph* gene with outer primer) and another amplicon at 106 bp (*pmph* gene with outer primer) for genovars L1-L3

Table 5: Comparison analysis of viral load of HIV infected women with and without *Chlamydia trachomatis* infection in Ilorin, Nigeria

Viral load (copies/µL)	No positive for *C. trachomatis* (%) (n=61)	No negative for *C. trachomatis* (%) (n=283)	Chi square (x^2)	*p* value
0	2 (3.2)	0	9.913	0.0419*
<20	33 (53.2)	167 (59.2)		
20-100	10 (16.1)	50 (17.7)		
101-1000	7 (11.3)	25 (8.9)		
>1000	9 (16.1)	41 (14.2)		

n = number of participants whose viral loads were available; * = statistically significant

Discussion:

Chlamydia trachomatis has been identified as an accelerator in the transmission and acquisition of HIV (11). Most of the women do not experience symptoms, however testing them helps to identify this infection. In this present study, 402 endocervical samples were collected and *C. trachomatis* was detected using conventional PCR technique. The overall prevalence of 18% is similar to the prevalence reported by Spinillo et al., (12) of 18.3% in HIV infected women, a study which was conducted in Italy. This is however lower than the prevalence reported in South Africa where a value of 39.2% obtained in HIV infected women (6). In Nigeria however, studies in various cities produce divergent reports of *C. trachomatis* prevalence. A study in Lagos reported a prevalence of 51% among pregnant and non-pregnant women (13), while Oloyede et al*.,* (14) reported a prevalence of 18.2% also in Lagos, Nigeria. Mawak et al., (15) reported a prevalence of 30.2%, while Ikeme et al., (16) in Enugu reported a prevalence of 29.4%. These variations may be due to differences in the population studied and laboratory detection methods used.

The age group with the highest prevalence of *C. trachomatis* infection in our study is 18-25 years (33.3%, n=1/3), which agrees with the study that reported younger age group < 25 years to be at greater risk of *C. trachomatis* infection because of the not yet fully developed cervix, which increase their susceptibility to infection (17). A previous study also reported that *C. trachomatis* infection is most common in people aged less than 25 years due to greater exposure to high-risk sexual behaviours (18). However, other studies in Africa have reported higher prevalence of 35.6% in women aged 25-45 years, and a study in Mpumalanga Province reported high prevalence of 52.8% in female miners aged 46-49 years (6).

Age at first intercourse is one of the risk factors of *C. trachomatis* infection. This is the case in our study where women who had their first sexual intercourse between age 18 and 25 years had significantly higher prevalence of *C. trachomatis i*nfection of 25.0% compared to women who had their first intercourse between age 26 and 49 years, with prevalence of 16.6% (p=0.000), but disagrees with the study by Mafokwane et al., (6) who reported higher prevalence of 35.6% among women aged 25–45 years (6), and a previous study in southeast Nigeria that reported higher prevalence of 43.9% among women between the age of 26–30 years attending gynaecological clinics (13).

Previous studies have also reported

that unprotected sex is one of the major risk factors for acquisition of sexually transmitted infections. Although there was a significant association between use of protection and *C. trachomatis* infection in our study (p=0.000), the prevalence of infection was highest among those who used unknown means of protection (100%, n=5/5), followed by those who used condoms (20.8%, n=40/192) and lowest among those who did not use any protection (13.3%, n=27/203). This contradicts the study by Dubbink et al., (19) who reported a high infection prevalence of 40% in people who did not use condoms. However, our study findings may indicate that the use of condom may not protect against STDs, which may likely be due to inappropriate use of condoms during sexual intercourse in our participants, while those using unapproved or unvalidated means of protection are at high risk of *C. trachomatis* infection.

Of the *C. trachomatis* detected in the 72 infected women, only 48 could be genotyped by the MAS-PCR method used, with genovars D-K identified in 31 (43.0%) and genovars L1-L3 in 17 (23.6%) cases. This is similar to a study by Lesiak-Markowicz et al., (20) who identified and reported serovars D-F as the most recognised serovars worldwide. Foschi et al., (4) also identified serovars D-K as the most prevalent serovar in their study where they reported an overall prevalence of 83%. Our inability to type the remaining 24 *C. trachomatis* by MAS-PCR assay may have been due to low bacterial load in the endocervical specimens and technical limitations of multiplex PCR.

Although there were 17 *C. trachomatis* genovars L1-L3 in our study, only 2 of the 72 (2.8%) women with *C. trachomatis* infection had history of vaginal sores and blisters, indicating that most of the women with genovars L1-L3 infection are asymptomatic. This is similar to the report of a study by Haro-cruz et al., (5) on endocervical samples of infertile women in Mexico, where genovar L2 strain was found in (8.7%) of the infertile women and these women did not present with or had history suggestive of a sexually transmitted infection or ulcers or sores at the time of specimen collection.

Lymphogranuloma venereum (LGV) is a sexually transmitted infection caused by serovars L1, L2 and L3 of *C. trachomatis*. It is endemic in Africa, India, South America, Southeast Asia and the Caribbean. Studies have reported that women are more likely to become infected with LGV (21). The classical picture of LGV (buboes and lymphadenopathy) was not present in the women that had L1-L3 genovars in our study, which may mean that they were most likely asymptomatic. Gomes et al., (21) reported the detection of 7 LGV from specimens collected from men and women who had no clear LGV symptoms. Another explanation for this may be that point mutations are present in the genome of L2 variants that prevent the development of symptoms of LGV or proctitis (21).

In agreement with our study and others in the literatures (13,21) in relation to the symptomatic course of most *C. trachomatis* infection, a proportion of HIV seropositive women do not have any symptom or sign of infection on physical examination. Many cases without *C. trachomatis* infection in our study also presented with symptoms such as lower abdominal pain, dysuria, dyspareunia and vaginal discharge. This may be due to the presence of possible other STD pathogens aside *C. trachomatis* such as *Ureaplasma urealyticum*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, *Candida albicans* and others. This will require further molecular epidemiological study of these other STD pathogens.

Conclusion:

Our study provides information on the prevalence, risk factors and epidemiological distribution of genovars of *C. trachomatis* infection among women with HI`V/AIDS in Ilorin, Nigeria using MAS-PCR assay. The genovars D-K and L1-L3 identified are good guide for appropriate management of *C. trachomatis* infections, including complications, contact tracing and prevention.

Contributions of authors:

ASA was involved in the conceptualization of study, data and sample collection, coordinated the research work, ensured compliance with ethical standards and study protocols, and secured funding through grants. AAA reviewed the literature. TSS developed the methodology used in the study and reviewed the literature. OAA contributed to the optimisation of research protocol, literature review and drafting of initial manuscript. AB reviewed the literature. All authors approved the manuscript submitted for publication.

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Conflict of interest:

Authors declare no conflict of interest

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Original Article **Open Access**

Antibiotic resistance profile of Non-Fermenting Gram-negative bacteria recovered from clinical specimens in a tertiary care teaching hospital, Hyderabad, India

*Kumuda, A., and Pavani, G.

Department of Microbiology, Apollo Institute of Medical Sciences and Research, Hyderabad, India
*Correspondence to: kumudarumugam@gmail.com; +918805647838

Abstract

Background: Non-Fermenting Gram-negative bacilli (NFGNB), including *Pseudomonas aeruginosa*, *Acinetobacter* species, and *Burkholderia* species, are emerging as significant opportunistic pathogens, especially in immunocompromised and hospitalized patients. These organisms pose a major therapeutic challenge due to their multidrug-resistance (MDR) and intrinsic resistance mechanisms. The goal of this study is to determine antibiotic susceptibility pattern of NFGNB isolated from clinical specimens of patients in a tertiary care teaching hospital in Hyderabad, India.
Methodology: A descriptive cross-sectional observational study of patients with clinical infections was conducted between December 2024 and April 2025, to identify NFGNB from their clinical specimens. Different clinical samples (blood, sputum, urine, pus and others) were collected from patients of both sexes and all age groups for bacterial isolation using automated Bact/Alert for blood culture and conventional method for other samples. Identification of NFGNB isolates and antimicrobial susceptibility testing (AST) of the isolates against selected antibiotics were performed using the VITEK 2 Compact system, and AST interpretations were made in line with the Clinical and Laboratory Standards Institute (CLSI) guidelines.
Results: During the study period, clinical samples of 1575 patients were processed, 359 were positive for bacterial isolates on cultures, and 32 NFGNB were identified from clinical samples of 32 patients with clinical infections. Among the 32 isolates, *Pseudomonas* accounted for 78.0% (n=25) followed by *Acinetobacter* (13.0%, n=4) and *Burkholderia* (9.0%, n=3). NFGNB infections were more frequent in males (78.0%) and patients aged 51-75 years. Majority (78.0%) of isolates were from inpatients. *Pseudomonas* showed high resistance to fluoroquinolones (36.0%) but sensitive to piperacillin/tazobactam (84.0%) and β-lactam combinations (76.0%). *Acinetobacter* isolates exhibited 75.0% resistance to multiple antibiotics but 50.0% were sensitive to carbapenems and β-lactam/β-lactamase inhibitor combinations. *Burkholderia* isolates showed complete resistance to cefepime, cotrimoxazole and amikacin, but were 100.0% sensitive to piperacillin/tazobactam.
Conclusion: The study underscores the alarming levels of antimicrobial resistance among NFGNB, particularly in hospitalized and elderly patients. The findings highlight the necessity of continuous antimicrobial resistance surveillance, stringent infection control, and robust antibiotic stewardship to combat the growing threat of multidrug-resistant organisms.

Keywords: Non-Fermenting Gram-negative bacilli; Multi-drug resistance; Clinical specimens

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Profil de résistance aux antibiotiques des bactéries Gram-négatives Non-Fermentantes issues d'échantillons cliniques d'un hôpital universitaire de soins tertiaires, Hyderabad, Inde

*Kumuda, A., et Pavani, G.

Département de Microbiologie, Institut Apollo des Sciences Médicales et de la Recherche, Hyderabad, Inde
*Correspondance: kumudarumugam@gmail.com; +918805647838

Résumé

Contexte: Les bacilles Gram-négatif Non-Fermentants (BGNF), notamment *Pseudomonas aeruginosa*, *Acinetobacter* et *Burkholderia*, apparaissent comme des pathogènes opportunistes importants, notamment chez

les patients immunodéprimés et hospitalisés. Ces organismes représentent un défi thérapeutique majeur en raison de leurs mécanismes de multirésistance aux antibiotiques (MDR) et de résistance intrinsèque. L'objectif de cette étude est de déterminer le profil de sensibilité aux antibiotiques du NFGNB isolé à partir d'échantillons cliniques de patients d'un hôpital universitaire de soins tertiaires à Hyderabad, en Inde.
Méthodologie: Une étude observationnelle descriptive transversale a été menée auprès de patients atteints d'infections cliniques entre décembre 2024 et avril 2025 afin d'identifier le NFGNB à partir de leurs échantillons cliniques. Différents échantillons cliniques (sang, expectorations, urine, pus et autres) ont été prélevés chez des patients des deux sexes et de tous âges afin d'être isolés bactériennement à l'aide du système automatisé Bact/Alert pour les hémocultures et de la méthode conventionnelle pour les autres échantillons. L'identification des isolats de NFGNB et les tests de sensibilité aux antimicrobiens (AST) des isolats contre certains antibiotiques ont été réalisés à l'aide du système VITEK 2 Compact. Les interprétations des AST ont été réalisées conformément aux recommandations du Institut des Normes Cliniques et de Laboratoire (CLSI).
Résultats: Au cours de la période d'étude, des échantillons cliniques de 1575 patients ont été traités, 359 étaient positifs pour les isolats bactériens sur les cultures et 32 NFGNB ont été identifiés à partir d'échantillons cliniques de 32 patients atteints d'infections cliniques. Parmi les 32 isolats, *Pseudomonas* représentait 78,0% (n=25), suivi par *Acinetobacter* (13,0%, n=4) et *Burkholderia* (9,0%, n=3). Les infections à NFGNB étaient plus fréquentes chez les hommes (78,0%) et les patients âgés de 51 à 75 ans. La majorité (78,0%) des isolats provenaient de patients hospitalisés. *Pseudomonas* a montré une résistance élevée aux fluoroquinolones (36,0%) mais une sensibilité aux combinaisons pipéracilline/tazobactam (84,0%) et β-lactamines (76,0%). Les isolats d'*Acinetobacter* présentaient une résistance de 75,0 % à de multiples antibiotiques, mais 50,0% étaient sensibles aux carbapénèmes et aux associations β-lactamines/inhibiteurs de β-lactamases. Les isolats de Burkholderia présentaient une résistance complète au céfépime, au cotrimoxazole et à l'amikacine, mais une sensibilité de 100,0% à la pipéracilline/tazobactam.
Conclusion: Cette étude souligne les niveaux alarmants de résistance aux antimicrobiens chez les patients atteints de NFGNB, en particulier chez les patients hospitalisés et les personnes âgées. Ces résultats soulignent la nécessité d'une surveillance continue de la résistance aux antimicrobiens, d'un contrôle rigoureux des infections et d'une gestion rigoureuse des antibiotiques pour lutter contre la menace croissante des organismes multirésistants.

Mots-clés: Bacilles à Gram-négatif Non-Fermentants; Multirésistance aux antibiotiques; Échantillons cliniques

Introduction:

Non-Fermenting Gram-negative bacilli (NFGNB) represent a varied group of bacteria that either utilize oxidative pathways to utilize carbohydrates for energy or are incapable of metabolizing them. These organisms have become causes of infections linked to healthcare, especially in immunocompromised patients (1). The two most frequently encountered non-fermenters that are pathogenic to humans are *Pseudomonas* and *Acinetobacter*. Other NFGNB species such as *Elizabethkingia meningoseptica*, *Burkholderia cepacia*, and *Stenotrophomonas maltophilia* are less frequent but are becoming increasingly reported, especially in immunocompromised patients, and those who have been hospitalised for an extended period (2).

These organisms are primarily saprophytic and were once regarded as mere contaminants or harmless commensals with minimal clinical importance (3). In the recent times however, NFGNB are responsible for several infections, including urinary tract infection (UTI), meningitis, wound infections, osteomyelitis, septicemia, and pneumonia (4). These infections are challenging to treat because of their multidrug-resistance (MDR) and rapid onset of high level MDR to different antibiotic classes such as fluoroquinolones, aminoglycosides and beta-lactam drugs (5). These organisms are often resistant to several antibiotics due to their capacity to produce enzymes such as metallo-beta-lactamase (MBL) and extended-spectrum beta lactamase (ESBL) which inactivate many beta-lactam drugs (5).

The increasing antibiotic resistance among NFGNB is a significant global concern, potentially leading to adverse clinical outcomes such as therapeutic failure, progression to bacteremia, the need for intravenous treatment, hospital admission, and prolonged hospital stays. A wide array of causes, such as improper antibiotic usage in humans and animals, environmental contamination, poor sanitation, and innate characteristics of bacteria, contribute to the complex, constantly evolving issue of antimicrobial resistance (6). Understanding the various antibiotic susceptibility pattern among NFGNB isolated from various clinical specimens was the goal of this study.

Materials and method:

Study setting, design and specimen collection:

This study was carried out in the Department of Microbiology of Apollo Institute of Medical Sciences and Research, Hyderabad, India. The study was a descriptive cross-sectional observational design carried out between December 2024 and April 2025 on patients who had Non-Fermenting Gram-negative bacilli (NFGNB) isolated from their clinical samples.

Specimens such as sputum, wound swabs, blood, urine, and others were collected from patients of both sexes and all ages, presenting with suspected infections in both

inpatient and outpatient departments. The samples were promptly transported to the microbiology laboratory for microbiological analysis.

Bacterial isolation:

To perform blood culture, 2-3 ml of paediatric blood and 5-10 ml of adult blood were aseptically collected into Bact/Alert PF Plus culture bottles for children and Bact/Alert FA Plus culture bottles for adults. The bottles were incubated in Bact/Alert 3D-60 automated system (BioMérieux SA) and monitored for a maximum of five days. An aliquot from every culture positive bottle was subcultured onto standard media (Chocolate, Blood and MacConkey agar plates) for bacteria isolation. Conversely, specimens such as urine, pus, sputum were directly seeded onto these media and subsequently incubated at 37℃ for 18 to 24 hours.

Bacterial identification and antibiotic susceptibility testing:

Non-Fermenting Gram-negative bacilli were identified based on conventional microbiological procedures such as colony morphology, Gram staining, and motility. Using the Vitek® 2 Compact system (BioMérieux, SA), Gram-negative identification card (ID-GN) was used for bacterial species identification, and AST-N406 susceptibility card was used for antibiotic susceptibility test (AST) in accordance with the manufacturer's standard protocol against piperacillin/tazobactam, levofloxacin, ciprofloxacin, ceftriaxone, ceftazidime, cefepime, cefoperazone/sulbactam, imipenem, meropenem, amikacin, minocycline, and trimethoprim-sulphamethoxazole (cotrimoxazole).

The turbidity of the inoculum prepared from bacterial colonies was adjusted to 0.5 MacFarland standard using DensiCHEK device (BioMérieux SA), after which the standardized inoculum was suspended in 3 ml sterile 0.45% sodium chloride solution. The results of AST were interpreted using the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Results:

Distribution of Non-Fermenting Gram-negative bacilli (NFGNB) isolates:

Over the period of the study, clinical samples of 1575 patients were processed in the microbiology laboratory, and 359 were positive for bacterial isolates on cultures. Of these culture-positive samples, 32 Non-Fermenting Gram-negative bacilli (NFGNB) were identified from samples of 32 patients. Fig 1 shows that among the 32 NFGNB isolates, *Pseudomonas* accounted for 25 (78.0%), followed by *Acinetobacter* species with 4 isolates (13.0%), and *Burkholderia* species with 3 isolates (9.0%).

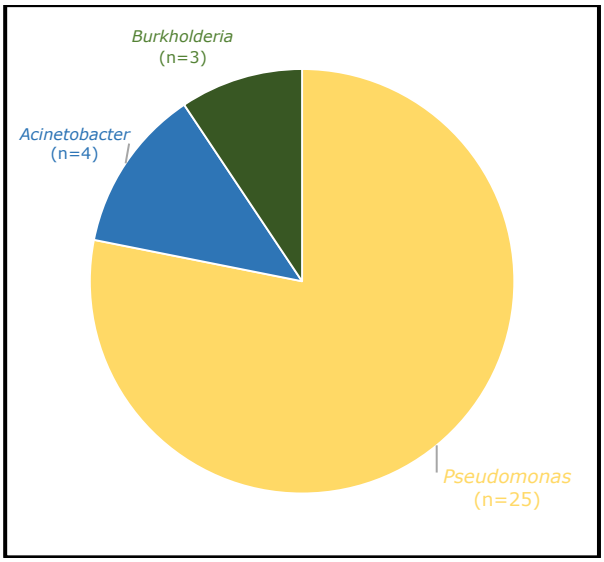

Fig 1: Frequency distribution of Non-Fermenting Gram-negative bacilli isolates from clinical samples of 32 patients

A higher frequency of NFGNB (n=25, 78.0%) were isolated on cultures from male patients compared to 7 (22.0%) from female patients (Table 1). The most frequently affected age group was between 51-75 years (n= 15, 47.0%) followed by 26-50 years group (n=14, 44.0%) and 1-25 years group (n=3, 9.0%). Twenty-five isolates (78.0%) were recovered from in-patient setting, while 7 (22%) were isolated from out-patient setting.

Table 1: Distribution of Non-Fermentative Gram-negative bacilli isolates with respect to sex, age group and location of patients

Variable	Frequency	Percentage
Age group (years)		
1-25	3	9.0
26-50	14	44.0
51-75	15	47.0
Sex		
Male	25	78.0
Female	7	22.0
Patient location		
In-patient	25	78.0
Out-patient	7	22.0

Antibiotic susceptibility test results of NFGNB isolates:

Antibiotic susceptibility results of the 25 *Pseudomonas* isolates revealed highest resistance to levofloxacin and ciprofloxacin (36.0%, n=9 each), followed by ceftazidime and imipenem (28.0%, n=7 each). In contrast, the isolates showed high sensitivity to piperacillin/tazobactam (84.0%, n=21), followed by cefepime, cefoperazone/sulbactam, meropenem and amikacin (76.0%, n=19 each) (Fig 2).

Fig 3 shows that among the 4 *Acinetobacter* isolates, resistance was most frequently observed against amikacin, cefepime,

ceftriaxone, ciprofloxacin, and cotrimoxazole (75.0%, n=3 each). However, 50.0% of the isolates (n=2) were sensitive to each of piperacillin/tazobactam, imipenem, meropenem, and cefoperazone/sulbactam.

For the 3 *Burkholderia* isolates, they were completely resistant (100.0%, n=3) to cefepime, cotrimoxazole and amikacin, but completely sensitive to piperacillin/tazobactam (100.0%, n=3), and 66.7% (n=2) sensitive to meropenem, ceftazidime and cefoperazone/sulbactam as shown in Fig 4.

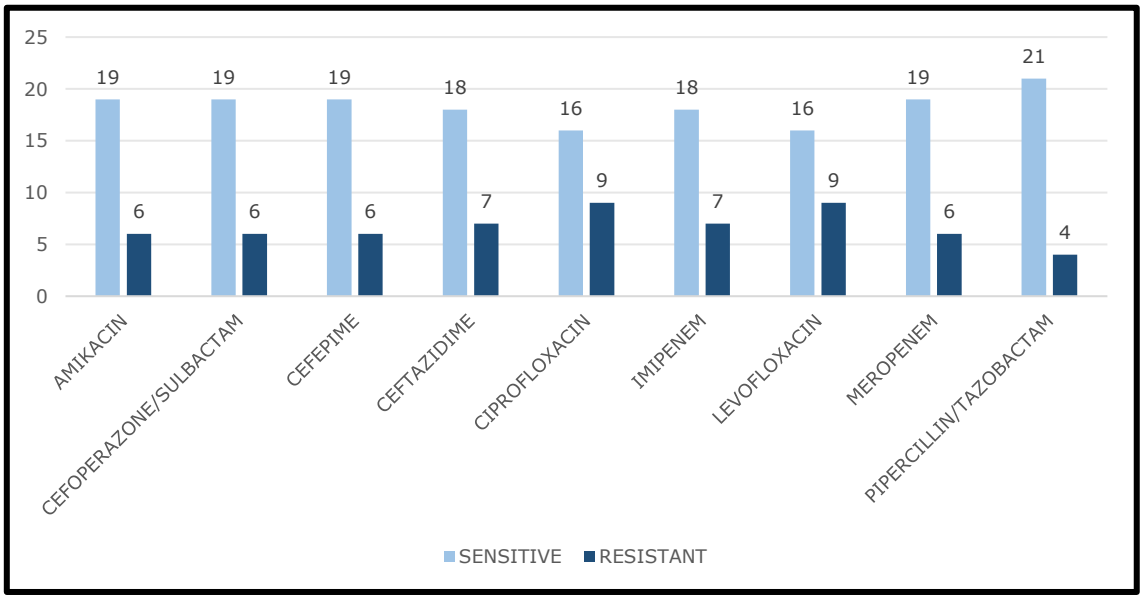

Fig 2: Antibiotic susceptibility of *Pseudomonas* isolates

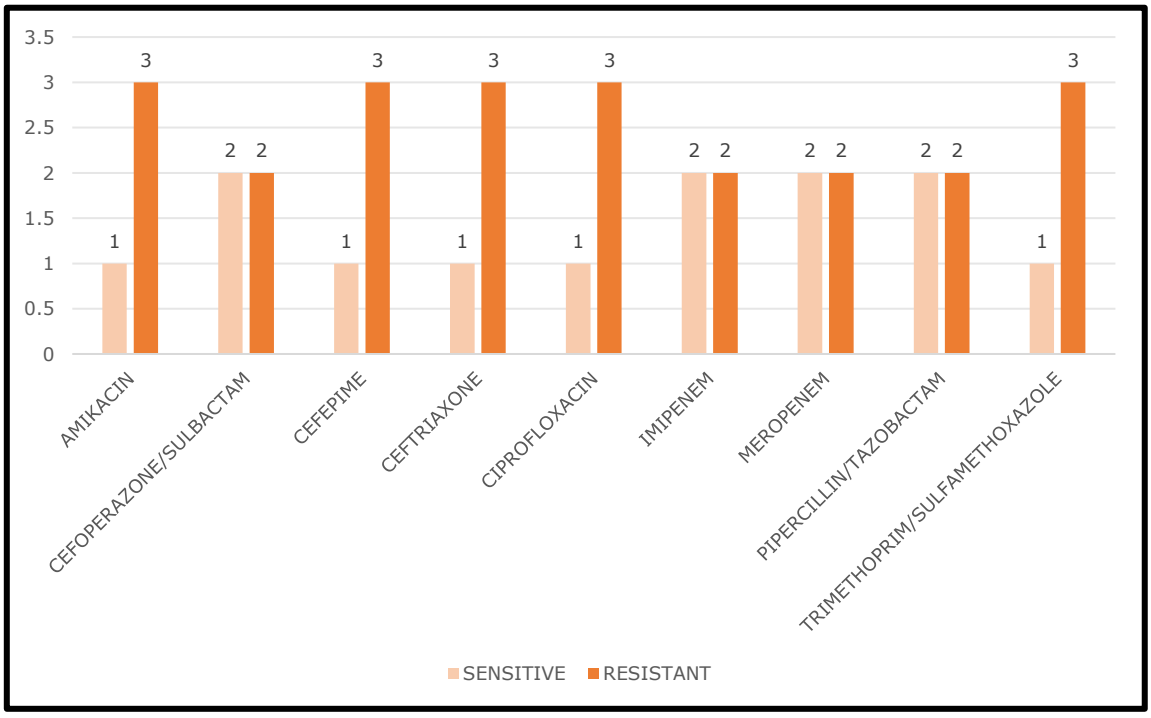

Fig 3: Antibiotic susceptibility of *Acinetobacter* isolates

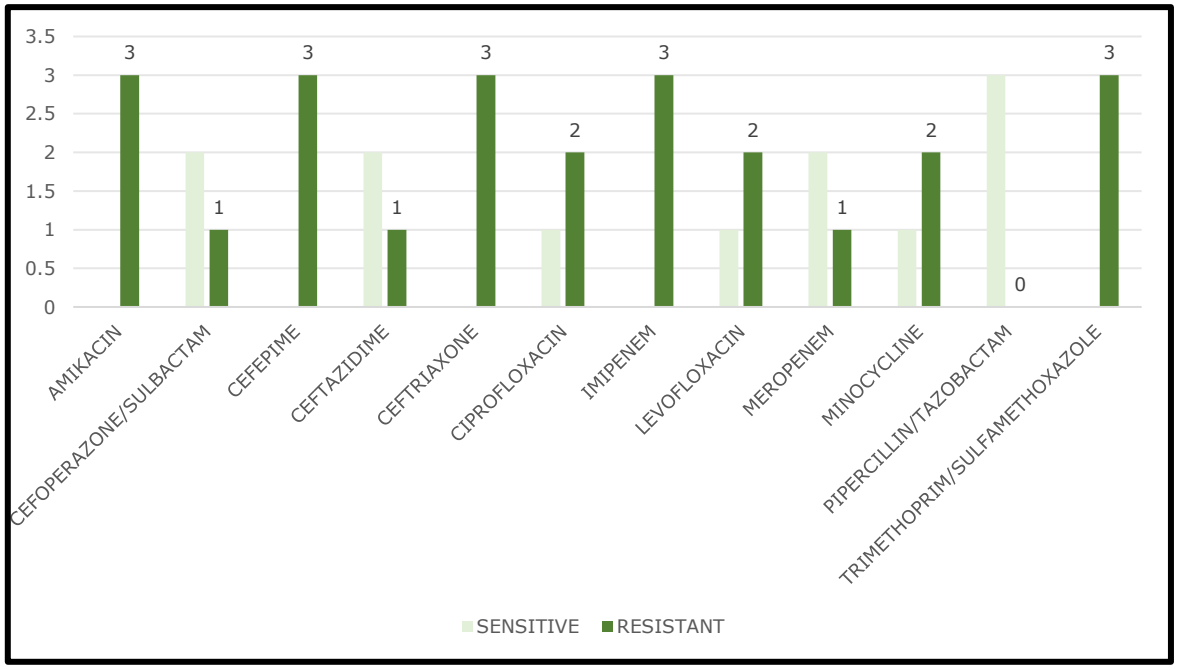

Fig 4: Antibiotic susceptibility of *Burkholderia* isolates

Multi-drug resistance among NFGNB isolates:

Multi-drug resistance (MDR), defined as resistance to at least three antibiotics in different classes of antimicrobial agents, is seen 10 of 25 (40.0%) *Pseudomonas* isolates, 3 of 4 (75.0%) *Acinetobacter* isolates, and all 3 (100.0%) *Burkholderia* isolates.

Discussion:

In our study, a higher proportion of NFGNB were isolated from males (78.0%) compared to females (22.0%). This male dominance is consistent with results from the findings of the study by Ghanshani et al., (7) who reported that 68% of their patients were men. Gender-based differences such as genetic and hormonal factors may account for this disparity (8). The present study showed that the most affected age group was 51-75 years (47.0%), followed by 26-50% (44.0%). This trend is consistent with findings by Taj et al., (9) where most of the patients with infections were in the 51-60 years group. Age-related decrease in immunity and increased risk of co-morbidities may explain the higher prevalence in older adults.

In terms of patient settings, 22.0% of isolates were from the outpatient and 78.0% came from inpatient. This distribution illustrates how hospitalized patients, are more likely to have infections caused by NFGNB as a result of factors such as invasive surgeries and prolonged hospital stay (10). *Pseudomonas aeruginosa* isolates in this study exhibited highest resistance to levofloxacin and ciprofloxacin (36%), which is comparable to the findings of a study by Tiwari et al., (12) of 43% and Kanthakumar et al., (11) of 33.2%. Resistance to imipenem and ceftazidime of 28.0% also aligned with other regional data, such as imipenem resistance rates of 26.2% and 16.2% reported by Kanthakumar et al., (11) and Bindu et al., (13) respectively. Piperacillin/tazobactam retained good efficacy (84.0% sensitivity), comparable to 86.5% reported by Kanthakumar et al., (11) and 74.0% by Tiwari (12). Sensitivity to cefepime, cefoperazone/sulbactam, meropenem and amikacin of 76.0% each aligns closely with the data reported by Bindu et al., (13) where sensitivity to cefepime and amikacin were 75.7% and 78.4% respectively.

In our study, *Acinetobacter baumannii* showed 75.0% resistance each to ciprofloxacin, cotrimoxazole, cefepime, amikacin and ceftriaxone. A study by Verma et al., (14) reported resistance rates of 72.16% to amikacin and 86.08% to ciprofloxacin, while high resistance was also reported by Gaur et al., (15), with over 80.0% of isolates resistant to cephalosporins and 81.0% to ciprofloxacin. The sensitivity of *Acinetobacter* isolates was 50.0% to each of cefoperazone/sulbactam, imipenem, meropenem and piperacillin/tazobactam, which is comparable to the findings of the studies by Gaur et al., (15) and Sivaranjani et al., (16), which reported some variations in susceptibility, with imipenem resistance ranging from 9.1% to 26.2%.

All three isolates of *Burkholderia* in this study showed 100.0% resistance to cefepime, cotrimoxazole and amikacin. A study conducted by Ibrahim et al., (17) reported 100.0% resistance to cefepime and 91.8% to amikacin, but 100.0% sensitivity to piperaci-

llin/tazobactam, followed by 67.0% sensitivity to meropenem, ceftazidime, and cefoperazone/sulbactam. This aligns with a study by Sethi (18) where *Burkholderia* isolates showed sensitivity rates of 89.0% to cotrimoxazole, 65.0% to ceftazidime and 43.0% to meropenem. These results mirror resistance trends that underscores the critical need for ongoing antimicrobial surveillance and judicious antibiotic use to maintain the effectiveness of current treatment options.

The resistance observed may be due to multiple factors, such as β-lactamase production, active efflux mechanisms, and genetic mutations at antibiotic target sites, all of which enhance the organism's resilience against several drug classes. These observations also point to variations in resistance patterns among NFGNB, reinforcing the necessity for region-specific monitoring and targeted antimicrobial stewardship.

Conclusion:

The high prevalence and resistance to antibiotics of clinical isolates of NFGNB, which includes *Pseudomonas*, *Acinetobacter* and *Burkholderia* species, are highlighted in this study. The predominance of infections in older males and in hospitalized patients emphasizes these group's vulnerability. High levels of resistance, especially to commonly used antibiotics such as fluoroquinolones, cephalosporins and aminoglycosides were observed, with some sensitivity retained to carbapenems and β-lactam/β-lactamase inhibitor combinations.

The concerning patterns of resistance, remarkably complete resistance observed in *Burkholderia* isolates, underscore the pressing need for strong infection control practices, antimicrobial resistance surveillance, and establishment of effective, community-based antibiotic stewardship initiatives to stop the spread of multidrug-resistant infections and guide rational therapeutic choices.

Contributions of authors:

Both authors contributed significantly to this study. KA and PG designed the study, performed the procedures and collected data, analyzed the data and drafted the manuscript. Both authors reviewed, revised, and approved the final version of the manuscript.

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Conflict of interest:

Authors declare no conflict of interest.

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Original Article **Open Access**

Acinetobacter baumannii infections among patients in intensive care unit at the University College Hospital, Ibadan, Nigeria

*[1]Sanusi, A. A., [2,3]Okesola, A. O., and [2,4]Odusanya, O. O.

[1]Department of Anaesthesia, College of Medicine, University of Ibadan/University College Hospital, Ibadan, Nigeria
[2]Department of Medical Microbiology, College of Medicine, University of Ibadan, Ibadan, Nigeria
[3]Department of Medical Microbiology University College Hospital, Ibadan, Nigeria
[4]AllTalentz LLC
*Correspondence to: arinolasanusi@yahoo.com; +2348033250788; ORCiD number: 00000003-1725-9097

Abstract:

Background: Healthcare associated infections (HAIs), also known as nosocomial infections, pose a major safety risk for both patients and healthcare workers globally. *Acinetobacter* species are Gram-negative bacteria primarily associated with HAIs and have been implicated in outbreaks of infections such as ventilator-associated pneumonia, urinary tract infections, and septicaemia. The multi-drug resistance (MDR) nature of *Acinetobacter* species coupled with ability to survive for long periods in harsh conditions have made them of global health concern. Patients in intensive care units (ICUs) are highly susceptible to *Acinetobacter* infection. This study was designed to determine the prevalence and antibiotic resistance profiles of *Acinetobacter* isolates in infections among patients in the ICU of the University College Hospital, Ibadan, Nigeria.
Methodology: A total of 120 consecutive patients were recruited into this study between April and December 2021 from whom blood samples, tracheal aspirates and catheter urine samples were obtained. Samples were obtained from patients who had spent a minimum of 48 hours in the ICU at the time of sample collection. The samples were cultured, and bacterial isolates were identified using standard microbiological methods. Antibiotic susceptibility testing was performed on all isolates by the disc diffusion method in line with the Clinical and Laboratory Standards Institute (CLSI) guideline. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 26.0.
Results: From the samples obtained from 120 enrolled patients during the study period, only 5 were positive for *Acinetobacter* species, giving a prevalence of 4.2%. Four (80.0%) of the isolates were *Acinetobacter baumannii* while 1 (20.0%) was *Acinetobacter haemolyticus.* Four (80.0%) isolates were from the tracheal aspirates, 1 (20.0%) was from the blood culture, while the catheter urine specimen yielded no *Acinetobacter* isolate. The isolates were 80.0% resistant to amikacin, 60.0% to gentamicin, 60.0% to ceftazidime, 40.0% to ciprofloxacin, 40.0% to levofloxacin, 40.0% to meropenem, and 0% to colistin. The 4 *A. baumannii* isolates were multi-drug resistant (resistant to three or more classes of antibiotics) while the only *A. haemolyticus* was mono-drug resistant.
Conclusion: The need for continued infection prevention and control, surveillance of multi-drug resistant *Acinetobacter* spp and antimicrobial stewardship practices in the ICU is hereby emphasized.

Keywords: *Acinetobacter baumannii*, intensive care unit, multi-drug resistance

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Infections à *Acinetobacter baumannii* chez les patients de l'unité de soins intensifs de l'Hôpital Universitaire d'Ibadan, Nigéria

*[1]Sanusi, A. A., [2,3]Okesola, A. O., et [2,4]Odusanya, O. O.

[1]Service d'Anesthésie, Faculté de Médecine, Université d'Ibadan/Hôpital Universitaire, Ibadan, Nigéria
[2]Service de Microbiologie Médicale, Faculté de Médecine, Université d'Ibadan, Ibadan, Nigéria
[3]Service de Microbiologie Médicale, Hôpital Universitaire, Ibadan, Nigéria
[4]AllTalentz LLC
*Correspondance à: arinolasanusi@yahoo.com; +234 803 325 0788; Numéro ORCiD: 00000003-1725-9097

Résumé:

Contexte: Les infections associées aux soins (IAS), également appelées infections nosocomiales, représentent

un risque majeur pour la sécurité des patients et des professionnels de santé dans le monde entier. Les espèces d'*Acinetobacter* sont des bactéries à Gram négatif principalement associées aux IAS et ont été impliquées dans des épidémies d'infections telles que la pneumonie acquise sous ventilation mécanique, les infections urinaires et la septicémie. La multirésistance aux médicaments (MDR) des espèces d'*Acinetobacter*, associée à leur capacité à survivre longtemps dans des conditions difficiles, en fait une préoccupation sanitaire mondiale. Les patients en unité de soins intensifs (USI) sont très sensibles aux infections à *Acinetobacter*. Cette étude visait à déterminer la prévalence et les profils de résistance aux antibiotiques des isolats d'*Acinetobacter* lors d'infections chez les patients en USI de l' Hôpital Universitaire d'Ibadan, Nigéria.
Méthodologie: Au total, 120 patients consécutifs ont été recrutés pour cette étude entre avril et décembre 2021. Des échantillons de sang, d'aspirations trachéales et d'urine ont été prélevés par cathéter. Français Des échantillons ont été obtenus auprès de patients ayant passé au moins 48 heures en USI au moment du prélèvement. Les échantillons ont été mis en culture et les isolats bactériens ont été identifiés à l'aide de méthodes microbiologiques standard. Un test de sensibilité aux antibiotiques a été effectué sur tous les isolats par la méthode de diffusion sur disque, conformément aux directives de l'Institut Normes Cliniques et de Laboratoire (CLSI). Les données ont été analysées à l'aide du logiciel statistique pour les sciences sociales (SPSS) version 26.0.
Résultats: Parmi les échantillons obtenus auprès de 120 patients inclus pendant la période d'étude, seuls 5 étaient positifs pour les espèces d'*Acinetobacter*, soit une prévalence de 4,2%. Quatre (80,0%) des isolats étaient *Acinetobacter baumannii* tandis qu'un (20,0%) était *Acinetobacter haemolyticus*. Quatre (80,0%) isolats provenaient des aspirats trachéaux, un (20,0%) provenait de l'hémoculture, tandis que l'échantillon d'urine du cathéter n'a donné aucun isolat *Acinetobacter*. Français Les isolats étaient résistants à 80,0% à l'amikacine, à 60,0% à la gentamicine, à 60,0% à la céftazidime, à 40,0% à la ciprofloxacine, à 40,0% à la lévofloxacine, à 40,0% au méropénème et à 0% à la colistine. Les 4 isolats d'*A. baumannii* étaient multirésistants (résistants à trois classes d'antibiotiques ou plus) tandis que le seul *A. haemolyticus* était monorésistant.
Conclusion: La nécessité d'une prévention et d'un contrôle continus des infections, d'une surveillance des espèces d'Acinetobacter multirésistantes et de pratiques de gestion des antimicrobiens dans l'unité de soins intensifs est ici soulignée.

Mots-clés: *Acinetobacter baumannii,* unité de soins intensifs, multirésistance

Introduction:

Infection is a frequent and major cause of morbidity and mortality in the intensive care units (ICU) (1,2). *Acinetobacter* is one of the important Gram-negative pathogens causing serious healthcare-associated infections (HAIs) and this is a major public health concern (3,4). *Acinetobacter baumannii* is reportedly ubiquitous, responsible for serious infections in hospitalized patients, with a high propensity to develop resistance to antimicrobial agents (5). Vincent et al., (6) reported that HAIs affect about 5% to 15% of hospitalised patients on regular wards and can have a rate of 50.0% or more in patients in intensive care units. *Acinetobacter baumannii* has been reported in ventilator associated pneumonia with high morbidity and mortality (6).

Acinetobacter spp are considered as part of normal flora of the skin, mucosa of the pharynx and the human respiratory secretions (7). Furthermore, *A. baumannii* is frequently isolated from reusable medical equipment such as ventilator tubings, monitoring devices, humidifiers and respirometers as documented by Kanafani & Kary (8). According to Odewale et al., (9)*, Acinetobacter* is known to persist and survive on surfaces under a wide variety of environmental conditions, therefore it is a common cause of outbreak.

The study by Ayobami et al., (10) showed that there is evidence of increasing *A. baumannii* outbreaks in healthcare facilities across sub-Saharan Africa and Eastern Mediterranean countries. *Acinetobacter baumannii* strains have been identified to have acquired multiple drug resistance and are isolated or identified in numerous hospitals throughout the world in studies by Abbo et al., (11) and Kanafani and Kary (8). High prevalence of *Acinetobacter* infection has been reported worldwide, 19.2% in Asia, 17.1% in Eastern Europe, 14.8% in Africa, 13.8% in Central and South America (12). Therefore, frequent review of antibiotic susceptibility pattern of clinical location specific isolates is necessary for proper prevention and control of healthcare associated infections and hospital acquired outbreaks.

The general objective of this study was to determine the prevalence and antimicrobial susceptibility of *A. baumannii* isolates among patients in the intensive care unit (ICU) of the University College Hospital (UCH), Ibadan, Nigeria. The specific objectives are to determine the prevalence of *A. baumannii* infections among ICU patients of the hospital, evaluate the distribution of *Acinetobacter* spp in the different specimen obtained from these patients and determine the antimicrobial susceptibility and resistance profiles of the isolates from these patients.

Materials and method:

Study design and setting:

This was a descriptive, cross-sectional study involving patients who had spent at least 48 hours in the ICU of the hospital as at the time of data collection. The study was carried out in the department of medical microbiology and the ICU, UCH, Ibadan, Nigeria from April 1, 2021 to September 30, 2021. The ICU is a 12-bed facility, with an average monthly admission of 30-40 patients.

Study participants:

The study participants were patients, who at the time of data collection, had been on admission at the ICU for up to or more than 48 hours and included those on mechanical ventilation and those who had urinary catheter *in situ.* The reasons for ICU admission from the Emergency Department included pneumonia, respiratory failure and sepsis while respiratory failure was the reason for admission from the general ward. Postoperative admissions into the ICU were due to respiratory insufficiency and/or cardiovascular instability. Patients who had been on ICU admission less than 48 hours and those with poor condition or *in-extremis* were excluded.

Determination of sample size and sampling method:

The minimum sample size was determined using the Fisher's formula; $n=z^2(1-p)p/d^2$, where n is minimum sample size, p is prevalence of 8.5% in a previous study (9), z is 1.96, and d is tolerance/error of 5%. This gave a calculated minimum sample size of 120. A random sampling technique was used to select the participants included in this study. Consecutive admission into the ICU from the start of the study, that met inclusion criteria until the minimal sample size was achieved at the end of the period.

Specimen collection:

Tracheal aspirate, blood and catheter urine specimens were obtained by aseptic procedure from the patients and for each specimen. For blood culture, about 5ml of venous blood was obtained from each patient, into a BD BACTEC aerobic blood culture bottle for incubation. Tracheal aspirate was obtained using sterile mucus extractor connected to the suction machine. Aspirate was then transferred into a sterile universal bottle and immediately transported to the microbiology laboratory for further processing.

For catheter urine, the urethral catheter was disconnected from the urine bag and clamped for 10-20 minutes after which the urine collecting in the catheter was allowed to drip into a sterile universal bottle. All biological samples were promptly sent to the microbiology laboratory, for microscopy, culture and sensitivity testing.

Specimen culture for *Acinetobacter* isolation:

Blood, catheter urine and tracheal aspirate samples were inoculated on MacConkey agar (Mac, Oxoid) and Chocolate agar plates and incubated for 18-24 hours at 35-37^{0}C and examined for bacterial growth. All non-lactose fermenters on MacConkey agar plate were subcultured to obtain pure isolates.

Phenotypic identification of *Acinetobacter* isolates:

Identification of *Acinetobacter* isolate was achieved by examination of colony morphology on culture plates, Gram staining, and biochemical tests which included citrate, catalase, indole and oxidase tests. Gram negative coccobacilli that were non-lactose fermenting, citrate and catalase positive, and oxidase and indole negative were presumptively considered as *Acinetobacter baumannii*. The Microbact 24E (MB24E) system was used to phenotypically confirm *Acinetobacter baumannii* following approved procedure described by Ling et al., (13).

Antimicrobial susceptibility test:

Antimicrobial susceptibility test (AST) was performed by the modified Kirby-Bauer test disc diffusion method. The inoculum was prepared as a suspension of the isolate by picking 2 or 3 colonies of the isolate with a sterile straight wire and making an emulsion of it in peptone water. This suspension was standardized by comparing it with 0.5 McFarland turbidity standard.

Using a sterile swab stick, sterile Mueller-Hinton (MH) agar plates were inoculated with the broth cultures. After about 3 minutes, antibiotic-impregnated discs (amoxicillin-clavulanic acid 30µg, amikacin 30µg, meropenem 10µg, ciprofloxacin 5µg, gentamicin 10µg, levofloxacin 5µg, ceftazidime 30µg, colistin 10 µg) were placed on the surface of the agar and incubated at 35-37^{o}C for 24 hours.

The diameter of the zones of inhibition were measured with a standard calibrated meter rule and interpreted as sensitive or resistant in line with standard interpretative CLSI charts as described by Benkova et al., (14).

Statistical analysis:

Data were entered into Microsoft Excel and analyzed using the Statistical Package for the Social Sciences (SPSS) version 26. Descriptive statistics were employed to summarize the data. Variables such as age group, source of admission, type of biological samples, and antimicrobial susceptibility patterns were summarized using frequencies and percentages. Chi square was performed to examine associations between categorical variables. A significance level of $p<0.05$ was considered statistically significant.

Results:

Characteristics of the participants:

The age of the 120 participants ranged from 8 to 82 years, with 94 (78.3%) females and 26 (21.7%) males. Ninety-nine patients (82.5%) were admitted from the Accident and Emergency department while 11 (9.2%) and 10 (8.3%) were from the general wards and operating theatres of the hospital respectively (Table 1). Blood samples were collected from

40 (33.3%) of the participants, tracheal aspirates from 36 (30.0%) and catheter urine from 44 (36.7%) (Table 1).

Prevalence of *Acinetobacter* infection:

Acinetobacter species were isolated from samples of 5 participants (4 *A. baumannii* and 1 *A. haemolyticus*), giving a prevalence of 4.2%, with 1 (2.5%) isolate from the 40 blood culture samples, 4 (11.1%) isolates from the 36 trachea aspirates, and none (0%) from the 44 urine samples.

The prevalence of *Acinetobacter* isolation was not significantly associated with age or sex of participants (p>0.05) but was significantly associated with site of isolation, with significantly higher rate of isolation from respiratory tract (11.1%) than from blood (2.5%) and urinary tract (0%) (p=0.038) (Table 2).

Table 1: Characteristics of participants

Characteristics	Frequency	Percentage
Age group (years)		
<10	2	1.7
10 – 29	26	21.7
30 – 39	45	37.5
40 – 49	31	25.8
50 – 59	5	4.2
≥ 60	11	9.2
Sex		
Males	94	78.3
Females	26	21.7
Admission sources		
Accident & Emergency	99	82.5
General ward	11	9.2
Operating theatre	10	8.3
Biological samples		
Blood	40	33.3
Tracheal aspirate	36	30.0
Catheter urine	44	36.7
Total	**120**	**100.0**

Table 2: Prevalence of *Acinetobacter* infection by age, sex and site of isolation

Variables	No of patients	No infected with *Acinetobacter*	Percentage	x^2	p value
Age group (years)					
<10	2	0	0	1.203	0.9446^{ns}
10 – 29	26	1	12.5		
30 – 39	45	2	4.8		
40 – 49	31	2	6.4		
50 – 59	5	0	0		
≥ 60	11	0	0		
Sex					
Male	94	5	5.3	0.4184	0.5177^{ns}
Female	26	0	0		
Site of isolation					
Blood	40	1	2.5	6.539	0.038^{s}
Respiratory	36	4	11.1		
Urinary	44	0	0		
Total	**120**	**5**	**4.2**		

ns = not statistically significant; s = statistically significant (p<0.05)

Table 3: Antimicrobial susceptibility and resistance of *Acinetobacter* isolates

Antibiotics	Gen	Amk	Cef	Lev	Cipro	Mer	Col
Isolate 1	R	R	R	R	S	S	S
Isolate 2	R	R	R	S	R	S	S
Isolate 3	R	R	S	S	S	R	S
Isolate 4	S	R	R	S	R	S	S
Isolate 5	S	S	S	S	S	R	S
% Resistant	**60.0**	**80.0**	**60.0**	**20.0**	**40.0**	**40.0**	**0**

R = Resistant; Isolates 1 – 4: *A baumannii;* Isolate 5: *A. haemolyticus;* G Gen = Gentamicin; Amk = Amikacin; Ceft = Ceftazidime; Lev = Levofloxacin; Cipro = Ciprofloxacin; Mer = Meropenem, Col = Colistin

Table 4: Antimicrobial resistant profiles of *Acinetobacter* isolates

Isolates number	Isolate	Resistant profile	
1	*Acinetobacter baumannii*	$Gen^R/Amk^R/Cef^R/ /Lev^R$	MDR (3)
2	*Acinetobacter baumannii*	$Gen^R/Amk^R/Cef^R/Cipr^R$	MDR (3)
3	*Acinetobacter baumannii*	$Gen^R/Amk^R/Mer^R$	MDR (3)
4	*Acinetobacter baumannii*	$Amk^R/Cef^R/Cip^R$	MDR (3)
5	*Acinetobacter haemolyticus*	Mer^R	Mono-resistant

MDR = Multi-drug resistant; Gen = Gentamicin; Amk = Amikacin; Ceft = Ceftazidime; Lev = Levofloxacin; Cipro = Ciprofloxacin; Mer = Meropenem

Antimicrobial susceptibility of *Acinetobacter* isolates:

All (100%) the *Acinetobacter* isolates were susceptible to colistin, 4 (80.0%) isolates were resistant to amikacin, 3 (60.0%) isolates were resistant to gentamicin and ceftazidime, 2 (40.0%) to ciprofloxacin and meropenem, and 1 (20.0%) isolate was resistant to levofloxacin (Table 3). Except for *A. haemolyticus*, all the *A baumannii* isolates were multi-drug resistant (Table 4).

Discussion:

This study was undertaken to determine the prevalence of *Acinetobacter baumannii* among patients admitted to the intensive care unit of the University College Hospital Ibadan, a tertiary health facility in Oyo State of Nigeria. The prevalence of *Acinetobacter* spp in ICU in this study was 4.17%. This was lower than a previously reported prevalence of 79.0% in the same hospital in 2014 by Nwadike et al., (15). This could be due to difference in sample size as Nwadike et al had a sample size of 400 as against 120 samples in this study.

