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Epidemiology and Molecular Characterisation of *Blastocystis* sp. in Nigeria

Dawaki S.S.[1,2] AbdulSalam, A.M.[2], Al-Mekhlafi, H.M.[2], Ithoi, I.[2], and Ado U.A.[3]

[1] Department of Animal and Environmental Biology, Faculty of Life Science, Federal University Dutse, Jigawa State, Nigeria.
[2] Department of Parasitology, Faculty of Medicine, University of Malaya. 50603 Kuala Lumpur, Malaysia.
[3] Aminu Kano Teaching Hospital, Kano.

*Corresponding Author: saldawaki@gmail.com

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Abstract

Blastocystis sp. is a common intestinal protist with global distribution, yet data on its prevalence and genetic diversity in Northern Nigerian communities remain limited. This study aimed to determine the prevalence and identify the subtypes (STs) of *Blastocystis* sp. circulating in this region. In this cross-sectional community-based study, 551 stool samples were collected from asymptomatic residents of five villages in Kano State, Nigeria. Samples were analysed by direct smear microscopy. Microscopy-positive samples were then cultured in Jones' medium, and subsequent molecular analysis was performed on positive cultures. This involved DNA extraction, PCR amplification of the small subunit ribosomal RNA (SSU-rRNA) gene, cloning, and sequencing to determine the subtypes. Microscopy revealed a *Blastocystis* prevalence of 29.2% (161/551), with higher infection rates in males and participants aged ≤15 years. Of the 161 microscopy-positive samples, 54 (32.3%) were successfully cultured and subtyped. Four subtypes were identified: ST1 (42.6%), ST3 (31.5%), ST4 (13%), and ST2 (7.4%). Three samples indicated mixed infections. ST1, ST3, and ST4 were more prevalent in male respondents, while all mixed infections were observed in males. The frequencies of all subtypes were generally higher in adults. Sequence analysis of cloned PCR products showed high homology with reference sequences in GenBank. This study confirms a high prevalence and genetic diversity of *Blastocystis* sp. in Northern Nigeria, with ST1 being the most dominant subtype. The occurrence of mixed infections highlights the complex epidemiology of this protist in the study population.

Introduction

Blastocystis sp. is among the most common organisms that colonise the human gastrointestinal tract. The enteric protistan Stramenopile infects an estimated 1,000,000,000 people globally; however, its pathogenicity has been a significant controversy among several authors (Idris et al., 2010; Jantermtor et al., 2013; Cabrine-Santos et al., 2015; Abu-Madi et al., 2015; Scanlan & Stensvold, 2013; Noël et al., 2005; Tan, 2008; Tan et al., 2010; Mirza et al., 2012). *Blastocystis* has been reported in asymptomatic humans, while some studies linked this organism with intestinal and extra-intestinal diseases (Zuel-Fakkar et al., 2011; Jimenez-Gonzalez et al., 2012; Abu-Madi et al., 2015). The protozoan has

been known to infect many other animals, including pigs, monkeys, guinea pigs, rodents, birds and reptiles (Abe et al., 2002; Tan, 2004; Petrášová et al., 2011). Following phylogenetic analysis of the SSU rRNA gene sequences of *Blastocystis*, 17 subtypes were identified (Abu-Madi et al., 2015). Humans acquire infection with only nine subtypes that were also found in other animals, indicating the zoonotic potential of *Blastocystis* (Boorom et al., 2008; Noel et al., 2005). Further studies are necessary to understand the epidemiology and pathogenesis of *Blastocystis* worldwide. Little is known about the prevalence and diversity of *Blastocystis* subtypes in Africa.

Generally, intestinal protozoan infections are prevalent worldwide, most conspicuously in developing countries due to lack of basic amenities and poor sanitation (Cabrine-Santos et al., 2015; Borges et al., 2009). In this context, the prevalence of Blastocystosis ranges between 30% and 60% in developing countries (Cabrine-Santos et al., 2015). However, in Nigeria, detection of *Blastocystis* sp. is not routinely done in most laboratories due to low awareness about its diagnosis and association with human disease. Moreover, data on *Blastocystis* subtypes (STs) are needed to increase our understanding of the genetic diversity of *Blastocystis* sp. in Nigeria. Therefore, this study aims to investigate the prevalence of *Blastocystis* sp. in apparently healthy Nigerians and to establish the distribution of subtypes among the subjects.