Different hospital prevalence of *A. baumannii* has been reported in many countries. In Kenya, 22.7% prevalence was reported (1), Ethiopia reported 2.5% (3), Tamil Nadu/India reported 11.17% (16), Honduras 34.5% (5) while in Saudi-Arabia, prevalence of 8.3% was reported (8) and 5.4% in Ibadan, Nigeria (17). These prevalence reports support the fact that *A. baumannii* is not only ubiquitous but worldwide. The details of the context and specimen sample however reveal the complexity of these reports. Specimen/sample for analysis included blood, urine, tracheal aspirate, sputum, surgical wound swab and the environment hence the wide variation in prevalence rates.

In this study, the prevalence of *Acinetobacter* isolation was 2.5% from blood, 11.1% from tracheal aspirate and 0% from urine. In Kenya (1), 7% was from the blood or tracheal aspirate and 12.1% from urine sample. A study from Dhaka, Bangladesh (4) reported 10.7% from the blood, 68% from respiratory samples and 40.3% from the urine, while the study from Ibadan, Nigeria (15), reported prevalence of 7% from blood, 7% from urine, and 86% from tracheal aspirates. These studies like ours showed higher prevalence of *Acinetobacter* isolation from tracheal/respiratory samples. This specimen-specific prevalence may suggest areas where the need exist for preventive interventions.

While prevalence deduced from systematic reviews and meta-analysis give a long-term measure of infection, country-wide or hospital-wide prevalence gives the impression of geographical and location-specific prevalence and challenge. The prevalence in specimens of catheter urine, tracheal aspirate and blood samples however suggest the risk posed by invasive techniques, ineffective/inadequate aseptic technique and instrumentation especially in ICU patients with lowered immunity. These need careful consideration and policy.

All the *A. baumannii* isolates from ICU patients in this study were from males. Garcia-Garmendia et al., (18) in their study posited that the male gender is at a higher risk of *A. baumannii* infections and reported a male: female ratio of 9:1. Also, gender disparity in *A. baumannii* infection was reported by Iregbu et al., (19) with 1.9: 1 male: female ratio from age 45 years and above. The age range of the patients in our study was 8 to 82 years. However, 4 (80.0%) of the isolates were recovered from male participants aged 30-49 years while 1 (20.0%) was from a participant in the 10-19 years age group. This age-group was lower than that reported by Odewale et al., (9).

The highest rates of infections were reported in the elderly patients by Al Samawi et al., (20) and Nwadike et al., (15). A wider age-range was reported by Unwigabiye et al., (21). Advancing age with several co-morbidities and lowered immunity may partially account for these findings. While our findings were closer to that of Mushtaqa et al., (22) who reported 6.2% in Pakistan, it is lower than that reported by Odewale et al., (9), in a hospital-wide study. The study by Nwadike et al., (15) ten years earlier in the same location as our study, reported a higher prevalence at the time of their study. Many factors interplay to determine prevalence of infection and could not have been due to time-trend alone. An improvement in infection control being observed in the hospital may be contributory.

Four of the *A. baumannii* were isolated from tracheal aspirates while only one from

blood samples and none from catheter urine. This is contrary to the findings by Dada-Adegbola et al (16) in a hospital-wide sampling where 41.0% of *Acinetobacter* species were isolated from blood samples, and 24.0% from wound swabs. Several studies have reported that respiratory tract produce the highest rate of *A. baumannii* infection as reported by Sileem et al., (23), Mathai et al., (24), and Patel et al., (25). A major risk factor for *A. baumannii* respiratory tract infection is mechanical ventilation and its duration; the longer a patient is on mechanical ventilation, the higher the risk of developing hospital-acquired ventilator-associated infection (6).

Acinetobacter species have shown increasing resistance to major antibiotic classes including β-lactams, aminoglycosides, and fluoroquinolones, hence, the World Health Organization (WHO) has included *A. baumannii* on the list of pathogens that should be given priority in the Research and Development of new antibiotics (26). Savov et al., (27), Mathai et al., (24) and Odewale et al., (9) all reported resistance of *A. baumannii* to currently known antibiotics (ampicillin, cephalothin, carbenicillin, gentamicin, amikacin, chloramphenicol, tetracycline, cephalosporins), therefore presenting a therapeutic dilemma for infection management the world over. In our study, all the isolates were susceptible to colistin, despite the multi-drug resistance to at least three other groups of antimicrobials. Increased susceptibility of *A. baumannii* isolates to colistin have been reported by Zafer et al., (28) and Odewale et al., (9). But for its toxicity, colistin alone or in combination therapy has been recommended for treatment of MDR *A. baumannii* infection.

Several interventions for *A. baumannii* infection control have been practiced. One such intervention is bacteriological surveillance in hospitals with daily review of antibiotic susceptibility of all clinical isolates as suggested by Urban et al., (29) and Mehtap et al., (30). Antibiotic stewardship committee has been suggested by Nwadike et al., (15) as well as adherence to and evaluation of infection prevention and control by Kipsang et al., (1). Finally providing adequate training periodically to hospital staff, observance of aseptic techniques during any medical procedure in addition to routine antimicrobial susceptibility test will reduce *A. baumannii* and other infective agents according to Kamal et al., (3).

The limitations of this study include the small number of patients who had *Acinetobacter* infection. This calls for caution in interpreting and extrapolating the results. Secondly, the phenotypic method used for identification of species is not as sensitive as the molecular technique which can both identify and detect the genetic basis of resistance in the isolates.

Conclusion:

The multi-drug resistant nature of *Acinetobacter* spp poses therapeutic challenge to the clinicians and patients. Antimicrobial stewardship and infection prevention control are critical measures to reduce the prevalence of *A. baumannii* (and other MDR pathogens) infections in the intensive care unit.

Contributions of authors:

OOA, OOO, and SAA contributed to the conceptualization and study design. OOO and SAA contributed to data collection. The laboratory analysis was performed by OOA and OOO. All authors contributed to the statistical analysis, manuscript writing, and approval of the final manuscript submitted for publication.

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Authors declare no conflict of interest

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Original Article **Open Access**

Phenotypic identification and species characterization of clinically significant coagulase-negative staphylococci isolates at Bouaké University Hospital, Côte d'Ivoire

*[1,2]N'Guessan, A. M., [1,2]Monemo, P., [1]Traoré, A., [1,2]Tadet, J. O. N., [1,2]Gawa, K. J., [1]Ouattara, A., and [1,2]Akoua Koffi, C.

[1]Bacteriology-Virology Laboratory, Bouaké University Hospital Center, Ivory Coast
[r2]Faculty of Medical Sciences, Alassane Ouattara University, Ivory Coast
*Correspondence to: mienmooh@gmail.com; +2250707669820

Abstract:

Background: Coagulase-negative staphylococci (CoNS) are now recognized as important agents of nosocomial infections, particularly in immunocompromised patients in the healthcare settings, due to their ability to form biofilms and their high resistance to methicillin. The aim of this study is to identify and characterize different species of CoNS isolated at the Bacteriology-Virology Laboratory of the Bouaké University Hospital, Côte d'Ivoire
Methodology: This was a laboratory-based study conducted at the Bacteriology-Virology Laboratory of the Bouaké University Hospital (CHUB) over a two-month period (November to December 2021). Coagulase negative staphylococci isolated from biological samples of hospitalized patients in the routine laboratory were first re-identified by Gram staining and conventional biochemical tests (catalase, coagulase) and further characterized into species using the API® Staph gallery (BioMérieux, France). Antibiotic susceptibility of the isolates was determined by the Kirby-Bauer disc diffusion method.
Results: A total of 100 CoNS were isolated from samples received from various departments of the CHUB during the period of the study. The main CoNS species identified among these were *Staphylococcus xylosus* (n=30), *Staphylococcus haemolyticus* (n=20), *Staphylococcus epidermidis* (n=18), *Staphylococcus lentus* (n=12), *Staphylococcus saprophyticus (*n=7), *Staphylococcus capitis* (n=4), and *Staphylococcus chromogenes (*n=3). The proportion of CoNS isolates resistant to penicillin was 91.0%, tetracycline 72.0%, cotrimoxazole 67.0%, cefoxitin 65.0%, kanamycin 61.0%, tobramycin 54.0%, levofloxacin 53.0%, and gentamicin 50.0%. The most frequent species, *S. haemolyticus* (100%) and *S. xylosus* (60.0%) were resistant to methicillin. Kanamycin, tobramycin and gentamicin (KTG) aminoglycoside resistance phenotypes were seen in 95.0% of S. *haemolyticus* and 86.0% of *S. saprophyticus* isolates. The constitutive MLS_B resistance phenotype was seen in 13.0% of *S. xylosus* and 11.0% of S. *epidermidis.*
Conclusion: A variety of CoNS species were isolated in this study, with *S. xylosus* and *S. haemolyticus* being the most frequently identified species. Methicillin resistance rate was 100.0% for *S haemolyticus* isolates which are likely involved in serious, potentially fatal nosocomial infections.

Keywords: Coagulase-negative staphylococci, phenotype, resistance, Côte d'Ivoire

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Identification phénotypique et caractérisation des espèces d'isolats de staphylocoques à coagulase négative cliniquement significatifs au CHU de Bouaké, Côte d'Ivoire

*[1,2]N'Guessan, A. M., [1,2]Monemo, P., [1]Traoré, A., [1,2]Tadet, J. O. N., [1,2]Gawa, K. J., [1]Ouattara, A., et [1,2]Akoua Koffi, C.

[1]Laboratoire de bactériologie-virologie, CHU de Bouaké, Côte d'Ivoire
[2]Faculté des Sciences Médicales, Université Alassane Ouattara, Côte d'Ivoire
*Correspondance à: mienmooh@gmail.com; +2250707669820

Résumé:

Contexte: Les staphylocoques à coagulase négative (SCN) sont désormais reconnus comme d'importants agents

d'infections nosocomiales, en particulier chez les patients immunodéprimés en milieu hospitalier, en raison de leur capacité à former des biofilms et de leur forte résistance à la méthicilline. L'objectif de cette étude est d'identifier et de caractériser différentes espèces de SCN isolées au laboratoire de bactériologie-virologie du CHU de Bouaké, en Côte d'Ivoire.
Méthodologie: Il s'agit d'une étude en laboratoire menée au laboratoire de bactériologie-virologie du CHU de Bouaké (CHUB) sur une période de deux mois (novembre à décembre 2021). Français Les staphylocoques à coagulase négative isolés d'échantillons biologiques de patients hospitalisés au laboratoire de routine ont d'abord été ré-identifiés par coloration de Gram et tests biochimiques conventionnels (catalase, coagulase) puis caractérisés en espèces en utilisant la galerie API® Staph (Bio Mérieux, France). La sensibilité aux antibiotiques des isolats a été déterminée par la méthode de diffusion sur disque de Kirby-Bauer.
Résultats: Au total, 100 CoNS ont été isolés d'échantillons reçus de différents services du CHUB pendant la période de l'étude. Les principales espèces de CoNS identifiées parmi celles-ci étaient *Staphylococcus xylosus* (n=30), *Staphylococcus haemolyticus* (n=20), *Staphylococcus epidermidis* (n=18), *Staphylococcus lentus* (n=12), *Staphylococcus saprophyticus* (n=7), *Staphylococcus capitis* (n=4) et *Staphylococcus chromogenes* (n= 3). Français La proportion d'isolats de CoNS résistants à la pénicilline était de 91,0%, à la tétracycline de 72,0%, au cotrimoxazole de 67,0%, à la céfoxitine de 65,0%, à la kanamycine de 61,0%, à la tobramycine de 54,0%, à la lévofloxacine de 53,0% et à la gentamicine de 50,0%. Les espèces les plus fréquentes, *S. haemolyticus* (100.0%) et *S. xylosus* (60,0%), étaient résistantes à la méthicilline. Des phénotypes de résistance à l'aminoside kanamycine, tobramycine et gentamicine (KTG) ont été observés chez 95,0% des isolats de *S. haemolyticus* et 86,0% des isolats de *S. saprophyticus.* Le phénotype de résistance constitutif au MLS_B a été observé chez 13,0% de *S. xylosus* et 11,0% de *S. epidermidis*.
Conclusion: Diverses espèces de CoNS ont été isolées dans cette étude, *S. xylosus* et *S. haemolyticus* étant les plus fréquemment identifiées. Le taux de résistance à la méthicilline était de 100,0% pour les isolats de *S. haemolyticus,* probablement impliqués dans des infections nosocomiales graves, potentiellement mortelles.
Mots-clés: Staphylocoques à coagulase négative, phénotype, résistance, Côte d'Ivoire

Introduction:

Coagulase negative staphylococci (CoNS) are commensals of the skin and mucous membranes and have long been described as contaminants when routinely isolated in clinical bacteriology laboratories (1,2). Over the past few decades, their incidence has significantly increased, and CoNS have emerged as a cause of nosocomial infections (3). The distinct populations in which CoNS infections play a predominant role are premature newborns, patients with implanted medical devices, immunocompromised patients, and those with other comorbidities (4). CoNS form biofilms that allows bacteria to adhere to medical devices and, as a result, protects them from antibiotics (5). Infections caused by CoNS are numerous and can be very serious, and CoNS strains isolated from clinical samples are increasingly resistant to antibiotics, particularly methicillin (6).

The prevalence of methicillin resistance in CoNS strains isolated in hospitals is estimated at 75% in several countries around the world (7). New species have been described in recent years, while clinical information on most of these species is still scarce (8). Differentiating between clinically pathogenic strains and strains considered to be contaminants is one of the major challenges facing clinical biology laboratories (7). Identifying CoNS species is still difficult for most clinical laboratories (9) and are detected by conventional methods available in the laboratory. These methods include the use of Chapman agar for the isolation and enumeration of staphylococci, which allows the differentiation of species that ferment mannitol from those that do not ferment mannitol, and the coagulase test or a rapid slide agglutination test for the simultaneous detection of the affinity of fibrinogen, protein A, and capsular polysaccharides, which are the key markers of the bacterium.

Due to the emergence of infections caused by potentially pathogenic and antibiotic-resistant CoNS, these clinical strains should be properly identified and characterized. In our practice, any CoNS isolate was considered a contaminant and the result was reported as negative. The aim of our study is to provide information that will contribute to the identification and better understanding of CoNS species isolated from biological samples at the Bouaké University Hospital, Côte d'Ivoire.

Materials and method:

Study setting and design:

This was a laboratory-based study of CoNS isolated from clinical specimens of hospitalized patients and stored in the laboratory of the Bouake University Hospital (CHUB) during a 2-month period (November to December 2021). These strains were stored in heart-brain broth (HBB) containing glycerol at -80°C in the laboratory.

Ethical considerations:

Ethical approval was obtained from the Institutional Review Board of the Medical Scientific Directorate of Bouaké University Hospital. The study used samples from the laboratory's collection of isolates, therefore, there were no risks of physical harm to patients. Nevertheless, the data were anonymized to protect patient confidentiality.

Re-identification of CoNS isolates:

The clinical isolates were revived from a bacterial suspension in heart-brain broth,

then seeded on blood-enriched agar to purify them. The strains were then cultured on Chapman agar medium and identified on the basis of their cultural characteristics on Chapman agar medium and biochemical characteristics (coagulase test). Morphological characteristics were verified by Gram staining and catalase test, as well as purity.

Latex agglutination for the simultaneous detection of the affinity factor for fibrinogen, protein A, and capsular polysaccharides of *Staphylococcus aureus,* and detection of free staphylocoagulase on human citrated plasma were performed to differentiate *S. aureus* from CoNS species. The different species of CoNS were confirmed using the API Staph gallery, which was done following preparation of a homogeneous bacterial suspension equivalent to 0.5 McFarland by emulsifying an 18-24-hour colony in an ampoule of API Staph medium.

The microtubes were inoculated with the bacterial suspension to obtain colored turns according to the manufacturer's instructions. These reactions were read using the reading chart, and identification was obtained using the API Staph analytical catalog.

Antibiotic susceptibility testing of CoNS isolates:

Antibiotic susceptibility test (AST) of the CoNS isolates was performed by the Kirby Bauer disc diffusion method on Mueller Hinton agar medium (10) as recommended by the WHO. The AST was performed using young colonies incubated for 18 to 24 hours on Chapman agar to prepare inoculum suspension that was standardized to 0.5 McFarland turbidity standards.

Antibiotic discs were applied to Mueller Hinton (MH) agar medium that has been seeded with the standardized inoculum prepared from the pure young CoNS colonies. The antibiotic discs include penicillin G (P, 10IU), cefoxitin (CX, 30µg), vancomycin (VA, 30µg), kanamycin (K, 5µg), gentamicin (CN, 10µg), tobramycin (TOB., 10µg), fosfomycin (FF, 200 µg), ciprofloxacin (CIP, 5µg), norfloxacin (NOR, 10µg), erythromycin (E, 15µg), clindamycin (DA, 2µg), pristinamycin (PT, 15µg), fusidic acid (FA,10µg), and rifampicin (RA, 5µg).

After 18 to 24 hours of incubation in an aerobic incubator at 37°C, the zone diameters of inhibition of the different antibiotics were measured and compared to the reference diameters of the 2021 European Committee on Antimicrobial Susceptibility Testing (EUCAST) to determine sensitivity or resistance of each isolate to the selected antibiotics.

Statistical analysis:

Data entry was done on Microsoft Word version 2019 and Excel version 2019, and the analysis was performed using EPI INFO 7 software. The results were expressed as averages for quantitative variables and as percentages for qualitative variables.

Results:

Source of biological samples for CoNS isolates:

A total of 100 CoNS were isolated from clinical samples of hospitalized patients including 82.0% from blood, 10.0% from pus, and 3.0% from invasive medical devices (Fig 1). The CoNS isolates are mostly of hospital origin (87.0%), mainly from pediatrics (30.0%), internal medicine (17.0%), and intensive care (9.0%), compared to 13.0% that are of community origin (Fig 2).

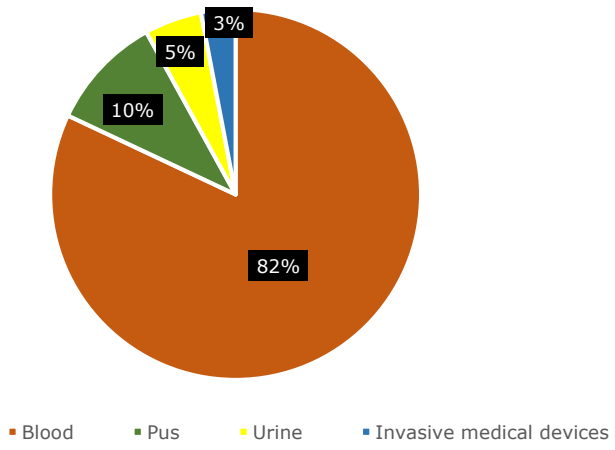

Fig 1: Distribution of coagulase negative staphylococci isolates by sample sources

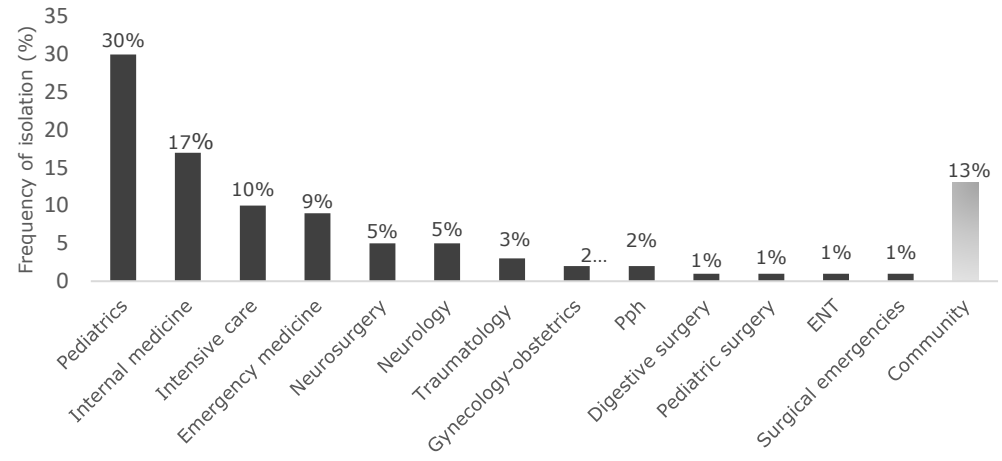

Fig 2: Frequency distribution of coagulase negative staphylococci isolates in hospital and community settings

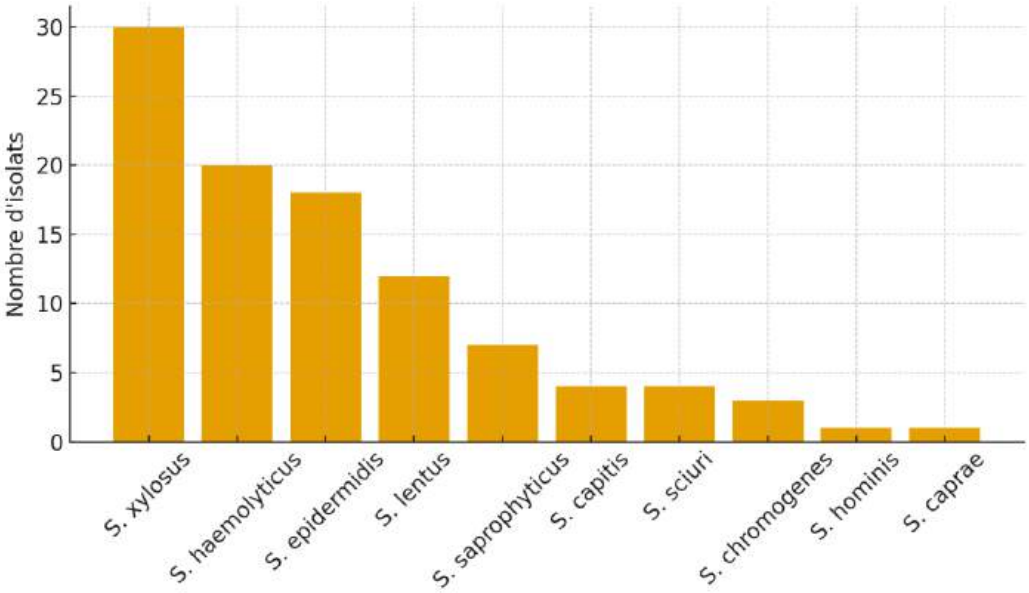

Fig 3: Frequency of coagulase negative staphylococci species identified

Frequency of CoNS species:

Ten CoNS species were identified (Fig 3). The main species were *S. xylosus* (30.0%), *S. haemolyticus* (20.0%), *S. epidermidis* (18.0%), and *S. lentus* (12.0%).

Distribution of CoNS isolates by associated pathologies:

Table 1 shows that a total of 45 CoNS were isolated from patients with infectious syndrome, with 16 *S. xylosus* (35.5%), 7 *S. epidermidis* (15.5%) and 6 *S. lentus* (13.3%) the main species isolated. In addition, *S. haemolyticus* was frequently isolated in patients with infectious syndrome, respiratory distress syndrome in premature babies, and neuro-encephalitic syndrome.

Antibiotic resistance profiles of CoNS isolates:

Of the 100 CoNS isolates tested, 91.0% were resistant to penicillin G, and 65% to cefoxitin, implying a high proportion of methicillin-resistant CoNS (MR-CoNS) isolates (Table 2). Cross-resistance to aminoglycosides of the kanamycin, tobramycin and gentamicin (KTG) phenotype was found in 95.0% of S*. haemolyticus* and 86.0% of *S. saprophyticus*. The constitutive resistance phenotype to MLS_B was found in 13.0% of S*. xylosus* and 11.0% of S*. epidermidis*.

Table 1: Distribution of coagulase negative staphylococci isolates by associated pathologies

Species	**Infectious syndrome (%) (n=45)**	**Suppuration (%) (n=3)**	**Burning sensation during urination (%) (n=1)**	**Neuro-encephalitic syndrome (%) (n=11)**	**Respiratory distress syndrome (%) (n=13)**
Staphylococcus xylosus	16 (35.5)	3 (75.0)	0	1 (9.1)	3 (23.1)
Staphylococcus saprophyticus	3 (6.6)	0	0	2 (18.2)	0
Staphylococcus epidermidis	7 (15.5)	0	1 (100.0)	1 (9.1)	4 (30.8)
Staphylococcus lentus	6 (13.3)	0	0	3 (27.3)	1 (7.7)
Staphylococcus hominis	1 (2.2)	0	0	0	0
Staphylococcus haemolyticus	5 (11.1)	0	0	2 (18.2)	4 (30.8)
Staphylococcus capitis	3 (6.6)	0	0	1 (9.1)	0
Staphylococcus chromogenes	2 (4.4)	0	0	1 (9.1)	0
Staphylococcus sciuri	2 (4.4)	1 (35.5)	0	0	1 (7.7)
Staphylococcus caprae	0	0)	0	0	0

Table 2: Antibiotic resistance of coagulase negative staphylococci isolates to selected antibiotics

Antibiotics	***S. xylosus*** (%) (**n=30**)	***S. lentus*** (%) (**n=12**)	***S. haemolyticus*** (%) (**n=20**)	**Total coagulase-negative staphylococci** (%) (**n=100**)
Penicillin G	29 (97.0)	10 (83.3)	20 (100.0)	91 (91.0)
Cefoxitin	17 (57.0)	5 (41.7)	20 (100.0)	65 (65.0)
Kanamycin	12 (40.0)	8 (66.7)	20 (100.0)	61 (61.0)
Gentamicin	9 (30.0)	6 (50.0)	20 (100.0)	50 (50.0)
Tobramycin	10 (33.3)	7 (58.3)	19 (95.0)	54 (54.0)
Chloramphenicol	5 (16.7)	1 (8.3)	1 (5.5)	12 (12.0)
Erythromycin	11 (36.7)	5 (41.7)	11 (55.5)	43 (43.0)
Clindamycin	4 (13.3)	4 (33.3)	4 (20.0)	16 (16.0)
Linezolid	8 (26.7)	5 (41.7)	3 (15.0)	16 (16.0)
Ciprofloxacin	9 (30.0)	8 (66.7)	17 (85.5)	48 (48.0)
Levofloxacin	11 (36.7)	7 (58.3)	18 (90.0)	53 (53.0)
Tetracycline	22 (73.3)	6 (50.0)	15 (75.0)	72 (72.0)
Cotrimoxazole	17 (57.0)	7 (58.3)	18 (90.0)	67 (67.0)
Vancomycin	3 (10.0)	1 (8.3)	2 (10.0)	12 (12.0)
Fosfomycin	11 (36.7)	2 (16.7)	8 (40.0)	27 (27.0)
Rifampicin	11 (36.7)	4 (33.3)	3 (15.0)	24 (24.0)
Fusidic acid	17 (57.0)	8 (66.7)	7 (35.0)	48 (48.0)
Pristinamycin	2 (6.7)	1 (8.3)	22 (10.0)	7 (7.0)
Minocycline	6 (20.7)	6 (50.0)	9 (45.0)	36 (36.0)

Discussion:

In our study, the main source of biological specimens analysed for the identification of CoNS was from blood cultures of hospitalized or outpatient (community) patients, which agrees with the finding of Zaineb et al., (11) who reported in their study that blood cultures were the most common biological specimens in which CoNS were frequently isolated but Mea (12) reported in his study that the predominant biological specimen was pus while Modibo (13) reported that urine was the main biological specimens in his study. The CoNS isolates were predominantly from the pediatrics department. This predominance could be explained by the high demand for specimen analysis due to the high patient loads and sensitivity of the department as well as the communication that exists between bacteriology-virology and the pediatric department. Zaineb et al., (11) also reported that pediatrics was the department where the isolation rate of CoNS was high.

The identification of CoNS isolates in our study was performed using the API Staph gallery, a miniaturized system that allows the identification of numerous strains of the genera *Staphylococcus, Micrococcus*, and *Kocuria.* We identified 100 CoNS isolates and identify several species such as *S. capitis, S. hominis, S. xylosus,* S*. epidermidis, S. haemolyticus, S.*

saprophyticus, S. caprae, S. chromogenes, S. lentus, and *S. sciuri.* The main species identified was S. *xylosus* (30.0%), followed by S. *haemolyticus* (20%), *S. epidermidis* (18.0%), and *S*. *lentus* (12.0%). Our results differ from those of Nanoukon (14) in Benin in 2017 and Mea (12) in Côte d'Ivoire in 2019, which had a predominance of *S. haemolyticus* (44.0%) and S*. epidermidis* (23.0%), respectively. This difference in CoNS distribution could be due to variability in strain distribution depending on geographical location or sample size.

Staphylococcus xylosus, a commensal of human and animal skin and naturally present in food, is like other CoNS species apart from *S. epidermidis*, increasingly isolated in laboratories. This high frequency of strain isolation has led to renewed clinical interest. A study conducted by Émilie et al., (2) on *S. xylosus* showed their genomic diversity, with most strains being commensal and useful in food, while others could be potentially dan gerous. Tselenis-Kotsowillis et al., (15) were the first to report a case of pyelonephritis caused by S. *xylosus,* Tompkins et al., (16) reported *S. xylosus* infection in a cancer patient. In addition, S*. xylosus* has also been implicated in nosocomial infections, particularly in pneumonia and septicemia in newborns (5, 16,17,18).

Bacteriological results showed that 82.0% of CoNS species in our study were isolated in positive blood cultures. Our results are similar to those of Nanoukon in Benin (14) and Zaineb et al., (11) who reported CoNS isolates in their studies highest from blood cultures. However, the most frequently implicated bacterium responsible for contaminating blood cultures is CoNS, often making it difficult to interpret, as reported by some researchers (3,19). This situation may be the result of prior contamination of the blood culture bottle at the time of collection by healthcare personnel. Our study did not evaluate the risks of blood culture contamination during collection. Although this is a significant limitation of our study, we chose to prioritize the policy of compliance with hygiene, infection prevention, and control measures in place at the CHUB and clinical-biological communication in order to accept or rule out the diagnosis of bacteremia.

The CoNS isolates identified in our series showed variability in antibiotic resistance profiles. The highest resistance rates were observed for tetracycline (72.0%), cotrimoxazole (67.0%), cefoxitin (65.0%), kanamycin (61.0%), tobramycin (54.0%), levofloxacin (53.0%), and gentamicin (50.0%). The CoNS species identified were 91.0% resistant to penicillin. Our results are similar to those of Mea (12) in Ivory Coast, and Nanoukon (14) in Benin, who reported that 94% and 92% of isolated species were resistant to penicillin, respectively.

Methicillin resistance was detected in 65.0% of the isolates identified. In his 2019 study, Mea (12) reported that 40.0% of these isolates were methicillin resistant, with methicillin resistance increasingly observed in CoNS. The main species identified were also resistant to methicillin; S*. xylosus (*57.0%), *S. epidermidis* (72.0%), and *S. haemolyticus* (100%). In our study, *S. haemolyticus* was 100% resistant to penicillin, cefoxitin, kanamycin, gentamicin, and 95.0% resistant to tobramycin. Maria et al., (20) made the same observation in their 2019 study on the comparative analysis of *S. haemolyticus*, revealing the key to hospital adaptation and pathogenicity of *S. haemolyticus. S. haemolyticus* is the second most frequently encountered CoNS species in hospitals and has the highest level of antibiotic resistance.

Multidrug resistance, particularly to methicillin and aminoglycosides, among CoNS is frequently encountered in hospitals. In this study, *S epidermidis* strains showed variable resistance to the antibiotics tested, with 78.0% resistance to penicillin and 72.0% to cefoxitin (methicillin resistant). Our results are similar to that of Modibo (13) in Mali, who reported that S*. epidermidis* strains were 80% resistant to methicillin. *Streptococcus lentus* isolates showed 83.0% resistance to penicillin and 42.0% to cefoxitin (methicillin resistant). A single isolate of *S. caprae* was isolated, which was sensitive (100.0%) to almost all antibiotics tested except penicillin, fosfomycin, and minocycline.

The CoNS species identified were associated different disease syndromes in patients colonized or infected with these strains. These included 45 cases of infectious syndrome, 13 cases of respiratory distress, and 11 cases of neuro-encephalic syndrome. *Staphylococcus haemolyticus* was frequently isolated in one diabetic patient with neuroendocrine syndrome and two premature newborns with respiratory distress syndrome. The increased pathogenicity of CoNS could explain the numerous cases associated with these identified species in our study. Ricarda et al., (4) in their study on CoNS reported that the incidence of CoNS has steadily increased in recent years in parallel with advances in medicine, especially with regard to the use of foreign body devices.

Premature newborns, patients with implanted medical devices, immunocompromised patients, and those with other relevant comorbidities are distinct populations in which CoNS infections play a prominent role. Furthermore, Noshak et al., (21) reported that CoNS are one of the main nosocomial pathogens and can cause several infections such as catheter-related bloodstream infections, skin infections, urinary tract infections, peritonitis, and endocarditis.

Conclusion:

Coagulase-negative staphylococci are commensals of the skin and mucous membranes of humans and animals, and evidence of their contamination of biological samples analyzed in microbiology laboratories is widely reported. Although considered as contaminants in microbiology laboratories, they have become a major cause of infections in immunocompromised persons including premature infants. Specific CoNS species such as *S. xylosus, S. haemolyticus, S. epidermidis,* and *S. lentus,* sometimes of animal origin, are known to be involved in nosocomial infections due to their ability to form biofilms and their high resistance to several classes of antibiotics.

Our study results showed varying rates of resistance to commonly used antibiotics. The emergence of MR-CoNS strains and the numerous resistance profiles found in these bacteria make them potentially lethal. Correct identification of CoNS strains associated with potential clinical infections and genomic surveillance of virulence and resistance factors would be an excellent prospect for CoNS at the Bouake University Hospital Center.

Contributions of the authors:

NAM and AKC designed the study; NAM, MP, TA, TJON, OA and GKJ performed the technical and laboratory analysis of the biological samples collected; NAM and MP analyzed the data and wrote the manuscript; AKC, MP, TJON, GKJ and TA participated in the critical review of the manuscript. All authors read and approved the manuscript submitted for publication.

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Conflict of interest:

Authors declare no conflict of interest.

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Original Article **Open Access**

Comparative evaluation of GenoType MTBDR*plus* line probe assay and solid culture for detection of drug-resistant tuberculosis in northcentral Nigeria

*[1,2]Eliya, T. T., [1,2]Ajide, B. A., [1]Bassey, E., [1,2]Ishaku, P., [1,5]Adeyanju, B. D., [1]Jonathan, K, and [1,3,4]John, B.

[1]Zankli Research Centre, Bingham University Karu, Km 26 Abuja-Keffi Expressway, Karu, Nigeria
[2]Biological Sciences Department, Bingham University Karu, Km 26 Abuja-Keffi Expressway, Karu, Nigeria
[3]Clinical Sciences, Liverpool School of Tropical Medicine, Pembroke Place, Liverpool, United Kingdom
[4]Community Medicine, Bingham University Karu, Km 26 Abuja-Keffi Expressway, Karu, Nigeria
[5]Institute of Human Virology, Plot 62 Emeritus Zone, Abuja, Nigeria
*Correspondence to: iliya.taryuta@binghamuni.edu.ng; +2347034229746

Abstract:

Background: Multi-drug resistant tuberculosis (MDR-TB) poses a great threat to the TB control programs worldwide. Early detection of rifampicin (RIF) and isoniazid (INH)-resistant *Mycobacterium tuberculosis* (MTB) is essential for effective treatment and control of MDR-TB. This study evaluated the performance of GenoType MTBDR*plus* Line Probe Assay (LPA) with the solid culture method for early diagnosis of MDR-TB.
Methodology: We enrolled a total of 112 consented and GeneXpert-confirmed MDR-TB participants from different health facilities in the States of northcentral Nigeria between January and December 2024 into the study. Sputum samples collected from the participants were transported using the triple sample packaging system in a cold chain to Zankli TB Reference Laboratory, Bingham University, Karu, and analysed by AFB microscopy, LPA, solid culture and drug susceptibility test (DST) methods. The diagnostic accuracy of LPA and solid culture for detection of MDR-TB was compared, with the DST as the 'gold standard'.
Results: Of the sputum samples from the 112 patients, 95.5% were positive for MTB on solid culture, 1.7% were negative, 1.7% were contaminated, and 1.1% were non-tuberculous mycobacteria (NTM). For LPA, 96.4% of the 112 patient samples were positive, 3.6% were negative, and 1.1% were NTM. The sensitivity for detection of mono-resistance to rifampicin and isoniazid, and resistance to both drugs (MDR) by LPA compared to the 'gold standard DST was respectively 95.7%, 72.9%, and 97.1%, while the specificity was respectively 88.4%, 88.7%, and 100.0%. The most frequently occurring mutation detected by LPA among the MDR strains was *rpoB* MUT2 and *katG* MUT2.
Conclusion: The LPA test was highly sensitive and specific for the detection of mono-resistance to rifampicin and isoniazid, and multi-drug resistance in sputum samples of TB patients. This makes it a valuable tool for early diagnosis of MDR-TB.

Keywords: MDR-TB, GenoType MTBDR*plus*, Drug Susceptibility Test, Line Probe Assay, Solid culture

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Évaluation comparative du test GenoType MTBDR*plus* sur sonde lignée et de la culture solide pour la détection de la tuberculose pharmaco-résistante dans le centre-nord du Nigéria

*[1,2]Eliya, T. T., [1,2]Ajide, B. A., [1]Bassey, E., [1,2]Ishaku, P., [1,5]Adeyanju, B. D., [1]Jonathan, K, et [1,3,4]John, B.

[1]Centre de Recherche Zankli, Université Bingham de Karu, Km 26, Autoroute Abuja-Keffi, Karu, Nigéria
[2]Département des Sciences Biologiques, Université Bingham de Karu, Km 26, Autoroute Abuja-Keffi, Karu, Nigéria
[3]Sciences Cliniques, École de Médecine Tropicale de Liverpool, Lieu Pembroke, Liverpool, Royaume-Uni
[4]Médecine Communautaire, Université Bingham de Karu, Km 26, Autoroute Abuja-Keffi, Karu, Nigéria
[5]Institut de Virologie Humaine, Parcelle 62, Zone Émérite Abuja, Nigéria
*Correspondance à: iliya.taryuta@binghamuni.edu.ng; +2347034229746

Résumé:

Contexte: La tuberculose multirésistante (TB-MR) représente une menace majeure pour les programmes de lutte contre la tuberculose dans le monde entier. La détection précoce de *Mycobacterium tuberculosis* (MTB) résistant à la rifampicine (RIF) et à l'isoniazide (INH) est essentielle pour un traitement et un contrôle efficace de la TB-MR. Cette étude a évalué les performances du test GenoType MTBDR*plus* sur sonde lignée (LPA) avec la méthode de culture solide pour le diagnostic précoce de la TB-MR.
Méthodologie: Nous avons recruté un total de 112 participants atteints de TB-MR, consentants et confirmés par GeneXpert, provenant de différents établissements de santé des États du centre-nord du Nigéria entre janvier et décembre 2024. Français Les échantillons d'expectorations prélevés sur les participants ont été transportés à l'aide du système d'emballage triple échantillon dans une chaîne du froid jusqu'au laboratoire de référence Zankli TB, Université Bingham de Karu, et analysés par microscopie AFB, LPA, culture solide et tests de sensibilité aux médicaments (DST). La précision diagnostique du LPA et de la culture solide pour la détection de la TB-MR a été comparée, le DST étant la «référence absolue».
Résultats: Sur les échantillons d'expectorations des 112 patients, 95,5% étaient positifs pour MTB sur culture solide, 1,7% étaient négatifs, 1,7% étaient contaminés et 1,1% étaient des mycobactéries non tuberculeuses (NTM). Pour le LPA, 96,4% des 112 échantillons de patients étaient positifs, 3,6% étaient négatifs et 1,1% étaient des NTM. La sensibilité de détection de la mono-résistance à la rifampicine et à l'isoniazide, et de la résistance aux deux médicaments (MR) par le test LPA, comparée au test de sensibilité aux médicaments de référence, était respectivement de 95,7%, 72,9% et 97,1%, tandis que la spécificité était respectivement de 88,4%, 88,7% et 100,0%. Les mutations les plus fréquemment détectées par le test LPA parmi les souches MR étaient *rpoB* MUT2 et *katG* MUT2.
Conclusion: Le test LPA s'est révélé très sensible et spécifique pour la détection de la mono-résistance à la rifampicine et à l'isoniazide, et de la multirésistance dans les échantillons d'expectorations de patients tuberculeux. Cela en fait un outil précieux pour le diagnostic précoce de la MR.

Mots-clés: MR-TB, GenoType MTBDR*plus*, test de sensibilité aux médicaments, test de sonde en ligne, culture solide

Introduction:

Tuberculosis (TB) is a serious infectious disease caused by members of the *Mycobacterium tuberculosis* complex (MTBC). It is one of the leading causes of death worldwide, with an estimated 1.5 million people dying from the disease in 2020 (1). Multidrug-resistant tuberculosis (MDR-TB) is a form of TB that is resistant to at least two of the most commonly used anti-TB drugs, rifampicin and isoniazid. MDR-TB is more difficult to treat and is associated with a higher mortality rate than drug-susceptible TB (1,2). MDR-TB poses a great threat to the TB control programs worldwide. Early diagnosis of rifampicin (RIF) and isoniazid (INH)-resistant MTB is essential for efficient treatment and control of MDR-TB (2).

MDR-TB develops when *M. tuberculosis* (MTB) mutates and become resistant to anti-TB drugs. This resistance often arises when TB patients do not complete their full course of treatment or when they receive sub-optimal doses of the drugs (3). The symptoms of MDR-TB are like those of drug-susceptible TB, and can include cough, fever, night sweats, weight loss, fatigue, and chest pain (4).

The diagnosis of MDR-TB can be challenging, as the symptoms are often like those of other respiratory infections. The most common methods for the diagnosis of MDR-TB include culture and molecular methods. Culture is the 'gold standard' method for the diagnosis of TB, but it is time-consuming, requires specialized equipment, and can take up to 8 weeks to produce results. Molecular tests are becoming increasingly available and are more accurate than sputum microscopy or culture for diagnosis of MDR-TB. Molecular tests can detect mutations in the TB bacteria that are associated with resistance to anti-TB (5,6).

The World Health Organization (WHO) has recommended the conventional drug susceptibility testing as the 'gold standard', but due to its long turnaround time, the treatment given to the patients is delayed, resulting in a higher risk of treatment failure and continuous transmission of MDR-TB (7). The choice of diagnostic method for MDR-TB will depend on the availability of resources and the clinical presentation of the patient. In resource-limited settings, sputum microscopy may be the only available option (7). In resource-rich settings, molecular tests are preferred, as they are more accurate. Early diagnosis of MDR-TB is important, as it allows for prompt treatment and reduces the risk of transmission to others. Although treatment for MDR-TB is long and complex, MDR-TB can be cured with the right combination of drugs. (5).

New methods such as the Line Probe Assay (LPA) have been developed to detect resistance faster and reliably using genotype rather than phenotype and have been endorsed by the WHO for fast and reliable detection of MTBC and its resistance to RMP and/or INH (8). Solid and liquid culture methods for drug susceptibility testing (DST) are time-consuming, requiring weeks to months for results, with culture and DST contamination challenges. This study aimed at evaluating the performance of GenoType MTBDR*plus* LPA with solid culture method for early diagnosis of MDR-TB in northcentral Nigeria and at detecting the mutation patterns associated with *rpo*B, *katG* and *inhA* genes.

Materials and method:

Study design:

This is a cross-sectional study of pulmonary TB (PTB) patients that compared the accuracy of the GenoType MTBDR*plus* line probe assay (LPA) and solid culture for early diagnosis of MDR-PTB.

Ethical considerations:

Ethical approval was obtained from the Bingham University Clinical Research Ethics Committee (NHREC/21/05/2005/01500). Informed consent was obtained from all participants before sample collection, ensuring their right to confidentiality and voluntary participation. Participants' data were anonymized using study codes with limited access to study personnel only.

Study participants:

The study consisted of 112 confirmed MDR-TB participants who were enrolled from January to December 2024 at different health facilities in the States of northcentral Nigeria. Participants were selected based on their confirmed MDR-TB status on the GeneXpert MTB/RIF platform at each facility.

Sample and data collection:

Sputum samples were collected from each consenting participant and packaged using the triple sample packaging system in a cold chain and shipped to Zankli TB Reference Laboratory, Bingham University, Karu, Nigeria. The sputum samples were analyzed using different diagnostic methods (8). The demographic data of each participant were also collected at enrolment with a designed form.

Decontamination of sputum and culture procedure:

The standard N-acetyl L-cysteine (NA LC)/sodium hydroxide (NaOH) method was used to process the sputum samples in a BSL-2 laboratory. To prevent the loss of mycobacterial viability, NaOH was neutralized by adding phosphate buffer after 15 min. The mixture was centrifuged at 3000g for 15 min, and the supernatant was discarded. The sediment was used for concentrated acid-fast bacilli (AFB) smear microscopy and inoculation on solid Lowenstein-Jensen (LJ) media (9,10,11).

The Ziehl-Neelsen (ZN) AFB staining was carried out on the sputum smears, and the results graded as scanty (1-9 AFB/100 fields), 1+ (10-99/100 fields), 2+ (1-10 AFB/field), or 3+ (more than 10 AFB/field) (11). Approximately 0.5 ml of the processed sample was inoculated onto prepared L-J slants and incubated at 37°C. The media were checked for growth weekly for 8 weeks. ZN staining was performed on the isolate from positive cultures to detect AFB, and MTBC SD Bioline was used to confirm the presence of MTBC.

Drug susceptibility test (DST):

The DST was performed on all positive cultures using standard proportion method (12), to first-line anti-TB drugs (isoniazid, rifampicin, and ethambutol), which classifies them as MDR-TB or drug-susceptible TB (DS-TB) strains (12).

Line Probe Assay (LPA):

DNA extraction and multiplex PCR amplification

DNA extraction was carried out on the sediments from the decontaminated samples using a commercial GenoLyse extraction kit, following the manufacturer's instructions. The GenoType MTB CM LPA speciation was then performed according to the manufacturer instruction which include master mix preparation, addition of templates, amplification, reverse hybridization, and interpretation of results (13).

Briefly, 10µl of amplification mix A (AM-A) was combined with 35µl of amplification mix B (AM-B), which contained deoxynucleotides, biotinylated primers, and dye. Following this, 5µl of the extracted DNA sample was added to the master mix in a separate room to avoid contamination. PCR amplification was carried out in a thermocycler under the following conditions; initial denaturation of 1 cycle at 95°C for 15 minutes, followed by first round amplification of 10 cycles at 95°C for 30 seconds, 58°C for 2 minutes, and 70°C for 40 seconds; second round amplification of 20 cycles at 95°C for 25 seconds, 53°C for 40 seconds, and 70°C for 40 seconds; and final extension of 1 cycle at 70°C for 8 minutes.

Reverse hybridization assay procedure:

The hybridization assay was performed using the GT-Blot 48® automated hybridizer (Hain Life Science, Nehren, Germany) following the manufacturer's guidelines. Preheated hybridization and stringent buffers, as well as diluted conjugate and substrate solutions, along with rinsing solutions and sterile distilled water, were loaded into their respective color-coded slots in the GT-Blot 48®. Suction heads were positioned into their corresponding color-coded solutions to ensure proper dispensing. Equal volumes (20µl) of the denaturing reagent and PCR amplicons were mixed in wells of a tray placed in the GT-Blot 48® and allowed to stand for 5 minutes at 25°C for denaturation.

After this, DNA test strips [that targets specific MTBC, wild type (WT) and mutation (MUT) sequences], labeled with sample identification numbers, were placed into the appropriate wells. The automated hybridizer then executed pre-programmed sequence of steps, including hybridization, stringent wash, rinsing, conjugate application, rinsing with sterile distilled water, substrate application, and final

rinse with sterile distilled water. Once the steps were completed, the test strips were dried using the integrated heating system of the GT-Blot 48®. The colour bands produced on the test strips are read and interpreted as presence or absence of resistance to rifampicin, isoniazid or both drugs, in line with the WHO guideline (13).

Data analysis:

The results obtained from AFB microscopy, LPA, solid culture and DST were recorded and compared. The sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) of the LPA and solid culture for MDR-TB diagnosis were calculated using SPSS version 26.0. The accuracy of LPA for detecting drug resistance was evaluated by comparing its result with the 'gold standard' DST method.

Results:

A total of 112 GeneXpert MTB/RIF confirmed positive pulmonary TB cases were enrolled in to the study; 68 (60.7%) were males, 13 (11.6%) were HIV positive, and 3 (2.7%) were age ≤ 14 years, with an average age of 44 years (Table 1). The AFB smear microscopy yielded a total of 87 (77.7%) positive cases with 34 (30.3%) 1^+, 23 (20.5%) 2^+, and 30 (26.7%) 3^+. On solid culture, 107 (95.5%) of 112 patient samples were positive, 2 (1.7%) were negative, 2 (1.7%) were contaminated and 1 (1.1%) grew non-tuberculous mycobacterium (NTM), while on LPA, 108 (96.4%) were positive, 4 (3.6%) were negative, and 1 (1.1%) was NTM as shown in Table 1.

LPA correctly identified MTB in 104 (97.2%) of the 107 MTB culture-positive patient samples but wrongly identified MTB in 4 (80.0%) of 5 MTB culture-negative or contaminated patient samples on solid cultures. One patient sample positive for NTM on the solid culture was negative on LPA by the absence of MTBC TUB control bands on the LPA test (specificity of LPA assay of 100%, 95% CI) as shown in Table 1.

Table 1: *Mycobacterium tuberculosis* detection by solid culture from sputum samples of GeneXpert-confirmed pulmonary tuberculosis patients in relation to LPA, AFB, gender, age, and HIV status

Variables		**MTB solid culture results**				
		MTB positive (%)	**MTB negative (%)**	**NTM (%)**	**Contaminated (%)**	**Total (%)**
LPA						
	Positive	104 (92.8)	2 (1.7)	0	2 (1.7)	108 (96.4)
	Negative	3 (2.6)	0	1 (1.1)	0	4 (3.6)
	Total	107 (95.5)	2 (1.7)	1 (1.1)	2 (1.7)	112 (100.0)
AFB						
	Negative	20 (17.8)	1 (1.1)	1 (1.1)	0	22 (19.6)
	Positive	87 (77.7)	1	0	2	90 (80.4)
	1+	34 (30.3)	0	0	1 (1.1)	35
	2+	23 (20.5)	0	0	1 (1.1)	24
	3+	30 (26.7)	1 (1.1)	0	0	31
	Total	107 (95.5)	2 (1.7)	1 (1.1)	2 (1.7)	112 (100.0)
Gender						
	Male	67 (59.8)	1 (1.5)	0	0	68 (60.7)
	Female	40 (35.7)	1 (2.3)	1 (1.1)	2 (1.7)	44 (39.3)
	Total	107 (95.5)	2 (1.7)	1 (1.1)	2 (1.7)	112 (100.0)
Age group (years)						
	≤14	3 (2.6)	0	0	0	3 (2.7)
	≥15	104 (92.8)	2 (1.7)	1 (1.1)	2 (1.7)	109 (97.3)
	Total	107 (95.5)	2 (1.7)	1 (1.1)	2 (1.7)	112 (100.0)
HIV status						
	Positive	12 (10.7)	0	0	1 (1.1)	13 (11.6)
	Negative	95 (84.8)	2 (1.7)	1 (1.1)	1 (1.1)	99 (88.4)
	Total	107 (95.5)	2 (1.7)	1 (1.1)	2 (1.7)	112 (100.0)

LPA=Line probe assay; AFB = Acid fast bacillus; HIV=Human immunodeficiency virus; MTB = *Mycobacterium tuberculosis*; NTM = Non-tuberculous mycobacteria

Table 2: Comparison of LPA and phenotypic DST results for rifampicin mono-resistance, isoniazid mono-resistance, and MDR-TB

Test method	Total positive samples	RIF mono-resistant	INH mono-resistant	MDR-TB (RIF + INH resistant)
LPA	108	29	19	38
DST	107	31	9	37

LPA=Line Probe Assay; DST= Drug Susceptibility Test; RIF=Rifampicin; MDR=Multi-drug resistant; INH: Isoniazid

For assessing the performance of the LPA test, all culture-negative and contaminated samples, LPA test reports with absent TUB control bands, or invalid LPA results were excluded. Only valid genotypic LPA and conventional phenotypic DST results were compared for the 112 samples. For the 108 LPA positive samples, 67 were rifampicin resistant (with 29 rifampicin mono-resistant), 57 were isoniazid resistant (with 19 isoniazid mono-resistant) and 38 were multi-drug resistant (rifampicin + isoniazid-resistant). Of the 107 positive solid cultures/DST, 31 were rifampicin mono-resistant, 9 were isoniazid mono-resistant and 37 were multi-drug resistant (Table 2).