Materials and methods

Sample Collection

This is a cross-sectional community-based study. A total of 551 samples were collected from residents of 5 villages in Kano State, Northern Nigeria. The participants ranged in age from 1 to 70 years old. Residents with any sign of intestinal distress were excluded from the study. Sample collection was carried out from May to June 2012. The collected stool samples were portioned into two. One half was preserved in 75% ethanol, while the other half was not preserved; however, normal saline was added to prevent desiccation and was used in the culturing of *Blastocystis*. All samples were shipped to the Parasitology Department of the University of Malaya.

Sample analysis

The prevalence of *Blastocystis* sp. was determined using the direct smear technique from the preserved samples. Corresponding microscopy positive samples from the unpreserved samples were cultured by inoculating approximately 50 mg of the stool specimen in 5 ml of Jones' medium supplemented with 10% horse serum and incubated at 37°C. The medium was replaced every other day, while the presence of *Blastocystis* sp. was observed daily for 14 days. Positive cultures were defined by the identification of any form of *Blastocystis* sp. (i.e., vacuolar, granular, amoeboid, and cystic forms). Cultures were considered negative when no growth was observed by the 14th day. Further molecular analysis, including DNA extraction, PCR, DNA purification and cloning, was carried out from the cultures that yielded growth only.

DNA extraction from cultures and PCR

Blastocystis positive cultures were washed in sterile phosphate-buffered saline (PBS) prior to DNA extraction. DNA was extracted using the QIAamp Fast DNA Stool mini kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The eluted DNA was stored at -20 °C till further use. For each sample, 5 μL genomic DNA was subjected to PCR analysis, using the primers forward Blast 505–532 (5′ GGA GGT AGT GAC AAT AAATC 3′) (Bőhm-Gloning et al., 1997) and reverse Blast 998–1017 (5′TGC TTT CGC ACT TGT TCATC 3′) (Santín et al., 2011), amplifying an approximately 500 base pairs (bp) fragment of the 1,800 bp small subunit ribosomal RNA gene (SSU-rDNA). The PCR mixture and cycle conditions were adapted from Abdulsalam et al. (2013b). PCR products confirmed by electrophoresis on a 1.5% agarose

gel were cut, and the desired DNA fragment was extracted using the QIAquick gel extraction kit (QIAGEN) and purified according to the manufacturer's description. The purified DNA was cloned in a vector pGEM®-T Vector (Promega, Madison, USA) and amplified in Escherichia coli JM109 competent cells (Promega). Successfully cloned DNA fragments were sequenced on both strands using M13 forward (5'GTA AAA CGA CGG CCA GT'3) and M13 reverse primers (5'GCG GAT AAC AAT TTC ACA CAG G'3). Sequencing was carried out using the ABI Big Dye® Terminator Cycle Sequencing Ready Reaction Kit v3.1, using the ABI PRISM® 3730xl DNA Analyser (Applied Biosystems, USA).

Phylogenetic analysis

Upon receiving the vector sequence chromatograms, the sequence of the inserted DNA fragment was isolated by Gene Runner software (version 3.05). These sequences were BLASTed in the Genbank database for confirmation. *Blastocystis* subtypes were identified by determining the exact match or the closest similarity against all known *Blastocystis* subtypes, as per the last classification by Stensvold et al. (2009b). Sequences downloaded from Genbank, as well as those obtained from this study, were aligned using ClustalW of BioEdit software (version 7.0.9.0) (Hall, 1999).

A phylogenetic tree was constructed using the neighbour-joining method carried out using the software MEGA version 4 (Tamura et al., 2007), and molecular distances were estimated using the Kimura two-parameter model (Kimura, 1980). Branch reliability was assessed using bootstrap analysis (1000 replicates). *Proteromonas lacerata* (U37108), an organism phylogenetically closely related to *Blastocystis* sp., was used as the out-group.

Ethical Approval

Ethical clearance was obtained from the University of Malaya, Malaysia, the Kano State Ministry of Health and the Kano State Hospitals Management Board, respectively.