From Table 3, the sensitivity for rifampicin and isoniazid monoresistance and that of MDR were 95.7%, 72.9%, and 97.1%, respectively, while their specificity was 88.4%, 88.7%, and 100%, respectively. Their PPV and NPV were 93.0%, 87.8%,98.5%, and 92.7%, 74.6%, 99.0%, respectively (Table 3).

All the 29 rifampicin-mono-resistant strains had mutation on the *rpoB* WT7, WT8, MUT1, MUT2, and MUT3 bands, while the 19 isoniazid mono-resistant strains had mutation on the *katG* WT, MUT1, MUT2, and the *inhA* WT1, WT2, MUT1, MUT2, and MUT3 bands. The most frequently occurring mutations among the MDR were *rpoB* MUT2 and *katG* MUT2 (Table 4).

Table 3: Diagnostic performance of LPA test in detecting resistance to rifampicin, isoniazid, and multi-drug resistance in comparison with the DST 'gold standard'

Drug/Diagnostic parameter	Rifampicin	Isoniazid	Rifampicin + Isoniazid
	Resistance (n=67) Sensitive (n=41)	Resistance (n=57) Sensitive (n=51)	Multi-drug resistance (n=38) Non-MDR (n=74)
Sensitivity (%)	95.7	72.9	97.1
Specificity (%)	88.4	88.7	100
PPV (%)	93.0	87.8	98.5
NPV (%)	92.7	74.6	99.0

MDR = multi-drug-resistance; NPV = negative predictive value; PPV = positive predictive value (values in parentheses are with 95% confidence intervals)

Table 4: Band patterns of drug-resistant *Mycobacterium tuberculosis* strains with the Line Probe Assay

Gene	Band	Gene region/mutation	RIF mono-resistant strain (n=29)	INH mono-resistant strains (n=19)	MDR-PTB strains (n=38)
rpoB					
	WT1	506–509	0	0	0
	WT2	510–513	1	0	1
	WT3	513–517	0	0	0
	WT4	516–519	0	0	0
	WT5	518–522	0	0	8
	WT6	521–525	0	0	10
	WT7	521–525	3	0	35
	WT8	53o- 533	28	0	35
	MUT1	D516V	28	0	24
	MUT2	H526 Y	29	0	38
	MUT3	S531 L	29	0	2
katG					
	WT	315	0	11	4
	MUT1	S315 T1	0	3	10
	MUT2	S315T2	0	2	23
inhA					
	WT1	-15/-16	0	4	9
	WT2	-8	0	1	2
	MUT1	C15T	0	18	10
	MUT2	A16G 0	0	19	1
	MUT3	T8C	0	12	22

RIF = rifampicin; INH = isoniazid; MDR-PTB = multidrug resistant-pulmonary tuberculosis; WT=Wildtype; MUT=Mutation

Discussion:

This study compared the accuracy of GenoType MTBDR*plus* LPA and solid culture for the early diagnosis of MDR-TB in north central Nigeria on a total of 112 GeneXpert-confirmed MDR-PTB patients. Our finding shows that LPA, compared to conventional solid culture, is reliable for the diagnosis of MDR-TB, with a sensitivity of 92.8%, meaning that it was able to correctly identify 92.8% of patients with MDR-TB as confirmed by solid culture. LPA also had a specificity of 88.4%, 88.7%, and 100,0% for rifampicin, isoniazid, and MDR-TB, respectively. The results of this study are consistent with a study conducted in India, which reported LPA sensitivity of 93.3% and specificity of 97.0% for the diagnosis of MDR-TB, while solid culture had a sensitivity of 85.7% and a specificity of 94.8% (14). Although our study showed that LPA correctly detected resistance to rifampicin and MDR-TB, it had a lower sensitivity (72.9%) for isoniazid resistance detection.

The specificity of LPA for detecting resistance to rifampicin, isoniazid, and MDR-PTB of 88.4%, 88.7%, and 100%, respectively, indicates that LPA had moderate specificity for rifampicin and isoniazid, but a high specificity for MDR-PTB. The India study (14) similarly reported that LPA had high sensitivity and specificity for detecting rifampicin resistance and MDR-TB, but lower sensitivity for detecting isoniazid resistance. The present study reports a lower sensitivity of 72.9% for isoniazid, while the India study (14), contrarily, reported a higher sensitivity of 92.0%. Additionally, the current study also reports moderate specificity for isoniazid resistance of 88.7%, while a meta-analytical study (15) reported high specificity of 99.0%. The differences in rates may be due to differences in sample sizes, study populations, or methodologies between studies, that could potentially contribute to the variations in these rates.

The positive predictive value (PPV) of a test indicates the probability that a positive test result accurately detects what is being tested for. In our study, the PPV of the LPA for detecting resistance to rifampicin, isoniazid, and MDR-TB was 93.0%, 87.8%, and 98.5%, respectively. This means that the LPA has a high probability of correctly identifying true resistant cases for rifampicin, isoniazid, and MDR-PTB. On the other hand, the negative predictive test (NPV) of a test indicates the probability that a negative test result correctly identifies absence of resistance. In this study, the NPVs of the LPA for detecting resistance to rifampicin, isoniazid, and MDR-PTB were 92.7%, 74.6%, and 99.0%, respectively. This means that LPA has a high probability of correctly identifying absence of rifampicin-resistant and MDR-TB cases, but a lower probability of such for isoniazid resistance. The results suggest that LPA, among molecular tests, offers a more comprehensive resistance profile within a short turnaround time compared to the culture method. Additionally, it has the added benefit of determining the appropriate drug regimen for patients with INH mono-resistance.

The findings from our study further revealed that most of the gene mutations were in the regions of *rpoB* H526 Y and 530-533 in rifampicin resistance, while those of INH resistance were in gene mutation regions *inhA* A16G0 and C15T. However, for the MDR strains, they were at the gene mutation regions *katG* S315T2, *inhA* T8C, and *rpoB* 530-533. A similar study (16) reported that the most common gene mutations were in codons 531 (34.0%), 526 (9.3%), and 516 (2.3%) in the rifampicin resistance-determining region (RR DR) of the *rpoB* gene, whereas the most associated mutations with rifampicin resistance was in codon 531, followed by 526 mutations. Another study (17) reported that up to 95%–98% of rifampicin-resistant strains exhibited mutations in the *rpoB* gene at codon 531. The evidence from our study did not show any relationship between specific mutation conferring rifampicin and isoniazid resistance, which is similar to other studies (14,18).

Our study is limited by the fact it was conducted in one specific region (northcentral) of Nigeria, with a relatively small sample size of 112 participants. Therefore, the findings may not be truly representative of the entire population or apply to other regions of the country. Our study also compared only LPA with solid culture and did not include other molecular tests for the diagnosis of MDR-TB. Therefore, the comparative performance of LPA with other molecular tests remains unknown. In addition, our study did not assess the cost-effectiveness of LPA compared to other diagnostic methods. Therefore, the financial implications of implementing LPA in resource-limited settings are not fully understood and need further investigation.

Conclusion:

This study provides evidence that the GenoType MTBDR*plus* LPA is accurate and reliable for the early diagnosis of MDR-TB, with a higher sensitivity and specificity for detecting MDR-TB. Therefore, it is recommended that TB programs should consider implementing LPA as part of their diagnostic protocol for MDR-TB.

Further research and validation studies should be conducted to confirm the findings of this study and to evaluate the performance of LPA in different settings and populations. This will help to establish the generalizability and reliability of LPA as a diagnostic tool

for MDR-TB.

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Contribution of authors:

TTE conceptualized the study, participated in conducting the laboratory work, analyzing data, and writing the first draft of the manuscript. ABA participated in the writing, proofreading, and editing of the manuscript. KJA analysed some data and participated in writing the manuscript. BE and IP participated in the laboratory work. BJS supervised the study and reviewed the manuscript. All authors approved the final copy of the manuscript.

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The dataset of this study is available from the corresponding author on reasonable request.

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Original Article **Open Access**

Prevalence and predictors of hepatitis B screening among pregnant women attending antenatal clinics in three primary health centres in Abeokuta, Nigeria: A cross-sectional study

*Aliu, O. O., Fowotade, A., and Omoruyi, C. E.

Department of Medical Microbiology, University College Hospital (UCH), Ibadan, Nigeria
*Correspondence to: aliu_my2004@yahoo.com

Abstract:

Background: Hepatitis B virus (HBV) infection remains a major public-health concern. Antenatal screening identifies maternal infection and enables interventions to prevent mother-to-child transmission. We assessed the prevalence and predictors of prior HBV screening among pregnant women attending three primary health centres in southwest Nigeria.
Methodology: This was a cross-sectional survey of 289 pregnant women consecutively enrolled at the antenatal clinics of the three selected health centres. A standardized questionnaire was interviewer-administered to each participant to collect information on socio-demographic characteristics, previous HBV screening and behavioral and healthcare- factors related to HBV screening. Data were analysed using SPSS. Associations of factors with prior HBV screening were determined using Chi-square and multivariable logistic regression estimated adjusted Odd ratio (AOR) with 95% confidence interval (CI).
Results: Overall, 31.0% of participants reported previous HBV screening. Prevalence of previous HBV screening was significantly associated with age, with highest in the 35–39-year age group (AOR≈6.40, p<0.001), previous hospitalization (AOR≈2.3, p=0.010) and receipt of clotting factors (AOR≈7.6, p=0.028). Education, occupation, marital status and trimester of pregnancy were not significant predictors.
Conclusion: Antenatal HBV screening coverage was suboptimal in the study. Healthcare touchpoints especially prior admissions and late-pregnancy visits offer opportunities to scale screening. Programs should target women with hospitalization history or coagulation treatment to strengthen HBV prevention of mother to child transmission (PMTCT).

Keywords: Hepatitis B; pregnancy; antenatal care; screening; southwest Nigeria

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Prévalence et facteurs prédictifs du dépistage de l'hépatite B chez les femmes enceintes fréquentant les consultations prénatales de trois centres de santé primaires à Abeokuta, au Nigéria: une étude transversale

*Aliu, O. O., Fowotade, A., et Omoruyi, C. E.

Département de Microbiologie Médicale, Centre Hospitalier Universitaire (CHU), Ibadan, Nigéria
*Correspondance à: aliu_my2004@yahoo.com

Résumé:

Contexte: L'infection par le virus de l'hépatite B (VHB) demeure un problème majeur de santé publique. Le dépistage prénatal permet d'identifier l'infection maternelle et de mettre en œuvre des interventions visant à prévenir la transmission mère-enfant. Nous avons évalué la prévalence et les facteurs prédictifs d'un dépistage antérieur du VHB chez les femmes enceintes fréquentant trois centres de santé primaires du sud-ouest du Nigéria.
Méthodologie: Il s'agit d'une enquête transversale menée auprès de 289 femmes enceintes inscrites consécutivement dans les consultations prénatales des trois centres de santé sélectionnés. Un questionnaire standardisé a été administré par un enquêteur à chaque participant afin de recueillir des informations sur leurs caractéristiques sociodémographiques, leurs antécédents de dépistage de l'hépatite B et les facteurs

comportementaux et liés aux soins de santé associés à ce dépistage. Les données ont été analysées à l'aide du logiciel SPSS. Les associations entre les facteurs et les antécédents de dépistage de l'hépatite B ont été déterminées par le test du χ^2 et une régression logistique multivariée a permis d'estimer les Odd ratio ajustés (ORa) avec leur intervalle de confiance (IC) à 95%.
Résultats: Au total, 31,0% des participants ont déclaré avoir déjà subi un dépistage de l'hépatite B. La prévalence de ces antécédents était significativement associée à l'âge, avec un taux maximal dans la tranche d'âge des 35-39 ans (ORa≈6,40, p<0,001), aux antécédents d'hospitalisation (ORa≈2,3, p=0,010) et à l'administration de facteurs de coagulation (ORa≈7,6, p=0,028). Le niveau d'études, la profession, la situation matrimoniale et le trimestre de grossesse n'étaient pas des facteurs prédictifs significatifs.
Conclusion: La couverture du dépistage prénatal de l'hépatite B était insuffisante dans cette étude. Les interactions avec les professionnels de santé, notamment les hospitalisations antérieures et les consultations de fin de grossesse, offrent des opportunités d'étendre le dépistage. Les programmes devraient cibler les femmes ayant des antécédents d'hospitalisation ou de traitement anticoagulant afin de renforcer la prévention de la transmission mère-enfant de l'hépatite B (PTME).

Mots-clés: Hépatite B; grossesse; soins prénatals; dépistage; sud-ouest du Nigéria

Introduction:

Hepatitis B virus (HBV) infection remains a significant global public-health concern, responsible for chronic liver disease, cirrhosis, and hepatocellular carcinoma. According to the World Health Organization (WHO), an estimated 296 million individuals are chronically infected worldwide, with sub-Saharan Africa representing a major burden region (1). Nigeria is hyperendemic, with national seroprevalence estimates ranging between 8–12% (2).

Perinatal transmission contributes substantially to the burden of chronic HBV infection, particularly in high-prevalence regions (3). Screening pregnant women during antenatal care (ANC) is crucial for identifying infections, initiating appropriate evaluation such as viral load testing, and administering antiviral prophylaxis to high-risk mothers to prevent mother-to-child transmission (PMTCT) (4).

Despite recommendations for routine antenatal HBV screening, implementation remains inconsistent in primary healthcare facilities across Nigeria. Reports indicate wide variations in HBV screening uptake among pregnant women, with many studies also highlighting structural, socio-demographic, and health-system barriers (5–8). This study aimed to assess the prevalence of prior HBV screening among pregnant women attending primary healthcare centres in Abeokuta and identify the key determinants influencing previous screening behaviors.

Materials and method:

Study design and setting:

We conducted a cross-sectional study in three primary health centers located in Abeokuta (Oba Ademola Hospital, Obantoko Hospital, and Osise Hospital), Ogun state, southwest Nigeria from June 2021 to June 2022.

Ethical consideration:

Ethical approval was obtained from the Ogun state Health Research Committee (SHREC), with approval number HPRS/381/242 and written informed consent was obtained from all participants, in accordance with the Declaration of Helsinki.

Study participants:

Pregnant women 15–49 years of age attending antenatal clinics, resident in the catchment areas and providing informed consent were eligible. Participants were consecutively enrolled to yield the sample size for the study.

Sample size determination:

The minimum required sample size for the study was determined using the Cochran formula for single-proportion population surveys appropriate for estimating prevalence in a defined population attending the three selected primary health centers; $n_o = Z^2 p(1-p)/d^2$, where n_0=minimum sample size, Z=standard normal deviate corresponding to 95% confidence (1.96), p=expected prevalence of hepatitis B infection of 9% (p=0.09) reported for national HBV prevalence by Olayinka et al., (2), and d=margin of error (precision) set at 5% (0.05). This gave a calculated sample size of 126, which was first adjusted for 15% attrition (non-response) to 148, and then adjusted for design effect (*Deff* of 1.9) of multi-center design, to give the adjusted sample size of 281, which was increased to 289.

Method of sampling:

The selection of the 289 study participants was done by proportional allocation using the average monthly patient load of each center to prevent sampling bias as shown in Table 1

Table 1: Proportional allocation for sampling of the study participants in the three health centers

Health Center	Proportion of Monthly Attendance (%)	No of allocated participants
Oba Ademola Hospital	40	116
Obantoko Hospital	35	101
Osise Hospital	25	72
Total	**100**	**289**

Data collection:

Data were collected using a standardized interviewer-administered questionnaire designed for the study. The instrument captured socio-demographic information (age, education, occupation, religion, marital status, family type); obstetric characteristics (trimester and parity); behavioral and clinical exposures (scarification practices, previous surgery, blood transfusion, clotting factor administration, HIV status, and prior hospitalization); and prior HBV screening history.

The questionnaire was pilot tested among 20 antenatal clinic attendees at Obantoko Primary Health Center to assess clarity and feasibility. Internal consistency and reliability of the multi-item exposure domain yielded a Cronbach alpha score of 0.81, indicating good reliability of the instrument for field use.

Data analysis:

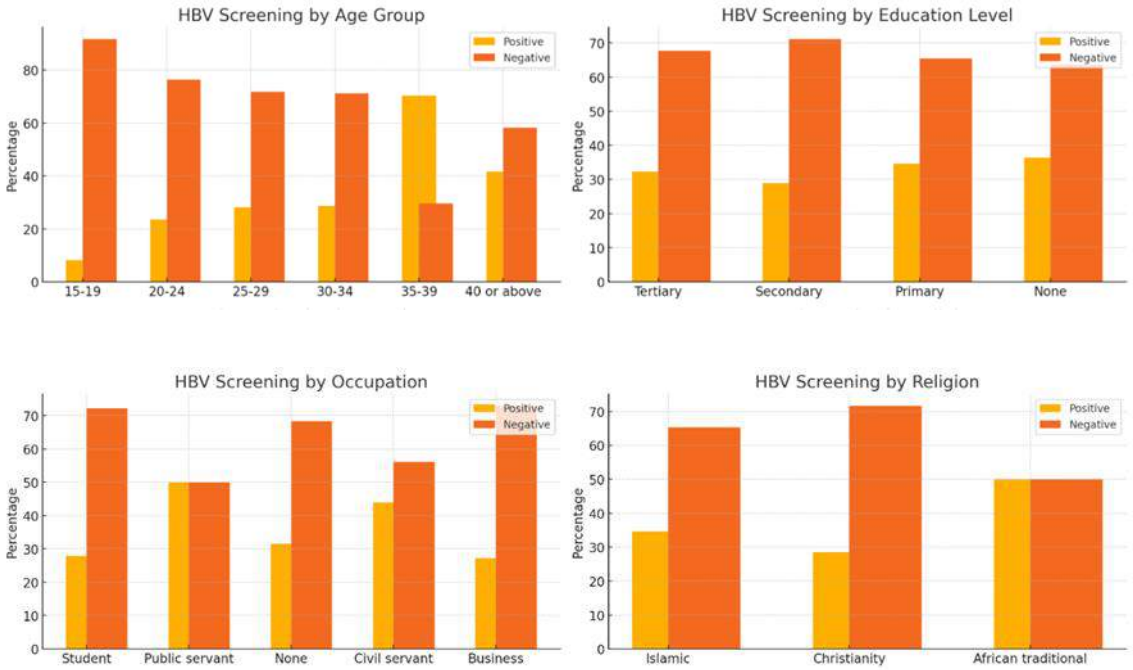
The primary outcome was self-reported prior HBV screening. Explanatory variables included age group, trimester of pregnancy, prior hospitalization, receipt of clotting factors, HIV diagnosis, and other relevant sociodemographic and clinical exposures.

Data were summarized using frequencies and percentages. Statistical analyses were performed using IBM SPSS Statistics version 25. The Chi square test was used to assess bivariate associations. Multivariable logistic regression was applied to estimate adjusted odds ratios (AORs) and corresponding 95% confidence intervals (CIs), adjusting for covariates selected a priori or those with $p<0.20$ in bivariate analysis. A two-sided $p<0.05$ was considered statistically significant.

Results:

Of the 289 pregnant women enrolled into the study, 90 (31.0%) reported previous HBV screening. The frequency distribution of the women with and without previous HBV screening with respect to socio-demographic characteristics is shown in Fig 1.

Analysis of socio-demographic characteristics shows that prevalence of previous HBV screening differed significantly by age ($\chi^2=24.994$, $p<0.001$) with highest prevalence (70.4%, n=19/27) in women aged 35–39 years. However, education, occupation, religion, marital status and family type were not significantly associated with prevalence of HBV screening among the participants (Table 2).



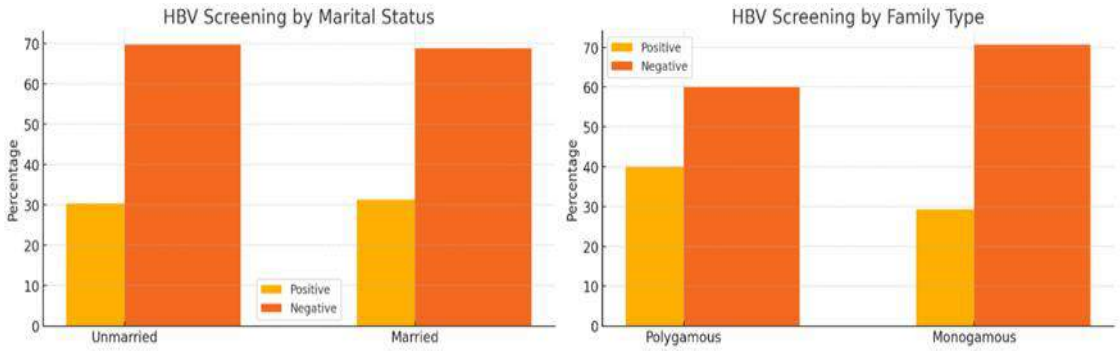


Fig 1: Frequency of hepatitis B virus screening among pregnant women by socio-demographic characteristics

Table 2: Prevalence of previous hepatitis B virus screening by socio-demographic characteristics of the pregnant women

Characteristics	Previous HBV screened		X^2	*p*-value
	Yes (n=90, 31.1%)	No (n=199, 68.9%)		
Age group (years)				
15-19	1 (8.3)	11 (91.7)	24.994	<0.001
20-24	13 (23.6)	42 (76.4)		
25-29	29 (28.2)	74 (71.8)		
30-34	23 (28.7)	57 (71.3)		
35-39	19 (70.4)	8 (29.6)		
≥ 40	5 (41.7)	7 (58.3)		
Educational level				
Tertiary	40 (32.3)	84 (67.7)	0.657	0.883
Secondary	37 (28.9)	91 (71.1)		
Primary	9 (34.6)	17 (65.4)		
None	4 (36.4)	7 (63.6)		
Occupation				
Student	12 (27.9)	31 (72.1)	7.300	0.121
Public servant	8 (50.0)	8 (50.0)		
Civil servant	18 (43.9)	23 (56.1)		
Business	46 (27.1)	124 (72.9)		
Unemployed	6 (31.6)	13 (68.4)		
Religion				
Islamic	41 (34.7)	77 (65.3)	1.638	0.441
Christianity	48 (28.4)	121 (71.6)		
African traditional	1 (50.0)	1 (50.0)		
Marital status				
Unmarried	10 (30.3)	23 (69.7)	0.012	0.912
Married	80 (31.3)	176 (68.8)		
Family type				
Polygamous	20 (40.0)	30 (60.0)	2.212	0.137
Monogamous	70 (29.3)	169 (70.7)		

The frequency distribution of the women with and without previous HBV screening with respect to behavioral and healthcare factors is shown in Fig 2 and Table 3. On bivariate analysis of behavioral/healthcare correlates, the prevalence of previous HBV screening was significantly higher among women reporting scarification (45.5%, χ^2=4.958, p=0.026), in third trimester of pregnancy (42.7%, χ^2= 19.912, p<0.001), with previous hospitalization (47.2%, χ^2=11.563, p=0.001), with HIV diagnosis (60.0%, χ^2=6.144, p=0.013), and who had received clotting factors (77.8%, χ^2=9.422, p=0.002) (Table 3).

However, in bivariate analysis, age was significantly associated with prior HBV screening (χ^2=24.994, p<0.001), with the highest prevalence observed among women aged 35–39 years (70.4%). After adjusting for covariates, only 35–39 years age group remained independently associated with prior HBV screening (AOR 6.40, 95% CI 2.70–15.20, p< 0.001). Independent predictors also included receipt of clotting factors (AOR 7.58, p=0.028) and prior hospitalization (AOR 2.29, p=0.010). Trimester of pregnancy, scarification, and HIV diagnosis were not significant in the final model (Table 4, Fig 3).

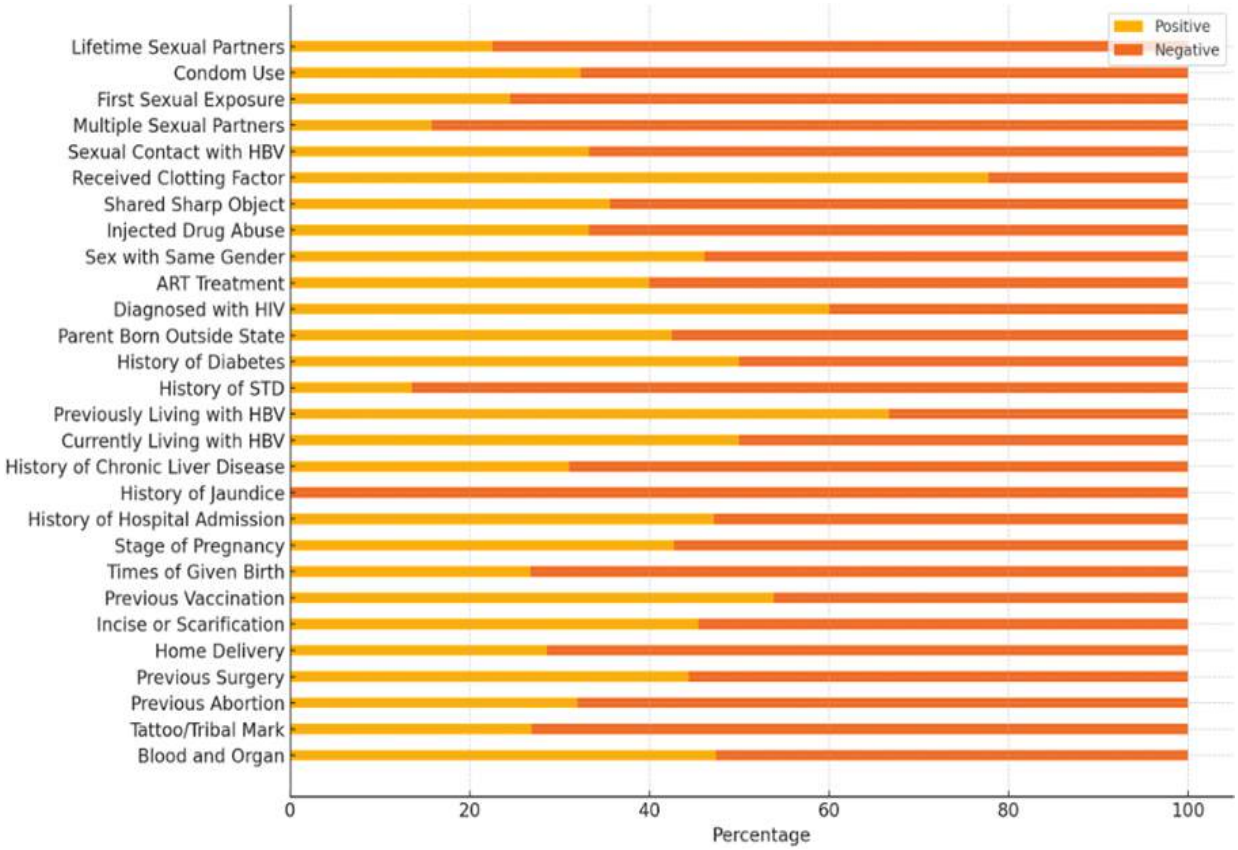


Fig 2: Frequency of hepatitis B virus screening among pregnant women by behavioral/healthcare factors

Table 3: Bivariate analysis of behavioral and health-care factors associated with prior HBV screening

Factors	Previous HBV screening		x^2	*p*-value
	Yes (n=90, 31.1%)	No (n=199, 68.9%)		
Blood and organ				
Yes	9 (47.4)	10 (52.6)	2.497	0.114
No	81 (30.0)	189 (70.0)		
Tattoo/tribal mark on the body				
Yes	25 (26.9)	68 (73.1)	1.161	0.281
No	65 (33.2)	131 (66.8)		
Previous abortion				
Yes	24 (32.0)	51 (68.0)	0.035	0.852
No	66 (30.8)	148 (69.2)		
Previous surgery				
Yes	12 (44.4)	15 (55.6)	2.458	0.117
No	78 (29.8)	184 (70.2)		
Home delivery				
Yes	6 (28.6)	15 (71.4)	0.070	0.792
No	84 (31.3)	184 (68.7)		
Incision or scarification				
Yes	20 (45.5)	24 (54.5)	4.958	0.026*
No	70 (28.6)	175 (71.4)		
Previous vaccination				
Yes	7 (53.8)	6 (46.2)	3.272	0.070
No	83 (30.1)	193 (69.9)		
No of previous child birth				
Primparous	27 (26.7)	74 (73.3)	2.627	0.453
1	25 (30.1)	58 (69.9)		
2	18 (33.3)	36 (66.7)		
>2	20 (39.2)	31 (60.8)		
Trimester of pregnancy				
First trimester	5 (26.3)	14 (73.7)	19.912	<0.001*
Second trimester	21 (17.5)	99 (82.5)		
Third trimester	64 (42.7)	86 (57.3)		
History of hospital admission				
Yes	34 (47.2)	38 (52.8)	11.563	0.001*
No	56 (25.8)	161(74.2)		
Currently living with positive HBV patients				
Yes	3 (50.0)	3 (50.0)	1.016	0.313
No	87 (30.7)	196 (69.3)		

Previously living with positive HBV patients			3.606	0.058
Yes	4 (66.7)	2 (33.3)		
No	86 (30.4)	197 (69.6)		
History of STD				
Yes	3 (13.6)	19 (86.4)	3.403	0.065
No	87 (32.6)	180 (67.4)		
History of diabetes				
Yes	3 (50.0)	3 (50.0)	1.016	0.313
No	87 (30.7)	196 (69.3)		
Parent born outside Ogun state				
Yes	17 (42.5)	23 (57.5)	2.793	0.095
No	73 (29.3)	176 (70.7)		
Diagnosed with HIV				
Yes	9 (60.0)	6 (40.0)	6.144	0.013*
No	81 (29.6)	193 (70.4)		
ART treatment				
Yes	1 (40.0)	4 (80.0)	0.295	0.587
No	89 (31.3)	195 (68.7)		
Sex with same gender				
Yes	6 (46.2)	7 (53.8)	1.431	0.232
No	84 (30.4)	192 (69.6)		
Injected (IV) drug abuse				
Yes	2 (33.3)	4 (66.7)	0.014	0.907
No	88 (31.1)	195 (68.9)		
Shared sharp object				
Yes	32 (35.6)	58 (64.4)	1.187	0.276
No	58 (29.1)	141 (70.9)		
Received clotting factor				
Yes	7 (77.8)	2 (22.2)	9.422	0.002*
No	83 (29.6)	197 (70.4)		
Sexual contact with HBV patients				
Yes	1 (33.3)	2 (66.7)	0.007	0.934
No	89 (31.1)	197 (68.9)		
History of jaundice				
Yes	0	5 (100.0)	2.301	0.129
No	90 (31.7)	194 (68.3)		
History of chronic liver disease				
Yes	0	0	-	-
No	90 (31.1)	199 (68.9)		
Multiple sexual partner				
Yes	3 (15.8)	16 (84.2)	2.235	0.135
No	87 (32.2)	183 (67.8)		
First sexual exposure				
≤15 years	12 (24.5)	37 (75.5)	1.373	0.241
>15 years	77 (33.0)	156 (67.0)		
Condom use				
Yes	24 (32.4)	50 (67.6)	0.077	0.781
No	66 (30.7)	149 (69.3)		
Lifetime sexual partner				
1	83 (32.2)	175 (67.8)	1.187	0.276
> 1	7 (22.6)	24 (77.4)		

Table 4: Multivariable logistic regression of predictors of prior HBV screening

Factor	**AOR**	**95% CI**	***p*-value**
Age group (vs ≥40 years)			
15–19 years	2.5	0.49–12.72	0.275
20–24 years	2.8	0.95–8.20	0.062
25–29 years	3.1	1.15–8.50	0.026*
30–34 years	0.9	0.48–1.70	0.72
35–39 years	6.4	2.70–15.20	<0.001*
Reference: ≥40 years	—	—	—
Scarification (Yes)	1.85	0.97–3.52	0.061
Clotting factor received (Yes)	7.58	1.25–45.80	0.028*
History of hospital admission (Yes)	229%	1.22–4.28	0.010*
Diagnosed with HIV (Yes)	247%	0.77–7.94	0.133
Trimester (3rd vs 1st)	188%	0.72–4.89	0.308
Trimester (2nd vs 1st)	57%	0.17–1.92	0.38

*Significant at $p < 0.05$; AOR=Adjusted Odd Ratio' CI = Confidence Interval.

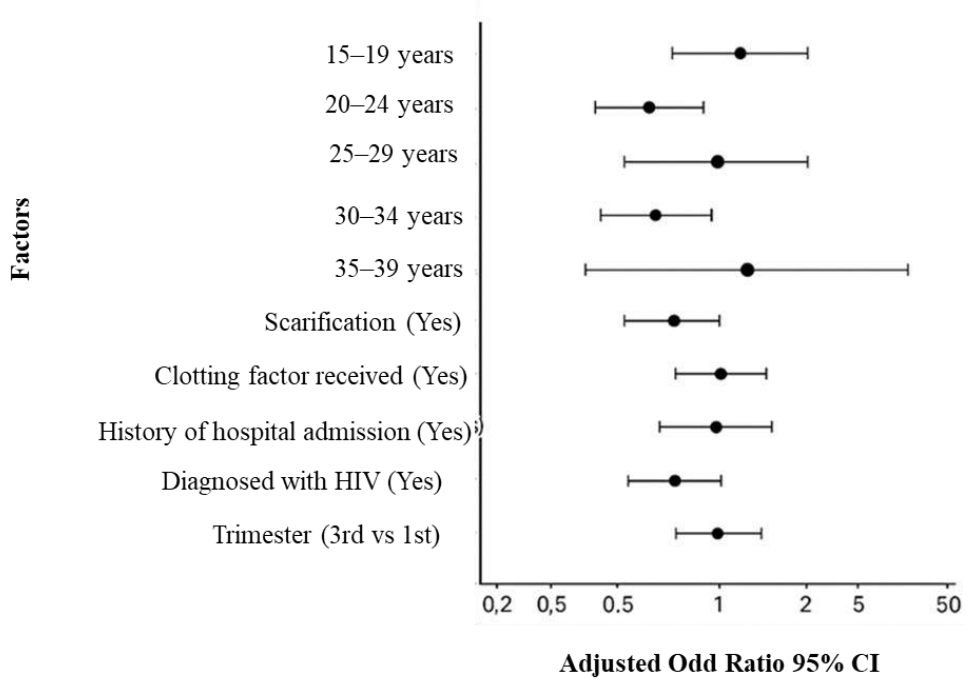


Fig 3: Forest plot of adjusted odds ratios (AORs) for predictors of prior hepatitis B virus screening among pregnant women

Discussion:

This study found that fewer than one-third of pregnant women in primary-care facilities had ever undergone HBV screening. Despite the high endemicity of HBV in Nigeria, this low uptake aligns with similar research from sub-Saharan Africa reporting persistent gaps in antenatal HBV screening coverage (5–7). Age demonstrated a strong association with screening likelihood. Women aged 35–39 years were significantly more likely to have been screened, consistent with previous studies showing older maternal age correlates with higher health-care engagement (8). Younger women, particularly adolescents, remain an underserved demographic in HBV screening programs.

Clinical factors including prior hospitalization and receipt of clotting factors were strongly predicted screening. These findings may suggest that HBV testing in these facilities is often event-triggered such as during hospital admission or use of blood products, rather than routinely integrated into ANC protocols. Strengthening provider-initiated testing and adopting a universal screening strategy could improve coverage substantially.

Despite strong bivariate associations, HIV diagnosis and trimesters were not significant in multivariable models, likely due to confounding by hospitalization or other clinical exposures. Nonetheless, integrating HBV screening with HIV PMTCT programs remains a high-yield strategy. A key implication is the urgent need to institutionalize first-trimester, universal HBV screening across all ANC facilities. Early identification enables timely viral load assessment and potential antiviral prophylaxis to prevent vertical transmission.

HBV screening uptake among pregnant women in primary health centres remains low. Age, hospitalization history, and clotting factor exposure were the main predictors of previous screening. Strengthening universal antenatal HBV screening, particularly at first trimester is essential to reduce undetected maternal infections and prevent perinatal transmission.

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Contributions of authors:

FA designed and supervised the research; AO analysed the data and wrote the manuscript; and OEC collected the data. All authors read and approved the manuscript submitted for publication

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Conflict of interest:

No conflict of interest is declared.

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Original Article **Open Access**

Knowledge, perception and uptake of human papillomavirus (HPV) vaccine among future healthcare providers: Insights from a medical school in Ogun State, Nigeria

*[1]Akinola-Fowotade, O., [2]Oluwole, T. O., [3]Omobowale, O., and [4]Fowotade, A.

[1]Benjamin S. Carson College of Health and Medical Sciences, Babcock University, Ogun State, Nigeria
[2]Department of Medical Microbiology, Babcock University, Ogun State, Nigeria
[3]Department of Community Medicine, College of Medicine, University of Ibadan, Nigeria
[4]Department of Medical Microbiology and Parasitology, College of Medicine, University of Ibadan, Nigeria
*Correspondence to: onaopemipofowotade@gmail.com; +2348108671326

Abstract:

Background: Human papillomavirus (HPV) is the primary cause of cervical cancer. Despite the availability of highly effective vaccines, awareness and uptake remain inadequate in many low- and- middle-income countries. Medical students, as future healthcare providers, play a crucial role in patient education, health promotion, and vaccine advocacy within their communities. Their knowledge, attitudes, and personal vaccination behaviors directly influence their ability to counsel patients and recommend HPV vaccination effectively. Understanding the knowledge levels, perceptions, and barriers to HPV vaccination among this population is essential for developing targeted educational interventions. The objective of this study is to assess the knowledge, perception, and uptake of HPV vaccination among female medical students in a tertiary institution in Nigeria.
Methodology: A descriptive cross-sectional study was conducted among 100 female medical students at Babcock University, Ilishan-Remo, Ogun State, Nigeria, using a structured, self-administered questionnaire to collect information on socio-demographic characteristics, knowledge, perceptions, uptake, and barriers related to HPV vaccination. Descriptive analysis was performed using frequencies and percentages. Association of HPV knowledge with practice of HPV prevention was determined using Chi square or Fischer's Exact test, and $p<0.05$ was considered statistically significant
Results: Majority of the participants (82.0%) were 18–23 years of age, and mostly (65.0%) of Yoruba tribe. Knowledge of HPV was categorized as adequate in 70.0% of the participants, with 87.0% having knowledge of HPV as being sexually transmitted, and 91.0% had knowledge of the link between HPV and cervical cancer. However, only 35.0% had received HPV vaccine. Knowledge of HPV was significantly associated with vaccine promotion, as 94.2% of participants with adequate knowledge would recommend vaccine to family and friends, compared to 70.0% of those with inadequate knowledge ($p=0.0002$). Knowledge was not significantly associated with HPV prevention practice ($p=0.17$). Major barriers to HPV vaccination highlighted by participants included lack of information, vaccine unavailability, and misconceptions such as infertility and depopulation conspiracies.
Conclusion: Although knowledge of HPV is high among the female medical students, vaccine uptake is low. Addressing misconceptions and barriers to HPV vaccination is vital to improving vaccine uptake.

Keywords: Knowledge; Perception, HPV vaccine; Medical student; Healthcare provider

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Connaissances, perception et adoption du vaccin contre le virus du papillome humain (VPH) par les futurs professionnels de santé: Études menées dans une faculté de médecine de l'État d'Ogun, au Nigéria

*[1]Akinola-Fowotade, O., [2]Oluwole, T. O., [3]Omobowale, O., et [4]Fowotade, A.

[1]Collège Benjamin S. Carson des Sciences de la Santé et de la Médecine, Université Babcock, État d'Ogun, Nigéria
[2]Département de Microbiologie Médicale, Université Babcock, État d'Ogun, Nigéria
[3]Département de Médecine Communautaire, Faculté de Médecine, Université d'Ibadan, Nigéria

[4]Département de Microbiologie Médicale et de Parasitologie, Faculté de Médecine, Université d'Ibadan, Nigéria
*Correspondance à: onaopemipofowotade@gmail.com; +234 810 867 1326

Résumé:

Contexte: Le virus du papillome humain (VPH) est la principale cause de cancer du col de l'utérus. Malgré la disponibilité de vaccins hautement efficaces, la sensibilisation et la couverture vaccinale restent insuffisantes dans de nombreux pays à revenu faible ou intermédiaire. Les étudiants en médecine, futurs professionnels de santé, jouent un rôle crucial dans l'éducation des patients, la promotion de la santé et la promotion de la vaccination au sein de leurs communautés. Leurs connaissances, leurs attitudes et leurs comportements personnels en matière de vaccination influencent directement leur capacité à conseiller les patients et à recommander efficacement la vaccination contre le VPH. Comprendre les niveaux de connaissances, les perceptions et les obstacles à la vaccination contre le VPH au sein de cette population est essentiel pour développer des interventions éducatives ciblées. L'objectif de cette étude est d'évaluer les connaissances, la perception et la couverture vaccinale contre le VPH chez les étudiantes en médecine d'un établissement d'enseignement supérieur au Nigéria.
Méthodologie: Une étude transversale descriptive a été menée auprès de 100 étudiantes en médecine de l'Université Babcock, à Ilishan-Remo, dans l'État d'Ogun, au Nigéria, à l'aide d'un questionnaire structuré et auto-administré pour recueillir des informations sur les caractéristiques sociodémographiques, les connaissances, les perceptions, la couverture vaccinale et les obstacles liés à la vaccination contre le VPH. Une analyse descriptive a été réalisée à l'aide de fréquences et de pourcentages. Français L'association entre la connaissance du VPH et la pratique de la prévention du VPH a été déterminée à l'aide du test du Chi carré ou du test exact de Fischer, et $p<0,05$ a été considéré comme statistiquement significatif.
Résultats: La majorité des participants (82.0%) étaient âgés de 18 à 23 ans et appartenaient majoritairement (65.0%) à la tribu Yoruba. Les connaissances sur le VPH ont été jugées suffisantes par 70.0% des participantes, dont 87.0% savaient que le VPH était sexuellement transmissible et 91.0% connaissaient le lien entre le VPH et le cancer du col de l'utérus. Cependant, seulement 35.0% avaient été vaccinées contre le VPH. La connaissance du VPH était significativement associée à la promotion de la vaccination comme 94,2% des participantes disposant de connaissances suffisantes recommanderaient le vaccin à leur famille et à leurs amis, contre 70.0% de celles dont les connaissances étaient insuffisantes ($p=0,0002$). La connaissance n'était pas significativement associée aux pratiques de prévention du VPH ($p=0,17$). Les principaux obstacles à la vaccination contre le VPH mis en avant par les participantes comprenaient le manque d'information, l'indisponibilité du vaccin et les idées fausses telles que l'infertilité et les théories du complot contre la dépopulation.
Conclusion: Bien que les étudiantes en médecine soient bien informées sur le VPH, la vaccination est faible. Il est essentiel de lutter contre les idées fausses et les obstacles à la vaccination contre le VPH pour améliorer la vaccination.

Mots-clés: Connaissances; Perception; Vaccin contre le VPH; Étudiante en médecine; Professionnelle de santé

Introduction:

Cervical cancer remains a significant public health concern globally, ranking as the fourth most common cancer affecting women, with an estimated 604,000 new cases and 342,000 deaths worldwide in 2020 alone (1). The burden is disproportionately higher in low- and middle-income countries (LMICs), where approximately 90% of cervical cancer deaths occur (2). Nigeria, the most populous country in Africa, contributes substantially to this burden, with thousands of new cases diagnosed annually and limited access to screening and preventive healthcare (3).

Persistent infection with high-risk strains of the human papillomavirus (HPV), particularly types 16 and 18, is recognized as the primary aetiological factor for cervical cancer (4). HPV is the most common sexually transmitted infection globally (4), and while most infections are transient and asymptomatic, persistent infection with oncogenic types can lead to malignant transformation, particularly in the cervix (5).

In response to this, the World Health Organization (WHO) recommends vaccination of adolescent girls, typically aged 9-14 years, before the onset of sexual activity, as a key pillar in its global strategy to eliminate cervical cancer as a public health problem (6). Clinical trials and population-based studies have consistently demonstrated the safety, efficacy, and cost-effectiveness of the HPV vaccine in preventing cervical pre-cancer and cancer (7,8). Despite this, the uptake of HPV vaccination in Nigeria and many other LMICs remains unacceptably low due to a combination of factors, including limited awareness, vaccine hesitancy, misinformation, cost barriers, cultural and religious concerns, and inadequate integration into national immunization programs (9-11).

Healthcare professionals play a crucial role in influencing public attitudes and behaviors regarding vaccination. Medical students, in particular, represent a strategic group as they not only form a reservoir of future health workforce leaders and decision makers who will be involved in policy implementation (12). Their personal beliefs, knowledge base, and vaccination practices can either reinforce or undermine public trust in vaccines.

This study was conducted to assess the awareness, perception, and uptake of HPV vaccination among female medical students in Nigeria. By identifying knowledge gaps, prevailing attitudes, and barriers to vaccine up-

take, the findings aim to inform targeted educational campaigns in this key population.

Materials and method:

Study design and setting:

A descriptive, cross-sectional survey was conducted among 100 female medical students at Babcock University, Ilishan-Remo, Ogun State, Nigeria. Participants included female students in their 4th to 6th year of clinical study, who gave informed consent to participate in the study. The participants were recruited using a non-probability (convenience) sampling technique.

Data collection:

Data collection was done using a semi-structured, self-administered questionnaire designed to collect information on sociodemographic characteristics, knowledge of HPV and cervical cancer, perception of HPV vaccination, HPV vaccine uptake and willingness to be vaccinated, and barriers and myths influencing vaccine uptake. Knowledge and perception of the participants were assessed using single multiple-choice (SMC) method.

The questionnaire was piloted among 10 students from the same institution but outside of the medical school. The internal consistency of the questionnaire was assessed with an overall scale that yielded Cronbach's alpha score of 0.82, indicating good reliability.

Data analysis:

Data were entered, cleaned, and coded using Microsoft Excel before being imported into the Statistical Package for the Social Sciences (SPSS) version 25.0 for analysis. Descriptive statistics were used to summarize the data, including computation of frequencies, percentages, and proportions to describe participants' socio-demographic characteristics (age group, religion, tribe) as well as perceptions, and preventive practices regarding HPV vaccine uptake.

For knowledge assessment, the responses were grouped and analyzed to identify participants' knowledge level. The maximum obtainable score on the knowledge assessment was 21, and the mean score among participants was 19±1.2. Based on the scoring criteria, participants were classified as having adequate knowledge if the score is >19 and inadequate knowledge if the score is <19.

Reasons for not taking HPV vaccine were highlighted using the participants' exact words. Inferential statistical tests were performed using Chi square and Fischer's Exact test to determine association of knowledge with practice of HPV prevention. P value < 0.05 was considered statistically significant.

Results:

Sociodemographic characteristics of study participants:

Most of the participants are females aged 18-23 years (82.0%), with a mean age of 19.2 years. Majority are Christians (93.0%), and 65.0% belonged to the Yoruba tribe (Table 1).

Table 1: Socio-demographic characteristics of the study participants

Characteristics	Frequency	Percentage (%)
Religion		
Christian	93	93.0
Islam	7	7.0
Tribe		
Urhobo	2	2.0
Edo	5	5.0
Etsako	1	1.0
Hausa	1	1.0
Ibibio	1	1.0
Idoma	1	1.0
Igbo	19	19.0
Ika	2	2.0
Ikwere	2	2.0
Yoruba	66	66.0
Year of study		
400-Level	37	37.0
500-Level	42	42.0
600-Level	21	21.0
Age group (years)		
≤ 17 years	16	16.0
18 -23 years	82	82.0
≥ 24 years	2	2.0
Mean age (years)	19.2	
Total	**100**	**100.0**

Knowledge of HPV and cervical cancer among study participants:

Majority (87.0%) of the participants had the knowledge that HPV is primarily transmitted through sexual contact, while 91.0% of participants had the knowledge of the link between HPV and cervical cancer. On HPV vaccine effectiveness in preventing HPV infection, 95.0% had the knowledge that HPV vaccine is effective. Similarly, 96.0% had the knowledge that HPV vaccine is recommended for adolescents and young adults. However, only 21% knew that the appropriate age group of HPV vaccination was 9-14-years as recommended by the WHO (Table 2A).

Table 2B shows the knowledge level of the participants based on the scoring criteria, with 70.0% categorized as having 'adequate knowledge' (score > 19), and 30.0% classified as having 'inadequate knowledge' (score < 19).

Table 2A: Knowledge of HPV and cervical cancer among study participants

Variables	Frequency	Percentage (%)
HPV is primarily transmitted through sexual contact		
Yes	87	87.0
No	10	10.0
Maybe	3	3.0
HPV infection can lead to cervical cancer in women		
Yes	91	91.0
No	0	0.0
Maybe	9	9.0
HPV Vaccine is effective in preventing HPV infection		
Yes	95	95.0
No	5	5.0
Maybe	0	0
HPV vaccine is recommended for adolescents and young adults		
Yes	96	96.0
No	4	4.0
Maybe	0	0
Persistent HPV infections might be associated with cervical cancer		
Yes	76	76.0
No	0	0
Maybe	4	4.0
Recommended age group in Nigeria affected with HPV infections is 9-14 years?		
Yes	21	21.0
No	57	57.0
Maybe	22	22.0
Total	**100**	**100.0**

Table 2B: Knowledge levels of study participants

Knowledge level	Number of participants (%)
Inadequate (score <19)	30 (30.0)
Adequate (score > 19)	70 (70.0)
Total	**100 (100.0)**

Perception of HPV vaccine among study participants:

Ninety-four (94.0%) participants believed HPV is a significant health concern while 80.0% rated HPV vaccine as very important. Few (13.0%) respondents perceived there is high social stigma against receiving the HPV vaccine while 13.0% believed that cultural or religious beliefs could influence their views on HPV vaccination. Although 82.0% believed female students should be required to take the vaccine, fewer respondents (62.0%) supported the same for male students (Table 3).

Practice of HPV prevention:

Only 35.0% of the respondents reported having received HPV vaccine at the time of the survey, indicating low vaccine uptake. However, a large proportion (74.0%) expressed willingness to receive the vaccine in the future, and 83.0% would accept HPV vaccine if offered at no cost. Furthermore, 82.0% of participants are open to discussing HPV vaccination with their healthcare provider. Additionally, 87.0% would recommend HPV vaccination to friends or family members (Table 4).

Table 3: Perception of HPV vaccination among participants

Questions	Frequency	Percentage (%)
Do you believe HPV infection is a significant health concern?		
Yes	94	94.0
No	2	2.0
Maybe	4	4.0
Do you believe the HPV vaccine is safe?		
Yes	79	79.0
No	l5	5.0
Maybe	16	16.0
Do you think HPV vaccination is important?		
Yes	80	5.0
No	15	15.0
Maybe	5	80.0
Do you perceive the social stigma associated with HPV vaccination as high?		
Yes	13	13.0
No	24	24.0
Maybe	63	63.0
Are there any cultural or religious beliefs influencing your views on HPV vaccination?		
Yes	9	9.0
No	84	84.0
Maybe	7	7.0
Do you think female medical students should be required to receive the HPV vaccine?		
Yes	82	82.0
No	8	8.0
Maybe	10	10.0
Do you think male medical students should be required to receive the HPV vaccine?		
Yes	62	62.0
No	16	16.0
Maybe	22	22.0
Total	**100**	**100.0**

Statistical analysis showed significant association between participant's knowledge of HPV vaccine promotion practices with willingness to recommend HPV vaccine to family and friends (p=0.002), but no significant association with HPV vaccine receipt (OR=2.190, 95% CI=0.83-5.79, p=0.17) (Table 5).