Results and Discussions

Results

Identification and Prevalence of Microorganisms

Microscopic examination of the 551 stool samples collected revealed that 161 participants (29.2%) had Blastocystosis. The overall and demographic distribution of Blastocystosis is presented in Figures 1 and 2. The infection was more prevalent among males (33.5%) than among females (22.3%), and more so among young participants aged 15 and below than among older participants.

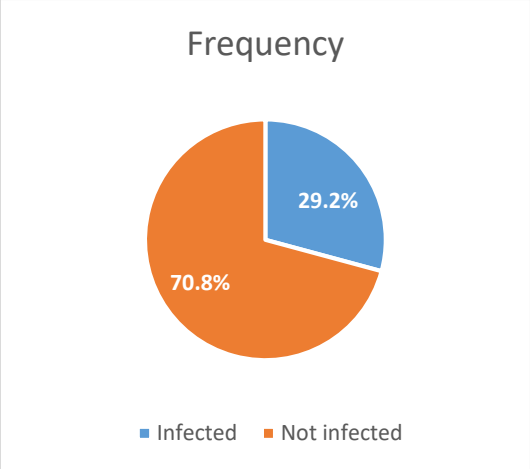

Figure 1. Overall prevalence of infection among the study participants

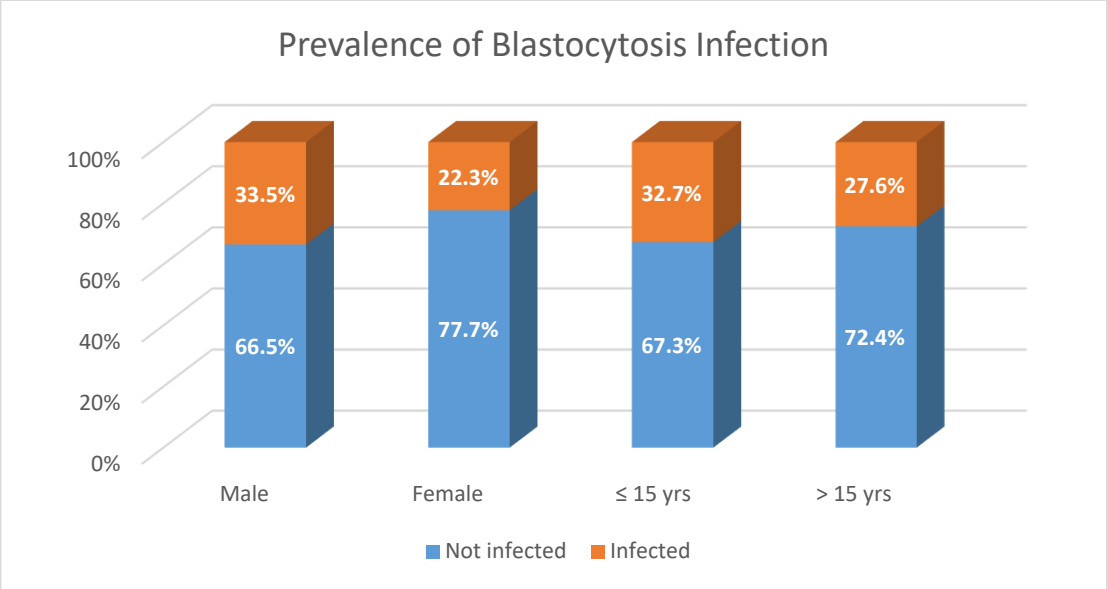

Figure 2. Distribution of *Blastocystis* infection among the study participants with regard to gender and age

Of the 161 microscopy-positive samples, only 54 (32.3%) were successfully cultured, and subsequent subtype analysis was executed. Data on the prevalence of *Blastocystis* subtypes in the study are summarised in Table 1. The cultured samples comprised 39 (34.2%) males, 15 (31.9%) females, 33 (31.4%) adults and 21 (37.5%) young respondents. In all four subtypes were identified containing ST1 (42.6%; 23/54), ST2 (7.4%; 4/54), ST3 (31.5%; 17/54) and ST4 (13%; 7/54). Three samples yielded ambiguous DNA sequences, indicating mixed infections with more than one strain. Subsequent analysis revealed the presence of ST4 and ST2, ST1 and ST3, and ST2 and ST1, respectively. Furthermore, ST1, ST3, and ST4 were recorded more among male (17, 12, and 5, respectively) respondents, but ST2 was of equal frequency (2). All the mixed infections were observed among male respondents. Similarly, frequencies of colonisation with all the subtypes were higher in adults (15, 11 and 4) than in children, except ST2, which was the same (2). However, mixed infections of ST4/ST2 and ST1/ST3 were only observed in children, while mixed infection of ST2/ST1 was found in an adult only.