Barriers to HPV vaccination updates:

Only 15 (15.0%) participants highlighted their reasons for not taking HPV vaccine as well as perceived misconceptions about the vaccine. These includes fear of infertility, high vaccine costs, lack of information, and access issues. Common misconceptions included 'vaccine causes infertility', 'Africans are used as test subjects', 'Only promiscuous or older women get HPV' and 'vaccine may cause the HPV associated disease' (Box 1).

Table 4: Practice of HPV prevention and vaccine promotion

Questions	Frequency	Percentage (%)
Have you received the HPV vaccine?		
Yes	35	35.0
No	65	65.0
Maybe	0	0
Would you consider getting vaccinated against HPV in the future?		
Yes	74	74.0
No	14	14.0
Maybe	12	12.0
Are you open to discussing HPV vaccination with your healthcare provider?		
Yes	82	82.0
No	3	3.0
Maybe	15	15.0
Would you recommend HPV vaccination to your friends or family members?		
Yes	87	87.0
No	4	4.0
Maybe	9	9.0
Would you consider getting vaccinated against HPV if it were offered to you for free?		
Yes	83	83.0
No	6	6.0
Maybe	11	11.0
Have you received any education on HPV and HPV vaccine prior to this survey?		
Yes	75	75.0
No	21	21.0
Maybe	4	4.0
Total	**100**	**100.0**

Box 1: Specified reasons for not receiving HPV vaccine by 15 respondents

Did not know where to get the vaccine
Fear of needles
I haven't had the opportunity
There isn't enough information on the long-term side effect
I don't see the need yet
I just found out about it
I just haven't found the time
I've never heard of the vaccine
It wasn't available when I wanted to get it
Just knowing about HPV for the first time
Just never had the chance
The cost of the vaccine is too expensive
The vaccine causes infertility
The vaccine has untoward side effects
The vaccine reduces female libido

Discussion:

The results of this study showed a high level of knowledge of HPV and cervical cancer among female medical students of Babcock University, with 70% categorized as having adequate knowledge. Majority of the students understood the sexual transmission route of HPV and its association with cervical cancer. These findings are consistent with previous studies in Nigeria and other sub-Saharan African countries, where medical students' generallly exhibit higher levels of health literacy compared to the general population (7,8). Despite this high level of knowledge of HPV, vaccine uptake remains low, with only 35.0% of the participants haven received the vaccine. This mirrors trends observed in similar studies across Africa, which point to a persistent gap between knowledge and preventive action (9,10).

Socio-cultural myths, limited access, and perceived side effects continue to obstruct the effective implementation of HPV vaccination (9,10), this is similar to findings from other studies on how sociocultural beliefs and myths affected knowledge of health conditions (13,14). The primary reasons for non-uptake among respondents in our survey included limited access, insufficient information about vaccine availability, and lingering safety concerns. Participants cited inaccessibility, unavailability of vaccine and affordability as well as fear of infertility as reason for not getting vaccinated against HPV. These findings highlight the need for intensified educational campaigns to debunk myths and promote scientific understanding.

Encouragingly, majority (83.0%) of the students expressed willingness to receive the vaccine in the future, particularly if it were made available at no cost. This reflects both a strong intent for preventive health behavior and recognition of financial constraints as a key barrier to vaccine access. Offering the vaccine at subsidized or no cost within University health services could therefore significantly improve uptake. Similar observations have been made from other African countries where policy-driven vaccine mandates and free services led to increased coverage (9,10). The acceptance of HPV vaccination can be improved among medical students through organizing interactive workshop and seminars with expert, delivering focused guest lectures, conducting campus-wide awareness campaigns, and encouraging participation in research projected dedicated to HPV prevention (11, 12).

Another notable finding is that 75.0% of the students had received prior education on HPV and vaccine. This level of exposure appears to have had a positive impact on their knowledge and perception, although this has not yet fully translated into behavior change. This points to the need for multi-level strategies that not only increase knowledge but also address access, affordability, and social support for vaccination. Furthermore, the perception of stigma related to HPV vaccination, reported by a majority of the students, indicates the influence of societal attitudes on vaccine acceptance. Addressing such stigma will

Table 5: Association of HPV knowledge and HPV prevention and vaccine promotion practices

Practice of HPV prevention		HPV knowledge		Total	x^2	OR (95% CI)	*p* value
		Adequate (%)	Inadequate (%)				
Would you recommend HPV vaccine to your friends or family members?	Yes	66 (94.2)	21 (70.0)	87 (87.0)	10.986	NA	0.0002*
	No	2 (2.9)	2 (6.7)	4 (4.0)			
	Maybe	2 (2.9)	7 (23.3)	9 (9.0)			
Have you received HPV vaccine?	Yes	28 (40.0)	7 (23.3)	35 (35.0)	1.884	2.190 (0.83-5.79)	0.17
	No	42 (60.0)	23 (76.7)	65 (65.0)			

HPV=Human papillomavirus; x^2-Chisquare; OR=Odd ratio; CI=Confidence interval; NA=Not applicable; *=Statistically significant

require peer-led health promotion, student-centered advocacy. There is the need to further explore the belief of the participants and how it serves a s a barrier to the practice of vaccination despite the high level of knowledge observed. Given that these students are future health professionals, equipping them with both the knowledge and motivation to advocate for HPV vaccination is essential to scaling up national vaccination programs.

This study underscores the complexity of HPV vaccine hesitancy, even among well-informed populations. It emphasizes the urgent need for a holistic approach that combines education, accessibility, cost-reduction, and cultural sensitivity to improve vaccine uptake among young women in Nigeria. However, the generalizability of our findings is limited because participants were selected by a non-probability (convenience) sampling method, with no sample size estimation at the onset of the study. Also, being a self-reported information given by the participants, information bias is a factor that may have significant effect on the quality level of evidence provided in the study.

Conclusion:

Despite high knowledge and positive perceptions among female medical students of Babcock University in Nigeria, actual HPV vaccine uptake is low. Multi-pronged strategies involving improved education, accessibility, cost reduction, and continuous social media-based advocacy are crucial. As future health professionals, the population group holds strategic potential to influence public health behavior, making targeted interventions both timely and impactful.

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Contributions of authors:

OAF designed the study protocol and administered the study questionnaire; TOO and OO contributed to the questionnaire design and also supervised the survey; and AF edited the study protocol. All authors contributed to the preparation, editing and approval of the manuscript submitted for publication.

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Conflict of interest:

Authors declare no conflict of interest

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Original Article **Open Access**

Aetiologic agents of cutaneous mycoses at the parasitology-mycology laboratory of University Center Hospital Joseph Ravoahangy Andrianavalona (CHU JRA), Antananarivo, Madagascar

*[1,2]Zafindraibe, N. J., [2]Andriambao, H. M. R., [2]Rasamindrakotroka, A., and [2]Razanakolona, L. R. S.

[1]Bacteriology, Parasitology-Mycology Laboratory, University Center Hospital Joseph Ravoahangy Andrianavalona (CHU JRA), Antananarivo, Madagascar
[2]Department of Biology, Faculty of Medicine, Antananarivo University, Madagascar
*Correspondence to: juliemail_21@yahoo.fr; njzafindraibe@gmail.com

Abstract:

Background: Superficial and cutaneous fungal infections are among the most common dermatological conditions worldwide, particularly in tropical and subtropical regions. Caused by a variety of fungi (yeasts, dermatophytes, and molds), they can result in significant morbidity. In resource-limited settings such as Madagascar, their true burden remains underestimated due to limited access to diagnostic facilities and frequent empirical treatment. Local epidemiological data are essential for improving diagnosis and management strategies. The objectives of this study are to determine the prevalence, species distribution, affected anatomical sites and demographic characteristics of patients with superficial and cutaneous mycoses diagnosed at the University Center Hospital Joseph Ravoahangy Andrianavalona (CHU JRA) in Antananarivo, Madagascar.
Methodology: This was a descriptive retrospective review of the register of all patients with cutaneous mycoses at the parasitology-mycology laboratory of CHU JRA over a period of 6 years (January 2015 to December 2020). Data on demographic and clinical characteristics of included patients were collected with designed form and these included age, sex, patient location/department (outpatient or inpatient), year of request for mycological examination, macroscopic examination results according to lesion topography, type of samples (pus, nails, scalp, scales), class of fungi (yeasts, dermatophytes, moulds) and results of mycological culture. Data were analysed on Microsoft Excel and EPI INFO. Chi-squared and Fisher's Exact tests were used to determine the relationship between categorical variables, and $p \leq 0.05$ was considered significant.
Results: Of the samples of 914 patients examined, 287 (31.4%) tested positive for cutaneous fungal infection, giving a prevalence of 31.4%. Yeasts accounted for 57.7% of isolates, dermatophytes for 25.8%, molds for 12.1% and others 4.4%. The most common fungi species involved in cutaneous infections were *Candida* spp (36.9%, n=106), *Trichophyton* spp (21.6%, n=62), and *Aspergillus* spp (5.9%, n=16). The most commonly affected sites were glabrous skin and nails, with *Trichophyton* spp and non-*albicans Candida* species being predominant, respectively. Clinically, the most frequent cutaneous findings were scaly lesions (skin), onycholysis (nails), and tinea capitis (scalp).
Conclusion: Superficial fungal infections are a major health concern in Madagascar. Improved clinical awareness and systematic mycological testing are critical for accurate diagnosis and management.

Keywords: Cutaneous mycoses; *Candida* spp; *Trichophyton* spp; Prevalence; Madagascar

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Agents étiologiques des mycoses cutanées au laboratoire de parasitologie-mycologie du Centre Hospitalier Universitaire Joseph Ravoahangy Andrianavalona (CHU JRA), Antananarivo, Madagascar

*[1,2]Zafindraibe, N. J., [2]Andriambao, H. M. R., [2]Rasamindrakotroka, A., et [2]Razanakolona, L. R. S.

[1]Laboratoire de Bactériologie, Parasitologie -Mycologie, Hôpital Universitaire Joseph Ravoahangy Andrianavalona (CHU-JRA), Antananarivo, Madagascar
[2]Département de Biologie, Faculté de Médecine, Université d'Antananarivo, Madagascar
*Correspondence to: juliemail_21@yahoo.fr; njzafindraibe@gmail.com

Résumé:

Contexte: Les infections fongiques superficielles et cutanées comptent parmi les affections dermatologiques les plus courantes dans le monde, en particulier dans les régions tropicales et subtropicales. Causées par une variété de champignons (levures, dermatophytes et moisissures), elles peuvent entraîner une morbidité importante. Dans les pays à ressources limitées comme Madagascar, leur véritable fardeau reste sous-estimé en raison de l'accès limité aux installations de diagnostic et des traitements empiriques fréquents. Les données épidémiologiques locales sont essentielles pour améliorer le diagnostic et les stratégies de prise en charge. Les objectifs de cette étude sont de déterminer la prévalence, la distribution des espèces, les sites anatomiques affectés et les caractéristiques démographiques des patients atteints de mycoses superficielles et cutanées diagnostiquées au Centre Hospitalier Universitaire Joseph Ravoahangy Andrianavalona (CHU JRA) à Antananarivo, Madagascar.
Méthodologie: Il s'agissait d'une revue rétrospective descriptive du registre de tous les patients atteints de mycoses cutanées au laboratoire de parasitologie-mycologie du CHU JRA sur une période de 6 ans (janvier 2015 à décembre 2020). Les données sur les caractéristiques démographiques et cliniques des patients inclus ont été recueillies à l'aide d'un formulaire conçu et celles-ci comprenaient l'âge, le sexe, la localisation/les services du patient (ambulatoire ou hospitalisé), l'année de la demande d'examen mycologique, les résultats de l'examen macroscopique selon la topographie des lésions, le type d'échantillons (pus, ongles, cuir chevelu, squames), la classe de champignons (levures, dermatophytes, moisissures) et les résultats de la culture mycologique. Les données ont été analysées sur Microsoft Excel et EPI INFO. Les tests du Chi carré et exact de Fisher ont été utilisés pour déterminer la relation entre les variables catégorielles, et $p \leq 0,05$ a été considéré comme significatif.
Résultats: Sur les échantillons de 914 patients examinés, 287 (31,4%) ont été testés positifs pour une infection fongique cutanée, ce qui donne une prévalence de 31,4%. Les levures représentaient 57,7% des isolats, les dermatophytes 25,8%, les moisissures 12,1% et les autres 4,4%. Les espèces fongiques les plus fréquemment impliquées dans les infections cutanées étaient *Candida* spp (36,9%, n=106), *Trichophyton* spp (21,6%, n=62) et *Aspergillus* spp (5,9%, n=16). Les sites les plus fréquemment touchés étaient la peau glabre et les ongles, les espèces *Trichophyton* spp et *Candida* non *albicans* étant respectivement prédominantes. Sur le plan clinique, les signes cutanés les plus fréquents étaient des lésions squameuses (peau), une onycholyse (ongles) et une teigne (cuir chevelu).
Conclusion: Les infections fongiques superficielles constituent un problème de santé majeur à Madagascar. Une meilleure connaissance clinique et des tests mycologiques systématiques sont essentiels pour un diagnostic et une prise en charge précis.

Mots-clés: Mycoses cutanées; *Candida* spp; *Trichophyton* spp; Prévalence; Madagascar

Introduction:

Fungal infections are widespread globally, causing a diverse range of diseases, including opportunistic and chronic infections, particularly in immunocompromised individuals (1). In recent years, their rising incidence, driven by the indiscriminate use of antibiotics, anticancer therapies, and immunodeficiency conditions such as acquired immune deficiency syndrome (AIDS), has garnered increased attention from clinicians and microbiologists (2).

Superficial mycoses, affecting the skin and its appendages, are caused by keratinophilic fungi and typically elicit minimal inflammatory response (3). Among the most common infections worldwide, their prevalence is estimated at 20–25%, with an increasing trend (4,5). They are particularly prevalent in tropical regions and represent a major cause of dermatological consultations in many African countries (6).

Over the past decade, environmental changes, lifestyle shifts, pet ownership, aging population, and increased use of immunosuppressive therapies have altered the epidemiology and clinical presentation of superficial mycoses (7). Although often benign, these infections cause significant morbidity, recurrence, and cosmetic disfigurement (2,8).

Accurate mycological identification of causative agents is critical to guide appropriate antifungal treatment (9,10). Given their growing clinical importance, potential complications, especially in immunocompromised patients, their recurrent and treatment-resistant nature, and the scarcity of recent epidemiological data, we conducted a retrospective study of superficial cutaneous mycoses at the Parasitology-Mycology Laboratory of Joseph Ravoahangy Andrianavalona University Hospital Centre from 2015 to 2020. This study aimed to determine the prevalence of superficial mycoses among patients referred for mycological examination and to characterize their clinical and biological profiles.

Materials and method:

Study design and setting:

This was a retrospective descriptive analysis of laboratory records of patients at the Parasitology-Mycology Laboratory within

the Paraclinic Unit of Research and Training (UPFR) of the Joseph Ravoahangy Andrianavalona University Hospital Centre (CHU-JRA), 4th Arrondissement of Antananarivo Renivohitra, Analamanga region, Madagascar .

The laboratory, part of the hospital's microbiology department, processes parasitological and mycological samples from medical and surgical departments of Antananarivo's university hospitals, as well as from private clinics and medical practices. The study spanned six years (January 2015 to December 2020)

Ethical consideration:

Consent of the head of the Parasitology-Mycology Laboratory at CHU-JRA and the director of establishment of the University Center Hospital were obtained prior to commencement of the study. The confidentiality of the contents and information obtained were respected and all information received was treated in strict confidentiality. As the study involved retrospective analysis of records, ethical review and approval was not required.

Study population and participants:

The study population included all patients who had mycological examinations and were registered in the results register of the Parasitology–Mycology laboratory. The study participants included all patients with superficial mycoses who were registered in the mycology results register between 2015 and 2020. All patients with negative mycological examination results were excluded.

A mycological examination of patients was considered positive if on direct examination, there is presence of mycelial filaments or spores or yeasts, and on culture, there is presence of various pathogenic fungal colonies on the plate (dermatophytes*, Candida* spp, *Malassezia* spp. etc*...*).

Data collection:

The study variables collected from the laboratory register included patient age, sex, year of request for mycological examination, clinical information according to lesion topography, macroscopic examination results according to lesion topography, prescribing departments (outpatient or inpatient), type of samples (pus, nails, scalp, scales) and their topography, class of responsible species (yeasts, dermatophytes, moulds and others) and mycological culture results.

Sample collection:

Between January 2015 and December 2020, samples from patients with suspected superficial fungal infections (SFIs) were collected at the CHU-JRA Parasitology-Mycology Laboratory. For each patient, demographic data (age, sex), infection site, underlying conditions, and prior medication history were recorded. Infected areas were cleaned before sampling. Scales, hair, and nail samples were collected using sterile scalpels, hair pluggers, or nail clippers and stored in sterile Petri dishes. For suspected pityriasis versicolor, adhesive tape was used to collect samples from affected areas. Samples were divided for direct examination and culture.

Direct microscopic examination of samples:

Samples were processed based on lesion type. Scales, hair, and nail specimens were placed on sterile slides and examined using 10–20% potassium hydroxide (KOH) or lactophenol (for hair) with a compound light microscope. Observations included hyphae, branching hyphae, spores, budding cells, or pseudohyphae. For pityriasis versicolor, adhesive tape samples were examined for short hyphae and round yeasts in grape-like clusters.

Culture of sample:

Samples were inoculated onto two media: Sabouraud's dextrose agar with chloramphenicol (50 mg/L) and cycloheximide (500 mg/L) (SCC), and Sabouraud's glucose agar without cycloheximide (SC). Cultures were incubated at 25–35°C for up to four weeks and monitored daily for colony growth. Mucoid colonies indicated yeast fungi, while filamentous growth suggested moulds.

Fungi identification:

Fungal genera were identified based on macroscopic and microscopic characteristics. Macroscopic features included colony texture, topography, margins, pigmentation (obverse and reverse), and growth rate. Microscopic identification involved tease mounts and slide cultures observed under light microscopy (40×). Microconidia, macroconidia, and accessory structures were assessed. Yeast infections were identified by the presence of budding cells, pseudohyphae, or yeasts, while mold infections were characterized by septate or non-septate, hyaline or dematiaceous hyphae, or arthroconidia.

Statistical analysis:

Demographic and clinical data were entered into Microsoft Excel and analyzed using EPI INFO version 7.2.6. Descriptive statistics were used to summarize patient characteristics and fungal profiles. Associations between fungal genera and demographic variables were assessed using the Chi-square test or Fisher's exact test, with a p-value ≤ 0.05 considered statistically significant.

Results:

Prevalence of superficial mycosis:

From January 1, 2015 to December 31, 2020, 914 patients had mycological examinations at the Parasitology-Mycology Laboratory of the Joseph Ravoahangy Andrianavalona University Hospital Centre (CHU-JRA). Of these, 287 (31.4%) had confirmed superficial mycoses, defined by positive direct examination and/or culture results. Direct examination was positive in 97.2% (n=279/287) of cases, while culture was positive in 80.1% (n=230/287), with 77.4% (222/287) of cases positive on both direct examination and cultures, and 2.8% (n=8/230) of culture-positive cases negative on direct examination (Table 1).

A total of 272 fungal species were isolated from 230 culture positive samples with multiple species isolated from some samples. The prevalence of superficial mycoses peaked in 2016, accounting for 30.0% (n=86/287) of cases, with another peak in 2018 (Fig 1).

Table 1: Correlation between direct examination and culture results in mycological examination of superficial mycoses

Direct Microscopic Examination (DME)	**Culture**		**Total (%)**
	Number positive (%)	Number negative (%)	
Number positive (%)	222 (77.4)	57 (19.9)	279 (97.2)
Number negative (%)	8 (2.8)	0	8 (2.8)
Total (%)	**230 (80.1)**	**57 (19.9)**	**287 (100.0)**

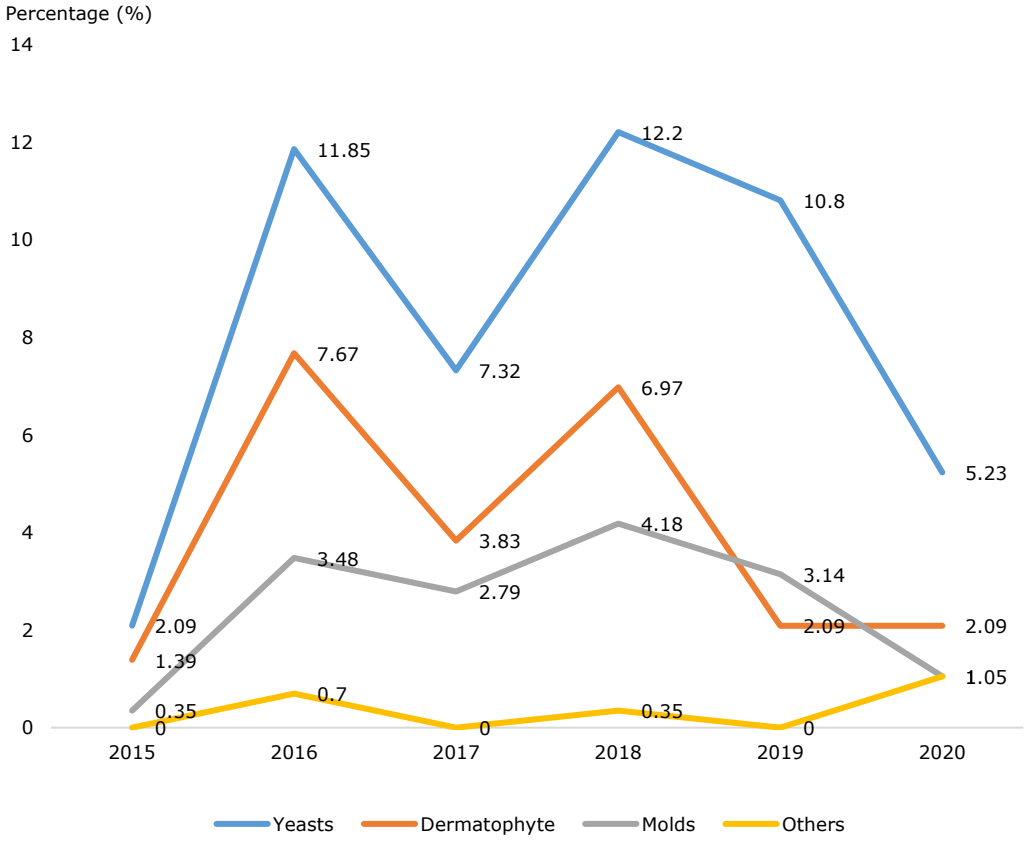


Fig 1: Temporal distribution of superficial mycoses by fungal agent classes over the study period

Socio-demographic characteristics of patients with superficial mycosis:

The age of patients with superficial mycosis ranged from 1 to 78 years (mean age: 31 years). Females predominated (58.9%, n=169/287) compared to males (40.4%, n= 116/287), with a male:female ratio of 2:3. The highest frequency was observed in women aged 31–60 years (24.7%) and men in the same age group (15.0%). Gender data were missing for 0.7% (n=2) of cases (Fig 2).

Mycological examination results:

The most frequently affected site was hairless skin (glabrous, large and small skin folds) (52.4%, n=154/287), followed by nails (29.9%, n=86/287), scalp (12.2%, n=35/ 287), and undetermined (6.4%, n=12/287) (Fig 3), with multiple-site involvement common in the upper limbs, lower limbs, and thorax (Table 2).

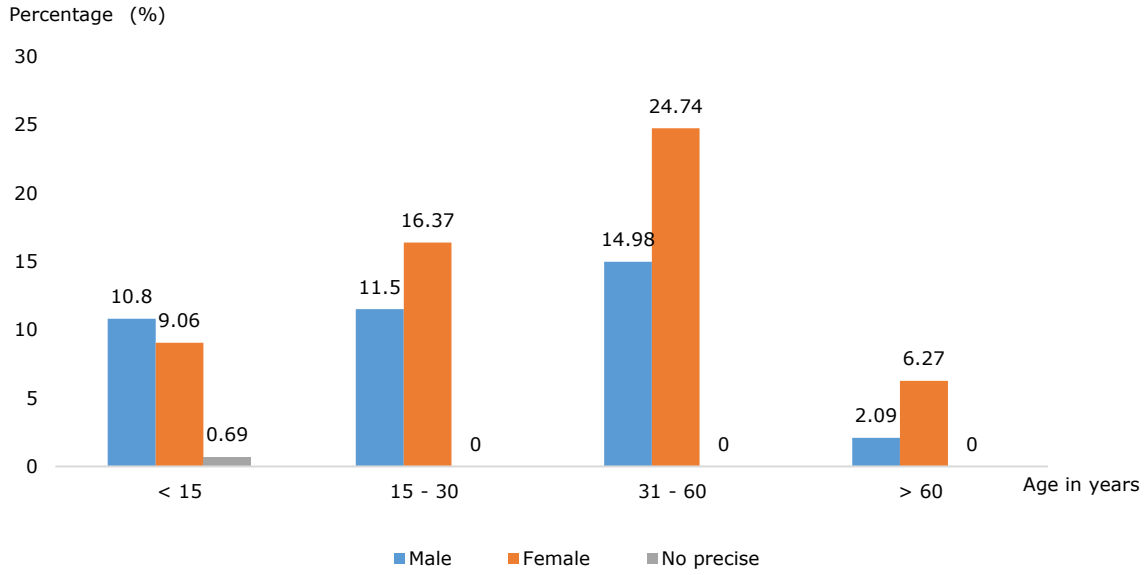
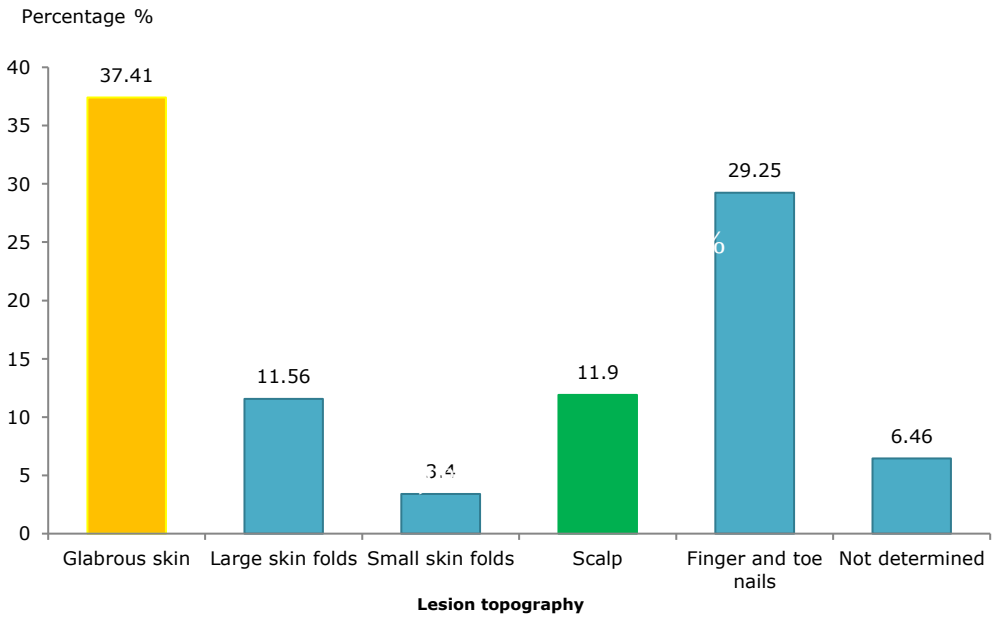


Fig 2: Distribution of superficial and cutaneous fungal infections according to age and gender



Large skin folds (axillary, inguino crural, mammary, intergluteal); Small skin folds (between the fingers, between the toes)

Fig 3: Frequency distribution of superficial mycoses by different body sites of patients

Table 2: Distribution of superficial mycoses based on topography and involvement of multiple body sites

Sampling sites	Frequency (%)	Frequency of involvement of multiple sites (%)
Face	10 (3.4)	7 (7.2)
External auditory canal	20 (6.8)	0
Thorax	16 (5.4)	13 (13.4)
Upper limbs	27 (9.2)	16 (16.5)
Lower limbs	26 (8.8)	13 (13.4)
Axilla	7 (2.4)	4 (4.1)
Inguinal	17 (5.8)	12 (12.4)
Mammary	4 (1.4)	1 (1.0)
Elbows	1 (0.3)	0
Scalp	35 (11.9)	3 (3.1)
Not precise	19 (6.5)	2 (2.1)
Abdomen	9 (3.0)	6 (6.2)
Neck	2 (0.7)	2 (2.1)
Inter gluteal fold	5 (1.7)	1 (1.0)
Finger nail	50 (17.0)	8 (8.2)
Toe nail	36 (12.2)	8 (8.2)
Intertrigo of the small folds	10 (3.4)	1 (1.0)
Total	**294 (100.0)**	**97 (100.0)**

Yeasts were the most commonly isolated fungi pathogens (57.7%; n=157), followed by dermatophytes (25.8%; n=70), moulds (12.1%; n=33), and other fungi (4.4%; n=12) (Fig 4). Among the yeasts, *Candida* spp were predominant (36.9%, n=106/287), with *Candida albicans* and non-*albicans Candida* species contributing significantly, followed by *Rhodotorula* spp (12.5%, n=36/287), *Trichosporon* spp (3.1 %, n=9/287), and *Malassezia* spp (2.1 %, n=6/287).

Among the dermatophytes, *Trichophyton* spp accounted for 21.6% (n=62 /287) of infections, followed by *Microsporum* spp (2.1%, n=6/287). Moulds included *Aspergillus* spp (5.9%, n=17/287), black moulds (3.1%, n=9/287), *Penicillium* spp (1.3%, n=4/287), *Scytalidium* spp (0.7%, n=2/287), and *Cladosporium* spp (0.7%, n=2/287) (Table 3).

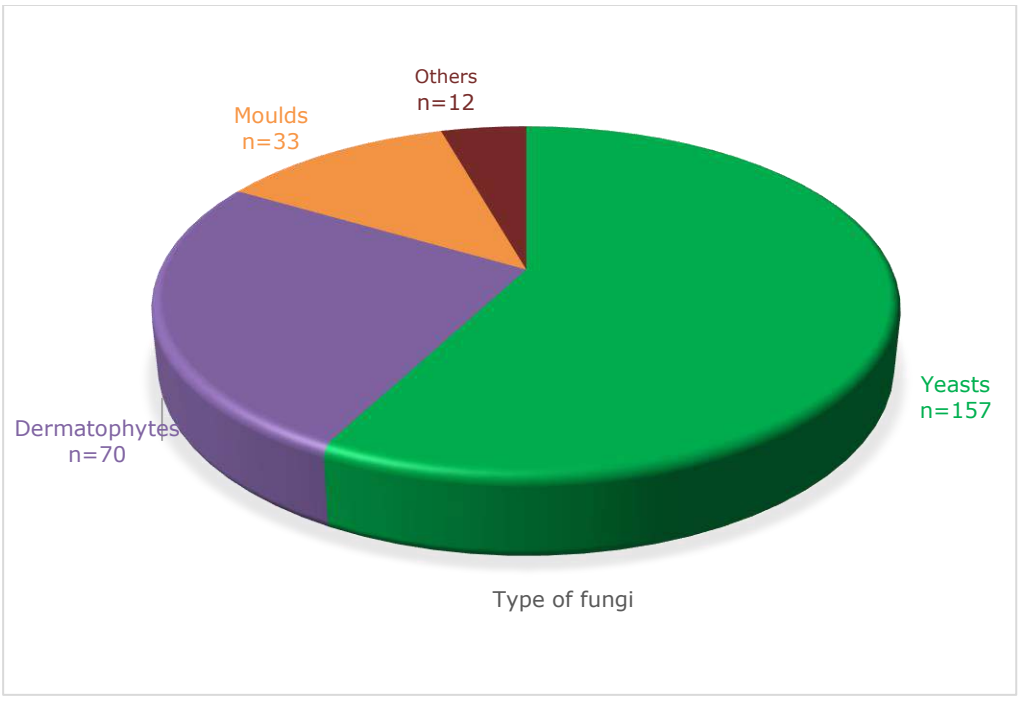


Fig 4: Frequency of fungi class isolated from patients with superficial mycoses

Table 3: Distribution of fungal species identified by mycological cultures according to body sites affected by superficial mycoses in CHUJRA, Antananarivo, Madagascar

Class of fungi	**Fungi species**	**Number of fungi isolated from affected superficial sites (%)**						**Total (%)**
		Glabrous skin	**Scalp**	**Nails**	**Large Folds**	**Small Folds**	**External canal auditory**	
Yeast	*Malassezia* spp	1 (1.3)	1 (4.6)	-	-	-	-	2
	Malassezia furfur	4 (5.3)	-	-	-	-	-	4
	Candida spp	1 (1.3)	-	8 (5.9)	-	-	-	9
	Non albicans Candida	12 (15.8)	6 (27.2)	34 (25.0)	2 (11.2)	1 (14.3)	2 (14.3)	57
	Candida albicans	9 (11.8)	1 (4.6)	29 (21.3)	1 (5.9)	-	-	40
	Rhodotorula spp	14 (18.4)	4 (18.2)	10 (7.6)	5 (29.4)	1 (14.3)	2 (14.3)	36
	Trichosporon spp	4 (5.3)		4 (2.9)	-	1 (14.3)	-	9
	Subtotal	**45 (59.2)**	**12 (54.5)**	**85 (62.5)**	**8 (47.1)**	**3 (42.9)**	**4 (28.6)**	**157 (57.7)**
Dermatophytes	*Microsporum* spp	2 (2.6)	-	1 (0.7)	-	-	-	3
	Trichophyton rubrum	1 (1.3)	-	1 (0.7)	1 (5.9)	-	1 (7.1)	4
	Trichophyton mentagrophytes	3 (4.0)	-	1 (0.7)	1 (5.9)	2 (28.6)	-	7
	Trichophyton tonsurans	1 (1.3)	2 (9.1)	-	1 (5.9)	-	-	4
	Trichophyton violaceum	-	1 (4.6)	2 (1.5)	-	-	-	3
	Trichophyton verrucosum	-	-	-	1 (5.9)	-	-	1
	Trichophyton spp	15 (19.7)	2 (9.1)	20 (14.7)	3 (17.7)	2 (28.6)	1 (7.1)	43
	Epidermophyton floccosum	-	-	-	1 (5.9)	-	-	1
	Microsporum persicolor	-	-	-	1 (5.9)	-	-	1
	Microsporum langeronii	-	3 (13.6)	-	-			3
	Subtotal	**22 (28.9)**	**8 (36.4)**	**25 (18.4)**	**9 (52.9)**	**4 (57.1)**	**2 (14.3)**	**70 (25.8)**
Molds	*Aspergillus* spp	-	-	7 (5,2)	-	-	3 (21,4)	10
	Aspergillus fumigatus	-	-	2 (1,5)	-	-	3 (21,4)	5
	Aspergillus flavus	-	-	-	-	-	-	-
	Aspergillus niger	-	-	-	-	-	1 (7,1)	1
	Black molds	2 (2.6)	-	6 (4,4)	-	-	1 (7,1)	9
	Scytalidium dimidiatum	1 (1.3)	-	1 (0,7)	-	-	-	2
	Penicillium spp	-	-	4 (2,9)	-	-	-	4
	Cladosporium spp	1 (1.3)	-	1 (0,7)	-	-	-	2
	Subtotal	**4 (5.3)**	**0**	**21 (15.4)**	**0**	**0**	**8 (57.1)**	**33 (12.1)**
Others	Not determined	4 (5.3)	2 (9.1)	3 (2.2)	-	-	-	9
	Prototheca zopfii	-	-	1 (0.7)	-	-	-	1
	Prototheca spp	1 (1.3)	-	1(0.7)	-	-	-	1
	Subtotal	**5 (6.6)**	**2 (9.1)**	**5 (3.7)**	**0**	**0**	**0**	**12 (4.4)**
Total (%)		**76 (27.9)**	**22 (8.1)**	**136 (50.0)**	**17 (6.3)**	**7 (2.6)**	**14 (5.2)**	**272 (100.0)**

Frequency distribution of isolated fungi species by anatomical sites:

The distribution of the identified fungal species is detailed in Table 3. On the hairless skin, *Trichophyton* spp (19.7%), *Rhodotorula* spp (18.4%), non-*albicans Candida* (15.8%), and *Candida albicans* (11.8%) were the most frequent isolates. On the scalp, non-*albicans Candida* (27.3%), *Rhodotorula* spp (18.2%), and *Microsporum langeronii* (13.6%) predominated. On the nails (onychomycosis), non-*albicans Candida* (25.0%), *Candida albicans* (21.3%), *Trichophyton* spp (14.7%), and *Rhodotorula* spp (7.4%) were most common.

On large folds (axillary, inguinal, mammary, elbow, intergluteal), *Rhodotorula* spp (n=5), *Trichophyton* spp. (n=3), and non-*albicans Candida* (n=2) were frequently isolated. On the interdigital and intertriginous spaces, *Trichophyton* spp (n=2) and *Trichophyton mentagrophytes* (n=2) were the primary isolates. On the external auditory canal, *Aspergillus fumigatus* (n=3) and *Aspergillus* spp (n=3) were the most common (Table 3).

Discussion:

Superficial mycoses are a significant yet underrecognized public health issue in Madagascar, particularly in tropical climates conducive to fungal proliferation (10,11). In this study, 31.4% (n=287/914) of patients undergoing mycological examination at the CHU-JRA Parasitology-Mycology Laboratory had confirmed superficial mycoses, consistent with global prevalence estimates of 20–25% (5). However, prevalence varies widely across studies (14.3–52.2%), influenced by sample size, diagnostic techniques, and regional factors (1,7). Madagascar's tropical environment, coupled with socioeconomic challenges such as poor hygiene and limited access to healthcare, likely exacerbates the burden of these infections (11,13).

In our study, direct examination was more sensitive (97.2%, n=279/287) than culture (80.1%, n=230/287) in detecting the causative fungal agents in patients with superficial mycosis, with 77.6% (n=222/287) concordance between the two methods. Notably, 19.9% of samples were positive by direct microscopy but culture-negative, possibly due to low fungal load or prior antifungal treatment (14,15). Similar findings were reported in Turkey with 97% positivity rate with combined microscopy and culture (14) and in France (41.6% vs. 36.3% for microscopy vs. culture) (15). Direct examination detects non-viable fungal structures, complementing culture results, but its accuracy depends on proper sample collection and meticulous analysis (16). The use of conventional phenotypic identification in this study limited species-level differentiation, highlighting the need for advanced diagnostic tools.

Among the patients with superficial mycoses, females (58.9%, n=169/287) were more frequently affected than males (40.4%, n=116/287), particularly in the 31–60-year age group (39.8%: females 24.7% vs males 15.0%), and the 15–30-year group (27.9%: females 16.4% vs males 11.5%). This aligns with studies in Senegal (29.5% prevalence in women aged 31–60 years) (12), China (17), Saudi Arabia (18), Brazil (19), India (20), and Nigeria (21). Female predominance may be attributed to household activities involving prolonged water exposure, increasing maceration risk, and greater dermatological consultation rates due to aesthetic concerns (12,21). The high prevalence in active age groups (15–60 years) likely reflects increased environmental exposure, hormonal influences, and hygiene practices (21–23).

Hairless skin (37.4%) and nails (29.3%) were the most affected sites, with multiple-site involvement common in the upper limbs, lower limbs, and thorax. Similar patterns were observed in Nigeria (24), India (2,25), Nepal (26), and Brazil (16). Exposed body parts are prone to environmental contamination, trauma, and chemical exposure, while poor hygiene and socioeconomic conditions in Madagascar amplify these risks (13, 16). Yeasts dominated (57.7%), followed by dermatophytes (25.8%) and moulds (12.1%). This contrasts with global trends where dermatophytes are typically predominant (12, 24,29), but aligns with studies in Brazil (59.9–82.9% yeasts) (23,27) and Egypt (46.0%) (28). Among the yeasts, *Candida* spp (36.9%) were most common, followed by *Rhodotorula* spp (11.2%). *Trichophyton* spp (21.6%) led among dermatophytes, consistent with their anthropophilic nature (29–31), while *Aspergillus* spp (5.9%) were the primary moulds (28). The high yeast prevalence may reflect Madagascar's humid climate and frequent water exposure, favoring opportunistic *Candida* spp (32).

Fungal species varied by site. Hairless skin infections were primarily caused by *Trichophyton* spp (14.6%), *Rhodotorula* spp (12.7%), and *C. albicans* (8.2%), while scalp infections were caused by non-*albicans Candida* (17.1%), *Rhodotorula* spp (11.4%), and *Microsporum langeronii* (8.6%). Onychomycosis was dominated by non-*albicans Candida* (27.0%), *Candida albicans* (20.0%), and *Trichophyton* spp (15.9%), consistent with studies in Côte d'Ivoire (32,33,34), Serbia (35), and Lithuania (36). Otomycoses were primarily caused by *Aspergillus fumigatus* and other *Aspergillus* species. The site-specific distribution reflects environmental, occupational, and hygiene-related risk factors, with *Candida* spp thriving in moist conditions and *Trichophyton* spp spreading through direct contact (4,25).

Our study provides critical epidemiological data on superficial mycoses in Madagascar, highlighting a high prevalence (31.4%) and a unique predominance of yeasts, particularly *Candida* spp compared to global dermatophyte-driven trends. These findings underscore the need for enhanced diagnostic capacity, including molecular techniques, to improve species identification and guide targeted antifungal therapy. The high burden among females and active age groups suggests that public health interventions should focus on improving hygiene education, particularly for women engaged in water-intensive household tasks. Strengthening healthcare access and training clinicians in mycological diagnostics could reduce recurrence and morbidity. Additionally, this study contributes to the limited literature on fungal infections in Madagascar, providing a baseline for future research and regional comparisons in Africa (10,12,13).

There are some limitations to our study. Selection bias may arise as the sample was drawn from the laboratory register, which may not fully represent the Malagasy population. Information bias due to missing or incomplete data in the register may have influenced the results.

Conclusion:

Superficial mycoses are prevalent in Madagascar, with a crude prevalence of 31.4% in this study, driven by yeasts (57.6%), dermatophytes (25.5%), and moulds (15.9%). The high burden, particularly in females and active age groups, underscores the influence of tropical climate, socioeconomic challenges, and limited healthcare access.

To address this public health issue, we recommend increasing public awareness of dermatological consultations, enhancing physician training on mycological diagnostics, and improving access to rapid, effective care. Future studies should incorporate molecular diagnostics to improve species identification and guide targeted antifungal therapy.

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Contributions of authors:

All authors contributed to the study conception, design, and manuscript preparation. NJZ and RHA performed data collection and laboratory analyses. RHA performed statistical analysis. All authors interpreted the data, drafted the manuscript, and approved the final version for submission.

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Conflicts of interest:

Authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Original Article **Open Access**

Antifungal resistance pattern of *Candida* species isolated from clinical specimens of patients in selected healthcare facilities in Yenagoa, Nigeria

[1]Baraikio, D., [2]Daokoru-Olukole, C., and [3]Abdu, A. B.

[1]Department of Pharmaceutical Microbiology and Biotechnology, Faculty of Pharmacy, Niger Delta University, Wilberforce-Island, Bayelsa State, Nigeria
[2]Department of Microbiology, Faculty of Science, Niger Delta University, Wilberforce-Island, Bayelsa State, Nigeria
[3]Department of Medical Microbiology and Parasitology, Faculty of Basic Clinical Sciences, Niger Delta University, Wilberforce-Island, Bayelsa State, Nigeria
*Correspondence to: simply4didi@yahoo.com; +2348035391089

Abstract:

Background: *Candida* species are normal flora of humans that can be pathogenic and cause candidiasis. Candidiasis is a public health problem due to its high prevalence, mortality, and increased costs of care associated with serious systemic *Candida* infections. This study was designed to identify the antifungal resistance pattern of *Candida* species isolated from different clinical specimens in Yenagoa, Bayelsa State, Nigeria, and to detect resistant genes associated with the *Candida* species.
Methodology: A total of 500 patients from 5 different health facilities in Yenagoa, Bayelsa State, Nigeria, with clinical features of urinary tract infection (candiduria), cutaneous candidiasis, oral thrush, lower respiratory tract infection and HIV/TB were selected in the study. Urine, skin, sputum and mouth samples were obtained from the patients and cultured on Sabouraud Dextrose agar and Brilliance *Candida* agar. The isolates were phenotypically identified using standard mycological methods and genotypically confirmed by polymerase chain reaction (PCR) amplification of the internal transcribed spacer (ITS) region of the rRNA genes and sequencing. The semi-solid agar antifungal susceptibility testing method was performed on the isolates against commonly used antimycotic agents. The known antifungal resistance gene *Erg11* gene, was amplified by PCR.
Results: From clinical samples obtained from a total of 500 patients, 170 clinical isolates of *Candida* species were identified, giving the prevalence of *Candida* isolation of 34.0% in the study. *Candida albicans* was the predominant species (64.70%, n=110), followed by *Pichia kudriavzevii* (16.47%, n=28), *Nakaseomyces glabratus* (12.35%, n=21), *Candida akabanensis* (5.29%, n=9) and *Candida tropicalis* (1.17%, n=2). Female patients were more prone to *Candida* infection (36.6%, n=102/279) and age group 28-37 years (40.2%, n=41/102) had the highest prevalence of *Candida* species, but no statistically significant difference in *Candida* prevalence with respect to sex (p=0.2069) and age (p=0.1743). The three antimycotic agents to which *Candida* isolates exhibited the highest rates of resistance were griseofulvin (61.8%, n=105), ketoconazole (52.9%, n=90), and itraconazole (51.8%, n=88). Less than half of the isolates were resistant to fluconazole (n=70, 41.2%) and terbinafine (n=62, 36.5%). Nystatin had the lowest rates of resistance (19.4%, n=33). *Erg II* gene was detected in *P. kudriavzevii*, *C. albicans*, and *C. tropicalis* isolates.
Conclusion: Increased antifungal resistance by *Candida* species is a public health problem that can lead to failure in treatment, spread of infection and possibly death especially, in immune compromised individuals**.** *Erg* 11 genes contribute to *Candida* species resistance to antifungal drugs

Keywords: *Candida;* Normal flora; Antifungal agents; Anti-fungal resistance gene; PCR

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Profil de résistance aux antifongiques des espèces de *Candida* isolées à partir d'échantillons cliniques de patients dans certains établissements de santé à Yenagoa, au Nigéria

[1]Baraikio, D., [2]Daokoru-Olukole, C., et [3]Abdu, A. B.

[1]Département de Microbiologie Pharmaceutique et de Biotechnologie, Faculté de Pharmacie, Université du Delta du Niger, Wilberforce-Island, État de Bayelsa, Nigéria
[2]Département de Microbiologie, Faculté des Sciences, Université du Delta du Niger, Wilberforce-Island, État de Bayelsa, Nigéria

[3]Département de Microbiologie Médicale et de Parasitologie, Faculté des Sciences Cliniques Fondamentales, Université du delta du Niger, Wilberforce-Island, État de Bayelsa, Nigéria
*Correspondance à: simply4didi@yahoo.com; +2348035391089

Résumé:

Contexte: Les espèces de *Candida* constituent la flore normale de l'homme et peuvent être pathogènes et provoquer des candidoses. La candidose est un problème de santé publique en raison de sa prévalence élevée, de sa mortalité et des coûts accrus des soins associés aux infections systémiques graves à *Candida*. Cette étude a été conçue pour identifier le profil de résistance antifongique des espèces de Candida isolées à partir de différents échantillons cliniques à Yenagoa, dans l'État de Bayelsa, au Nigéria, et pour détecter les gènes de résistance associés aux espèces de *Candida*.
Méthodologie: Un total de 500 patients provenant de 5 établissements de santé différents à Yenagoa, dans l'État de Bayelsa, au Nigéria, présentant des signes cliniques d'infection des voies urinaires (candidurie), de candidose cutanée, de muguet buccal, d'infection des voies respiratoires inférieures et de VIH/TB ont été sélectionnés dans l'étude. Des échantillons d'urine, de peau, d'expectorations et de bouche ont été prélevés sur les patients et cultivés sur gélose Sabouraud Dextrose et sur gélose Brilliance *Candida*. Français Les isolats ont été identifiés phénotypiquement à l'aide de méthodes mycologiques standard et confirmés génotypiquement par amplification par réaction en chaîne par polymérase (PCR) de la région de l'espaceur interne transcrit (ITS) des gènes d'ARNr et séquençage. La méthode de test de sensibilité antifongique en gélose semi-solide a été réalisée sur les isolats contre des agents antimycosiques couramment utilisés. Le gène de résistance antifongique connu Erg11 a été amplifié par PCR.
Résultats: À partir d'échantillons cliniques obtenus auprès d'un total de 500 patients, 170 isolats cliniques d'espèces de *Candida* ont été identifiés, ce qui donne une prévalence d'isolement de *Candida* de 34,0 % dans l'étude. Français *Candida albicans* était l'espèce prédominante (64,7%, n=110), suivie de *Pichia kudriavzevii* (16,5%, n=28), *Nakaseomyces glabratus* (12,4%, n=21), *Candida akabanensis* (5,3%, n=9) et *Candida tropicalis* (1,2%, n=2). Les patientes étaient plus sujettes à l'infection à *Candida* (36,6%, n=102/279) et la tranche d'âge 28-37 ans (40,2%, n=41/102) présentait la prévalence la plus élevée d'espèces de *Candida*, mais aucune différence statistiquement significative dans la prévalence de *Candida* en fonction du sexe (p=0,2069) et de l'âge (p=0,1743). Les trois agents antimycosiques auxquels les isolats de *Candida* présentaient les taux de résistance les plus élevés étaient la griséofulvine (61,8%, n=105), le kétoconazole (52,9%, n=90) et l'itraconazole (51,8%, n=88). Moins de la moitié des isolats étaient résistants au fluconazole (41,2%, n=70) et à la terbinafine (36,5%, n=62). La nystatine présentait les taux de résistance les plus faibles (19,4%, n=33). Le gène *Erg* II a été détecté chez les isolats de *P. kudriavzevii, C. albicans* et *C. tropicalis*.
Conclusion: L'augmentation de la résistance antifongique des espèces de *Candida* est un problème de santé publique pouvant entraîner l'échec du traitement, la propagation de l'infection et potentiellement le décès, en particulier chez les personnes immunodéprimées. Les gènes *Erg* 11 contribuent à la résistance des espèces de *Candida* aux antifongiques.

Mots-clés: *Candida*; Flore normale; Agents antifongiques; Gène de résistance aux antifongiques; PCR

Introduction:

Twenty of the more than 150 *Candida* species are known to infect humans. Ignacio et al., (1) reported that adults and paediatric patients are mostly infected by *Candida albicans*, the fungus responsible for candidiasis. Among all types of sepsis, including those caused by fungus and bacteria, *C. albicans* has the highest death rate, at over 40% in the USA alone (2). Due to their rising prevalence as the cause of candidiasis, non-*albicans Candida* species like *Nakaseomyces glabratus* (formerly *Candida glabratus*), *Candida tropicalis, Candida parapsilosis, Candida kefyr, Candida famata, Candida auris*, and others have gained clinical significance (1).

Antimycotic drugs used for treatment of diseases caused by *Candida* species include polyenes, echinocandins, pyrimidine analogs and azoles (3). Azoles work by blocking the enzyme, lanosterol 14-demethylase (encoded by *Erg11*p gene), which is responsible for converting lanosterol to ergosterol (4). Echinocandins function by inhibiting β-1-3-glucan synthase, encoded by the *fks1* and *fks2* genes (5). Polyene binds to the sterol and causes permeability change in the membrane and disrupts membrane potential. They create transmembrane channels, which then act as an exit for the cell constituents such as Na^+ ions and K^+ until lyses of takes place. As a result of the low solubility and high toxicity to host, polyene use is limited (1,5). Flucytosine (5FC) interferes with pyrimidine metabolism thus inhibiting synthesis of DNA and RNA (5).

In recent years, the emergence of resistant strains to antimycotic agents, including strains resistant to multiple drugs, has been increasingly published (6,7). Also, it has been shown that phenotypes resistant to antibiotics can arise throughout an infection (8). Antifungal resistance is a major global public health issue that threatens the wellbeing of both human, crops, and the environment (9). As a result, antifungal resistance can lead to treatment failures, prolong illnesses and increased mortality (10). Antifungal resistance is also associated with increased healthcare costs for patients, healthcare system, and the country as a whole (11).

The objectives of this study are to determine the species composition and relative frequencies of *Candida* isolates recovered from

clinical specimens of patients with suspected fungi infections attending selected healthcare facilities in Yenagoa, Bayelsa State, Nigeria, and to evaluate their susceptibility profiles against commonly used antifungal agents.

Materials and method:

Study area:

The research was carried out in selected health facilities in Yenagoa, Bayelsa State, Nigeria from 2021 to 2023. The health facilities were; (i) Tuberculosis, Buruli Ulcer and Leprosy referral Center, (ii) Diete-Koki Memorial hospital, (iii) Niger Delta University Teaching Hospital, (iv) Federal Medical Center, and (v) Primary Health Center.

Ethical consideration:

Ethical approval with reference number BSHREC/Vol.1/21/12/005 was obtained from the Bayelsa State Ministry of Health, Nigeria. Informed consent was obtained from each study participant.

Study participants, sample size and method of sampling:

The study participants were symptomatic patients for suspected clinical infections including cough, chest pain, fever, frequent urination, skin rash, vaginal itching, weight loss and white patches on the tongue, with high suspicion for *Candida* infections. Sample size was determined using Yamane's formula as described by Adzani et al., (12); $n=N/(K+N(e)^2$, where 'n' is the calculated sample size, 'N' is the population of study, 'K' is a constant (1), and 'e' is the degree of error expected. This gave a sample size of 500.

Participants were selected by simple random sampling from the five health facilities; Tuberculosis, Buruli Ulcer and Leprosy referral Center (n=300), Diete-Koki Memorial hospital (n=100), Niger Delta University Teaching Hospital (n=30), Federal Medical Center (n=20), and Primary Health Center (n=50).

Specimen collection:

Specimens were collected from oral cavity, skin surface and urinary tract using appropriate techniques. Oral specimens were obtained using sterile swabs. Briefly, the inner cheek, the gum and the tongue surface were swabbed with a sterile cotton swab following the protocol described by Meagan et al., (13). For sputum collection, participants were asked to cough deeply into a wide-necked sterile container, early in the morning before the mouth was washed (14).

Skin specimens were collected in accordance with the technique outlined by the Center for Disease Control and Prevention (15). The soft end of the swab was firmly rubbed 3-5 times at the collection site. While swabbing the groin, the swab was rubbed over the surface of the left and right groin five times per hip crease, focusing on the inguinal crease, the skin fold that separates the leg and pelvis.

Clean catch early morning midstream urine sample was collected for microbiological analysis. In the case of females, participants were asked to to wash their hands and clean the urethral opening area, allowing it to dry before urine collection with the labia held apart. Samples were placed in leak-proof plastic bags were immediately transported to the laboratory for analysis. However, when there is a delay, samples were refrigerated at 4-6^{0}C.

Isolation and phenotypic identification of *Candida* species:

The clinical samples were inoculated on Sabouraud Dextrose agar, incubated at 37^{0}C and examined for 24-72 hours. *Candida albicans* appeared white, creamy, soft, smooth, and wrinkled colonies. *Nakaseomyces glabratus* were small, shiny, round, smooth, and creamy-colored aromatic colonies. *Pichia kudriavzevii* on the other hand, were off-white, dull, and creamy colonies, with a rough surface and slightly raised with the production of wet, mucoid appearance. *Candida tropicalis* appeared white to a creamy, shiny, texture, and a smooth, domed appearance. *Candida akabanensis* appeared as white to cream, soft, smooth and glistening appearance.

Isolated *Candida* species were further sub-cultured on SDA to obtain pure colonies. Phenotypic identification was based on microscopy using KOH mount, Gram staining, Germ tube test and chromogenic media (brilliance *Candida* agar). KOH mount revealed budding yeast cells, pseudo-hyphae and hyphae. Microscopic examination revealed Gram positive cells that were purple colored while cells positive for Germ tube test showed filamentous outgrowth.

On brilliance *Candida* agar, *C. albicans* appeared green in colour, and *C. tropicalis, N. glabratus,* and *P. kudriavzevii* appeared dark blue, beige/brown/yellow and brown or pink colors respectively. *Candida akabanensis* was identified genotypically.

Genotypic identification of *Candida* species:

Genotypic identification to confirm the isolated *Candida* was done by PCR amplification of the internal transcribed spacer (ITS) region of the 16S rRNA gene, followed by sequencing.

DNA extraction:

Genomic DNA was extracted using the Inqaba, South Africa's ZR fungal/bacterial DNA mini-prep extraction kit. The pure culture of the *Candida* isolates was suspended during rapid growth in a ZR Bashing Bead Lysis tube with 200µl of isotonic buffer, and then 750µl of lysis solution was added to the combination.

For 5 minutes at full speed, the tubes were treated in a bead beater with a 2ml tube holder attachment. The tube with the ZR smashing bead lysis was spun at 10,000xg for 1 minute. Following its passage into a collecting tube containing 400µl of supernatant, the liquid in question was subjected to one-minute spinning at 10,000 rpm using an orange-topped Zymo-Spin IIIF spin filter. To make the filtrate volume in the collecting tubes 1600µl, 1200µl of fungal/bacterial DNA binding buffer was added. Later on, 800µl was moved to a Zymo-Spin IIC column in an independent collecting tube and spun at 10,000xg for one minute. Afterwards, the collecting tube had its flow through removed.

The DNA pre-wash buffer and Zymo-spin IIC were mixed in a new collection tube using 200µl of each. Then, for one minute, the mixture was spun at 10,000g. Prior to centrifugation at 10,000xg for 1 minute, 500µl of fungal/bacterial DNA Wash Buffer was added to the tube. Next, 100 µl of DNA elution buffer was added to the column matrix, and the Zymo-spin IIC column was centrifuged at 10,000g for 30 seconds. The column was then transferred to a fresh 1.50 µl centrifuge tube. Subsequent procedures requiring ultra-pure DNA were then executed and stored at -20^{o}C.

DNA quantification:

The amount of genomic DNA that was extracted was measured using a Nanodrop 1000 spectrophotometer. The software for the device was activated by double-clicking the Nanodrop icon. The device was initialized with two milliliters of sterile distilled water and blanked out with normal saline. After depositing two microliters of DNA extract onto the base pedestal, the top pedestal was lowered until it made contact with the DNA. The DNA concentration was quantified by pressing the "measure" button.

PCR amplification of ITS region of rRNA genes:

The ITS region of the rRNA genes of the isolates was amplified using the ITS1F: 5'-CTTGGTCATTTAGAGGAAGTAA-3' and ITS4: 5' TCCTCCGCTTATTGATATGC-3, primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 50µl for 35 cycles. In the PCR mix, the template was extracted DNA, and the primers were X2 Dream Taq Master mix (Taq polymerase, dNTPs, $MgCl_2$) from Inqaba, South Africa, with a concentration of 0.4M.

The following were the PCR reaction cycle; denaturation at 95^{o}C for 5 mins, and continues for another 30 seconds, annealing at 53^{o}C for 30 seconds, and extension at 72^{o}C for 30 seconds, for 35 cycles, with final extension at 72^{o}C for 5 minutes. The 550 bp product was visualized using a blue light transilluminator after 15 minutes of resolution on 1% agarose gel at 200V.

Ribosomal RNA sequencing:

Sequencing of the ITS region of the rRNA gene amplicon was performed by Inqaba Biotechnological of Pretoria, South Africa in a 3510 ABI sequencer that was outfitted with the BigDye Terminator kit for the sequencing process. The following components were used in the sequencing process; 10 µl of BigDye® terminator v1.1/v3.1, 2.25 ul of 5 x BigDye sequencing buffer, 10 µM of primer PCR, and 2-10 ng of PCR template per 100 bp. Sequencing was performed under the following conditions; 32 cycles of once at 96°C for 10 sec, once at 55°C for 5 sec, and once at 60°C for 4 minutes.

The sequences were edited using the bioinformatics algorithm Trace Edit. This was used for base calling and sequence trimming after which the clean sequences were copied to the NCBI domain to check for sequences of organisms with the highest percentage similarity. The evolutionary history was inferred using the Neighbor-Joining method in MEGA 6.0. The bootstrap consensus tree inferred from 500 replicates was taken to represent the evolutionary history of the taxa analyzed. The evolutionary distances were computed using the Jukes-Cantor method (16).

PCR amplification of *Erg 11* gene:

The *Erg* gene fragment was amplified using ErgF: 5'-GTAGAAATACTGAACGTCCGATAA-3' and ErgR: 5'-CCAATTCCACATTGTTTCGGTCTAA-3' primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 30µl for 35 cycles. The X2 Dream Taq Master mix (Taq polymerase, dNTPs, $MgCl_2$) supplied by Inqaba Biotec, South Africa, was used, with the extracted DNA as a template, and primers added at a concentration of 0.5µM to the PCR mix.

The following PCR conditions were applied; denaturation start at 95^{o}C for 5 sec followed by additional 30 sec at 95°C, annealing at 56°C for 30 seconds, and extension at 72^{o}C for 30 sec, for 35 cycles, with final extension at 72°C for 5 minutes. After 30 minutes of resolution on 1% agarose gel at 130 V, the gel was visualized in blue light transilluminator.

Antifungal susceptibility test:

Candida isolates were tested for their *in vitro* susceptibility to the azoles (fluconazole, itraconazole, and ketoconazole), allylamines (terbinafine), polyene (nystatin), and polyketide metabolite (griseofulvin). The isolates were suspended in 5ml sterile water, and the turbidity was adjusted to 0.5 McFarland standards. Antifungal susceptibility of *Candida* species isolates was tested using semi-solid agar antifungal susceptibility (SAAS) testing method. In this method, Brain Heart infusion broth (BHIB) with 0.5% Brain Heart infusion agar (BHIA) was used. The drugs were dissol-

ved either in water or dimethyl sulphoxide (DMSO). Five milliliters of semi-solid agar were transferred to different test tubes. Two-fold serial dilutions of the drugs were made in the different test tubes with semi-solid agar. Drug free media were prepared as positive control. The drug and drug free media were inoculated with a standard platinum loopful (0.001ml) of inoculum suspension by inserting the loop deep within the semisolid agar. The tubes were incubated at 35^0C for 24-48 hours (17).

The standard protocol described by the Clinical and Laboratory Standards Institute (18) was used to determine the minimum inhibitory concentration (MICs) of the anti-fungi agents against the *Candida* isolates. The interpretive breakpoints for sensitivity were as follows; fluconazole ≤8µg/ml; itraconazole ≤ 0.125µg/ml; ketoconazole ≤0.125µg/ml; nystatin ≤4µg/ml; terbinafine ≤4µg/ml; and griseofulvin ≤4µg/ml (18).

Statistical analysis:

Data were analyzed using SPSS Windows version 15.0. Descriptive statistics were used to express the data in percentages. Association of *Candida* prevalence and distribution with socio-demographic and other variables was determined by Chi square test, with statistical significance set at $p \leq 0.05$.

Results:

Prevalence distribution of *Candida* isolation from participants by gender and age:

Of the total 500 selected participants from the healthcare facilities, 221 (44.2%) were males and 279 (55.8%) females. *Candida* species were isolated from 170 (34.0%); 68 of 221 (30.8%) males, and 102 of 279 (36.6%) females. The age range of the participants was between 18-67 years. The age group 28–37 years had the highest *Candida* isolation rate (40.2%, 41/102), followed by age groups 48–57 (37.9%, 36/ 95), age group 58-67 (34.7%, 33/95), and age group 38–47 (33.0%, 33/100). The age group 18–27 years had the lowest prevalence, with isolation rate of 25.0% (27/108) (Table 1).

Prevalence distribution of *Candida* isolates by health facilities:

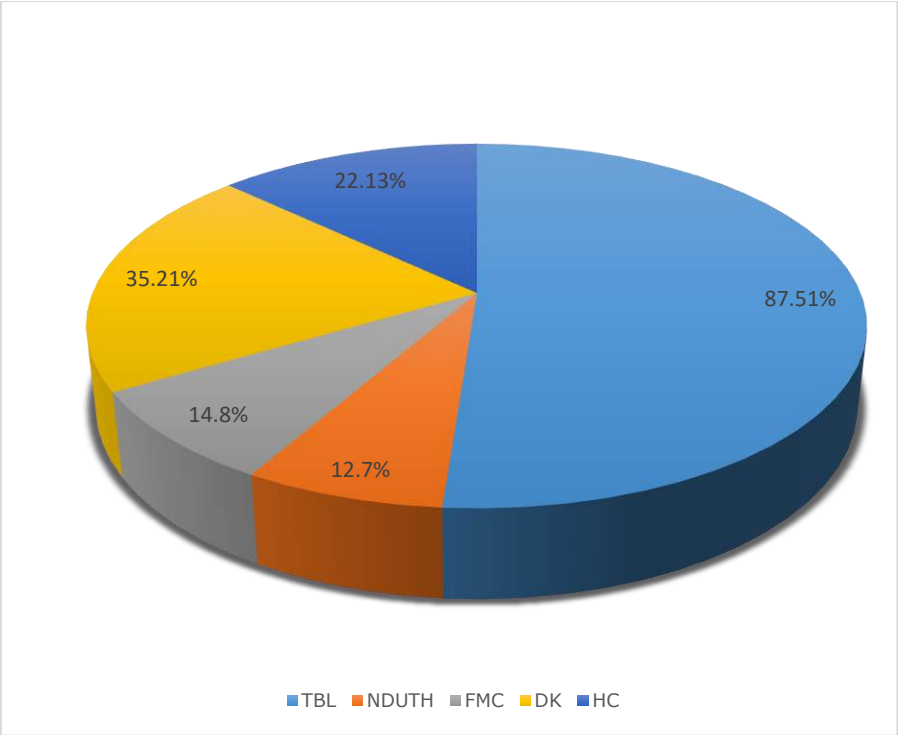
A total of 170 clinical isolates were recovered from the 500 selected patients giving a *Candida* prevalence of 34.0%. These include 110 *C. albicans,* 28 *P. kudriavzevii*, 21 *N. glabratus*, 9 *C. akabanensis* and 2 *C. tropicalis*. Of the 170 *Candida* isolates, 87 (29.0%) were from samples of 300 patients attending the Tuberculosis Buruli Ulcer and Leprosy Referral Hospital in Igbogene, 35 (35.0%) were from samples of 100 patients attending Diete-Koki Hospital in Opolo, 22 (44.0%) were from samples of 50 patients attending Primary Health Center, 14 (70.0%) were from samples of 20 patients attending Federal Medical Centre, and 12 (40.0%) were from samples of 30 patients attending Niger Delta University Teaching Hospital (Fig 1).

There was a statistically significant difference (x^2=17.647, p=0.0014) in *Candida* isolation rate between the health facilities, with the highest isolation rate of 70.0% in Primary Health Center and lowest isolation rate of 29.0% in Tuberculosis Buruli Ulcer and Leprosy Referral Hospital.

Table 1: Prevalence distribution of *Candida* isolation among participants with respect to sex and age

Variable	Total number examined	No with *Candida* isolated (%)	No without *Candida* isolated (%)	x^2	*p* value
Sex					
Male	221	68 (30.8)	153 (69.2)	1.593	0.2069^{ns}
Female	279	102 (36.6)	177 (63.4)		
Age group (years)					
18-27	108	27 (25.0)	81 (75.0)	6.353	0.1743^{ns}
28-37	102	41 (40.2)	61 (59.8)		
38-47	100	33 (33.0)	67 (67.0)		
48-57	95	36 (37.9)	59 (62.1)		
58-67	95	33 (34.7)	62 (65.3)		
Total	**500**	**170 (34.0)**	**330 (66.0)**		

ns = not statistically significant



TBL: Tuberculosis Buruli Ulcer and Leprosy Referral Hospital, NDUTH: Niger Delta University Teaching Hospital, FMC: Federal Medical Centre, DK: Diete-Koki Memorial Hospital, HC: Primary Health Centre

Fig. 1: Distribution of *Candida* isolates by health facilities

Frequency of *Candida* species isolated from patients:

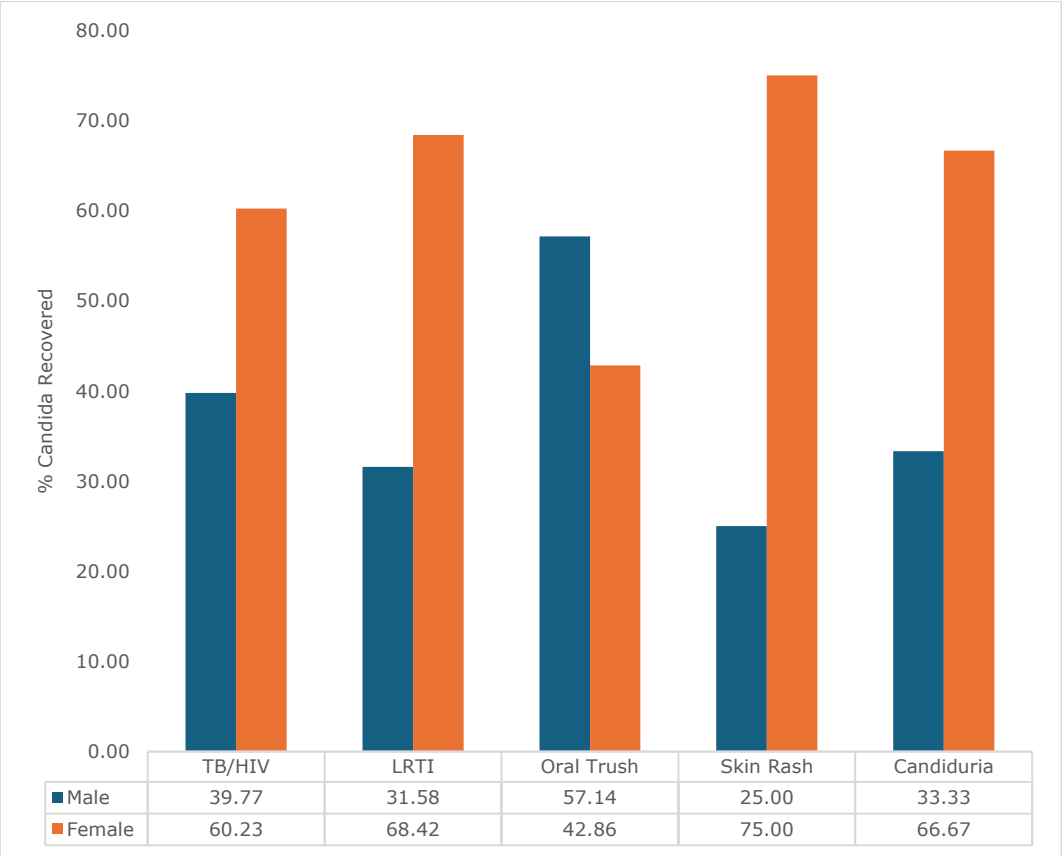
Out of 170 *Candida* species isolated clinical samples, *Candida albicans* was the most frequent, with 110 isolates (64.7%). Of these, 95 (86.4%) were isolated from sputum, 12 (10.9%) from urine, and 3 (2.7%) from skin. From the mouth, 28 species of *Pichia kudriavzevii* were identified, accounting for 16.5% of the total isolates, 21 (13.4%) and 9 (5.3%) were *Nakaseomyces glabratus* and *Candida akabanensis* from sputum respectively. *Candida tropicalis* was the least recovered, with only 2 isolates (1.17%); 1 (50.0%) isolated from the skin and 1 (50.0%) from sputum (Table 2).

Distribution of *Candida* species based on clinical conditions of patients:

A total of 88 *Candida* isolates were isolated from patients with HIV and TB diagnosis, with 35 (39.8%) in males and 53 (60.2%) in females. A total of 38 *Candida* isolates (26 from females and 12 from males) were recovered from individuals with lower respiratory tract infections. Patients with oral infections had 28 *Candida* isolates recovered from them, male and 12 from females. Twelve *Candida* isolates were recovered from individuals with candiduria; 4 from in males and 8 from females. Four species of *Candida* were identified from patients with skin rash, three of whom were females and one a male (Fig 2).

Table 2: Frequency of *Candida* species isolated from samples of patients

Sample type	**Clinical isolates (%)**				
	C. albicans	***N. glabratus***	***C. tropicalis***	***C. akabanensis***	***P. kudriavzevii***
Mouth	-	-	-	-	28 (100.0)
Sputum	95 (86.4)	21 (100.0)	1 (50.0)	9 (100.0)	-
Urine	12 (8.7)	-	-	-	-
Skin	3 (2.2)	-	1 (50.0)	-	-
Total	**110 (64.7)**	**21 (12.4)**	**2 (1.2)**	**9 (5.3)**	**28 (16.5)**



	TB/HIV	LRTI	Oral Trush	Skin Rash	Candiduria
Male	39.77	31.58	57.14	25.00	33.33
Female	60.23	68.42	42.86	75.00	66.67

TB/HIV: Tuberculosis/Human Immunodeficiency Virus; LRTI: Lower Respiratory Tract infection; Candiduria: Urinary Tract infection.

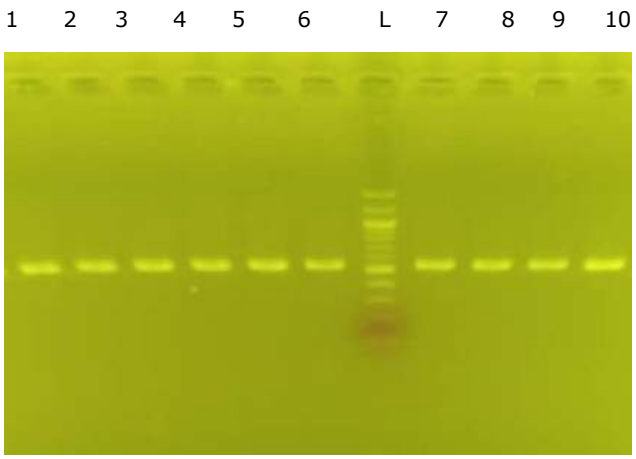
Fig 2: Recovery of *Candida* species from clinical conditions

ITS 16S rRNA amplification and sequencing of *Candida* isolates:

The 550 bp product of the PCR amplified ITS rRNA region of representative *Candida* isolates is shown in Fig 3. A Megablast search for remarkably similar ITS sequences from the National Centre for Biotechnology Information' (NCBI) database showed perfect match with the ITS sequences obtained from the *Candida* isolates as shown in the phylogenetic tree in Fig 4.

Detection of *Erg II* gene amplicon:

The 400 bp PCR amplicon of *Erg11* gene for *P. kudriavzevii*, *C. albicans*, and *C. tropicalis* isolates is shown in Fig 5.



Lanes 1-10 represent the ITS bands at 500bp while lane L represents the 100bp molecular ladder.

Fig. 3: Agarose gel electrophoresis showing the amplified ITS fragment of 16S rRNA gene of representative *Candida* isolates

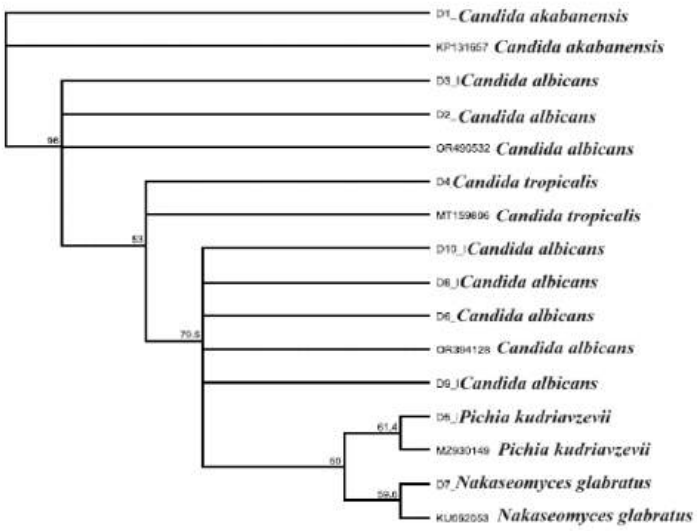
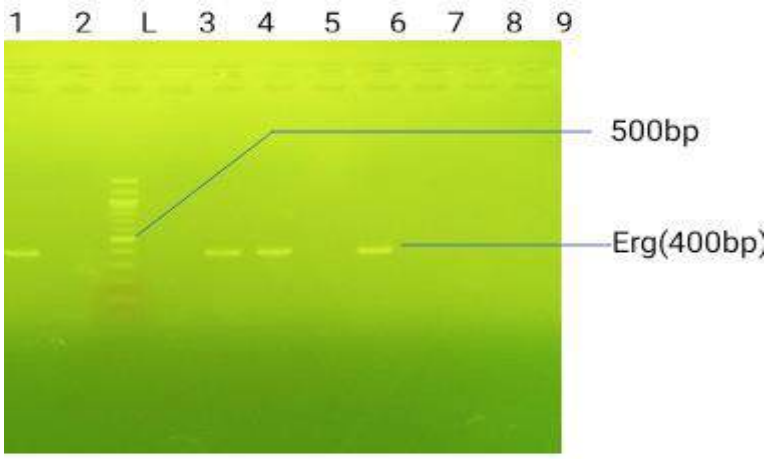


Fig.4: Phylogenetic tree showing the evolutionary distance between the fungal isolates



Lanes 1, 4, 5 and 7 represent the *Erg11* genes bands at 400bp while lane L represents the 100bp molecular ladder.

Fig. 5: Agarose gel electrophoresis showing the amplified *Erg11* gene of some *Candida* isolates

Antifungal susceptibility profiles of isolated *Candida species:*

Table 3 shows the results of *in vitro* antifungal susceptibility testing of the *Candida* isolates to six antifungal agents. Of the 110 *C. albicans* tested, 69 (62.7%) were susceptible to fluconazole, 51 (46.4%) to itraconazole, 50 (45.5%) to ketoconazole, 86 (78.18%) to nystatin, and 72 (65.5%) to terbinafine. Isolates were more resistant to griseofulvin (71.8%, n=79), with only 31 (28.8%) isolates susceptible.

Fifteen (71.4%) of 21 *N. glabratus* were sensitive to fluconazole, and 18 (85.7%) to nystatin. Resistance was high for the other drugs, for example, 12 (57.1%) isolates were resistant to itraconazole, 14 (66.7%) to terbinafine, 15 (71.4%) to griseofulvin, and 11 (52.3%) to ketoconazole. Five of 9 (55.6%) *C. akabanensis* isolates were sensitive to fluconazole and all 9 (100.0%) isolates were susceptible to nystatin, terbinafine and itraconazole. Griseofulvin and ketoconazole showed less activity, with percentage susceptibility of 44.4% and 33.3% respectively.

All 2 *C. tropicalis* isolates were susceptible (100.0%) to terbinafine and nystatin, but resistant to itraconazole. On the other hand, 50.0% of the isolates were resistant to fluconazole, ketoconazole and griseofulvin. *Pichia kudriavzevii* isolates were more susceptible to nystatin (n=22, 78.6%), followed by terbinafine (n=18, 64.3%), and itraconazole (n=15, 53.5%). However, resistance to other antifungal agents was high, for example, 23 (82.1%) of the 28 isolates were resistant to griseofulvin, 16 (57.14%) to ketoconazole, and 18 (64.3%) to fluconazole.

Table 3: *In vitro* susceptibility of clinical *Candida* isolates to selected antifungal agents

Antifungal agent		*C. albicans* (n=110)	*N. glabratus* (n=21)	*C. tropicalis* (n=2)	*C. akabanensis* (n=9)	*P. kudriavzevii* (n=28)	Total isolates (n=170)
FLU	S	69 (62.7)	15 (71.4)	1 (50.0)	5 (55.6)	10 (35.7)	100 (58.8)
	R	41 (37.3)	6 (28.5)	1 (50.0)	4 (44.4)	18 (64.3)	70 (41.2)
ITR	S	51 (46.4)	9 (42.9)	0	9 (100.0)	13 (46.4)	82 (48.2)
	R	59 (53.6)	12 (57.1)	2 (100.0)	0	15 (53.6)	88 (51.8)
KET	S	50 (45.5)	10 (47.6)	1 (50.0)	3 (33.3)	16 (57.1)	80 (47.1)
	R	60 (54.5)	11 (52.4)	1 (50.0)	6 (66.7)	12 (42.9)	90 (52.9)
TER	S	72 (65.5)	7 (33.3)	2 (100.0)	9 (100.0)	18 (64.3)	108 (63.5)
	R	38 (34.5)	14 (66.7)	0	0	10 (35.7)	62 (36.5)
NYS	S	86 (78.2)	18 (85.7)	2 (100.0)	9 (100.0)	22 (78.6)	137 (80.6)
	R	24 (21.8)	3 (14.3)	0	0	6 (21.4)	33 (19.4)
GRI	S	31 (28.2)	6 (28.6)	1 (50.0)	4 (44.4)	23 (82.1)	65 (38.2)
	R	79 (71.8)	15 (71.4)	1 (50.0)	5 (55.6)	5 (17.9)	105 (61.8)

FLU= Fluconazole, ITR=Itraconazole, KET=Ketoconazole, TER=Terbinafine, NYS=Nystatin, GRI=Griseofulvin, S=Sensitive R=Resistance, N=Number of isolates tested.

Discussion:

The purpose of this study was to evaluate how isolated *Candida* species respond to common antimycotic drugs *in vitro*. Overall, the isolated *Candida* species were more sensitive to nystatin (80.6%), followed by terbinafine (63.5%), and fluconazole (58.8%) in the current study. This agrees with the earlier findings of Alkhars et al., (19) who reported 94.4% susceptibility of *Candida* species among mother-child dyads, and Kessler et al., (20). Terbinafine demonstrated remarkable *in vitro* efficacy against *C. albicans* and *C. tropicalis* in their studies. However, our study finding does not agree with findings of several studies. For example, Lawal et al., (21) reported that *Candida* species were more susceptible to ketoconazole, itraconazole, and nystatin. Mahnaz et al., (22) reported terbinafine resistance was highest in *Candida* species. This finding implies that when *Candida* species are resistant to azoles, nystatin and terbinafine may be used as an alternative to treat candidiasis in this setting.

The resistance patterns of *C. albicans* to the azoles showed that, except for fluconazole, which has a comparatively lower resistance rate of 37.3%, the isolates were highly resistant to ketoconazole (54.5%) and itraconazole (53.6%). The susceptibility patterns documented in earlier research by Berkow and Lockhart (23), which reported that fluconazole resistance in *C, albicans* was minimal, ranging from 0.5 to 2% among isolates from the United States of America, are corroborated by our findings but the resistance rate in our study surpasses theirs. Our study contrasts with a 10-year analysis of isolates from vulvovaginal infections by Sobel (24), which reported lower fluconazole resistance in *C. albicans* of 22.8%, despite variations in the annual trends of resistance. Fluconazole resistance, for instance, was 33.3% in 2017 and increased to 35.7% at the end of 2021, four years later, which is consistent with the results of the current study.

Fluconazole resistance was seen in 64.3% of *P. kudriavzevii* isolates tested in our study, ketoconazole resistance in 42.85%, and itraconazole resistance in 46.42%. These patterns of resistance exhibited by *P. kudriavzevii* isolates from Yenagoa, Nigeria to azoles are similar to those reported from other regions of the world. As reported by Seyoum et al., (25) and Chen et al., (26), *P. kudriavzevii* is thought to be naturally resistant to fluconazole, and this result is in line with those findings.

Low *in vitro* activity of the azoles; fluconazole (n=5, 55.6%) and ketoconazole (n=3, 33.3%) was seen in the majority of the *C. akabanensis* isolates in this study. This result agrees with the study by Adogo et al., (27), who reported that every isolated *C. akabanensis* from northcentral Nigeria was resistant to both fluconazole and voriconazole. Nevertheless, itraconazole was found to be effective against the *C. akabanensis* isolates as all (100.00%) the isolates were susceptible to it. As a result, itraconazole should be one of the preferred antimycotic for treating candidiasis that are identified to be *C. akabanensis.*

Our study found that one each of the two *C. tropicalis* (50.0%) isolates were resistant to fluconazole and ketoconazole. In comparison, all 2 (100%) were resistant to itraconazole. The high level of resistance to azoles among *C. tropicalis* is comparable to that reported by a study in Taiwan (28) but contrasted the low levels of fluconazole and voriconazole resistance reported by studies in Middle East (29), Italy and Spain (30). Emerging antifungal resistant isolates are primarily caused by prior and extended exposure to antifungal agents (31,32). Together with the potential connection between antibiotic prophylaxis and fluconazole-resistant strains (33), there is also the inference that either the host conditions could be caused by alternative pathways leading to resistance (34) or the azoles-resistant

strains were obtained from the hands, especially the contaminated hands of healthcare workers (35). On the other hand, a study conducted in Taiwan found that fruit-related azole-resistant *C. tropicalis* isolates grouped with the fluconazole non-susceptible blood isolates. This was observed at the same time that the country's use of fungicides in agricultural applications increased fourfold (28). Therefore, as previously reported by (28,36), we opined that the isolates in our study might have acquired azoles resistance from the environment.

None of the *C. akabanensis* and *C. tropicalis* isolates were resistant to terbinafine, however, *P. kudriavzevii* (35.7%, 10/28) and *C. albicans* (34.5%, 38/110) showed low resistance. Cross-resistance between terbinafine and azoles has already been reported by other researchers (37,38). Our study suggests that cross-resistance to the azoles may also be the cause of resistance observed in *P. kudriavzevii* and the high degree of resistance reported in the isolates of *N. glabratus*. Similar to the earlier study by Yassin et al., (39), terbinafine proved to be the most active *in vitro* against *C. akabanensis*, *C*. *tropicalis*, and *C. albicans* in our study, despite the high prevalence of multidrug-resistant *Candida* strains. As a result, before starting medical therapy, it is recommended that hospitals perform antifungal sensitivity testing.

Majority of the *Candida* species (61.8%) were resistant to griseofulvin, with *C. albicans* (71.8%, 79/110), *N. glabratus* (71.4%, 15/21), *C. akabanensis* (66.7%, 6/9), and *C. tropicalis* (50.0%, 1/2) being resistant, and 23 of 28 (82.1%) *P. kudriavzevii* were also resistant to this agent. This could be explained by the fact that griseofulvin is easily obtained over the counter and is commonly prescribed to treat dermatophytes (40), which makes it easy for people to misuse the prescription as a self-medication. However, a study indicates that a combination of azoles and griseofulvin might be effective in infection due to *Candida* species (41).

Our study show high susceptibility of *Candida* isolates to nystatin (80.6%), with *P. kudriavzevii* (78.6%, 22/28), *N. glabratus* (85.7%, 18/21), and *C. albicans* (78.2%, 86/110), but this high nystatin susceptibility is lower when compared with previous studies, such as De-la-Torre et al., (42) with 95.0%, Nguyen et al., (43) with 93.7% and Zida et al., (44) with 93.7%. These results also show that some strains of *Candida* can express resistance to nystatin (although at a relatively low percentage). Numerous researchers have reported the benefits of using nystatin to treat candidiasis (45,46,47). Although most of the isolates in our study exhibited low resistance to nystatin, it is noteworthy that all the isolated *C. akabanensis* (100.0%, 9/9) and *C. tropicalis* (100.0%, 2/2) were totally sensitive to nystatin. As a result, nystatin is considered an effective medication and is advised to be used either as a first-line treatment or as a "backup" treatment for candidiasis in Yenagoa.

Azoles inhibit ergosterol production pathway by blocking cytochrome P450-dependent enzyme, lanosterol 14-alpha-demethylase, encoded by *Erg11* in yeasts. Inhibiting this pathway impedes ergosterol synthesis, resulting in the accumulation of toxic sterol intermediates that undermine membrane integrity and block fungal growth. The modification of the target 14-alpha-demethylase constitutes a mechanism of azole resistance. These modifications result in diminished affinity of 14-alpha-demethylase for azoles or provoke conformational changes that hinder the antifungal agent access to the active site, hence azoles cannot perform their inhibitory role. The modifications arise from point mutations in the *Erg11* gene, which encodes 14-alpha-demethylase (48). Over expression of *Erg11* genes can occur as a result of gain-of-function mutation (49). Our study found that azoles resistance may have occurred via this mechanism as *Erg11* were detected by PCR in *P. kudriavzevii, C. albicans,* and *C. tropicalis* isolates.

Conclusion:

Though, considered normal flora of humans, *Candida* species can cause candidiasis in humans with compromised immunity. Increased antifungal resistance by *Candida* species is a public health challenge that can lead to failure of treatment, spread of resistant infections and potential mortality from such infections.

Erg11 genes detected in some *Candida* species in our study encode resistance to anti-fungal agents and can complicate treatment of candidiasis in the study setting. Nystatin is recommended as a first-line or as a backup treatment for candidiasis.

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Contribution of authors:

BD was involved in sample collection from medical centers and in laboratory bench work, CDO was involved in writing the manuscript and laboratory bench work, and ABA was the project supervisor and also analyzed data. All authors approved the manuscript submitted for publication.

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Original Article **Open Access**

Morphological and molecular characterization of haloalkaliphilic fungal isolates from hot-spring shores of Lake Magadi, Kenya

*[1,2]Kiboi, N., [3]Abonyo, C., [4]Mwiti, B., [5]Marera, D., [4]Ngugi, M. P., and [6]Were, T

[1]Department of Medical Laboratory Sciences, South Eastern Kenya University, Kitui, Kenya
[2]Department of Medical Laboratory Sciences, Masinde Muliro University of Science and Technology, Kakamega, Kenya
[3]Department of Medical Laboratory Sciences, Alupe University, Busia, Kenya
[4]Department of Biochemistry, Microbiology and Biotechnology, Kenyatta University, Nairobi, Kenya
[5]Department of Human Anatomy, Maseno University, Maseno, Kenya
[6]Department of Pathology, Masinde Muliro University of Science and Technology, Kakamega, Kenya
*Correspondence to: nathankiboi@gmail.com; +254 718-145-100; ORCiD: https://orcid.org/0000-0002-2417-6399

Abstract:

Background: The extensive saline-alkaline ecosystem is increasingly gaining recognition as a rich source of haloalkaliphilic fungal biota whose ecological roles remain less explored. Equally, hot water and saline releasing lakes are being appreciated as untapped natural sources of fungal extrolites bearing unique chemical bioactivities. Existing literature reveals scanty evidence on fungal biodiversity in East African Rift-valley soda lakes. This study sought to bio-prospect Lake Magadi in Kenya as a source of extremophilic fungal microbial diversity.
Methodology: A laboratory-based experimental study was conducted using simple random sampling to select 9 mark-points in the lake for establishment of fungal morphological and molecular characterization. Wet sediments, surface water and soil samples were collected from each sampling site in triplicate and cultured on Sabouraud's Dextrose Agar (SDA), Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) plates at 25^0C and 41^0C for 1-3 weeks, to isolate and identify fungi by conventional macro and microscopic morphology. The ITS-1 and ITS-4 universal fungal primer pairs were used to amplify extracted fungal DNA from isolates, followed by Sanger sequencing. The NCBI BLASTn was used for molecular identification and evolutionary relationships were inferred using neighbor-joining method with 1000 bootstrap support. The salinity, pH, electrical conductivity (EC) and total dissolved solids (TDS) of the samples were measured onsite using portable pH meter and double interface electrical-chemical analyzer.
Results: A total of 30 fungal isolates were recovered from the samples using culture based and molecular techniques. Fungal sporulation was observed at pH 8–9 and salinity of 9.6–10.8 ppm. Growth was more evident at 25^0C (n=25) compared to 41^0C (n=5), and further supported on SDA (n=16) relative to MEA (n=10) and PDA (n=4). Wet sediments accrued highest diversity of fungal biota (n=15). Phylogenetically, isolates were affiliated to 9 genera with *Cladosporium* (n=8), *Aspergillus* (n=5) and *Penicillium* (n=4) displaying dominance. The genera *Aspergillus* and *Penicillium* alongside *Fusarium* and *Sarocladium* exhibited close evolutionary affiliation as sister groups with overlapping clades.
Conclusion: The findings of this study validate existence of diverse fungal community species discerned through observed distinct phenotypic features vis-a-vis molecular identities. Diversity and taxonomic richness are significantly influenced by pH, salinity, temperature and variable cultivation media types. Metagenomic tools including DNA pyrosequencing and whole genome sequencing can enhance heightened detection of diverse microbial biota across East African saline-alkaline ecosystems.

Keywords: Haloalkaliphilic, Characterization, Saline-alkaline, Extremophiles, Hot-spring, Kenya

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Caractérisation morphologique et moléculaire d'isolats fongiques haloalcaliphiles provenant des rives des sources chaudes du lac Magadi, au Kenya

*[1,2]Kiboi, N., [3]Abonyo, C., [4]Mwiti, B., [5]Marera, D., [4]Ngugi, M. P., et [6]Were, T

[1]Département des Sciences de Laboratoire Médical, Université du Sud-Est du Kenya, Kitui, Kenya
[2]Département des Sciences de Laboratoire Médical, Université des Sciences et Technologies Masinde Muliro, Kakamega, Kenya

[3]Département des Sciences de Laboratoire Médical, Université Alupe, Busia, Kenya
[4]Département de Biochimie, Microbiologie et Biotechnologie, Université Kenyatta, Nairobi, Kenya
[5]Département d'Anatomie Humaine, Université de Maseno, Maseno, Kenya
[6]Département de Pathologie, Université des Sciences et Technologies Masinde Muliro, Kakamega, Kenya
*Correspondance à: nathankiboi@gmail.com; +254 718-145-100; ORCiD: https://orcid.org/0000-0002-2417-6399

Résumé:

Contexte: Le vaste écosystème salin-alcalin est de plus en plus reconnu comme une riche source de biote fongique haloalcaliphile dont les rôles écologiques restent peu explorés. De même, les lacs libérant de l'eau chaude et de l'eau salée sont considérés comme des sources naturelles inexploitées d'extrolites fongiques présentant des bioactivités chimiques uniques. La littérature existante révèle peu de données sur la biodiversité fongique dans les lacs sodiques de la vallée du Rift est-africain. Cette étude visait à bioprospecter le lac Magadi, au Kenya, comme source de diversité microbienne fongique extrémophile.
Méthodologie: Une étude expérimentale en laboratoire a été menée par échantillonnage aléatoire simple afin de sélectionner 9 points de repère dans le lac pour établir une caractérisation morphologique et moléculaire des champignons. Des échantillons de sédiments humides, d'eau de surface et de sol ont été prélevés en triple sur chaque site d'échantillonnage et mis en culture sur des plaques de gélose dextrose de Sabouraud (SDA), de gélose dextrose de pomme de terre (PDA) et de gélose à l'extrait de malt (MEA) à 25°C et 41°C pendant 1 à 3 semaines, afin d'isoler et d'identifier les champignons par morphologie macro et microscopique conventionnelle. Les paires d'amorces fongiques universelles ITS-1 et ITS-4 ont été utilisées pour amplifier l'ADN fongique extrait des isolats, puis le séquençage Sanger a été réalisé. Le NCBI BLASTn a été utilisé pour l'identification moléculaire et les relations évolutives ont été déduites par la méthode de «jointure entre voisins» avec un support bootstrap de 1000. La salinité, le pH, la conductivité électrique (CE) et les solides dissous totaux (SDT) des échantillons ont été mesurés sur site à l'aide d'un pH-mètre portable et d'un analyseur électrochimique à double interface.
Résultats: Au total, 30 isolats fongiques ont été récupérés à partir des échantillons par des techniques de culture et moléculaires. La sporulation fongique a été observée à un pH de 8 à 9 et une salinité de 9,6 à 10,8 ppm. La croissance était plus marquée à 25°C (n=25) qu'à 41°C (n=5), et a été confirmée par la SDA (n=16) par rapport à la MEA (n=10) et à la PDA (n=4). Les sédiments humides ont présenté la plus grande diversité de biotes fongiques (n=15). Phylogénétiquement, les isolats étaient affiliés à neuf genres, *Cladosporium* (n=8), *Aspergillus* (n=5) et *Penicillium* (n=4) étant dominants. Les genres *Aspergillus* et *Penicillium*, ainsi que *Fusarium* et *Sarocladium*, présentaient une étroite filiation évolutive en tant que groupes frères avec des clades se chevauchant.
Conclusion: Les résultats de cette étude valident l'existence d'espèces fongiques diverses, distinguées par des caractéristiques phénotypiques distinctes observées par rapport aux identités moléculaires. La diversité et la richesse taxonomique sont significativement influencées par le pH, la salinité, la température et les différents types de milieux de culture. Les outils métagénomiques, notamment le pyroséquençage de l'ADN et le séquençage du génome entier, peuvent améliorer la détection de biotes microbiens diversifiés dans les écosystèmes salins-alcalins d'Afrique de l'Est.

Mots-clés: haloalcalin, caractérisation, salin-alcalin, extrémophiles, source chaude, Kenya

Introduction:

Saline-alkaline soda lakes within Kenyan Rift-valley comprise of Lake Magadi, Bogoria, Elementaita, Nakuru, and Sonachi (1). Besides exclusive topographical and geological alignments, large deposits of sodium carbonate (Na_2CO_3) complexed with insoluble Ca^{2+} and Mg^{3+} salts facilitate existence of varied microbial biota under alkaline conditions (2,3). Likewise, substantial saline deposits in these ecosystems favors thriving of extremophile species displaying halotolerant and alkaliphilic attributes (4). The pH levels of Lake Magadi range between 8–12, whereas total salts are estimated between 5-10% (5). Among widely profiled hypersaline and alkaliphilic lake catchments comprise of Solar Salterns (Puerto Rico), *Chott* Melghir (Algeria), Caspian Sea, Great Salt Lake (Utah), Wadi Natrun (Egypt) and Lake Bogoria (Kenya) (6-10).

Halotolerant and alkaliphilic fungal species are ecologically defined rather than taxonomically classified, with further categorization as either obligate or facultative (11, 12). They are isolated by diverse cultivation methods using specific or modified fungal media, however, does not distinguish between facultative strains and terrestrial habitat contaminants (13). Yet still their isolation including cultivation from natural ecosystems requires careful picking of the fungal host or supporting material such as sponges, algae, sea grasses, sediments, soils and water media (14).

Growth of obligate extremophilic fungal biota ensues entirely within hypersaline ecosystems, whereas their facultative counterparts exhibit sporulative cycles, based primarily on their physiological adaptive mechanisms which confers tolerance to abiotic stressors inclusive of high salinity, hydrostatic pressures, elevated temperatures and pH fluctuations (7,9). They produce substantial amounts of extremozymes and extremolytes that enhances their tolerability (15).

Regardless of the harsh saline-alkaline ecosystem, appreciable fungal biodiversity produces a repertoire of natural products

with varied bioactivities. Bio-prospecting Kenya's marine-like ecological setting through morphological and molecular characterization provides crucial evidence-based material and knowledge for critical research founded decision-making practices in the country's conservancy and natural protected sites. Profiling of fungal diversity within East-African soda lakes also forms a steady basis for supplementary exploration of potent natural bioactive compounds from extremophilic fungal species that may reveal capacity for potential medicinal applicability.

Materials and method:

Study site, design and sampling technique:

This was a laboratory-based experimental study using random sampling in 9 mark-points at study site in Lake Magadi, Kenya. It covers an estimated 100 square km in size and lies 2° 00' 0'' S and 36° 00' 0'' E of the equator at an elevation of 600 meters (16). Saline emitting hot-springs (heating up to 86°C) rest along the North-western and Southern shore-lines and release into alkaline lagoons along the lake margins (3). Saline deposits also enhance thriving of a fish species (*Cichlid alcolapia graham)* that occupies the lake's basin (17).

Ethical consideration:

This study was approved by Masinde Muliro University of Science and Technology Institutional Scientific and Ethics Review Committee (MMU/COR: 403012 vol-2). Consent for participation was not required for this study.

Physico-chemical indices of the lake sampling points:

The pH levels were measured onsite using portable digital pH meter (Oakton® pH 110) and universal indicator strips (range 1-14) (Merk®, USA). The *in-situ* and sampling points temperature was obtained using digitized water thermometer (Hanna; model HI-98509, Europe). The total dissolved solids (TDS), electrical conductivity (EC) and salinity levels were assessed using double interphase electrical-chemical analyzer (Jenway™-3405 series, England). Geographical positionning of site sampling locations with regards to elevation, latitude and longitude were determined using global positioning system (Garmin, eTrex 22x®, USA).

Sample collection and processing:

About 30g each of wet sediments and soil material were collected into zip-lock bags, while 50ml surface water was aspirated using sterile syringes and transferred into dry, leak-proof screw capped bottles. All samples were obtained in triplicates, transported under dry ice and stored at -20^{0}C overnight until processing.

One gram each of the pooled sediment and soil samples were suspended in sterile universal bottles containing 10ml distilled water. Contents were vortexed for 5 min and allowed to settle before undergoing a ten-fold (1:10) dilution sequence. 100µl of highest dilution (10^{-1}) from each sample was inoculated by spread plate method into sterile Sabouraud's Dextrose Agar (SDA), Potato Dextrose Agar (PDA) and Malt Extract Agar (MEA) plates.

Fungal media preparation and fungi isolation:

Varied agar media types (SDA, PDA, MEA) were used for fungal isolation with reconstitution based on pH levels measured at sampling site, while adhering to manufacturer's directions for culture media preparation. Media contents were sterilized at 121^{0}C, 15 lbs for 15 min. Streptomycin and carbenicillin (Duchefa Biochemie®) stock solutions were prepared at equal ratios (0.1g), with 100µl being pipetted into growth medium cooled to molten state at 50^{0}C. Contents were swirled gently before plating onto 9mm plates.

Approximately 100µl of the sample aliquots were pipetted into respective molten agar plates and allowed to solidify before incubating in an inverted position aerobically at room temperature (RT; 25^{0}C) and 41^{0}C for 1-3 weeks, with evaluation of mycelial growth. Resultant pure culture isolates were aseptically cut as agar plugs and seeded onto freshly prepared media. Mycelial growth duration was evaluated as mean growth rate (colony diameter; mm), while recording initial and final growth duration in days.

Morphological identification of fungal isolates:

Pure fungal isolates were identified up to genera level on the basis of visualized discrete morphological characteristics. Macroscopic features including colour, shape, pigmentation, texture, ridges, consistency, elevation, margins and opacity were carefully assessed and recorded. Microscopic properties were ascertained after lactophenol cotton blue (LPCB) staining. Micrographs were generated using mounted digital camera at 100x magnification.

Macro and micro-morphological properties of isolates were compared against fungal identification keys and reference illustration tables (18), aided by MycoBank fungal stock descriptions (19). Microscopic identification features comprised of conidial shape, hyphae, aerial mycelia, vesicles and phialides.

Molecular characterization of fungal isolates:

DNA extraction:

Genomic DNA was extracted using Solarbio® kit (Solarbio Life Sciences©, Beijing,

China) consistent with the manufacturer protocol. Briefly, 100mg fungal mycelium was resuspended in 200µl DNAse-free water and contents crushed. 200µl of solution A (50mM Tris pH 8.5; 5mM EDTA pH 8.0 and 25% sucrose) and 2µl of RNase A were pipetted into mixture containing 100mg bashing beads. Contents were vortexed at high speed and incubated for 30min at RT for proper lysis. 20µl proteinase K was added and digestion done in a 55^{0}C pre-heated water bath for 30 min.

Tube contents were centrifuged at 12,000rpm for 2min and the supernatant transferred. 200µl solution B (10mM Tris pH 8.5; 5mM EDTA pH 8 and 1% sodium dodecyl sulfate) was added with further incubation. 200µl of anhydrous ethanol followed by 600µl wash buffer was added to adsorption column with centrifugation at 12,000rpm for 2min while discarding the filtrate. 50µl elution buffer was then pipetted into adsorption column matrix and centrifugation repeated at 12,000 rpm for 2min. DNA pellet deposited at bottom of tube was stored at -20^{0}C until purity assessment.

DNA quantification and purity assessment:

Nucleic acids exhibit maximal spectral absorbance at 260 nm, whereas proteins and residual extraction salts absorb at 280 nm (20). Absorbance ratios at 260/280 nm, 260/230 nm and DNA yield (ng/µl), were quantified using Nanodrop One© (Thermo-Fisher™, USA). Briefly, 2µl blanking solution was pipetted onto the Nanodrop sensor and 30 sec allowed for optical density reading. Sample pedestal was then cleaned before loading 2µl genomic DNA samples in successive order. DNA bands quality control check was done using 3µl template on 1.5% agarose gel under UV visualization.

PCR amplification:

PCR amplification targeted the ITS gene region using primer pairs ITS-1 (5'-TCC GTAGGTGAACCTGCGG-3') and ITS-4 (5'-TCC TCCGCTTATTGATATGC-*3'*) as previously described (21). Briefly, 25µl reaction volume contained 5 µl Taq 5x (New England Biolabs™, USA), 1 µl ITS-1 forward primer, 1 µl ITS-4 reverse primer, 15 µl molecular-grade water and 3 µl DNA template. The control lacked DNA template. The Realplex thermal cycler was configured at 1 cycle initial denaturation (2 min; 95^{0}C), 34 cycles final denaturation (30 sec; 95^{0}C), annealing (1 min; 56^{0}C), initial extension (1 min; 68^{0}C) and final extension at (7 min; 68^{0}c).

Gel electrophoresis and DNA cleaning:

About 5µl of amplicons volume each were carefully loaded onto 1.5% agarose gel and run at 100 volts for 45 min. DNA bands were stained with SYBR™ green, with 100 base pair (bp) ladder used as size standard. Image visualization utilized UVIDOC HD6 (Cleaver Scientific®, England). Target fragments were purified from the gel slice using Pure Link™ DNA clean-up kit (Thermo-Fisher™, USA) as per manufacturer's guidelines, followed by repeated quality-check electrophoresis prior to submission for sequencing.

Sequencing and phylogenetic analysis:

The PCR products were sequenced using Sanger sequencing (Macrogen®, Europe) on Applied Biosystems genomics platform. Multiple sequences were aligned with ClustalW (Bio Edit v7.2) while MEGA v11 performed phylogenetic analysis. Aligned sequences were deposited in NCBI database for accession numbers generation. Sequences with >90% similarity using BLASTn progressed to phylogenetic analysis, with 1000 bootstrap support. Evolutionary relationships were inferred using neighbor-joining method following Kimura two-parameter with Gamma distribution (K2+G). Interactive Tree of Life (iTOL) conducted tree visualization.

Results:

Physico-chemical parameters of the sampling sites:

The physico-chemical parameters of the lake and sampling sites are summarized in Table 1. Temperatures at sampling points ranged from 76.9 (MP-08) to 83.1 ^{0}C (MP-05) while *in situ* recordings fluctuated between 34.1 and 35.2 ^{0}C for MP-09 and MP-01, respectively. The pH levels were moderately alkaline varying between 8.3 (MP-09) to 9.1 (MP-01). The EC readings ranged between 32.0 (MP-08) and 34.8 (MP-01) mS/cm and TDS between 17.8 and 19.7 ppm for MP-08 and MP-05 mark-points. Salinity values ranged from 8.7 (MP-08) to 10.8 (MP-01) ppm. Site elevation was spread across 612 (MP-02) and 645 (MP-01) meters.

Characteristic fungal isolation profiles in varying culture media types:

A total of 30 isolates grew on variable media types, SDA (n=16) showed higher isolation capacity relative to PDA (n=4) and MEA (n=10). Optimal growth occurred at RT of 25^{0}C (n=25) compared to 41^{0}C (n=5). Wet sediments (n=15) had higher isolates recovery compared to soils (n=13) and surface water (n=2). Sampling points MP-04 (n=9) and MP-08 (n=1) recorded highest and least growth of isolates, respectively. Results are summarized in Table 2 and Supplementary Table (https://afrjcem.org/supplementary-materials/)

Table 1: Physico-chemical parameters of sampling sites in Lake Magadi, Kenya

Sampling Points (Mark point)	Elevation (Metres, M)	Temperature (^{0}c)		pH	EC (mS/cm)	TDS (PPM)	Salinity (NaCl) (PPM)
		Environment (*In situ*)	Sample points				
MP-01	645	35.2	82.1	9.1	34.8	19.4	10.8
MP-02	612	34.8	81.8	8.7	33.9	18.1	92
MP-03	613	34.8	80.5	8.9	33.6	18.9	10.1
MP-04	615	34.6	76.1	8.6	32.1	18.5	9.4
MP-05	617	34.7	83.1	8.8	34.1	19.7	10.5
MP-06	618	34.9	81.7	8.7	32.7	18.4	9.3
MP-07	618	34.4	77.6	8.5	32.3	18.8	9.9
MP-08	620	34.5	76.9	8.5	32.0	17.8	8.7
MP-09	621	34.1	77.3	8.3	32.4	18.6	9.6

Mark-point (MP); Total dissolved solids (TDS); Electrical conductivity (EC); milli Siemens per cm (mS/cm); Potential hydrogen (pH); Degree Celsius (^{0}c); Meters (M); Parts per million (PPM)

Table 2: Characteristic fungal isolation profiles on growth media types

Sampling Points	Sample Type	Sample Isolate	Growth Temp Range (°C) RT (25°C); 41°C	Growth Media (SDA, PDA, MEA)
MP-01	Sediment	-		
	Soil	-		
	Water	-		
MP-02	Sediment	**P17**, 41°C, SDA		
	Soil	-		
	Water	**P21**, 41°C, SDA		
MP-03	Sediment	**P5**, RT, PDA; **P10**, RT, MEA; **P12**, RT, SDA; **P14**, RT, MEA; **P27**, RT, SDA; **P28**, RT, SDA		
	Soil	-		
	Water	-		
MP-04	Sediment	-		
	Soil	**P2**, RT, PDA; **P4**, RT, SDA; **P9**, RT, SDA; **P11**, RT, SDA; **P15**, RT, SDA; **P20**, RT, MEA; **P26**, RT, PDA; **P30**, RT, MEA		
	Water	**P19**, RT, SDA		
MP-05	Sediment	**P6,**41°C, SDA		
	Soil	**P13**, 41°C, SDA		
	Water	-		
MP-06	Sediment	-		
	Soil	**P7**, RT, MEA; **P8**, RT, MEA; **P18**, RT, MEA; **P24**, RT, MEA		
	Water	-		
MP-07	Sediment	-		
	Soil	-		
	Water	-		
MP-08	Sediment	**P29**, 41°C, SDA		
	Soil	-		
	Water	-		
MP-09	Sediment	**P1**, RT, PDA; **P3**, RT, MEA; **P16**, RT, SDA; **P22**, RT, SDA; **P23**, RT, SDA; **P25**, RT, MEA		
	Soil	-		
	Water	-		

Mark-point (MP), Sabouraud's dextrose agar (SDA), Potato dextrose agar (PDA), Malt extract agar (MEA), Degrees Celsius (°C), Temperature (Temp), Room temperature (RT), Fungal isolate code number (P)

Morphological identification of fungal isolates:

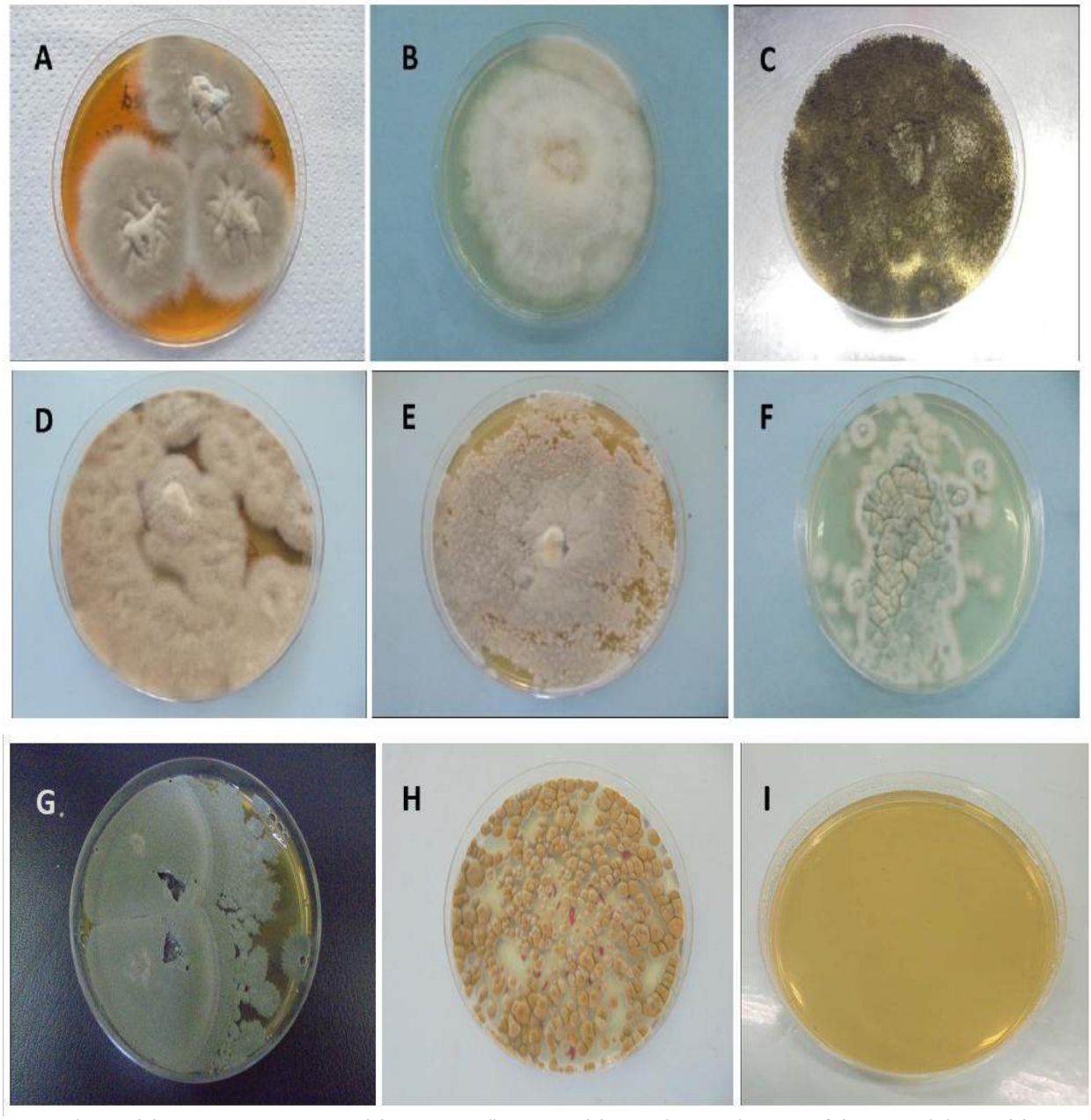
Macroscopic morphological description:

Colonial features varied from front and reverse angle view in 30 culturable isolates. Fungal isolate P19 (*Alternaria alternata*) exhibited raised brown velvet-like mycelia with round shape, entire margins, smooth non-powdery texture with opaque opacity (Fig 1). Sample isolate P21 (*Aspergillus niger*) displayed brownish black filamentous mycelia with filiform margins, rough texture, non-powdery consistency and opaque opacity. Isolate P12 (*Fusarium equiseti*) appeared filamentous with cotton white mycelia, filiform margins, rough texture, non-powdery consistency and translucent opacity.

Microscopic features of fungal isolates:

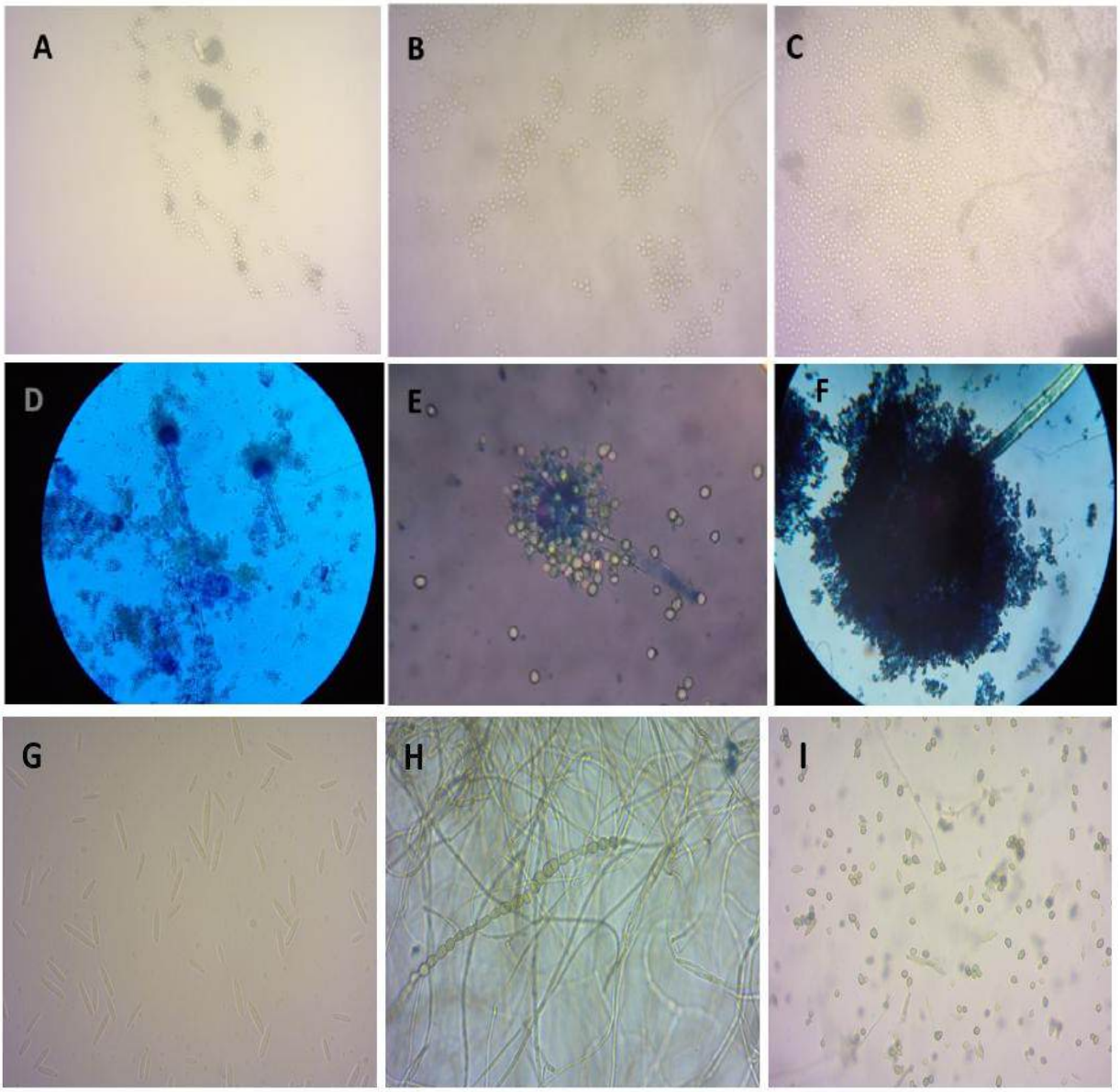
Isolate P7 (*Fusarium incarnatum*) displayed phenotypic features of elongated slightly curved tapering apical cells with 3-5

septate, numerous hyaline macroconidia and phialoconidium (Fig 2). Isolate P26 (*Penicillium expansum* presented brush-shaped conidiophores with multiple conidia in chains and septate mycelial hyphae that appeared hyaline. Isolate P16 (*Aspergillus versicolor*) exhibited long septate hyphae, pale brown upright and loosely radiating conidiophores with mature vesicles bearing biseriate phialides. Isolate P13 (*Aspergillus fumigatus*) showed spiked conidiophores with greenish conidia arranged in column chains and supported by phialide structures with clavate vesicles (Fig 2).



P29, *Bipolaris* sp. (**A**); P12, *Fusarium equiseti* sp. (**B**); P21, *Aspergillus niger* sp. (**C**); P19, *Alternaria alternata* sp. **(D**); P9, *Sarocladium* sp. (**E**); 26, *Penicillium expansum* sp. (**F**); P30, *Penicillium chrysogenum* sp. (**G**); P3, *Aspiosporina morbosa* sp. (**H**); Negative Control **(I**).

Fig 1: Macroscopic morphological properties of fungal isolates from Lake Magadi, Kenya



Magnification at x100. P2, *Penicillium chrysogenum* sp. strain EG 13-1 (**A**); P6, *Penicillium chrysogenum* sp. isolate KP-114 (**B**); P26, *Penicillium expansum* sp. (**C**); P13, Aspergillus *fumigatus* sp. (**D**); P16, *Aspergillus versicolor* sp. (**E**); P21, *Aspergillus niger* sp. (**F**); P7, *Fusarium incarnatum* sp. (**G**); P19, *Alternaria alternata* sp. (**H**); P20, *Cladosporium cladosporioides* sp. (**I**).

Fig 2: Microscopic features of study isolates following lactophenol cotton blue (LPCB) staining

Molecular characterization of fungal isolates:

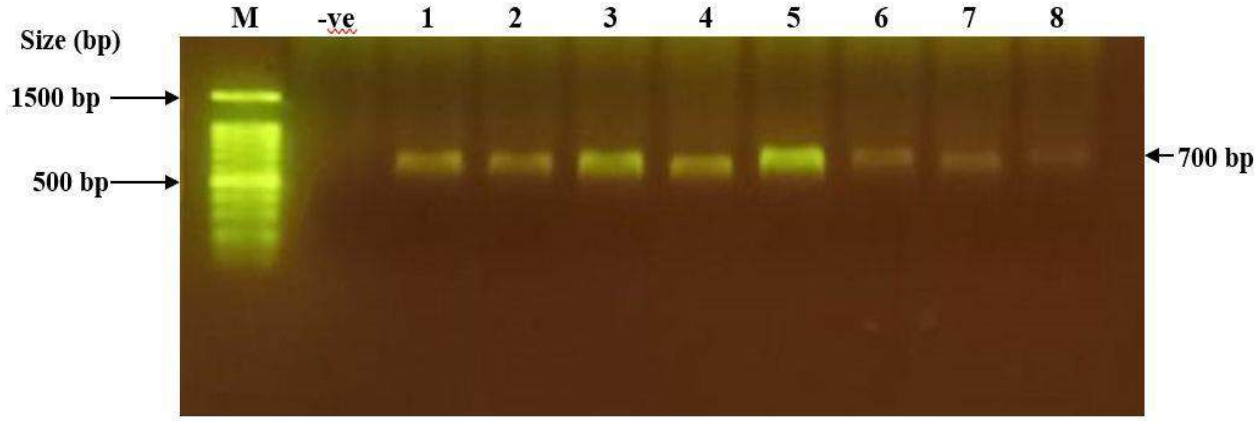
ITS gene sequence analysis and phylogenetic diversity of study isolates:

Amplified ITS genes produced band fragments of approximately 700 base pairs (bp) for representative isolates P7, P12, P14, P18, P19, P21, P27 and P29 alongside 100 bp molecular ladder as shown in Fig 3. DNA yield ranged between 101.5-446.6 ng/µl, while purity assessment readings averaged 1.8-2.0 for bulk of samples. Sequence data detected fungal clusters with high percentage identity (≥90%) classified into 9 clades (*Cladosporium, Aspergillus, Fusarium, Penicillium, Sarocladium, Curvularia, Bipolaris, Alternaria*, and *Dothideomycetes*) (Table 3 and Fig 4).

The genera *Cladosporium, Aspergillus, Penicillium* and *Fusarium* dominated, with numerous individual strains. For instance, *C. cladosporioides, C. oryzae*, *C. parahalotolerans*, *C. oxysporum* and *C. perangustum* were affiliated to genus *Cladosporium,* whereas *A. fumigatus*, *A. niger* and *A. versicolor* were linked to genera *Aspergillus.* The genera *Fusarium* and *Penicillium* strains included *F. incarnatum*, *F. oxysporum* and *F. equiseti* alongside *P. chrysogenum* and *P. expansum*, respectively. Minor strains comprised *Sarocladium* sp. voucher GJB; *Phoma* sp. Sin 154W03 and *Curvularia mebaldsii* Bi22.

Evolutionary relationship of taxa with

good bootstrap support of 1000 replicates showed genera *Aspergillus* and *Penicillium; Curvularia*, *Alternaria* and *Dothideomycetes as well as Fusarium* and *Sarocladium* as sister groups with overlapping clades that bear close evolutionary affiliation.



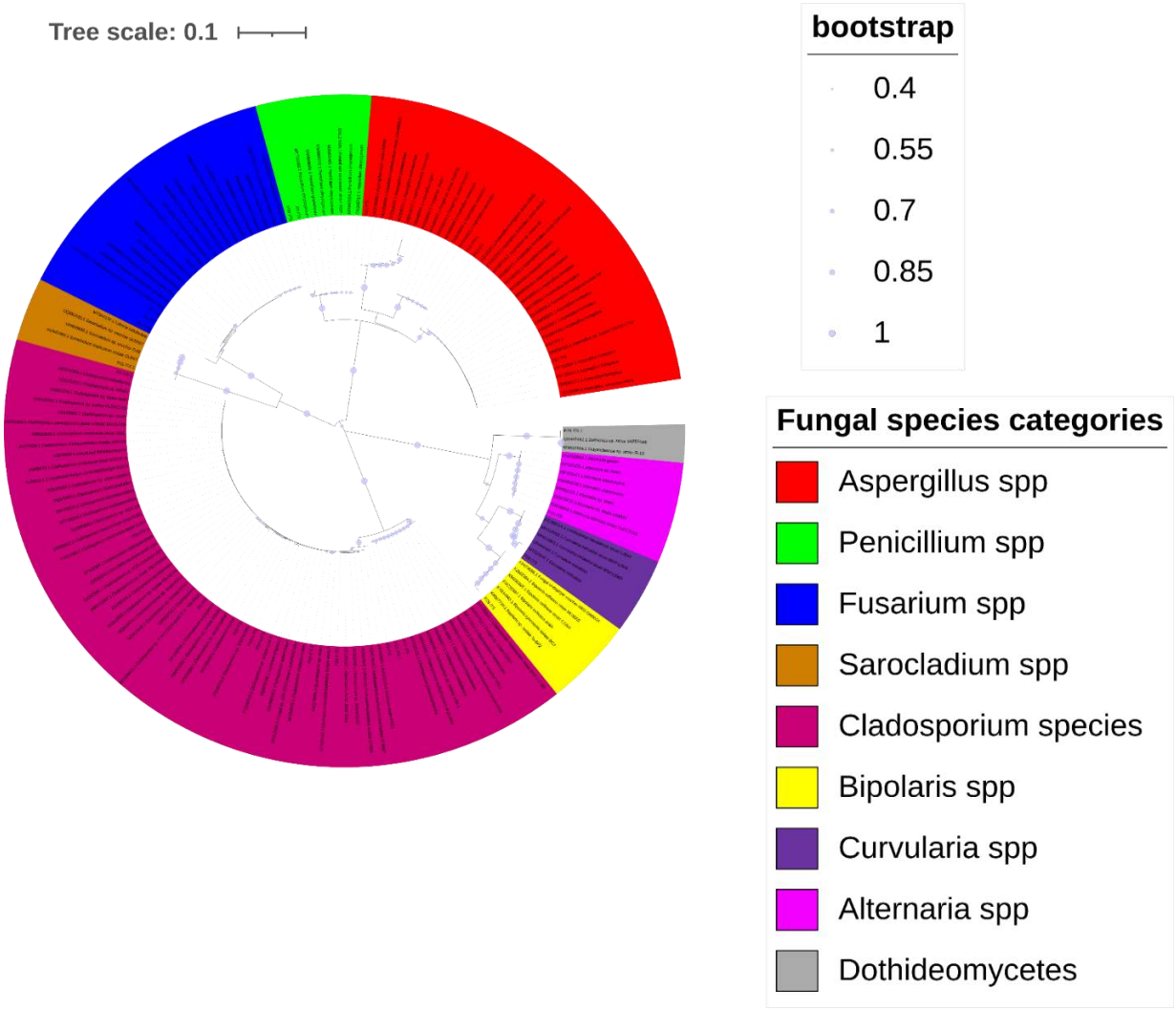
700 bp fragments targeting ITS region. Lane M; 100 bp ladder. -ve; negative control. Lanes 1 – 8; isolates P7, P12, P14, P18, P19, P21, P27 and P29. Base pairs (bp).

Fig 3: Agarose gel with PCR products of study isolates

Table 3: ITS gene sequence analysis results of fungal isolates using BLASTn

Sample ID (Query Sequence)	Sequence Accession Number	Percentage (%) Identity	Fungal Species Identified
P1	MK140706.1	99.79	*Cladosporium oxysporum* isolate MN 12
P2	MT730080.1	97.81	*Penicillium chrysogenum* strain EG 13-1
P3	AF493982.1	93.99	*Aspiosporina morbosa* strain PFC1 ITS
P4	LN834380.1	99.58	*Cladosporium perangustum*
P5	KX387685.1	99.79	*Cladosporium* sp. XQ3-13 isolate J29
P6	OQ826664.1	100.00	*Penicillium chrysogenum* isolate KP-114
P7	KX523892.1	95.70	*Fusarium incarnatum* isolate F31
P8	KF914438.1	100.00	*Fusarium oxysporum* sp. *lycopersici* strain FO43
P9	MH890606.1	99.43	*Sarocladium* sp. voucher GJB
P10	KT192295.1	99.81	*Aspergillus fumigatus* strain 40a3-2
P11	MH890606.1	99.43	*Sarocladium* sp. voucher GJB
P12	KY436192.1	91.84	*Fusarium equiseti* isolate ATS37
P13	MT994683.1	91.89	*Aspergillus fumigatus* isolate AA
P14	MT786364.1	97.62	*Cladosporium cladosporioides* strain JZY1-25
P15	MG018196.1	100.00	*Phoma* sp. 1KM-2017 isolate Sin154W03
P16	PP076803.1	99.42	*Aspergillus versicolor* isolate E42
P17	OW984430.1	99.61	*Aspergillus fumigatus*
P18	MG385089.1	98.96	*Cladosporium oryzae* isolate ihWWF158
P19	KJ410043.1	100.00	*Alternaria alternata strain* DUCC5021
P20	AF455442.1	99.79	*Cladosporium cladosporioides* isolate wb413
P21	MK840965.1	99.04	*Aspergillus niger* isolate F5
P22	MT229245.1	100.00	*Curvularia mebaldsii* strain Bi22
P23	MN859971.1	99.19	*Cladosporium halotolerans* isolate DC03
P24	MF688676.1	99.15	*Cladosporium cladosporioides* isolate VGCC15-3
P25	MN837894.1	99.78	*Didymellaceae* sp. strain SL13
P26	MK907949.1	97.85	*Penicillium expansum* isolate EF171
P27	OP237121.1	98.94	*Cladosporium parahalotolerans* strain 299N1
P28	MN837894.1	99.78	*Didymellaceae* sp. strain SL13
P29	KX867789.1	99.00	*Bipolaris* sp. isolate Ta-BP2
P30	MT730080.1	97.63	*Penicillium chrysogenum* strain EG 13-1

Internal transcribed spacer (**ITS**); Nucleotide basic local alignment search tool (**BLASTn**).



Google Drive Link: https://drive.google.com/file/d/12DYGu_B1ZqzQ1RAtj7YyuiSO6pDXrEe/view?usp=drive_link

Fig 4: Tree diagram of fungal species targeting ITS gene region

Discussion:

Physico-chemical indices are formerly shown to impact on survivability of diverse microbial biota including extremophilic fungi in hypersaline ecosystems (22). In particular, salinity levels (range 8.7–10.8 ppm) in our study conform with earlier studies reporting mean levels of 10 mg/L (4,5). Sporulation and metabolites production are propagated in sodium chloride and glucose enriched catchments (23). Our isolates were predominantly alkaliphiles which portrays adaptive resilience towards abiotic stressors (5). The sampling site temperatures (range 76.1–83.1^{0}C) in our study parallel previous studies in hot-springs of Rift-valley soda lakes recording measurements between 77.7–84.6^{0}C (6).

Regarding *in situ* temperatures, the bulk of haloalkaliphiles exhibit optimal growth range between 20–45^{0}C (9,24), which coincides with our study, with a range of 34.1-35.2^{0}C. Lower TDS values averaging 0.64–0.65 mg/l were documented from Lake Bogoria sampling points compared to our study findings of 17.8–19.7 ppm. Lake Magadi is supplied by run-off waters bearing eroded material from higher adjacent ground areas which consequently propels and deposits large number of dissolved solids in form of silt into hot-spring rivulets (25).

Additionally, our study agrees with previous research using culture-based techniques and assorted media types for fungal isolation (7,26). Cultures of related fungal genera thrive best on media types with comparable growth requirements which enhances cultivation from their natural ecosystems (27). SDA showed highest suitability for fungal isolation among profiled media types, attributable to differences in concentration of growth nutrient composition as constituted by media manufacturer brands.

The fungal isolates in our study were phylogenetically clustered into 9 genera, maj-

ority of which have formerly been isolated in hypersaline habitats sharing ecological preference to extreme conditions. In particular, genus *Penicillium, Aspergillus, Cladosporium, Fusarium, Chaetomium, Phoma, Talaromyces, Ectophoma, Emericella, Phaeosphaeria* and *Acremonium* have reported isolation from sediments in Rift valley halotolerant ecosystems (3). Importantly, genera *Aspergillus, Penicillium* and *Cladosporium* exhibit higher colonization rates among *Ascomycota* compared to other minor classes (8,28). Consistent pattern is experienced from hypersaline waters of *Chott* Melghir (Algeria), Solar Salterns (Puerto Rico) and Caspian Sea especially involving numerous strains of *Aspergillus, Penicillium* and *Cladosporium* (9,10). Phylogenetic and molecular diversity observations in our study concurs with previous studies from solar salterns that reported genera *Aspergillus* and *Penicillium* as sister groups with joint evolutionary ancestry (10,29). Likewise, genus *Curvularia* and *Alternaria* reveal close relatedness and shared ancestry in *Pleosporaceae* family (30).

Nonetheless, our study has some limitations. The study did not critically evaluate seasonal variability patterns that may positively impact on total fungal community from the study setting. Morphological and molecular identities of study isolates may not represent entire fungal biota across Kenyan saline-alkaline ecosystems. DNA pyrosequencing and whole genome sequencing were not incorporated for heightened detection of fungal species in the catchment setting.

Conclusion:

Notwithstanding we observed significant difference in morphological and molecular identification among the isolates in our study that was discerned through observed distinct phenotypic features relative to molecular identities. Importantly, *C. cladosporioides* and *A. fumigatus* were most predominant species among all profiled isolates. Fungal diversity and taxonomic richness are significantly influenced by salinity levels, pH, temperature and variable culture media types.

There is need to design studies that will optimize culture growth conditions for numerous fungal species due to lack of an independent growth medium ideal for cultivation of all fungal groups. Adoption of next generation sequencing to enhance characterization scope of halotolerant and alkaliphilic fungal species in Kenyan extreme environments.

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Contributions of authors:

NK, TW and MPN conceived and designed the study. NK, CA and BM performed the laboratory experiments and statistical analyses. NK, TW, and DM drafted the manuscript. TW, DM and MPN critically reviewed, edited and validated the manuscript. All authors read and approved the final manuscript version.

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Conflict of interest:

The authors have no known competing financial or personal interests to declare.

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Original Article **Open Access**

Effects of fruit and vegetable smoothie formulations on serum electrolytes, haematological indices and faecal bacteriology: An *in vivo* experiment in female Wistar albino rats

Ekong, M. O., *Anashie, M. A., Ekpenyong, G. A., Nnaji, G. C., Asuquo, J. O., and Archibong, E. A.

Department of Microbiology, University of Cross River State (UNICROSS), Calabar, Nigeria
*Correspondence to: mildredanashie310@gmail.com; 08143509138

Abstract:

Background: The global demand for fruits and vegetables consumed as smoothies is increasing, driven by a growing population and heightened awareness of their health benefits. However, the nutritional significance and health effects of smoothies remain underexplored. Chemotherapeutic agents are widely used in managing various illnesses but are associated with the dysregulation and destruction of healthy cells, raising concerns in clinical settings. This study aimed to assess the potential of smoothie formulations in regulating serum electrolytes, haematological indices, and faecal bacteriology in an experimental animal model.
Methodology: Thirty female Wistar albino rats (*Rattus norvegicus*) were divided into 5 groups of 6 rats each, using a permuted-block randomized design, with the 5th group serving as the positive control. Smoothie formulations of banana/watermelon (BNWM), watermelon/cucumber (WMCC), pineapple/orange (PPOR), and banana/cucumber (BNCC) were prepared at 10g/100ml concentrations. The smoothies were administered to the rats orally at 1ml/kg body weight daily for 30 days, while the control group received no smoothie formulations. Faecal count and bacterial identity were carried out twice during the experiment using standard bacteriological techniques. Serum electrolytes and haematological indices were determined by standard methods on blood samples collected through cardiac puncture from anaesthetized rats on day 30 of the experiment. Experimental data were expressed as mean±SEM and percentages. Comparisons between groups were performed using Student's *t*-test and ANOVA. Significance difference was defined at $p<0.05$.
Results: Residual feed intake (RFI) values ranged from 33.8±5.09 to 36.9±3.66, with groups 3–5 rats showing progressive weekly increases while groups 1 and 2 rats fluctuated. Residual water intake (RWI) was highest in the control group (80.8±22.66ml) compared to smoothie-fed groups (59.2–67.8ml). Body weight reductions occurred across all groups but were not statistically significant ($p=0.1507$). Faecal bacteriology showed variable trends, with group 2 rats exhibiting a marked increase, group 3 rats with slight decrease, and group 1 rats having no detectable growth at the second sampling. Overall, Gram-negative isolates (51.2%) were slightly more frequent than Gram-positive (48.8%), with *Vibrio* spp (19.5%), *Staphylococcus* spp (17.1%), and *Bacillus* spp (14.6%) being most common. Electrolytes remained within normal ranges, although bicarbonate was lower in smoothie-fed rats (18.7–22.7mEq/L) compared to control (24.3mEq/L). Hematological indices showed significantly elevated white blood cells, lymphocytes, mean corpuscular volume, and platelets in smoothie-fed groups relative to the control, while haemoglobin, packed cell volume, mean corpuscular haemoglobin, and mean corpuscular haemoglobin concentration showed non-significant variations.
Conclusion: This *in vivo* animal experimental findings suggest that smoothie formulations have potential in promoting weight management, maintaining electrolyte balance, enhancing haematological parameters, and reducing the pathogenic impact of gut microbiota without inducing symptoms. Further research is recommended to explore the incorporation of smoothies as dietary supplements, particularly for health conditions requiring electrolyte normalization and immune support, offering a safer alternative to synthetic therapies.

Keywords*:* Albino rats, faecal bacteriology, smoothie formulations, electrolytes, haematological indices

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Effets des préparations de smoothies aux fruits et légumes sur les électrolytes sériques, les indices hématologiques et la bactériologie fécale: une expérience *in vivo* chez des rats albinos femelles Wistar

Ekong, M. O., *Anashie, M. A., Ekpenyong, G. A., Nnaji, G. C., Asuquo, J. O., et Archibong, E. A.

Département de Microbiologie, Université de l'État de Cross River (UNICROSS), Calabar, Nigéria
*Correspondance: mildredanashie310@gmail.com; 0814350913

Résumé:

Contexte: La demande mondiale de fruits et légumes consommés sous forme de smoothies est en hausse, stimulée par la croissance démographique et une sensibilisation accrue à leurs bienfaits pour la santé. Cependant, l'importance nutritionnelle et les effets des smoothies sur la santé restent peu étudiés. Les agents chimiothérapeutiques sont largement utilisés dans la prise en charge de diverses maladies, mais ils sont associés à une dysrégulation et à la destruction des cellules saines, suscitant des inquiétudes en milieu clinique. Cette étude visait à évaluer le potentiel des préparations de smoothies dans la régulation des électrolytes sériques, des indices hématologiques et de la bactériologie fécale dans un modèle animal expérimental.
Méthodologie: Trente rats albinos Wistar femelles (*Rattus norvegicus*) ont été répartis en cinq groupes de six rats chacun, selon un dispositif randomisé en blocs permutés, le cinquième groupe servant de témoin positif. Des préparations de smoothies banane/pastèque (BNWM), pastèque/concombre (WMCC), ananas/orange (PPOR) et banane/concombre (BNCC) ont été préparées à des concentrations de 10g/100 ml. Les smoothies ont été administrés aux rats par voie orale à raison de 1 ml/kg de poids corporel par jour pendant 30 jours, tandis que le groupe témoin n'a reçu aucune préparation de smoothie. La numération fécale et l'identité bactérienne ont été réalisées à deux reprises au cours de l'expérience à l'aide de techniques bactériologiques standard. Les électrolytes sériques et les indices hématologiques ont été déterminés par des méthodes standard sur des échantillons de sang prélevés par ponction cardiaque chez des rats anesthésiés au 30e jour de l'expérience. Les données expérimentales ont été exprimées en moyenne ± écart-type et en pourcentages. Les comparaisons entre les groupes ont été réalisées à l'aide du test '*t*' de Student et d'une ANOVA. La différence de significativité a été définie à $p<0,05$.
Résultats: Les valeurs de la consommation alimentaire résiduelle (RFI) variaient de 33,8±5,09 à 36,9±3,66, les rats des groupes 3 à 5 présentant une augmentation hebdomadaire progressive, tandis que les rats des groupes 1 et 2 fluctuaient. La consommation d'eau résiduelle (RWI) était la plus élevée dans le groupe témoin (80,8±22,66 ml) par rapport aux groupes nourris au smoothie (59,2 à 67,8ml). Français Des réductions de poids corporel ont été observées dans tous les groupes, mais n'étaient pas statistiquement significatives ($p=0,1507$). La bactériologie fécale a montré des tendances variables, les rats du groupe 2 présentant une augmentation marquée, les rats du groupe 3 une légère diminution et les rats du groupe 1 n'ayant aucune croissance détectable lors du deuxième prélèvement. Globalement, les isolats Gram négatifs (51,2%) étaient légèrement plus fréquents que les Gram positifs (48,8%), les *Vibrio* spp (19,5%), *Staphylococcus* spp (17,1%) et *Bacillus* spp (14,6%) étant les plus courants. Les électrolytes sont restés dans les plages normales, bien que le bicarbonate était plus faible chez les rats nourris au smoothie (18,7–22,7mEq/L) que chez le témoin (24,3mEq/L). Les indices hématologiques ont montré une augmentation significative des globules blancs, des lymphocytes, du volume globulaire moyen et des plaquettes dans les groupes nourris au smoothie par rapport au groupe témoin, tandis que l'hémoglobine, l'hématocrite, l'hémoglobine corpusculaire moyenne et la concentration corpusculaire moyenne en hémoglobine n'ont montré aucune variation significative.
Conclusion: Ces résultats expérimentaux in vivo sur des animaux suggèrent que les préparations à base de smoothies pourraient favoriser la gestion du poids, maintenir l'équilibre électrolytique, améliorer les paramètres hématologiques et réduire l'impact pathogène du microbiote intestinal sans induire de symptômes. Des recherches complémentaires sont recommandées pour explorer l'intégration des smoothies comme compléments alimentaires, notamment pour les pathologies nécessitant une normalisation électrolytique et un soutien immunitaire, offrant ainsi une alternative plus sûre aux thérapies synthétiques.

Mots-clés: Rats albinos, bactériologie fécale, préparations à base de smoothies, électrolytes, indices hématologiques

Introduction:

Smoothies are a popular and convenient means of consuming various fruits, vegetables, and other nutrient-rich ingredients in liquid form. It is a versatile platform for combining vitamins, minerals, fibers, antioxidants, and essential fatty acids for a valuable, balanced diet (1). It serves as health enthusiasts, easy meal replacement, providing hydration, energy, and nourishment, aiding in weight management, and improving immune function (2). The high fiber content from fruits and vegetables can help regulate blood sugar levels, promote satiety, and enhance digestive health. Antioxidants from ingredients like berries can combat oxidation, stress, and inflammation, supporting cardiovascular health and reducing the risk of chronic diseases (3).

The versatility of smoothies has gained popularity as a healthful beverage convenience option and plays a vital role in the gut microbiome. Recent studies have highlighted the profound effect diet has on the gut microbiome, a community of microorganisms that plays a crucial role in digestion, metabolism, and immune function (4).

In albino rats, research into the con-

sumption of smoothies has shown promising results in modulating the gut microbiome (5). The fiber content in smoothies, especially from fruits and vegetables, acts as prebiotics, feeding beneficial bacteria in the gut. This can increase microbial diversity, improving gut health and potentially enhancing the rats overall physiological functions.

A balanced microbiome in these rats has been associated with improved digestion, reduced inflammation, and better nutrient absorption, thus demonstrating the potential therapeutic benefits of smoothies for gut health. The present study aims to investigate the effects of smoothie consumption on serum electrolytes, haematological indices, and faecal bacteriology in experimental albino rats.

Materials and method:

Study setting:

This experimental study was conducted at the Animal House of the Physiology Department, University of Calabar, Calabar, Nigeria. The facility is equipped for housing and handling laboratory animals under controlled environmental conditions suitable for in vivo experiments. All procedures involving the animals were carried out in accordance with institutional guidelines and the principles outlined in the Guide for the Care and Use of Laboratory Animals (6).

Fruits and smoothie preparations:

Five different fruits; banana (*Musa paradisiaca*), watermelon (*Citrullus lanatus*), cucumber (*Cucumis sativus*), pineapple (*Ananas census*) and orange (*Citrus sinensis*), were purchased fresh within Ekpo Abasi Junction fruit market in Calabar south and were aseptically wrapped using sterile foil paper. The identities of the fruits were confirmed by the Faculty Botanist, Professor Samuel Udoh, of the Department of Plant Science and Biotechnology, University of Cross River State (UNICROSS), Nigeria before being transported to the Department of Microbiological Laboratory, UNICROSS, for analysis.

The fruits were washed using running tap water several times, peeled with a sterile knife, and diced into smaller pieces. Ten g of each fruit was weighed using a digital compact scale (Atom A123) weighing balance and 20 g of the combined fruits were blended in 100 ml distilled water with a Moulex juicer blender (Model JB-70B) for 5 minutes.

Four different fruit combinations; banana/watermelon (BNWM), watermelon/cucumber (WMCC), pineapple/orange (PPOR), and banana/cucumber (BNCC), were used to prepare different types of smoothies. The prepared smoothies were emptied into sterile bottles and administered orally within 5 minutes of preparation to the experimental rat groups.

Albino rats for the study:

Thirty, 74-day old female Wistar albino rats (*Rattus norvegicus*), were purchased from the Faculty of Basic Medical Science, University of Calabar (UNICAL) on April 12, 2024. The rats were randomly divided into 5 groups of 6 rats each. The individual groups were housed in a standard laboratory cage placed in the well-ventilated laboratories with an average temperature of (27±2^{o}C), 85% relative humidity with an alternative 12 hours of natural light and 12 hours of dark or night cycle. The laboratory cages were properly disinfected with Morigad veterinary Lysol solution a few days before the arrival of the experimental animals.

Animals were adequately cared for in compliance with the principles of laboratory animal care (6). The cages were fitted with feeders containing fresh commercially prepared growers' mash (Vital Feeds, Grand Cereals Limited, Ibadan, Nigeria) and clean drinking tap water daily. The animals were allowed to acclimatize in the experiment site for seven days before each experiment commenced.

Experimental design:

The experimental design used was a permuted-block randomized design with four treatment groups; banana/watermelon (BNWM), watermelon/cucumber (WMCC), pineapple/orange (PPOR), and banana/cucumber (BNCC), and a control group. Rats in each treatment group was given smoothies based on body weight (1ml/kg) daily within the 30 days of experimental trials, except for the control group (group 5). The control group (group 5 rats) was fed with 100% commercially prepared growers mash (Vital Feeds, Grand Cereals Limited, Ibadan, Nigeria) and clean drinking water, in place of smoothie from the beginning to the end of the experiment.

General observations of animals:

The experimental animals were adequately observed for abnormalities in behavioral and physical changes such as eyes, skin, posture, fur, and response to handling. Time started, and a lasting period in such situations was recorded and documented. Residual daily feed/ water intake, changes in body weight, mortality, and morbidity were also examined.

Growth examination study:

The growth examination study was carried out based on the method described by Pugalenthi et al., (7).

Residual feed-intake (RFI):

Daily feed consumption was ascertained by subtracting the initial quantity (g) of feed, known as residual feed intake (RFI), from the leftover (LOF); $\mathrm{RFI} - \mathrm{Leftover}\ \mathrm{x}100$

Residual water-intake (RWI):

The residual daily water intake was calculated by subtracting the initial volume (mL) of water (IVO) from the leftover volume of water (LVO); $\text{RWI} - \text{leftover x}100$

Bacteriological analysis of faecal sample of albino rats:

The faecal samples of the rats were taken twice at intervals of four weeks during the experimental period. A sterile spatula was used to pick the rat faeces (pallets) into a universal bottle and transported to the University of Cross River State (UNICROSS) Microbiology laboratory for analysis within an hour. Approximately 1g weight of the faecal pallet was dissolved in 9ml sterile normal saline and shaken vigorously and intermittently for 30 minutes until the pallets were completely dissolved. Taking 1ml of the pallet solution, a ten-fold serial dilution down to 10^{-10} was done.

Bacterial cell count, isolation and identification:

Bacterial cell count was carried out using a standard bacteriological technique; a pour-plating method was employed for easy morphological characterization. A 1ml of 10^{-7} and 10^{-8} of the pallet solutions were placed in a clean Petri dish; then, 20ml of prepared semi-solid, molten Nutrient or MacConkey agar (RDM-NA-01 or RDM-MCA-02) were added. The solution was also cultured using *Salmonella-Shigella* (SS agar) (CA92121UAS) and thiosulfate-citrate-bile-salt (TCBS agar) (CM1 025) as differential media for the identification of *Salmonella-Shigella* and *Vibrio* species respectively.

The Petri dishes were gently swirled after adding the semi-solid molten agar until the contents were properly mixed. The plates were allowed to set before incubation at 37^{o}C in a humidified incubator for 24 hours, after which different morphological characterizations were observed, and emergent colonies were counted and recorded. The purification of discrete colonies was done by three successive sub-cultures and re-isolation on nutrient agar, characterized by standard bacterial techniques as described in Cheesbrough (8).

Haematology and serum electrolyte determination:

Blood sample was collected from the rats according to the method described by Oguwike et al., (9). At the end of the 30 days trial, all the rats were anesthetized with urethane at 6mg/kg (0.6 ml) per rat. About 2ml of blood was collected by cardiac puncture into ethylene-diamine tetra acetic acid (EDTA) bottle for electrolyte determination and 2ml into lithium heparin bottle for haematological analysis.

The haematological parameters such as white blood count (WBC), neutrophils, lymphocytes, monocytes, eosinophils, basophils, atypical lymphocyte (ALY), large immature cell (LIC), red blood count (RBC), haemoglobin (Hb), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH), mean corpuscular haemoglobin concentration (MCHC), and platelet (PLT) were determined by the procedure described by Sastry (10). The electrolyte parameters; Sodium (mmol/l), potassium (mmol/l), chloride (mmol/l) and bicarbonate (mEq/L) were determined using Audiom (AZ) automated electrolyte machine.

Statistical analysis:

Experimental data were expressed as mean±SEM and as percentages. Comparisons between 2 groups were performed using Student's *t*-test, and between more than 2 groups using ANOVA, with Bonferroni multiple comparisons. Statistically significant difference was defined as $p<0.05$. Graphs were generated using ORIGIN® version 6.1 and Microsoft Excel™.

Results:

Residual feed and water intake:

The mean residual feed intake (RFI) values were 35.9±3.27, 33.8±5.09, 36.9± 3.66, 35.5±5.94, and 36.1±6.25 for rats in groups 1–5, respectively. Across the experimental period, rats in groups 1 and 2 showed more fluctuations in RFI compared to the other groups. In contrast, rats in groups 3–5 demon strated a progressive increase in RFI over the weeks, with the highest values observed in week 4 of the trial (Table 1, Fig 1).

The residual water intake (RWI) values across the experimental groups are presented in Table 2. The mean RWI was highest for rats in group 5 (80.8 ± 22.66 ml), followed by rats in group 3 (67.8 ± 5.51 ml), while rats in groups 1, 2, and 4 recorded lower mean intakes (59.6±5.59 ml, 62.7±9.32 ml, and 59.2±11.00 ml, respectively). Across the four weeks of the experiment, fluctuations in intake were observed in rats for most groups rather than a consistent progressive increase (Table 2, Fig 2). Figs 1 and 2 showed that RFI and RWI of the rats varied among the group with no commensuration between both within the experimental period.

Table 1: Residual feed intake (RFI) for all the rats in the experimental and control groups

Rat groups	Σ of RFI for Week 1	Σ of RFI for Week 2	Σ of RFI for Week 3	Σ of RFI for Week 4	Mean ± SE
Group 1	32.9	29.6	36.4	44.8	35.93±3.27
Group 2	34.4	24.8	28.0	47.8	33.75±5.09
Group 3	30.0	32.5	38.5	46.4	36.85±3.66
Group 4	25.0	27.7	38.1	51.2	35.50±5.94
Group 5	20.8	34.1	38.1	51.2	36.05±6.25

Group 1=Banana and Watermelon (BNWM), Group 2=Watermelon and Cucumber (WMCC), Group 3=Pineapple and Orange (PPOR), Group 4=Banana and Cucumber (BNCC), Group 5=Control; SE = Standard error

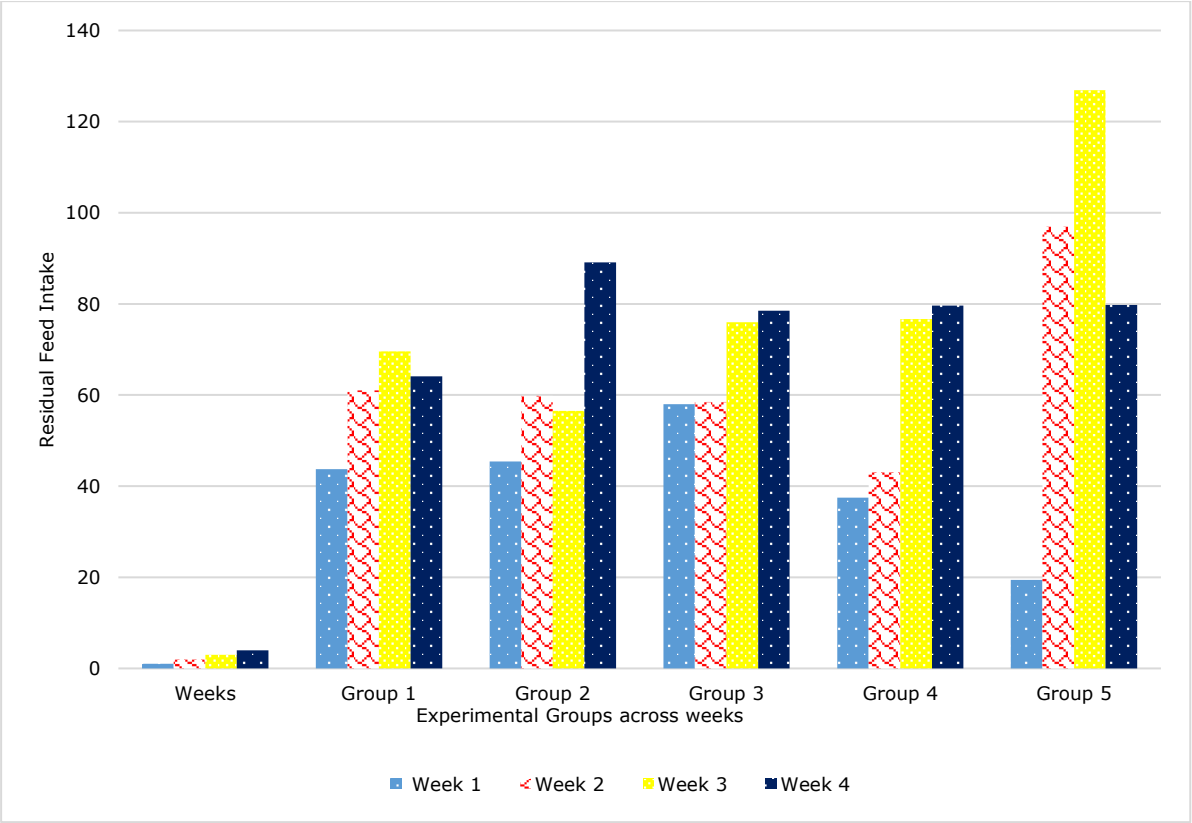

Fig 1: Variations in residual feed intake within the experimental period

Table 2: Residual water intake (RWI) for all rats in the experimental and control groups

Rat groups	Σ of RWI for Week 1	Σ of RWI for Week 2	Σ of RWI for Week 3	Σ of RWI for Week 4	Mean ± SE
Group 1	43.7	61	69.6	64.1	59.6±5.59
Group 2	45.4	59.8	56.5	89.1	62.7±9.32
Group 3	58	58.5	76	78.5	67.8±5.51
Group 4	37.5	43.1	76.7	79.6	59.2±11.00
Group 5	19.4	97	126.9	79.8	80.8±22.66

Group 1=Banana and Watermelon (BNWM), Group 2=Watermelon and Cucumber (WMCC), Group 3=Pineapple and Orange (PPOR), Group 4=Banana and Cucumber (BNCC), Group 5=Control; SE = Standard error

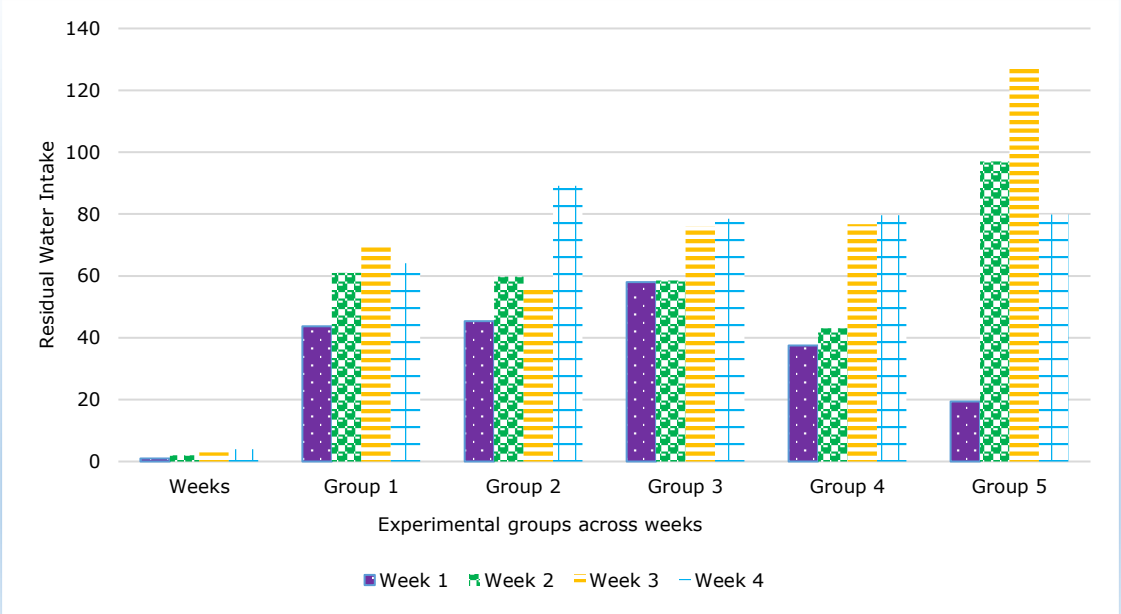

Fig 2: Variations in residual water intake within the experimental period

Table 3: Body weight changes for all the rats in the experimental and control groups

Rat groups	Σ of body weight change for Week 1	Σ of body weight change for Week 2	Σ of body weight change for Week 3	Σ of body weight change for Week 4	Mean± SE	*p* values
Group 1	27.2	12.7	5.3	8	13.3± 4.88	0.0302
Group 2	8.8	9.7	5.9	8.3	8.2± 0.81	0.5006
Group 3	9.8	8.2	7.7	9.6	8.8±0.52	0.7717
Group 4	8.8	7.2	7.8	22.3	11.5±3.61	0.1982
Group 5	14.9	15.8	7.2	23.7	15.4±3.37	0.4344
***p* values**	0.1507	0.1806	0.7287	0.2553		

Group 1=Banana and Watermelon (BNWM), Group 2=Watermelon and Cucumber (WMCC), Group 3=Pineapple and Orange (PPOR), Group 4=Banana and Cucumber (BNCC), Group 5=Control; SE = Standard error

Body weight of rats:

The general body weight assessment revealed varied reductions across all experimental groups, including the control. In group 1, the reduction rate was progressive throughout the four weeks of the experiment, with weekly cumulative changes of 27.2g, 12.7g, 5.3g, and 8.0g, respectively. The mean (±SE) body weight changes were 13.3±4.88g, 8.2± 0.81g, 8.8±0.52g, 11.5±3.61g, and 15.4± 3.37g for groups 1–5, respectively.

Statistical analysis showed no significant differences in the mean body weight change among the groups (p=0.1507), although rats in group 1 exhibited the largest variability over time. Weekly comparisons indicated that body weight changes did not differ significantly between groups in weeks 1–4 (p-values ranging from 0.1507 to 0.7287) (Table 3).

Bacteriological analysis of the faecal contents of the rats:

The faecal bacteriology of each experimental group was assessed at two intervals within the 30-day experimental trial. The highest bacterial cell count during the first sampling was 2.0–3.9×10⁷ CFU/ml for group 3 while the lowest was 1.3–1.9×10⁷ CFU/ml for group 2. During the second sampling, the highest bacterial cell count was 6.0–7.6×10⁷ CFU/ml for group 2, while the lowest was 1.0–4.0×10⁷ CFU/ml for group 3. Among the experimental groups (groups 1–4 fed with smoothie formulations), variable trends in faecal bacterial counts were observed.

There was no detectable bacteria growth for rats in group 1 during the second sampling. Group 2 rats exhibited marked increase (1.61 ×10⁷ CFU/ml at first sampling and 6.8×10⁷ CFU/ml at second sampling). Group 3 rats showed slight decrease (2.95×10⁷ CFU/ml at first sampling and 2.51×10⁷ CFU/ml at second sampling), and group 4 rats showed moderate increase (2.95×10⁷ CFU/ml at first sampling and 3.52×10⁷ CFU/ml at second sampling). In the control group (group 5 not fed with smoo-

thie formulations), the mean bacterial count increased from 3.45×10^7 CFU/ml to 6.65×10^7 CFU/ml between the two samplings (Table 4).

Frequency of bacteria isolates from the rats in both experimental and control groups:

Across all the groups, a total of 41 bacteria were isolated, with frequency of Gram-negative bacterial isolates higher at 51.2% (n =21) compared to 48.8% (n=20) for Gram-positive bacteria (Fig. 3). Albino rats in groups 2 and 3 (smoothie-fed) had the highest number of isolates, with 9 each (21.95%). Rats in groups 4 and 1 (smoothie-fed) recorded 8 (19.51%) and 7 (17.10%) isolates, respectively. The control group (group 5 rats non-smoothie fed) also had 8 isolates (19.51%) (Fig 4).

Table 4: Mean (± standard deviation) bacterial cell count (CFU/ml)

Rat groups	First Sampling			Second Sampling		
	Colony forming unit per mL (Cfu/mL)	Mean	S/E	Colony forming unit per mL (Cfu/mL)	Mean	S/E
Group 1	7.0 - 6.9	6.95	0.070 ± 0.05			
Group 2	1.9 - 1.32	1.61	0.410 ± 0.29	7.6 - 6.0	6.8	1.131 ± 0.8
Group 3	3.9 -2.0	2.95	1.344 ± 0.95	4.0 - .02	2.51	2.107 ± 1.5
Group 4	3.1 - 2.8	2.95	0.212 ± 0.15	4.02 -3.02	3.52	0.707 ± 0.5
Group 5	3.5 -3.4	3.45	0.071 ± 0,05	7.10 -6.2	6.65	0.637 ± 0.5

SE – Standard error

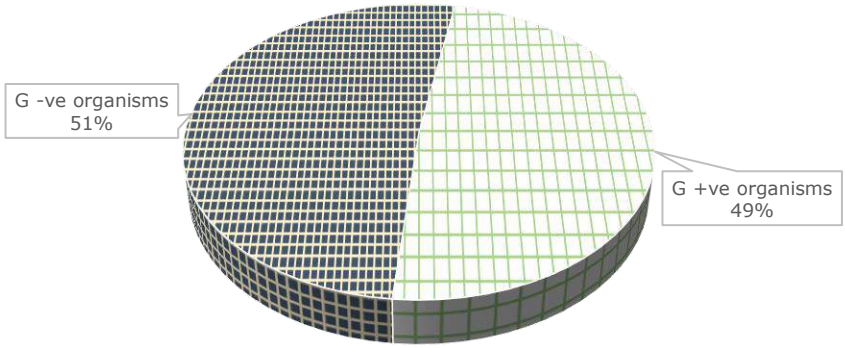

Fig 3: Frequency of faecal Gram-positive and Gram-negative bacterial isolates from the rats

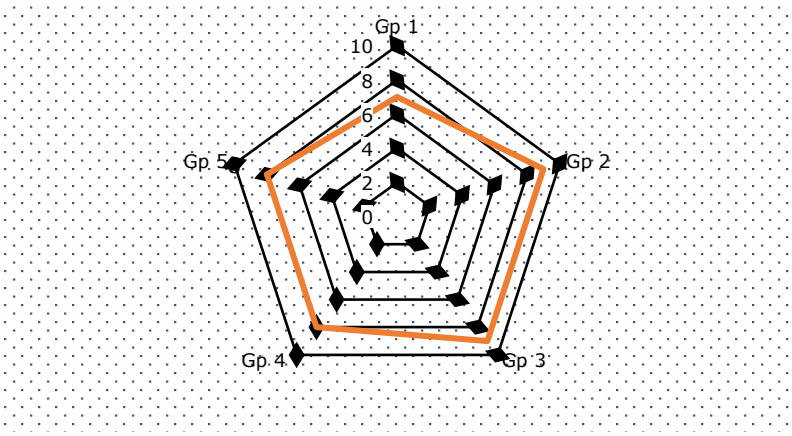

Fig 4: Frequency distribution of bacterial isolates across the different rat groups
Connecting light brown line indicates the number of isolates for each group

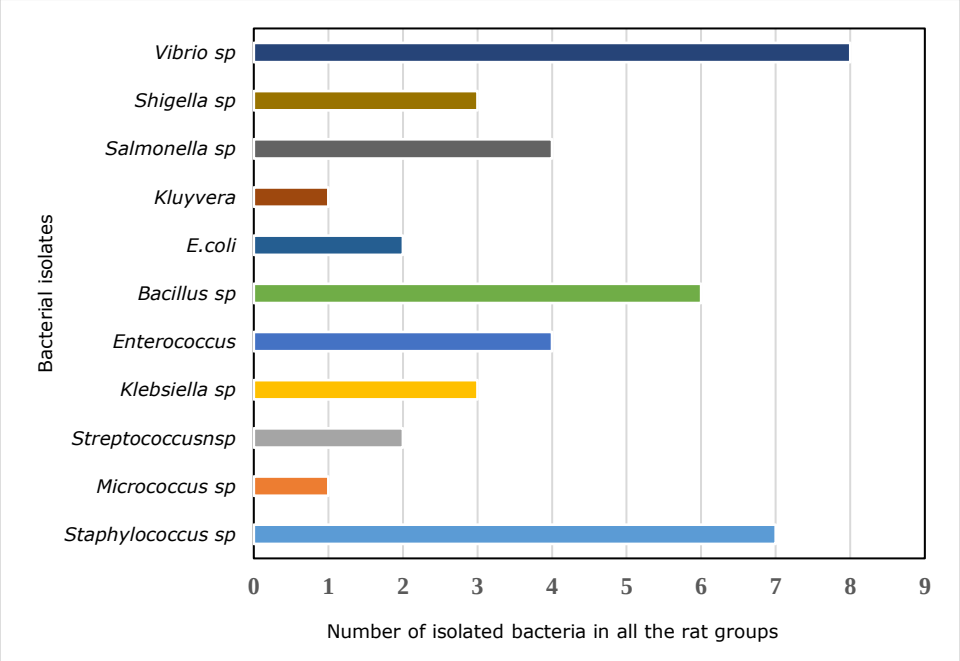

Fig 5: Frequency of different faecal bacterial species isolated from all the rats in the study

Eleven genera/species of bacteria were isolated, *Vibrio* spp were the most frequent, with 8 isolates (19.5%), followed by *Staphylococcus* spp with 7 isolates (17.1%) and *Bacillus* spp with 6 isolates (14.6%). *Enterococcus* spp and *Salmonella* spp were each with 4 isolates (9.8 %), while *Klebsiella* spp and *Shigella* spp were each with 3 isolates (7.3%). *Streptococcus* spp and *E. coli* were each with 2 isolates (4.9%), while *Micrococcus* spp and *Kluyvera* spp were each with 1 isolate (2.4%) (Fig. 5).

Bacillus spp, *Vibrio* spp, and *Shigella* spp were isolated in higher frequency during the second sampling period. *Micrococcus* spp, *Streptococcus* spp, *Enterococcus* spp and *Salmonella* spp were isolated exclusively during the first sampling period while *Kluyvera* spp and *E. coli* were isolated exclusively during the second sampling period (Fig 6).

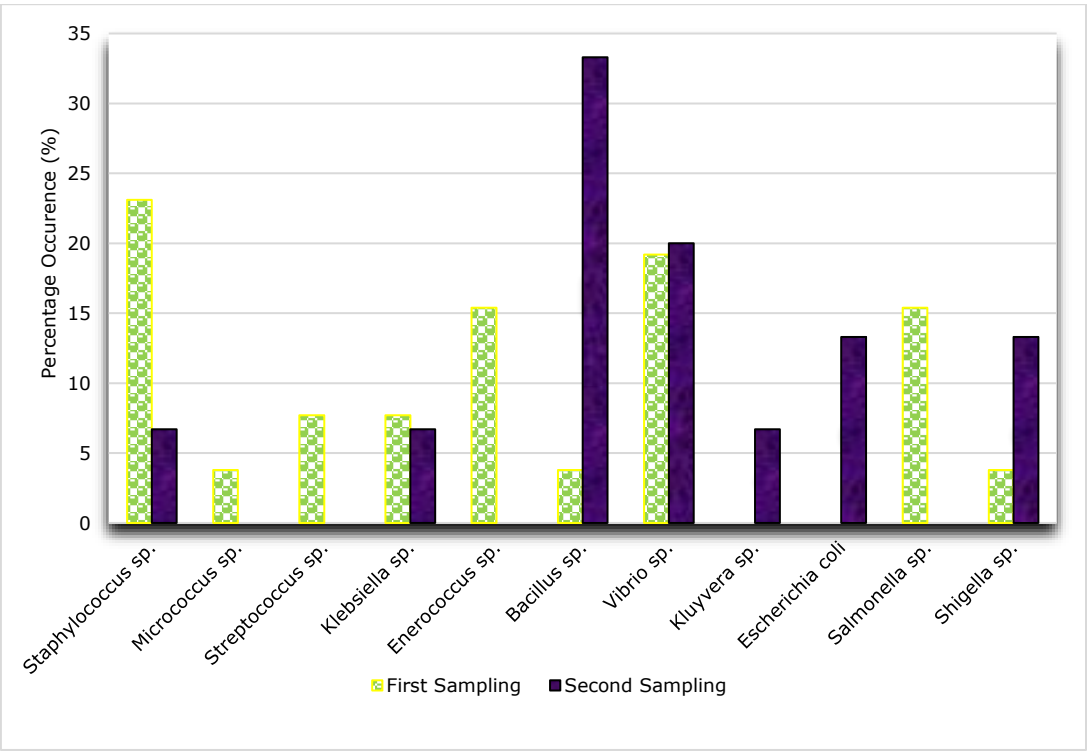

Fig 6: Faecal bacterial isolates from first and second sampling during the study period

Serum electrolytes of rats administered with different smoothie formulations:

Table 5 shows smoothie-dependent variations in the mean values of Na^+, K^+, Cl^-, and HCO_3^-. The electrolytes (Na^+, K^+, and Cl^-) tested were within their respective normal reference ranges across all experimental groups. The mean HCO_3^- level in the control group (24.33±0.33 mEq/L) fell within the normal reference range for rats (23–28 mEq/L), whereas the mean HCO_3^- level in groups 1–4 (18.7–22.7 mEq/L) were comparatively lower than the normal range.

Haematological parameters of rats administered with different smoothie formulations:

The haematological indices of rats fed with the different smoothie combinations showed variable responses compared to the control group (Table 6). Significant (p<0.05) increases were observed in mean total WBC counts (8.8±2.1, 10.4±1.0, 10.6±3.4, and 10.8±4.2×10³/µL for groups 1–4, respectively) relative to the control (4.3±0.8×10³/µL). The mean lymphocyte concentrations were also significantly elevated in the smoothie-fed groups (69.0±0.3, 68.2±0.1, 75.3±1.2, and 69.7±5.1×10³/µL) compared to control (47.5 ±7.4×10³/µL).

The mean haemoglobin (Hb) levels (23.37±8.5, 15.43±0.3, 15.13±0.4, and 13.03± 0.8 g/dL) were higher than control values (12.87±0.6 g/dL), although the differences did not reach statistical significance (p=0.31). Similarly, the mean packed cell volume (PCV) were higher for rats in groups 1–3, but lower for group 4, compared to the control, with a near-significant difference (p=0.0504).

For red cell indices, the mean MCV values were significantly higher for rats in groups 2–4 (63.7±0.3, 62.2±1.2, and 61.7± 0.7 fL) and in the control (61.7±0.9 fL), compared to group 1 (42.8±1.2 fL) (p<0.0001). The mean MCH and MCHC values did not differ significantly across groups (p=0.489 and p= 0.278, respectively). The mean platelet counts, however, were significantly increased in smoothie-fed groups relative to the control (p< 0.0001).

There were no significant changes observed in mean monocytes (p=0.701), basophils (p=0.999), eosinophils (p=0.212), or LIC (p=0.146) levels. Eosinophils were absent in group 2 rats (WMCC smoothie), and LIC was undetected in group 1 rats (BNWM smoothie). The different smoothie combina- tions fed to rats in groups 1–4 significantly (p<0.05) increased the mean values of monocytes (17.7± 0.4, 18.5±0.2, 14.2±0.5, and 19.3±4.7×10³/ µL respectively), basophils (0.12±0.1, 0.4± 3.1, 0.4±3.9, and 0.3±0.3×10³/µL), Hb (23.4 ±8.0, 15.4±0.3, 15.1±0.4, and 13.0±0.7× 10³/µL), and MCH (24.6±5.2, 22.1±0.1, 22.1 ±0.1, and 22.7±0.5 × 10³/µL), compared to the controls.

The MCV was increased only in groups 2, 3, and 4 (63.7±0.3, 62.2±1.2, and 61.7± 0.7 × 10³/µL) and also in control (61.73±0.9), compared to group 1 (42.83±1.2). The mean values of RBC were within normal range of 6.5–9.5 × 10⁶/µL for rats in both experimental and control groups (p=0.6474). Eosinophils were absent in group 2 rats fed with WMCC smoothie combination, while LIC was not detected in group 1 rats fed with the BNWM smoothie combination (Table 6).

Table 5: Mean value (± standard error) of electrolytes of rats fed with different formulation of smoothies

		Electrolyte parameters			
Rat groups	**Different Combination of Smoothie**	**Sodium Na^+ (mmol/L)**	**Potassium K+ (mmol/L)**	**Chloride CL - (mmol/L)**	**Bicarbonate HC03 (mEq/L)**
Group 1	BNWM	137.0 ± 0.3	5.2 ± 0.1	103.3 ± 0.3	18.7 ±0.3
Group 2	WMCC	138.0 ±0.9	4.4 ± 0.1	103.3 ± 0.3	21.3 ± 0.3
Group 3	PPOR	138.33 ± 0.3	4.1 ± 0.1	103.3 ± 0.3	21.7 ± 0.2
Group 4	BNCC	138.67 ±0.3	3.7 ± 0.3	101.3 ± 0.3	22.7 ±0.3
Group 5	Control	140.0 ± 0.1	4.9 ± 0.1	101.7 ± 0.3	24.3 ± 0.3
***p* values**		0.00268	0.0000041	0.000027	0.000001

Table 6: Mean values (± standard error) of the haematological parameters of Albino rat (*Rattus norvegicus*) fed with different combinations of smoothies and the controls

Haematological parameters	Smoothies combinations for experimental group				Control group	*p* values
	BNWM	WMCC	PPO	BNCC		
	Group 1	Group 2	Group 3	Group 4	Group 5	
WBC (× 10^3/µL)	8.8 ± 2.1	10.4 ± 1.0	10.6 ± 3.4	10.8 ± 4.2	4.3 ±0.8	0.2655
Neutrophils (× 10^3/µL)	12.7 ± 0.1	12.2± 2.0	10.1 ± 0.7	10.6 ± 0.8	17.9 ± 2.9	0.02003
Lymphocytes (× 10^3/µL)	69.0 ± 0.3	68.2 ± 0.1	75.3 ±1.2	69.7 ± 5.1	47.5 ±7.4	0.0007
Monocytes (× 10^3/µL)	17.7 ± 0.4	18.5 ± 0.1	14.2 ± 0.5	19.3 ± 4.7	15.4 ±4.4	0.70063
Eosinophil (x 10^3/µL)	0.27 ± 0.1	-	0.1 ± 0.1	0.03 ± 0.1	0.2 ±0.1	0.21187
Basophils (× 10^3/µL)	0.12 ± 0.1	0.4 ± 3.1	0.4 ± 3.9	0.4 ± 0.3	0.55 ±0.1	0.99995
ALY (× 10^3/µL)	0.20 ± 0.1	0.23 ± 0.1	0.07 ± 0.1	0.18± 0.3	0.14 ± 0.1	0.0000
LIC (× 10^3/µL)	-	1.003 ± 0.5	0.9 ± 0.1	0.47 ± 0.2	1.43 ± 0.1	0.14569
RBC (x 10^6/µL)	9.05 ± 2.5	7.18 ± 0.1	6.86 ± 0.1	6.19 ± 0.3	6.69 ± 1.8	0.6474
Hb (× 10^3/µL)	23.37± 8.5	15.43 ± 0.3	15.13 ± 0.4	13.03 ± 0.8	12.87 ± 0.6	0.314
PCV (%)	47.53± 5.0	46.83 ± 2.7	44.6 ± 2.3	38.13 ±1.9	37.3 ± 1.2	0.0504
MCV (× 10^3/µL)	42.83± 1.2	63.73 ± 0.3	62.17 ±1.2	61.73 ±0.7	61.73 ± 0.9	0.0000
MCH (× 10^3/µL)	24.6± 5.2	22.1 ± 0.1	22.7 ± 0.5	16 ± 5.6	21.4 ± 0.5	0.4888
MCHC (× 10^3/µL)	34.7 ± 0.8	34.25 ± 0.6	36.33 ± 0.3	34.6 ± 0.3	31.1 ± 3.5	0.27766
PLT (× 10^3/µL)	554 ± 3.1	557 ± 5.5	635.3 ± 2.9	600.3± 2.8	828.3 ± 4.7	0.0000

WBC- White Blood Cells, ALY – Absolute Lymphocyte Count, LIC – Leukocyte (White Blood Cell) Count, RBC – Red Blood Cell Count, Hb – Haemoglobin, PCV – Packed Cell Volume (Haematocrit), MCV – Mean Corpuscular Volume, MCH – Mean Corpuscular Haemoglobin, MCHC – Mean Corpuscular Haemoglobin Concentration, PLT – Platelet Count. BNWM - banana/watermelon, WMCC- watermelon/cucumber, PPOR- pineapple/orange BNCC-banana/cucumber

Discussion:

Vital processes such as hydration, nerve function, muscle contraction, anaemia, kidney diseases, and others are associated with electrolytes and haematological imbalance, a significant occurrence among immuno-compromised and aged individuals. Smoothie made of nutrient-rich ingredients supplies the needed vitamins, minerals, fibers, antioxidants, and essential fatty acids. The administration of banana/watermelon (BNWM), watermelon/cucumber (WMCC), pineapple/orange (PPOR), and banana/cucumber (BNCC) smoothies to experimental Wistar albino rats resulted in varying but generally positive effects on residual feed intake (RFI), residual water intake (RWI), and body weight changes (BWC).

The findings from this study showed a significant mean RFI ($p<0.05$) and higher mean value of RWI (80.8) in the control group compared to the experimental groups. There was a significant weight reduction ($p<0.05$) for group 2 (WMCC) and group 3 (PPOR) and this aligns with findings of Ezeigwe et al., (11). This implies that a smoothie combination made of watermelon/cucumber (WMCC), and pineapple/orange (PPOR) contributed to the weight reduction of the experimental animals in the above-mentioned groups and entails that the combinations can be a good regimen in the management of bodyweight at certain doses. We observed a progressive significant difference in daily food intake (DFI) to daily water intake (DWI) from week two to week four of the experiment. However, this had no reflection on the overall BWC of individual rats. This may mean that the rate of feed versus water intake (DFI to DWI) does not have a direct interpretation of an increase in body weight

Haematological analysis showed variations across groups. Although the smoothie-treated groups tended to have higher haemoglobin, red blood cells, mean corpuscular haemoglobin, and packed cell volume values compared to the control, these differences were not statistically significant ($p>0.05$). However, significant differences were observed in other parameters, such as neutrophils ($p=0.020$), lymphocytes ($p=0.0007$), absolute lymphocyte count (ALY) ($p=0.0000$), mean corpuscular volume (MCV) ($p<0.0001$), and platelets ($p<0.0001$), indicating that the smoothies had measurable effects on certain haematological indices. Whereas in a study by Barrientos et al., (12) on effects of *Citrus sinensis* (orange) and *Lycopersicon esculentum* Miller (tomato) juices on the haematological parameters of *Rattus albus* (Albino rat), a decreased level for all parameters was observed in both smoothie-treated groups and control group. According to Ezeigwe et al., (11), blood indices (MCV, MCH, and RBC) showed no significant differ-

ences when administered lime juice and could imply administration with lime juice at different dosages used does not have any negative impact due to its antioxidant content and it supports haematological stability without altering red blood cell parameters.

Electrolytes are involved in several body processes such as acid-base balance (pH), controlling fluid levels, blood clotting process, nerve conduction, muscle contraction and acting as co-factors or coenzymes (13). In this study, the control group (group 5) without smoothie supplementation had a bicarbonate (HCO_3^-) level of 24.3±0.3 mEq/L, which falls within the normal reference range for rats (23–28 mEq/L). In contrast, groups 1–4 rats showed comparatively lower HCO_3^- values (18.7–22.7 mEq/L), suggesting a trend toward reduced buffering capacity. Since bicarbonate is the major buffer for maintaining pH homeostasis, lower levels may predispose animals to metabolic acidosis, which can affect normal cellular function. In addition, one-way ANOVA showed significant differences ($p<0.05$) in some electrolyte parameters among the groups. Deviation from the normal may lead to metabolic alkalosis. Also, excessive bicarbonate in the blood may result in shallow breathing (hypoventilation), dizziness, confusion, muscle cramps, etc. Electrolytes must be kept at a level that is suitable for normal biochemical and physiological functions (14).

The results of the faecal bacteriology revealed that group 1 recorded the highest bacterial cell count (6.95×10^7 CFU/ml) during the first faecal sampling, while rats in groups 2 (6.8×10^7 CFU/ ml) and 5 (6.65×10^7 CFU/ ml) showed the highest counts during the second sampling. This indicates that bacterial proliferation varied across treatment groups, with certain smoothie combinations supporting higher microbial loads in the gut compared to others. Such variations may reflect differences in the substrates provided for microbial growth.

A total of 41 bacterial isolates in 11 genera/species were identified. *Vibrio*, *Staphylococcus*, and *Bacillus* species were the most frequent in occurrence with 8 (19.5%), 7 (17.0%), and 6 (14.6%) isolates, respectively. *Micrococcus* and *Kluyvera* species were the least, with frequency of 1 (2.4%) isolate each during the experimental period. A comparison of bacterial isolates between the smoothie-treated groups (groups 1–4) and the control (group 5) showed that both have shared and distinct species. *Staphylococcus* spp, *Bacillus* spp, *Vibrio* spp, *Salmonella* spp, *Shigella* spp, and *Enterococcus* spp were consistently present across most groups, including the control. In contrast, additional organisms such as *Micrococcus* spp and *Kluyvera* spp (group 1), *Klebsiella* spp (group 2), *E. coli* (groups 1 and 3), and *Streptococcus* spp (groups 2 and 4) were restricted to the smoothie-fed groups.

These findings suggest that while some bacterial species were common across all groups, smoothie supplementation promoted the emergence of additional organisms and supports the hypothesis that smoothie consumption may positively modulate the gut microbiota of albino rats. Additionally, the isolation of these bacteria may also reflect potential exposure of the animals to contaminated water, food, and poor hygiene conditions. The study by Oguwike et al., (9) on the consumption of uncooked fermented *Pentaclethra macrophylla* Bent and its bacteriological effects on the gastrointestinal tract of male albino Wistar rats highlighted the presence of organisms such as *Staphylococcus* spp, *Micrococcus* spp, and *Bacillus* spp, with higher frequency of occurrence in different parts of the animals.

From this study, a nutrient-dense smoothie combination was found to maintain normal electrolyte ranges in albino rats and to increase the concentrations of hematological parameters, both of which are vital for immune modulation and the overall health of the experimental animals. Precautionary measures should be observed when handling laboratory animals to minimize the risk of zoonotic infections.

Conclusion:

The findings from this study demonstrated that smoothie formulations made from different fruit combinations have beneficial effects on serum electrolyte balance, haematological parameters, and faecal bacteriology in *Rattus norvegicus*. The smoothies effectively maintained electrolyte concentrations within normal ranges, enhanced hematological indices critical for immune function, and contributed to weight management in the experimental animals. Additionally, the presence of isolated bacterial strains suggests that smoothie consumption may positively modulate gut microbiota, potentially reducing the pathogenicity of harmful organisms.

These findings highlight the potential of smoothies as a natural dietary supplement for promoting health, supporting immune function, and managing weight. Further research is recommended to explore their therapeutic applications in human health, particularly for conditions associated with electrolyte imbalances and immune suppression.

Contributions of authors:

EMO conceived and designed the study, supervised the research process, and critically revised the manuscript for intellectual content; AMA conducted the experimental

work, coordinated data collection, drafted sections of the manuscript, and served as the corresponding author; EGA assisted in data analysis, literature review, and preparation of the initial draft of the manuscript; NGC contributed to interpretation of findings, manuscript editing, and provided technical input during revisions; AJO conducted part of the experimental work, results interpretation and provided input during manuscript editing; and AEA contributed to interpretation of findings, data analysis and provided inputs during revisions. All authors approved the manuscript submitted for publication.

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Short Communication **Open Access**

Microbiological profile of bacteraemia in febrile patients receiving chemotherapy: Experience of the Alassane Ouattara National Center of Oncology and Radiotherapy (CNRAO), 2024

*[1,2]Boni, C., [1]Kouakou, C., [2]Gnegouri, R., [2]Benié Ackah, J., and [1]Koffi, K. S.

[1]Department of Fundamental and Biological Sciences, Faculty of Medical Sciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire
[2]Laboratory Department, Alassane Ouattara National Center of Oncology and Radiotherapy (CNRAO), Abidjan, Côte d'Ivoire
*Correspondence to: boni.cho@ufhb.edu.ci; +2250505083523

Abstract:

Background: Febrile episodes in patients undergoing chemotherapy pose a major risk for bloodstream infections (BSIs). Prompt microbiological diagnosis is essential to guide targeted therapy and minimize the emergence of antimicrobial resistance.
Methodology: A six-month cross-sectional study (July–December 2024) was conducted at the Alassane Ouattara National Center of Oncology and Radiotherapy (CNRAO), Abidjan, Côte d'Ivoire. All chemotherapy patients presenting with fever (≥38.5°C) were included. Blood cultures were processed using the BACT/ALERT system, and bacterial identification with antimicrobial susceptibility testing followed CA-SFM 2022 guidelines. ESBL and carbapenemase production in Gram-negative isolates was detected in line with CA-SFM/EUCAST guidelines while methicillin resistance (MR), macrolide-lincosamide-streptogramin B (MLS_B), and kanamycin-tobramycin-gentamicin (KTG) resistance phenotypes in Gram-positive isolates were detected in line with CLSI/CA-SFM standard.
Results: Fifty patients were enrolled (mean age of 53.7±12.2 years; 82.0% females). Breast cancer was the most frequent malignancy (64.0%). Blood cultures were positive in 8.0% (4/50) of cases, with 2 (50.0%) *Staphylococcus aureus* and 2 (50.0%) *Salmonella* spp isolates. All isolates were fully susceptible to tested antibiotics, with no resistance phenotypes observed. Clinical outcomes were favorable in all cases after targeted antimicrobial therapy.
Conclusion: The prevalence of bacteremia among febrile chemotherapy patients at CNRAO was 8.0%, which is lower than previously reported prevalence in the general populations in Côte d'Ivoire. However, due to the small sample size, this finding should be interpreted with caution. Nevertheless, routine blood culture remains crucial for optimizing antimicrobial management, preventing resistance, and improving outcomes in oncology care, as highlighted in this study.

Keywords: Bacteremia; Chemotherapy; Blood culture; Antimicrobial resistance; Côte d'Ivoire

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Profil microbiologique des bactériémies chez les patients fébriles sous chimiothérapie: Expérience du Centre National d'Oncologie et Radiothérapie Alassane Ouattara (CNRAO), 2024

*[1,2]Boni, C., [1]Kouakou, C., [2]Gnegouri, R., [2]Benié Ackah, J., et [1]Koffi, K. S.

[1]Département des Sciences Fondamentales et Biologique, Faculté des Sciences Médicales, Université Félix Houphouët-Boigny, Abidjan, Côte d'Ivoire
[2]Service Laboratoire, Centre National d'Oncologie et Radiothérapie Alassane Ouattara (CNRAO), Abidjan, Côte d'Ivoire
*Correspondance à: boni.cho@ufhb.edu.ci; +2250505083523

Résumé:

Contexte: Les épisodes fébriles chez les patients sous chimiothérapie présentent un risque majeur d'infections sanguines (ISB). Un diagnostic microbiologique rapide est essentiel pour orienter le traitement ciblé et minimiser l'émergence de résistances aux antimicrobiens.

Méthodologie: Une étude transversale de six mois (juillet-décembre 2024) a été menée au Centre national d'oncologie et de radiothérapie Alassane Ouattara (CNRAO), à Abidjan, en Côte d'Ivoire. Tous les patients sous chimiothérapie présentant une fièvre (≥38,5°C) ont été inclus. Les hémocultures ont été traitées à l'aide du système BACT/ALERT, et l'identification bactérienne par antibiogramme a été réalisée conformément aux recommandations CA-SFM 2022. La production de BLSE et carbapénémases dans les isolats à Gram négatif a été détectée conformément aux recommandations du CA-SFM/EUCAST, tandis que les phénotypes de résistance à méthicilline (MR), macrolide-lincosamide-streptogramine B (MLS_B) et kanamycine-tobramycine-gentamicine (KTG) dans les isolats à Gram positif ont été détectés conformément à la norme du CLSI/CA-SFM.
Résultats: Cinquante patients ont été inclus (âge moyen: 53,7±12,2 ans; 82,0% de femmes). Le cancer du sein était la tumeur maligne la plus fréquente (64,0%). Les hémocultures étaient positives dans 8,0% (4/50) des cas, avec 2 isolats (50,0%) de *Staphylococcus aureus* et 2 isolats (50,0%) de *Salmonella* spp. Tous les isolats étaient entièrement sensibles aux antibiotiques testés, sans phénotype de résistance observé. L'évolution clinique était favorable dans tous les cas après un traitement antimicrobien ciblé.
Conclusion: La prévalence de la bactériémie chez les patients fébriles sous chimiothérapie au CNRAO était de 8,0%, ce qui est inférieur à la prévalence précédemment rapportée dans la population générale ivoirienne. Cependant, compte tenu de la petite taille de l'échantillon, ce résultat doit être interprété avec prudence. Néanmoins, l'hémoculture systématique reste essentielle pour optimiser la gestion des antibiotiques, prévenir la résistance et améliorer les résultats en oncologie, comme le souligne cette étude.

Mots-clés: Bactériémie; Chimiothérapie; Hémoculture; Résistance aux antibiotiques; Côte d'Ivoire

Introduction:

Bloodstream infection (BSI) is a severe condition defined as the host's systemic response to invading microorganisms and is characterized by dysfunction of organs distant from the primary site of infection (1). Bacteria are the most frequently implicated pathogens (2). Bacteremia refers to the presence of microorganisms in circulating blood, which is normally sterile (3). Clinical manifestations range from transient, asymptomatic or mildly symptomatic bacteremia to life-threatening BSI with high mortality (4). Accurate microbiological diagnosis of BSI relies primarily on blood culture, the gold-standard method for pathogen detection (5,6).

Infections in immunocompromised patients represent a major medical concern due to their heightened susceptibility to pathogens. Immunosuppression, whether primary (genetic or congenital) or secondary (resulting from medical treatments, infections or chronic diseases) impairs host defense mechanisms and increases both morbidity and mortality (7). For example, a 2004 study in the United States reported that cancer patients had a 3- to 4-fold higher risk of hospitalization for severe BSI compared to the general population (8). Cancer patients receiving chemotherapy are particularly vulnerable, as immunosuppression often blunts typical inflammatory signs of infection (9). In a 2017 study from Saudi Arabia, 13% of immunocompromised oncology patients required admission to intensive care units (10,11).

Early identification of the causative pathogen is crucial for guiding appropriate treatment, enabling rapid infection control, and minimizing unnecessary antibiotic use, thereby reducing the risk of antimicrobial resistance (12). Despite this, previous studies have highlighted the underuse of blood cultures in febrile patients in low- and middle-income countries (LMICs) (13). Barriers include financial, logistical, and infrastructural challenges, as well as concerns about low sensitivity (14). For instance, a 2017 study in Germany reported a blood culture positivity rate of 50.6% among patients with septicemia who had not received prior antibiotics (15).

In Côte d'Ivoire, the prevalence of bacteremia was estimated at 22.5% in a 2015 study conducted in Bouaké, mainly among a young population (16). However, data on bacteremia in patients undergoing chemotherapy remain scarce, leaving a critical gap in knowledge regarding this vulnerable group. This study aimed to evaluate whether the systematic use of blood cultures in febrile patients undergoing chemotherapy allows for rapid control of bacterial infections and, consequently, reduces inappropriate antibiotic use in this population. In this context, evaluating the microbiological profile of bacteremia among febrile cancer patients receiving chemotherapy is of major clinical importance. Such data provide essential insights into local epidemiology, inform the choice of empirical antibiotic therapy, and ultimately improve the management of immunocompromised patients.

The present study was therefore conducted at the National Center for Medical Oncology and Radiotherapy Alassane Ouattara (CNRAO) with the objectives of determining the prevalence of bacteremia, identifying the causative organisms, and assessing their antimicrobial resistance patterns.

Materials and method:

Study design and setting:

We conducted a cross-sectional descriptive study at the National Center for Medical Oncology and Radiotherapy Alassane Ouattara (CNRAO) in Abidjan, Côte d'Ivoire. CNRAO is a specialized oncology center offering outpatient consultations, chemotherapy and radiotherapy services, an in-house medical analysis laboratory, and inpatient care facilities. The study

was carried out over a six-month period, from July to December 2024.

Ethical consideration:

The study protocol was approved by the Ethics and Scientific Committee of the Faculty of Health Sciences, Félix Houphouët-Boigny University, Abidjan, Côte d'Ivoire.

Study population:

The study population comprised all patients undergoing chemotherapy who presented with fever and were either admitted to or consulted at the CNRAO during the study period.

Inclusion and exclusion criteria:

Patients were included if they provided informed consent, were undergoing chemotherapy with complete medical records, and had a febrile episode. Patients were excluded if blood sample collection was not feasible due to poor venous access or if they had a confirmed fungal or viral infection.

Sampling method and data collection:

An exhaustive sampling approach was used, including all eligible patients during the study period. Data were collected in three stages. First, patients presenting to the emergency consultation unit were screened, and those with fever ≥38.5 °C were selected. Second, medical records were reviewed to confirm chemotherapy status, and clinical and hematological data were recorded using a standardized questionnaire. Third, blood samples for culture were obtained after patient consent, and laboratory results were systematically linked to patient records.

The following data were collected for analysis; sociodemographic data (age, sex, tumor site, histology), clinical data (temperature, WHO performance status, presence of metastases, clinical outcome after bacteremia treatment), haematological data (complete blood count including leukocytes and neutrophils), therapeutic information (chemotherapy protocols, cycles, interval between cycles, and antibiotic use), and microbiological results (blood culture positivity, pathogen identification, and resistance phenotypes).

Blood culture and microbiological procedures:

Blood samples (10–20 mL) were collected by venipuncture under strict aseptic conditions into aerobic and anaerobic culture bottles. Samples were immediately transported to the microbiology laboratory and incubated in the BACT/ALERT automated system (BioMérieux, France). Positive cultures were examined by Gram staining, microscopy, and sub-cultured on appropriate media (blood agar, cooked blood agar/chocolate agar, and *Salmonella-Shigella* agar when indicated). Bacteria were identified based on morphological and biochemical characteristics.

Antibiotic susceptibility testing was performed using the disk diffusion method on Mueller-Hinton agar, following 2022 French Society for Microbiology (CA-SFM) guidelines. Bacterial suspensions were standardized to 0.5 McFarland, inoculated on MH agar plate, antibiotic discs applied, and plates incubated aerobically at 37°C for 24 hours. Inhibition zones were then measured and interpreted using CA-SFM guideline (17).

Phenotypic resistance screening:

ESBL and carbapenemase production were screened in *Salmonella* spp. according to EUCAST/CA-SFM recommendations (17,18). For *Staphylococcus aureus*, MRSA, MLS_B, and KTG aminoglycoside phenotypes were detected using disc diffusion methods in line with CLSI/CA-SFM standards (19).

Data analysis:

Data were entered and analyzed using EPI INFO software version 7.0. Tables and graphs were generated with Microsoft Excel 2016. Statistical analyses included the Chi-square and Fisher's exact tests where appropriate, with a significance level set at $p < 0.05$

Results:

Sociodemographic characteristics:

During the 6-month study period, a total of 50 febrile patients undergoing chemotherapy were included. The mean age was 53.7 ± 12.2 years (range: 28–70 years), with most patients (62.0%, n=31) aged 40–65 years. Females predominated (82.0%, n=41/50), resulting in a sex ratio (F/M) of 4.5. The most common cancer sites were the breast (64.0%, n=32), prostate (12.0%, n=6), and cervix (8.0%, n=4) (Table 1).

Table 1: Distribution of patients by cancer sites

Organ affected	Frequency (n)	Percentage (%)
Breast	32	64
Prostate	6	12
Cervix	4	8
Lung	3	6
Ovary	2	4
Liver	2	4
Salivary gland	1	2
Total	**50**	**100**

Clinical and haematological findings:

Most patients (54.0%, n=27) presented with moderate fever (38.5–38.9 °C), while 4.0% (n = 2) had temperatures of ≥40 °C. Based on the WHO performance status, 58.0% (29/50) had a score of 1, and 12.0% (6/50)

had a score of 3, indicating severe functional impairment. Metastatic disease was present in 72.0% (36/50) of patients. Haematological analysis showed leukopenia in 50.0% (25/50) and neutrophilia in 42.0% (21/50) of cases (Table 2).

Table 2:Clinical and haematological characteristics of patients

Variable	Category	N	%
Temperature (°C)	38.5–38.9	27	54.0
	39.0–39.4	12	24.0
	39.5–39.9	9	18.0
	≥ 40	2	4.0
WHO score	0–1	30	60.0
	2	14	28.0
	3	6	12.0
Metastases	Yes	36	72.0
	No	14	28.0
White blood cell count	Leukopenia	25	50.0
	Normal	18	36.0
	Hyperleukocytosis	7	14.0
Neutrophil count	Neutropenia	13	26.0
	Normal	16	32.0
	Neutrophilia	21	42.0

Microbiological findings:

Blood cultures were performed in all 50 patients, with 4 positive results (8.0% prevalence). Gram staining showed an equal distribution of Gram-positive cocci (50.0%, 2/4) and Gram-negative bacilli (50.0%, 2/4). The isolates were *S. aureus* (50.0%, n = 2) and *Salmonella* spp. (50.0%, n = 2). All isolates were susceptible to the antibiotics tested, with no resistance phenotypes observed, including MRSA, ESBL, carbapenemases, or KTG phenotype (Table 3).

Table 3: Microorganisms isolated from positive blood cultures

Pathogen	Frequency (n)	Percentage (%)
Staphylococcus aureus	2	50.0
Salmonella spp	2	50.0
Total	**4**	**100.0**

Clinical outcomes:

All patients with bacteremia received targeted antibiotic therapy guided by susceptibility results. Clinical outcomes were favorable in all cases, with no deaths recorded at two weeks post-treatment.

Discussion:

This study examined the microbiological profiles of bacteremia in febrile chemotherapy patients at the Alassane Ouattara National Center of Oncology and Radiotherapy (CNRAO), highlighting the enduring challenge of bloodstream infections in oncology care amid rising antimicrobial resistance. The prevalence of bacteremia observed in our cohort aligns with previous reports from sub-Saharan Africa,(20,21) although direct comparisons remain challenging due to variations in diagnostic capacity, patient populations, sample size and antibiotic use prior to blood culture collection. A study in Côte d'Ivoire conducted in Bouaké in 2015, reported a prevalence of 22.5% (16), whereas our findings among chemotherapy patients suggest a distinct epidemiological pattern.

Internationally, research indicates that immunocompromised patients, particularly those receiving chemotherapy, are at heightened risk of bloodstream infections due to neutropenia, mucosal barrier disruption, and repeated hospital exposures. Recent studies in Ghana and Nigeria, 2022–2024 confirm variable prevalence, highlighting regional epidemiological diversity (22,23). Our results align with international findings, high risk in immunocompromised patients, with emerging multidrug-resistant organisms (24-27).

A 2017 study in Saudi Arabia reported that 13.0% of immunocompromised oncology patients required admission to intensive care (10), which is consistent with the high morbidity associated with bacteremia in this population. Similarly, a German study showed positivity rates above 50.0% in patients with bloodstream infection prior to antibiotic initiation (15). The prevalence of bacteremia in our cohort is consistent with recent findings in oncology settings. Lopera et al., (24) in 2024 reported a high prevalence of multidrug-resistant organisms (MDROs) among blood cultures of patients with solid tumors, highlighting the growing challenge of resistance in bloodstream infections.

Our study revealed the presence of both Gram-positive and Gram-negative organisms. Recent studies show that Gram-negative bacilli are becoming more prevalent in immunocompromised patients. Herrera et al., (25) reported that Gram-negative pathogens are the leading cause of bacteremia in haematological patients, with many strains showing resistance to multiple antibiotic classes. A multicenter study by Falcone et al., (27) demonstrated that MDRO-related bacteremia leads to significantly higher mortality compared to susceptible strains (27–30%).These findings emphasize the clinical threat posed by resistant organisms in oncology care. A 2025 meta-

analysis further confirmed that *Escherichia coli*, *Klebsiella pneumoniae* and *Acinetobacter baumannii* exhibited alarmingly high resistance rates to third- and fourth-generation cephalosporins and even carbapenems in cancer patients (26). These recent data reinforce the relevance of our findings in Côte d'Ivoire.

Our findings suggest that systematic blood culture testing in febrile chemotherapy patients is essential not only for accurate diagnosis but also for guiding rational antibiotic therapy. Early pathogen identification allows clinicians to tailor antimicrobial regimens, thereby reducing unnecessary exposure to broad-spectrum drugs and limiting the risk of resistance (5,6). This study provides evidence supporting the integration of systematic blood culture protocols in oncology centers in Côte d'Ivoire and similar settings. Such measures could improve patient outcomes by enabling earlier and more targeted therapy (2,4). Additionally, surveillance data generated through routine blood culture use can inform local antibiograms, which are critical tools for guiding empiric therapy (12).

This study was conducted in a single institution with a limited sample size, which may restrict generalizability and calls for caution in interpreting our findings. Prior antibiotic exposure likely reduced culture positivity rates, consistent with recent international findings (25,26). Additionally, resource constraints limited the detection of fastidious and anaerobic organisms, potentially underestimating pathogen diversity.

Conclusion:

This study showed the significant burden of bacteraemia among febrile patients on chemotherapy at CNRAO. Our findings mirror recent global trends of bacteremia in febrile chemotherapy patients caused by Gram-negative and Gram-positive bacteria, increasingly resistant to multiple antibiotic classes, and associated with high mortality.

The results reinforce the importance of systematic blood culture use for improving diagnosis, guiding treatment, and informing antimicrobial stewardship policies. Such measures are essential to improve patient outcomes, curb antimicrobial resistance, and align oncology care in Côte d'Ivoire with international best practices. Future multicenter studies with larger cohorts are needed to capture the full epidemiological spectrum of bacteraemia in oncology patients across Côte d'Ivoire and the wider West African region

Contributions of authors:

CB designed the study and supervised data collection. AK and YT performed the laboratory analyses. JN carried out the statistical analysis. All authors contributed to drafting and revising the manuscript and approved the final version.

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Conflicts of interest:

The authors declare no conflict of interest in relation to this study.

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Short Communication **Open Access**

Seroprevalence of hepatitis B virus infection at the Matam Communal Medical Center, Guinea-Conakry

[1,2]Traore, S., *[2,3]Conde, M. S., [2,3]Conde, M., [2]Youla, Y., [2,3]Diallo, A., [2,3]Camara, A. S., [2,3]Camara, A. A., [2,3]Soumah, M. L., [3]Diakite, Z., and [3]Sako, F. B.

[1]Centre Médical Communal de Matam, Conakry, Guinea
[2]Faculté des Sciences et Techniques de Santé Université Gamal Abdel de Conakry, Guinea
[3]Centre Hospitalier Universitaire Ignace Deen de Conakry, Guinea
*Correspondence to: mamadisaran93@gmail.com

Abstract:

Background: Hepatitis B remains a major public health problem in sub-Saharan Africa, particularly because of its high prevalence and serious complications. The objective of this study was to determine the seroprevalence of hepatitis B in patients seen at the Medical Biology Laboratory of the Communal Medical Center (CMC) in Matam, Guinea-Conakry.
Methodology: This was a descriptive cross-sectional study conducted over a period of 6 months (January to June 2025). All patients who came for biological examination were included, and seroprevalence of hepatitis B was determined by detecting HBsAg in the serum samples of the patients using rapid immunochromatographic test (ICT) method.
Results: Of the total 186 patients included, 15 tested positive for HBsAg, giving hepatitis B seroprevalence of 8.0%. The mean age of affected patients was 39±13 years. Males were more frequently affected (60.0%), with a male to female ratio of 1.5. Housewives (53.3%) and civil servants (26.7%) were the main socio-professional categories most frequently affected.
Conclusion: The seroprevalence of 8.0% indicates that hepatitis B virus is circulating in Matam, Guinea-Conakry. This result underlines the need to step up hepatitis B screening, awareness-raising and vaccination efforts in Matam area.

Keywords: Seroprevalence; HBsAg; Immunochromatographic; Screening; Guinea-Conakry

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Séroprévalence de l'infection par le virus de l'hépatite B au Centre Médical Communal de Matam, Guinée-Conakry

[1,2]Traore, S., *[2,3]Condé, M. S., [2,3]Condé, M., [2]Youla, Y., [2,3]Diallo, A., [2,3]Camara, A. S., [2,3]Camara, A. A., [2,3]Soumah, M. L., [3]Diakite, Z., et [3]Sako, F. B.

[1]Centre Médical Communal de Matam, Conakry, Guinée
[2]Faculté des Sciences et Techniques de Santé Université Gamal Abdel de Conakry, Guinée
[3]Centre Hospitalier Universitaire Ignace Deen de Conakry, Guinée
*Correspondance à: mamadisaran93@gmail.com

Résumé:

Contexte: L'hépatite B reste un problème majeur de santé publique en Afrique subsaharienne, notamment en raison de sa forte prévalence et de ses graves conséquences. L'objectif de cette étude était de déterminer la séroprévalence de l'hépatite B chez les patients vus au laboratoire de biologie médicale du Centre médical communal (CMC) de Matam, en Guinée-Conakry.
Méthodologie: Il s'agissait d'une étude transversale descriptive menée sur une période de 6 mois (janvier à juin 2025). Tous les patients venus pour un examen biologique ont été inclus, et la séroprévalence de l'hépatite B a été déterminée par la détection de l'HBsAg dans les échantillons sériques des patients à l'aide de la méthode du test immunochromatographique rapide (ICT).
Résultats: Sur un total de 186 patients inclus, 15 ont été testés positifs pour l'HBsAg, ce qui donne une séroprévalence de l'hépatite B de 8,0%. L'âge moyen des patients atteints était de 39±13 ans. Les hommes étaient plus fréquemment touchés (60,0%), avec un ratio homme/femme de 1,5. Les femmes au foyer (53,3%) et les fonctionnaires (26,7%) étaient les principales catégories socioprofessionnelles les plus fréquemment

touchées.
Conclusion: La séroprévalence de 8.0% indique que le virus de l'hépatite B circule à Matam, en Guinée-Conakry. Ce résultat souligne la nécessité d'intensifier les efforts de dépistage, de sensibilisation et de vaccination contre l'hépatite B dans la région de Matam.

Mots-clés: Séroprévalence; HBsAg; Immunochromatographie; Dépistage; Guinée-Conakry

Introduction:

Hepatitis B is a major viral infection in sub-Saharan Africa, where it represents a public health priority due to its high prevalence and serious complications, notably liver cirrhosis and hepatocellular carcinoma (1). Transmission of the virus is mainly maternal-fetal, sexual or blood-borne, which poses a particular challenge in regions where access to healthcare and screening tools remains limited. In addition, lack of awareness, social stigmatization and unequal access to care all contribute to delays in treatment, especially in low-and-middle-income countries. Hepatitis B is thus often described as a silent epidemic, diagnosed too late, when complications are already advanced (2).

The World Health Organization (WHO) estimates that worldwide in 2019, around 296 million people were carriers of chronic hepatitis B virus infection. Every year, 1.5 million new infections are reported, and nearly 820,000 deaths are attributed to complications of the disease, mainly cirrhosis and liver cancer. In Africa, the burden of disease is particularly high, with some 65 million people living with chronic infection, or around 5.4% of the regional population (3). In Europe, the prevalence of hepatitis B is generally below 2% in the general population, although it can reach or exceed 4-5% in certain regions of Eastern and Southern Europe, as well as in at-risk populations such as migrants, injecting drug users or incarcerated people (4).

More specifically in West Africa, a systematic review of epidemiological studies carried out in Burkina Faso between 1996 and 2017 estimated HBsAg overall prevalence at 11.21%, with variations according to group; 9.41% in the general population, 11.11% in pregnant women, 11.73% in blood donors and up to 12.61% in people living with HIV (5). In Senegal, prevalence was estimated at 8.2%, reflecting a worrying epidemiological situation despite prevention efforts (6), and in Guinea at 13%, making the country one of the most affected in the region (7). These data underline the urgent need to reinforce hepatitis B prevention, screening and treatment strategies in West Africa, notably by improving vaccination coverage, setting up targeted screening programs and expanding access to antiviral treatments.

Despite the availability of a safe and effective vaccine for several decades, hepatitis B remains a major cause of chronic infection, cirrhosis and liver cancer, particularly in sub-Saharan Africa. This situation raises questions about the effectiveness of prevention policies, vaccination coverage, screening and access to care. Such questions are; how can we explain the persistence of high hepatitis B prevalence in these countries, and what strategies could be implemented to improve prevention, diagnosis and management of this chronic viral infection? The aim of this study was to determine the prevalence of HBsAg carriage in patients seen at the medical biology laboratory of the Center Medical Communal (CMC) in Matam, Guinea-Conakry.

Materials and method:

The Medical Biology Laboratory of the Centre Medical Communal de Matam, Guinea-Conakry was the setting for this study. The study was a descriptive cross-sectional design conducted over a six-month period from January 1 to June 30, 2025. All patients presenting to the laboratory for biological examination were included and HBsAg detection was performed on serum samples collected from each patient using rapid immunochromatographic test (ICT) method.

Socio-demographic data of all patients who tested positive for HBsAg were collected using a proforma and the data were used exclusively for scientific purposes, with strict respect for patient confidentiality and anonymity.

Results:

Of the total 186 patients seen at the Medical Biology Laboratory of the Centre Medical Communal de Matam during the period of the study, 15 tested positive for HBsAg, giving a seroprevalence of 8%. The mean age of the positive patients was 39±13 years, with a range of 18-62 years (Fig 1). Gender distribution shows a predominance of males, with 9 (60%) males and 6 females (40.0%) with a male; female ratio of 1.5 (Fig 2).

The frequency distribution of affected patients by socio-professional category shows that housewives were the most frequently affected, with 8 cases (53.3%), followed by civil servants, with 4 cases (26.7%) and other workers with 3 cases (20.0%) (Table 1).

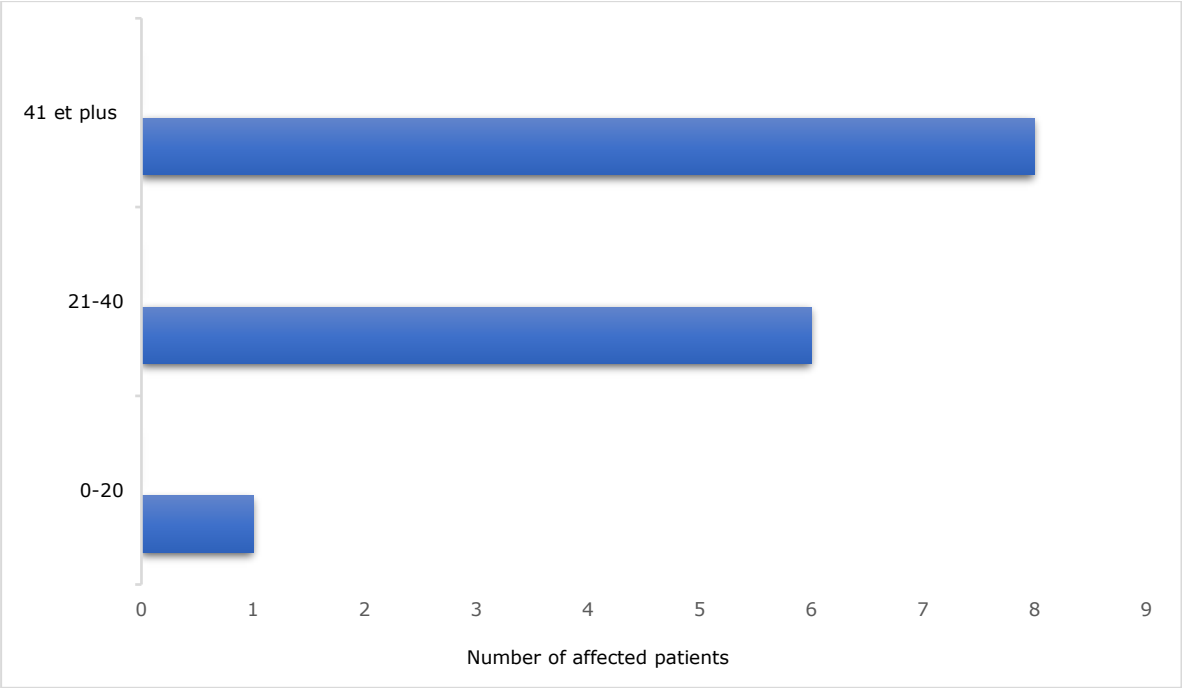

Fig 1: Frequency distribution of patients with hepatitis B virus infection by age group

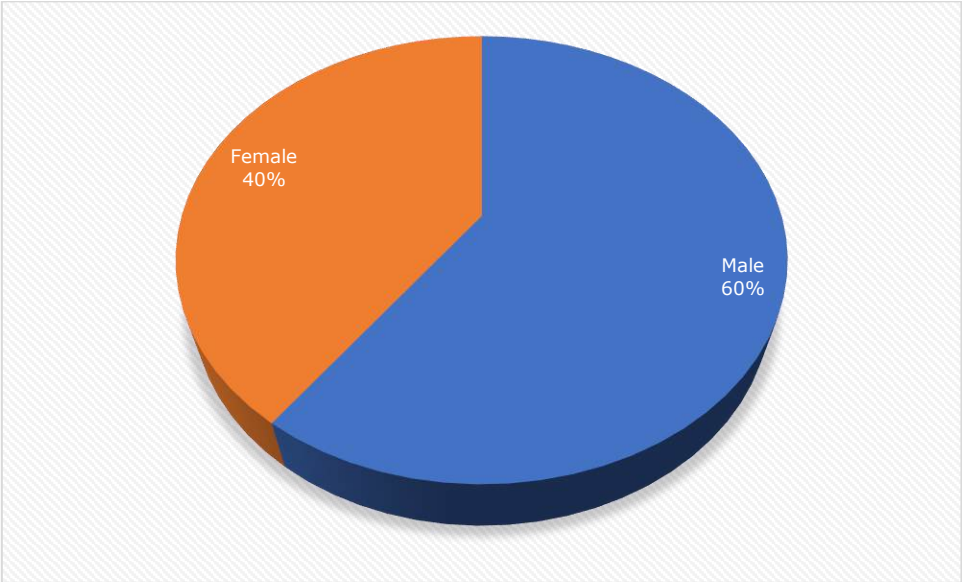

Fig 2: Frequency distribution of patients with hepatitis B virus infection by gender

Table 1: Frequency distribution of patients with hepatitis B virus infection by sociodemographic characteristics

Occupation	**Frequency**	**Percentage (%)**
House-keeping	8	53.3
Civil service	4	26.7
Other working group	3	20.0
Total	**15**	**100.0**

Discussion:

In our study, the prevalence of HBsAg among patients seen at the medical biology laboratory of the Centre Médical Communal de Matam was estimated at 8%. This rate, although moderate, indicates a significant presence and circulation of hepatitis B virus (HBV) in the population studied. To put this result into a national context, a meta-analysis including 22 studies carried out between 1996 and 2017 in Burkina Faso reported an overall prevalence of 11.2% (95% CI 11.0-11.4). Although our rate is slightly lower than this national estimate, it remains high, testifying to a sustained endemicity of HBV infection in the country (8).

For West Africa as a whole, a group of blood donor studies showed a mean prevalence of 6.9% (95% CI 5.95-7.97%), with a rate of 10.1% in West African countries. Our rate of 8% falls within this range, reflecting regional variability and the zone's intermediate-to-high situation. In a neighboring country, the Republic of Niger reported a prevalence of 5.7% among HIV-infected patients in Maradi. This difference may be explained by population selection (HIV patients vs. general population). In HIV-negative or general areas, rates are often higher (9).

The World Health Organization (WHO) classifies HBV prevalence in "high endemicity" zones at 8% (10). Our results therefore confirm that Matam is located in such a zone, justifying reinforced prevention and monitoring measures. This result may be interpreted as reflecting persistent transmission of HBV in the community, probably linked to factors such as inadequate vaccination, lack of knowledge of modes of transmission, or late recourse to healthcare structures. It is also possible that this prevalence is influenced by the specific characteristics of the population visiting the laboratory, which does not necessarily represent the general population as a whole. Furthermore, the lack of systematic screening and the stigma associated with this infection could limit the identification of asymptomatic cases, thus contributing to the underestimation of the true prevalence. This finding underscores the importance of stepping up HBV awareness, early screening and vaccination efforts, particularly in resource-limited areas such as Matam.

In our series, the mean age of patients with HBsAg infection was 39±13 years (range 18-62 years). This age profile is in line with several studies conducted in West Africa. For example, a survey conducted in Ouagadougou showed an average age of 29.7±14.7 years (range 1-78 years), with a high prevalence in the 31-40 age group (14.5%) (11). Another cross-sectional study covering 10 cities in Burkina Faso (2020-2022) reported an HBsAg prevalence of 8.8% in cohorts with a wide age range (1-97 years), underlining the importance of follow-up in active age groups (young to middle-aged adults) (12).

The gender breakdown shows a male predominance (male/female ratio of 1.5). This finding is widely confirmed by the literature. In 2017 in Ouagadougou, HBV prevalence was significantly higher in men (13.4%) than in women (7.8%; $p<0.001$). Also, in Burkina Faso, a national survey based on the DHS 2010-2011 found seroprevalence among men to be 10.5% versus 7.8% among women, a statistically significant trend ($p=0.0002$) (13). This male predominance is also found in other neighboring countries such as Senegal, where a study among military personnel shows an average age of around 39.8 years and a very high proportion of men, with seroprevalence rates of up to 16.9% in the 35-44 age bracket (14). These similarities between our data and those from the region suggest several explanatory hypotheses such as behavioral and exposure factors, biological or immune factors and access to hepatitis B screening. Because our sample size is relatively small, the findings should be interpreted with caution.

Conclusion:

This single center study carried out at the Medical Biology Laboratory of the Centre Médical Communal de Matam, enabled us to estimate the prevalence of hepatitis B at 8%, thus confirming the persistence of high level of hepatitis B virus circulation in Matam population. The infection mainly affected middle-aged adults, with a male predominance, and a marked affectation of housewives and civil servants among the socio-professional groups.

These results underline the importance of early and systematic screening, particularly among at-risk groups, as well as the need to reinforce community awareness of hepatitis B transmission modes and prevention methods. In addition, widespread vaccination, particularly of the birth dose, remains an essential strategy for reducing the incidence of infection. Complementary studies on a larger scale, including other health facilities and more detailed clinical data, would be useful to refine our understanding of the local epidemiology of the hepatitis B virus and guide public health policies adapted to the Matam context.

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Contributions of authors:

All authors contributed equally to data collection and acquisition, analysis and interpretation of results, writing and revision of the manuscript.

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Short Communication **Open Access**

Exploring the metabolic harmony: Probing the impact of VHBAX probiotics on glucose metabolism in a zebrafish model

Prabu, R., Prasad, S. V. S., Kodimule, S., and *Sunhare, R.

Development and Research Centre-Probiotics, Vidya Herbs Pvt Ltd, No. 102B & 105B, Pharmaceuticals SEZ Industrial area, KIDB, Hassan, 573201 Karnataka, India
*Correspondence to: raksha@vidyaherbs.com; +91-8600040075

Abstract:

Background: Probiotics alter gut microbiota composition and diversity. Research indicates that probiotic supplementation might enhance glucose homeostasis and reduce inflammation in patients with type Ⅱ diabetes mellitus. The aim of this study was to investigate how particular probiotic strains (VHBAX *Bacillus coagulans*) affect glucose homeostasis and appetite-related behaviours associated with metabolic illnesses such as obesity and type Ⅱ diabetes in a zebrafish model.
Methodology: An experimental laboratory-based investigation was carried out with zebrafish (*Danio rerio*) as the model organism. The zebrafish were divided into 3 groups of 6 zebrafish each; control (group I), diabetic-induced (group II), and diabetic-induced plus probiotic treatment (group III), with the latter receiving a diet supplemented with VHBAX probiotic strains. Glucose tolerance testing, and gene expression analysis for glucose metabolism were carried out in all the 3 groups. The composition of the gut microbiota was also analysed using 16S rRNA sequencing to assess microbial alterations related to probiotic administration. Data were analysed using SPSS 16.0 and presented as mean±SD. Difference between means of two groups was determined by Student *t*-test, and $p<0.05$ was considered statistically significant.
Results: When compared to diabetes-induced zebrafish (group II), diabetes-induced probiotic-treated zebrafish (group III) had enhanced glucose tolerance, altered expressions of appetite-regulating genes (AgRP, CNR1, CTSL1, Oxt, CCKAr, leptin A), and detectable alterations in gut microbiota composition, comparable to the control (group I). There was a significant association of VHBAX probiotic supplementation with microbial regulation and improved metabolic outcomes.
Conclusion: This experimental investigation shed light on the mechanisms by which VHBAX probiotics regulate glucose metabolism and appetite management through alteration of gut microbiota composition in zebrafish. The finding suggests that probiotic therapies can help manage or prevent metabolic disorders, including obesity and type Ⅱ diabetes.

Keywords: Zebra fish; Glucose metabolism; Diabetes; Microbiota; Metabolic disorder; Probiotics

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Exploration de l'harmonie métabolique: étude de l'impact des probiotiques VHBAX sur le métabolisme du glucose dans un modèle de poisson-zèbre

Prabu, R., Prasad, S. V. S., Kodimule, S., et *Sunhare, R.

Centre de Développement et Recherche sur les Probiotiques, Vidya Herbes Pvt Ltd, N° 102B et 105B, Zone Industrielle Pharmaceutique SEZ, KIDB, Hassan, 573201 Karnataka, Inde
*Correspondance à: raksha@vidyaherbs.com; +91-8600040075

Résumé:

Contexte: Les probiotiques modifient la composition et la diversité du microbiote intestinal. Des recherches indiquent qu'une supplémentation en probiotiques pourrait améliorer l'homéostasie du glucose et réduire l'inflammation chez les patients atteints de diabète de type II. L'objectif de cette étude était d'examiner l'impact de certaines souches probiotiques (*Bacillus coagulans* VHBAX) sur l'homéostasie du glucose et les comportements liés à l'appétit associés à des maladies métaboliques telles que l'obésité et le diabète de type II chez le poisson zèbre.

Méthodologie: Une étude expérimentale en laboratoire a été menée sur le poisson zèbre (*Danio rerio*) comme organisme modèle. Les poissons zèbres ont été divisés en trois groupes de six poissons zèbres chacun; témoin, diabétique induit et diabétique induit avec traitement, ces derniers recevant un régime alimentaire supplémenté en souches probiotiques VHBAX. Des tests de tolérance au glucose et une analyse de l'expression génique du métabolisme du glucose ont été réalisés dans tous les groupes. La composition du microbiote intestinal a également été analysée par séquençage de l'ARNr 16S afin d'évaluer les altérations microbiennes liées à l'administration de probiotiques. Les données ont été analysées avec SPSS 16.0 et présentées sous forme de moyenne ± écart type. La différence entre les moyennes des deux groupes a été déterminée par le test *t* Student, et une valeur de $p<0,05$ a été considérée comme statistiquement significative.
Résultats: Comparés aux poissons-zèbres diabétiques (groupe II), les poissons-zèbres diabétiques traités aux probiotiques (groupe III) présentaient une tolérance au glucose accrue, une expression altérée des gènes régulateurs de l'appétit (AgRP, CNR1, CTSL1, Oxt, CCKAr, leptine A) et des altérations détectables de la composition du microbiote intestinal, comparables à celles du groupe témoin (groupe I). Une association significative a été observée entre la supplémentation en probiotiques VHBAX et la régulation microbienne et une amélioration des résultats métaboliques.
Conclusion: Cette étude expérimentale a permis de mieux comprendre les mécanismes par lesquels les probiotiques VHBAX régulent le métabolisme du glucose et la gestion de l'appétit en modifiant la composition du microbiote intestinal chez le poisson-zèbre. Ces résultats suggèrent que les thérapies probiotiques peuvent contribuer à la prise en charge ou à la prévention des troubles métaboliques, notamment l'obésité et le diabète de type II.

Mots-clés: Poisson zèbre; Métabolisme du glucose; Diabète; Microbiote; Trouble métabolique; Probiotiques

Introduction:

Probiotics alter gut microbiota composition and diversity (1). Research indicates that probiotic supplementation might enhance glucose homeostasis and reduce inflammation in Type Ⅱ diabetes mellitus (DM) patients (2,3). Probiotics alter gut flora, creating beneficial molecules that demonstrate the importance of gut microbiota for human and animal health (4). Gut microbiota affects metabolism and energy balance for weight management. Rygan et al., (5) reported that probiotics can dramatically lower HbA1c and insulin levels in type Ⅱ diabetics without affecting lipid profile.

Many diabetic investigations on zebrafish larvae have shown that real-time PCR may detect PEPCK expression in anti-diabetic medicines (6). Zebrafish have genetic and physiological parallels to humans, making them an established system for metabolic and behavioural research. Zebrafish, as herbivores, can tolerate glucose better. The efficacy was connected to gut bacteria, specifically acetate-producing commensal *Cetobacterium somerae.* Acetate and *C. somerae* stimulated the parasympathetic nerve system, improving gut-brain glucose homeostasis (7). Impaired leptin signalling in mutant zebra fish may influence gut anorexigenic gene regulation (7).

The pandemic of obesity necessitates improved intervention techniques focusing on gut bacteria and metabolic products such as high-fat diets which favour *Firmicutes, Prevotella*, and *Methanobrevibacter* species growth. This leads to inflammation, increased hunger-suppressing hormones, dyslipidaemia, and reduced short-chain fatty acid (SCFA) expression (8). Understanding the connection between gut microbiota and glucose metabolism could lead to new diabetes and obesity prevention strategies (9).

The primary objective of this study is to evaluate the effects of probiotics (VHBAX *Bacillus coagulans*) on glucose metabolism. To achieve this, measuring and analysis of the expression of key genes involved in glucose metabolism pathways is needed. This gene expression analysis will provide insights into how probiotics may influence or modulate glucose metabolism in zebrafish under diabetic conditions.

Materials and method:

Study setting and ethical consideration:

This zebra fish study project proposal number SU/CLATR/IAEC/XXI/07/2023 entitled unveiling the impact of probiotics (VHBAX *Bacillus coagulans*) on glucose metabolism. Insights from a zebrafish animal model was approved by the IAEC of Satyabama Institute of Science and Technology, Chennai 600119, India.

Experimental and control animal:

Adult zebrafish from Chennai's Satyabama Institute of Science and Technology were housed in a semi-static system with charcoal-filtered tap water. Three groups of zebrafish (n=6 each) were housed in 3-liter recirculating tanks at 28.4°C under 14:10 hours light: dark conditions for the experiment. Facility water quality was checked regularly.

Each group was given different conditions to assess the VHBAX probiotic effects; Group I (control) received standard fish feed for a regular diet; Group II were induced diabetic zebrafish (100 mg alloxan per kg of zebrafish body weight) and kept in 10% sugar till the experiment ended after diabetes induction (28 days); Group III were induced diabetic zebrafish with probiotics administered by oral method. This group received sucrose induction and maintenance and *Bacillus coagulans* VHBAX probiotics daily till the experi-

ment ended (28 days) and were used to assess effects on glucose metabolism.

Determination of blood glucose as a measure of glucose tolerance in zebrafish:

The zebrafish were anaesthetized using hypothermia method by placing them in 17°C facility water and gradually reducing the temperature to 12°C by adding ice made from the facility water. Fish brought to stage II anaesthesia, allowed handling and still had opercular movements. The anaesthetized zebrafish were then decapitated by cutting through the pectoral girdle. Whole blood was collected for immediate analysis using test strips applied directly to the cardiac blood. Approximately 5-10µL of blood collected for blood glucose was accurately measured using Accu-Chek Compact Plus (1.5µL sample).

Histopathological examination of pancreatic tissues:

The zebrafish were euthanized by immersion in a freshly prepared buffered tricaine methane sulfonate (MS-222- RM2178 Hi Media) solution (200–300 mg/L, pH 7.0–7.5) until opercular movement ceased, followed by decapitation to confirm death before tissue collection. Whole mounts of control and experimental fish pancreas tissues were fixed, and the pancreas tissue slices were deparaffinized in xylene at 65°C for 20 minutes to dissolve paraffin wax. Both areas received two 10-minute xylene clearings after the initial treatment. Tissue slices were then immersed in alcohol solutions at 100%, 90%, 70%, 50%, and 30%. Each portion was hydrated with distilled water for 10 minutes.

Tissue slices were stained with Mayer's haematoxylin for 15 minutes to show cellular characteristics and the stain was removed by washing slides with tap water for 10 minutes and then counterstained with eosin for 2 mins. The slides were prepared for mounting after dehydrating and eliminating eosin stain. Two xylene changes followed 30%–100% alcohol soaking of the slides and light microscopic examination of DPX-mounted slices.

Measurement of glycolytic enzyme activity:

Liver tissue was collected immediately after sacrificing experimental animals for glycolytic enzyme measurement. The liver tissue was homogenised in ice-cold buffer (pH 7.6). The hexokinase homogenate was centrifuged at 9000 rpm for 100 min and glycolytic enzymes at 10000 rpm for 20 min. Pancreas from control (Group I) and experimental (Groups II and III) zebrafish were homogenised in 50mM Tris-HCl (pH 7.5), 4mM EDTA, 50mM sodium fluoride (NaF), 0.5mM phenylmethylsulfonyl fluoride, 500mM 1,4-dithiothreitol, and 250 mM sucrose.

The homogenate was centrifuged at 14000 rpm for 30 min at for hexokinase and fructose-1, 6-biphosphatase activities. After 30 minutes at 37ºC, the reaction was stopped by adding 10% TCA solution. The absorbance was 340 nm. The glycogen phosphorylase (GP) activity was determined by monitoring the reduction of oxidised NADP during glycogen breakdown at 340 nm, expressed in micromole NADP/min per gram of protein. The GP activity is defined as the conversion of 1µmol glycogen and orthophosphate into glucose-1 phosphate per minute at 30°C and pH 6.8 (11-12).

Quantitative real-time PCR assay for glucose metabolism genes:

Quantitative analysis of core glucose metabolism genes [CCKAr (cholecystokinin A receptor), CNR1 (cannabinoid receptor-1), CTSL1 (cathepsin L1), leptin A, AgRP (agouti-related peptide), and Oxt (oxytocin)] of the control and experimental zebrafish was done by real-time PCR assay. First, total RNA was isolated from the liver tissue and quantified according to the method of Chomczynski and Sacchi (13). A cDNA conversion kit (Thermo Scientific Inc.) created cDNA from total RNA after reverse transcription (RT).

About 0.5-1.0µg total RNA was combined with 1.5µL random hexamer primer (GAP DH-F:5′-TGCACCACCAACTGCTTAGC-3′ and GAPDH-R:5′-GGCATGGACTGTGGTCATGA-3′), incubated at 72°C for 10 minutes, and chilled immediately. This was then combined with 5.0µL premixed 10 mmol/L dNTP solution, 3.0 µL 10x M-MLV RT buffer, and 1.0 µL M-MLV RT in RNase/DNase-free water to reach 50µL. SYBR green fluorescent dye was used for BioRad CFX96 real-time PCR. Internal control was GAPDH.

Analysis of gut microbiota by 16S rRNA sequencing:

The gut microbiota (intestinal content) of zebrafish was analyzed using 16S rRNA gene sequencing. The whole gut was aseptically dissected from anaesthetized zebrafish, rinsed gently with sterile phosphate-buffered saline (PBS) and DNA extracted using QIAamp DNA Microbiome Kit. The hypervariable region (V3–V4) of the 16S rRNA gene was amplified using the universal bacterial primers 341-F (5-CCTACGGGNGGCWGCAG-3) and 805-R (5-GA CTACHVGGGTATCTAATCC-3). Amplicons were purified, quantified, and pooled in equimolar concentrations before sequencing on Illumina MiSeq platform.

Bioinformatic analysis of the 16S rRNA sequenced data was done by Shannon and Chao1 for alpha diversity, and by Principal Coordinates Analysis (PCoA) for beta diversity of the microbial communities of the control, diabetes-induced, and diabetes-induced probiotic-treated zebrafish groups. Functional prediction of the metagenome functions of the

microbial community based on the 16S rRNA sequencing profiles was carried out using PICRUSt2 (https://github.com/picrust/picrust2).

Statistical analysis:

Statistical analysis of data was done using SPSS 16.0. Data were presented as mean ± S.D (n=6). Difference between means of two groups was determined by Student *t*-test, and *p*-value below 0.05 was considered statistically significant.

Results:

Histopathological structures of pancreas in zebrafish in control and experimental groups:

Histological analysis of the pancreas tissue from both the control and experimental groups provided valuable insights into the effects of probiotic treatment on glucose metabolism (Fig 1). Compared to the control (group I) and diabetic-induced probiotic-treated fish (group III), the pancreatic tissue mass of diabetes-induced zebrafish (group II) showed disrupted pancreatic tissues.

Blood glucose levels in zebrafish in control and experimental groups:

Based on the study, elevation of glucose levels resulted in hyperglycemia in diabetes-induced zebrafish group (II) in comparison to the control (group I). The probiotic VHBAX significantly reduced the blood glucose levels of treated zebrafish in group III, bringing them close to those of the control group (as seen in Fig 2).

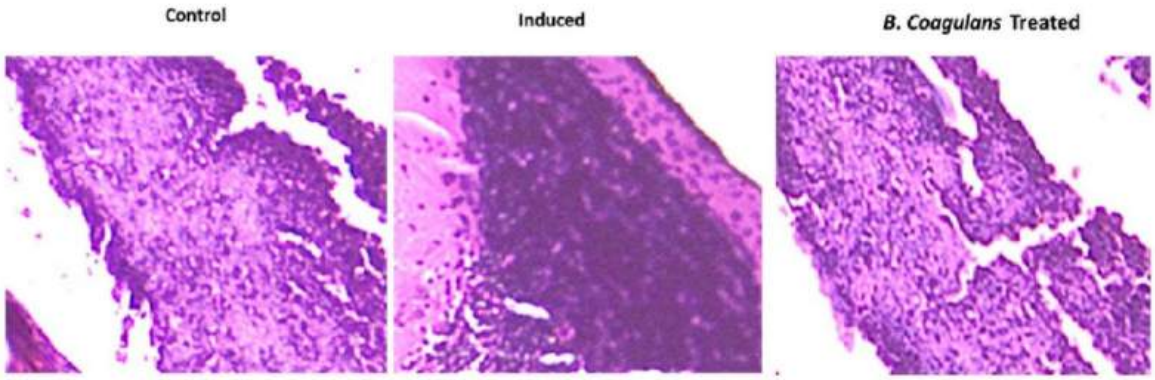

Fig 1: Microscopical observations of pancreas sections of control and experimental zebrafish groups

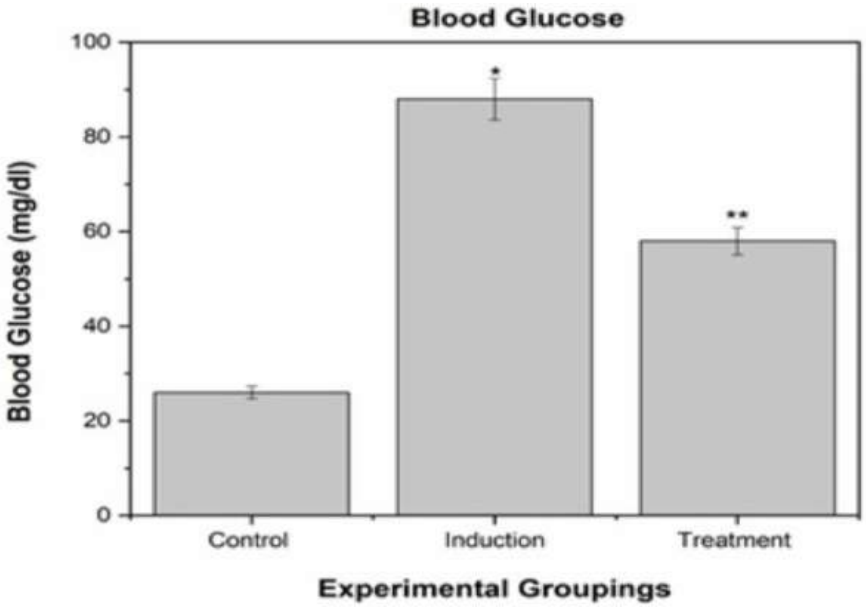

Fig 2: Quantification of blood glucose level in the control and experimental zebra fish groups

Glycolytic enzyme activity in zebrafish in control and experimental groups:

The activities of glycolytic enzymes, including hexokinase and phosphofructokinase-1 (PFK-1), were reduced in diabetes-induced zebrafish (group II) compared to the control (group I), while the activities considerably increased in group III zebrafish treated with VHBAX probiotics (Fig 3).

Metabolic gene expression profiles in zebrafish in control and experimental groups:

With respect to the gene expression profile, there was overexpression of AgRP, CNR1, CTSL1 and leptin A, and reduced expression of CCKAr and Oxt genes in diabetes-induced zebra fish (group II) compared to the control (group I) (Fig 4). VHBAX probiotic treatment of diabetes-induced zebrafish (group III) had significant effect on metabolic regulators such as AgRP, CNR1, and Oxt whereas the expression of CCKAr and Leptin A was decreased. These indicated the disturbed appetite and glucose signalling by suppressing orexigenic markers (AgRP, CNR1) and normalising anorexigenic genes (CCKAr, Leptin A) (Fig 4).

However, in probiotic treatment (group III), CCKAr expression to near-normal levels and supress leptin A expression to approximate control values, indicating that probiotic supplementation mitigated diabetes-related dysregulation of starvation and metabolic genes.

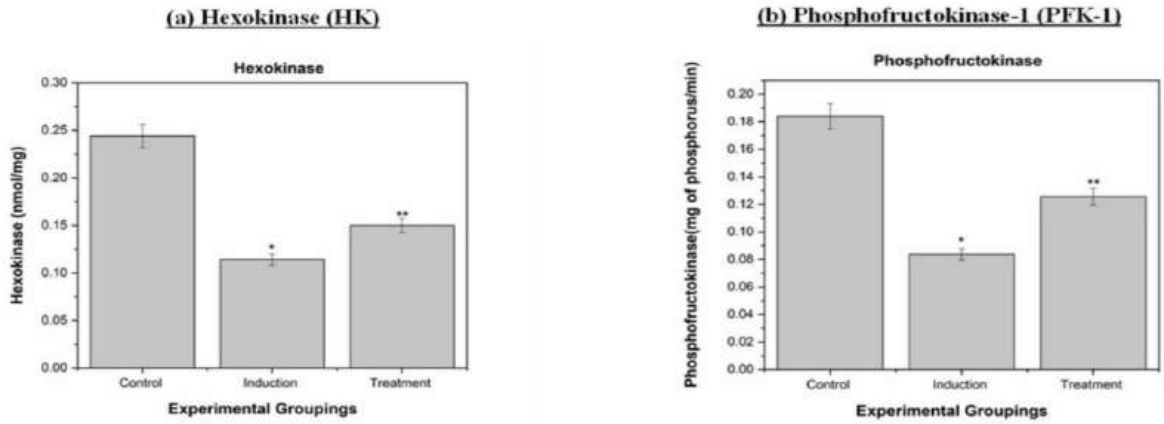

Fig 3: Quantification of key glycolytic enzymes of zebrafish in control and experimental groups

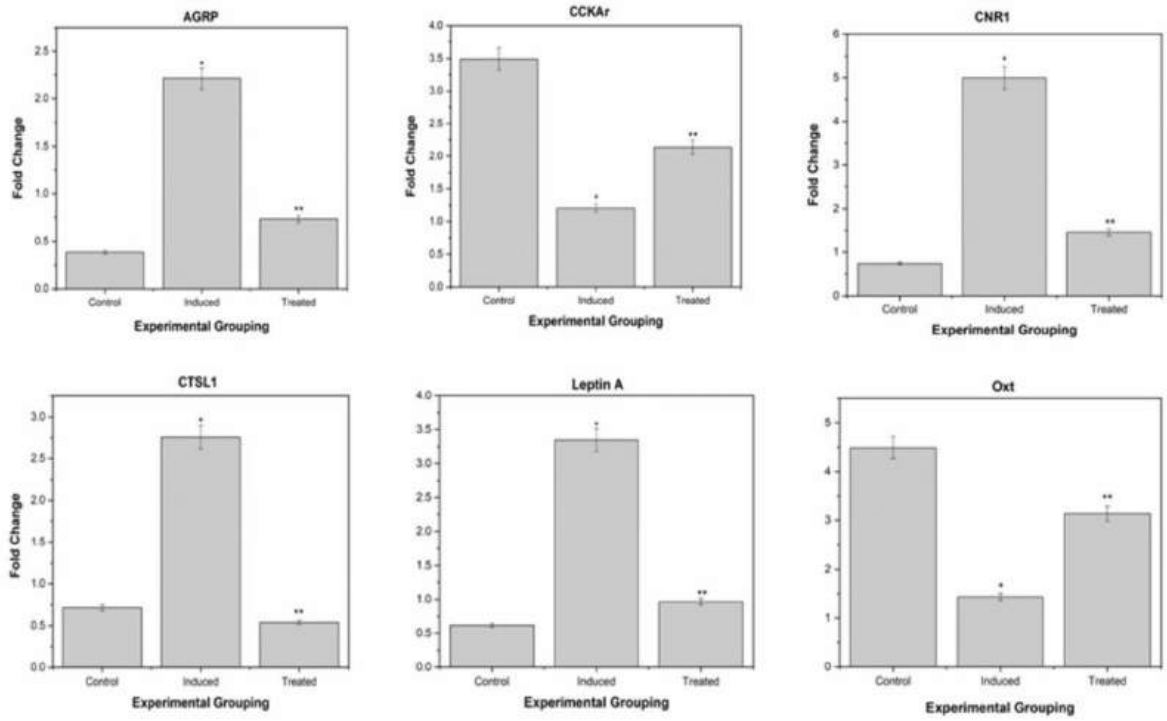

Fig 4: qPCR analysis of key genes involved in glucose metabolism of control and experimental zebrafish groups

Gut microbiota diversity of zebrafish in control and experimental groups:

The diabetes-induced zebra fish (group II) showed a lower alpha diversity (Shannon, Chao1) than the control (group I), which indicates that there was a less abundance of microbial species (Fig 5). The incorporation of VBHAX probiotics to diabetes-induced zebrafish in group III dramatically improved the diversity in line with the control (group I).

The results of the beta diversity analysis (PCoA) demonstrated that the groups were classified in a different manner. The microbial community demonstrated signs of recovery, as evidenced by the fact that diabetes-induced probiotic-treated zebrafish (group III) were placed closer to the control zebrafish (group I) and further away from diabetes-induced zebrafish (group II) when they were categorised into groups (Fig 6).

According to the results of functional prediction carried out using PICRUSt2, the diabetes-induced probiotic-treated zebrafish (group III) had a higher number of pathways for the production of short-chain fatty acids (SCFAs) and was more efficient at breaking down glucose.

Gut microbiome composition of zebrafish in control and experimental groups:

The result for the 16S rRNA sequencing showed that there were different microbiome patterns present in each of the groups. The microbial balance of the control zebrafish (group I) was in good condition, as evidenced by the fact that majority of their microbiomes were composed of *Cetobacterium* spp. This equilibrium was broken in diabetes-induced zebrafish (group II) which had increased number of opportunistic *Firmicutes* and *Proteobacteria*, an indication of dysbiosis.

The variety of healthy microbes was increased through the use of VHBAX treatment of zebrafish in group III, which accomplished this by increasing the number of beneficial commensals such *Bacteroides* spp while simultaneously reducing the number of pro-inflammatory taxa (Fig 6).

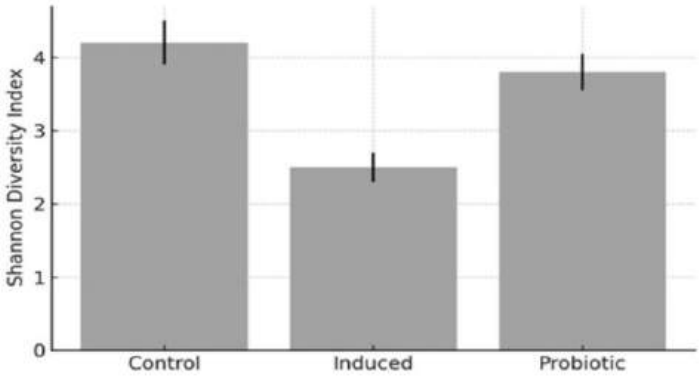

Fig 5: Alpha diversity (Shannon Index) showing microbiota richness in control, induced and probiotic treated zebrafish groups

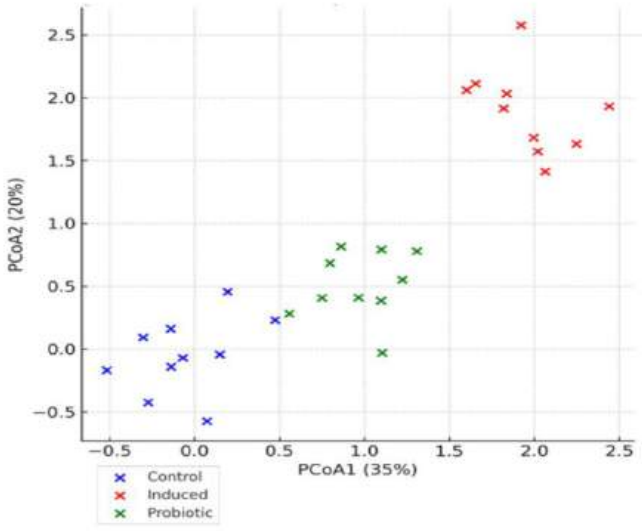

Fig 6: Beta diversity (PCoA plot) depicting distinct microbial community clustering among the three groups

Discussion:

This study compared metabolic processes in three zebrafish groups in different conditions, and the effects of VBHAX probiotics on zebra fish glucose metabolism were examined. In zebrafish in group III (diabetes-induced probiotic-treated), blood levels were lower than in the diabetes-induced zebrafish (group II) but higher than the control (group I). It appears that probiotic stabilised glucose metabolism and reduced diabetes in the zebrafish. Diabetes is caused by insulin sensitivity loss and high glycemia in early to late obesity (4). Gut microbiota may compensate for glucose metabolism in obese children, and genus *Haemophilus* and *Bifidobacterium longum* may alter glucose tolerance and insulin resistance (14).

The zebrafish in the diabetes-induced group had considerably reduced hexokinase (HK) and phosphofructokinase-1 (PFK-1) activity than the control group. Diabetic zebra fish have significant glucose metabolism failure, as shown by this enzyme activity reduction. Hexokinase and phosphofructokinase-1 activity rose considerably in probiotic-treated zebra fish. As shown by this exceptional enzyme activity increase, probiotics may control pancreatic enzymes to improve glucose metabolism. Gut microbes are known to regulate hunger. Probiotics altered zebra fish's gut microbiome, reducing appetite and blood sugar (15). A meta-analysis found that probiotics significantly lowered cholesterol, triglycerides, CRP, HbA1c, fasting plasma glucose, insulin, and systolic/diastolic blood pressure in type Ⅱ diabetic patients. Probiotics may be useful in treating type 2 diabetes by raising HDL levels (15).

Gene expression studies reveal probiotics increase glucose metabolism. CCKAr, CNR1, CTSL1 gene-encoded lysosomal cysteine protease, and neuropeptides such as leptin A, AgRP, and Neuropeptide Y (NPY) regulate glucose levels and metabolism. The glucose metabolism and regulation in the liver are explained by the CCKAr gene expression by creating an insulin-glucagon receptor protein that regulates blood glucose. These hormones (insulin and glucagon) stimulate CCKAr, which starts a cascade of events to customise blood glucose responses. CCKAr increases in treatment group show probiotics alter gene expression and receptor activation. Lysosomal cysteine protease (cathepsin L1) breaks down proteins and performs other cellular functions and can indirectly affect glucose metabolism through cellular nutrition sensing, autophagy, and lysosomal activity. The role of CTSL1 in glucose metabolism is unknown. The diabetes-induced zebrafish (group II) had increased CTSL1 expression, suggesting lysosomal and metabolic activity. The diabetes-induced probiotic treated zebrafish (group III) had low CTSL1 expression levels, indicating that probiotic inhibits CTSL1, which promotes glucose metabolism.

The cannabinoid receptor type 1 gene expression produces the CB1 receptor, which regulates signal transduction, cellular communication, and physiological regulation. G-protein coupled receptors in the central nervous system interact with endocannabinoids to regulate pain, hunger, and neuroprotection. Additionally, CB1 receptors impact peripheral tissue glucose control, inflammation, and energy balance. High CNR1 expression in the diabetes-induced zebrafish group in this study may indicate higher endocannabinoids signalling that regulates glucose and brain circuits. Reduced CNR1 expression levels in diabetes-induced probiotic-treated group indicate improved glucose metabolism.

In vertebrates like zebrafish, leptin A controls energy balance, hunger, and metabolism. Mostly produced by fat cells, it suppresses hunger and increases energy expenditure in the hypothalamus when body fat levels are sufficient. Leptin production is related to adipose tissue reserves, increases calorie burning, balances fat storage, and prevents obesity. Dysregulation can cause obesity and metabolic problems. Diabetes-induced zebrafish treated with probiotics had lower leptin A levels, leading to reduction in hunger and energy expenditure.

The hypothalamus produces AgRP, a neuropeptide that controls energy and feeding behaviour. AgRP targets brain receptors such the melanocortin-4 receptor (MC4R) to enhance hunger and decrease energy expenditure. AgRP dysregulation can cause obesity and metabolic diseases, hence understanding the AgRP system may lead to obesity treatments. lower AgRP levels in the diabetes-induced probiotic treated group in our study imply lower appetite stimulation and improved glucose management. The brain and peripheral organs are rich in neuropeptide Y (NPY) which controls glucose metabolism, hunger, and energy balance. Regulation of glucose metabolism by NPY is by regulating beta cell insulin secretion and peripheral insulin sensitivity. In diabetes, NPY promotes hunger and food intake, which may affect glucose management.

The 16S rRNA study showed the changes in the healthy microbiota had metabolic benefit on the system. According to previous studies, the replenishing of *Cetobacterium somerae* in zebrafish maintain their glucose levels by producing acetate (7). Likewise, increased *Bacteroides* spp may promote the synthesis of short-chain fatty acids (SCFAs) such as acetate, propionate, and butyrate in mammals for boosting glucose metabolism and reducing inflammation. Likewise, *Lactobacillus*

rhamnosus has been shown to replenish healthy gut bacteria, raise SCFA levels, change the expression of genes that lower glucose, reduce hunger, and lower blood glucose in zebrafish (4).

The results of these studies align with our current research that showed VHBAX probiotic supplementation induces microbial modifications and metabolic improvements. Finding from the current study suggests the two possible glucose metabolism pathways of VHBAX probiotics; first, by upregulating enzymes and modulating gene expression, and secondly, by modifying healthy microbiota that increased production of short-chain fatty acids, improving dysbiosis and promoting beneficial host signalling. Hence, VHBAX probiotics is suggested in the prevention and management of metabolic disorders, including type II diabetes and obesity. However, deep understanding of microbial diversity, SCFA metabolites, and predictive functional profiling are useful tools to provide broad understanding on the metabolic pathway which connect host and microbiota.

Conclusion:

Our study shed light on the mechanisms by which VHBAX probiotics regulate glucose metabolism and appetite management. The finding suggests that probiotic therapies can help manage or prevent metabolic disorders, including obesity and type 2 diabetes.

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Contributions of authors:

RS and PR conceptualized the study; SVSP and SK curated the data; SK and RS designed the methodology; RS and SVSP performed the experiment; PR and SK provided resources; PR and SVSP were involved with data validation; RS and PR were involved in data visualization; RS, SVSP, PR and SK were involved in manuscript writing. All authors read and approved the final manuscript submitted for publication.

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Conflict of interest

Authors declare no conflict of interest.

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CORRIGENDUM

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Female genital schistosomiasis in Matta health area, Western Cameroon Region: Prevalence, risk factors and biomarkers profile

*[1]Nguepi, M. S. D., [1]Ubre, S. I., [1]Tincho, M. B., [1]Egoko, M. D., [3]Nanfack, C. D., [2]Noubissi, P. A., [1]Agbor, F. I., and [2]Lambou, A. F.

[1]Department of Biochemistry and Molecular Biology, Faculty of Science, University of Buea, P. O. Box 63, Cameroon
[2]Department of Animal Biology and Conservation, Faculty of Science, University of Buea, P. O. Box 63, Cameroon
[3]Faculty of Medicine and Biomedical Sciences, Ministry of Health, University of Yaounde I,
P. O. Box 1364, Yaounde, Cameroon
*Correspondence to: syldong@yahoo.fr; Tel: 696510217

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Schistosomiase génitale féminine dans la zone de santé de Matta, région de l'Ouest du Cameroun: Prévalence, facteurs de risque et profil des biomarqueurs

*[1]Nguepi, M. S. D., [1]Ubre, S. I., [1]Tincho, M. B., [1]Egoko, M. D., [3]Nanfack, C. D., [2]Noubissi, P. A., [1]Agbor, F. I., et [2]Lambou, A. F.

[1]Département de Biochimie et Biologie Moléculaire, Faculté des Science, Université de Buéa, B.P. 63, Cameroun
[2]Département de Biologie Animale et Conservation, Faculté des Science, Université de Buéa, B.P. 63, Cameroun
[3]Faculté de Médecine et Sciences Biomédicales, Ministère de Santé, Université de Yaoundé I,
B.P. 1364, Yaoundé, Cameroun
*Correspondance à: syldong@yahoo.fr; Tél: 696510217

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