Two to four clones were randomly selected (namely a, b, c, d) from each isolate and subjected to DNA sequencing. Each sequence was then exploited in the ClustalW programme to establish multiple alignments, and it was found that nucleotide sequences in the four clones (a, b, c, d) from each of the 46 isolates and two clones (a and b; c and d) from each of the eight isolates were identical. A high homology was exhibited between the test sequences and the closest match reference sequences from Genbank (Data shown in Table 2).

Figure 3 shows the phylogenetic tree obtained with further analysis using *Proteromonas lacertae* (U37108) as out-group applying the rooted neighbour-joining tree; 9 clades were recognised (ST1- ST9) with moderate to strongly supported bootstrap values. Isolates from this study clustered under four subtypes, ST1, ST2, ST3 and ST4 and supported by good bootstrap values formed four independent monophyletic groups. The tree showed isolates of all three subtypes (ST1, ST2 and ST3) clustered in one group each. However, isolates belonging to ST4 had two distinct clades with well-supported bootstrap values (64% and 99%).

Table 1: Prevalence of *Blastocystis* Subtypes among the Respondents

Blastocystis subtypes	Overall N = 161	Gender Male n = 114	Females n = 47	Age groups Adults >15yrs n = 105	Children ≤ 15yrs n = 56
ST1	23	17	6	15	8
ST2	4	2	2	2	2
ST3	17	12	5	11	6
ST4	7	5	2	4	3
ST4 & ST2	1	1	0	0	1
ST1 & ST3	1	1	0	0	1
ST2 & ST1	1	1	0	1	0
Total (%)	54(32.3)	39(34.2)	15(31.9)	33(31.4)	21(37.5)

Table 2: *Blastocystis* subtypes and percentage homology with their closest match reference sequence from Genbank

Blastocystis Isolates clones	ST	Reference isolate (Accession no.)	Sequence homology %
KN60[a,b,c,d], KN200 [a,b,c,d], KN206 [a,b,c,d], KN231 [a,b,c,d], KN266[a,b,c,d], KN286[a,b,c,d], KN303[c,d], KN435[a,b], KN530[a,b,c,d], KN805[a,b,c,d] , KN1022 [a,b,c,d], KN1045[c,d], KN1049[a,b,c,d], KN1097[a,b],KN1102 [a,b,c,d], KN57 [a,b,c,d]	1	GU992416	99.7
KN320[a,b,c,d], KN334[a,b,c,d], KN354 [a,b,c,d], KN359[a,b,c,d], KN509 [a,b,c,d], KN518 [a,b,c,d]			97.0
KN215 [a,b,c,d], KN338 [a,b,c,d],	2	GU992412	99.0
KN415[a,b], KN1036 [a,b,c,d], KN1097[c,d], KN80 [a,b,c,d],			99.0
KN26[a,b,c,d], KN70[a,b,c,d], KN1012[a,b,c,d], KN1047[a,b,c,d], KN1187[a,b,c,d], KN88[a,b,c,d]			100
KN234[a,b,c,d], KN249[a,b,c,d], KN265 [a,b,c,d], KN288[a,b,c,d], KN315[a,b,c,d], KN329[a,b,c,d],KN341[a,b,c,d], KN435[c,d], KN521[a,b,c,d]	3	AB107963	99.7
KN1109 [a,b,c,d], KN1173 [a,b,c,d], KN87 [a,b,c,d]			99.5
KN415[c,d], KN522 [a,b,c,d], KN533 [a,b,c,d], KN1087[a,b,c,d]			100
KN241[a,b,c,d], KN433[a,b,c,d] KN313[a,b,c,d], KN374[a,b,c,d]	4	AY590114	99.7

Isolates from Kano (KN); clones (a, b, c, d); Subtype (ST); isolate clones with bold were used in phylogenetic tree construction.

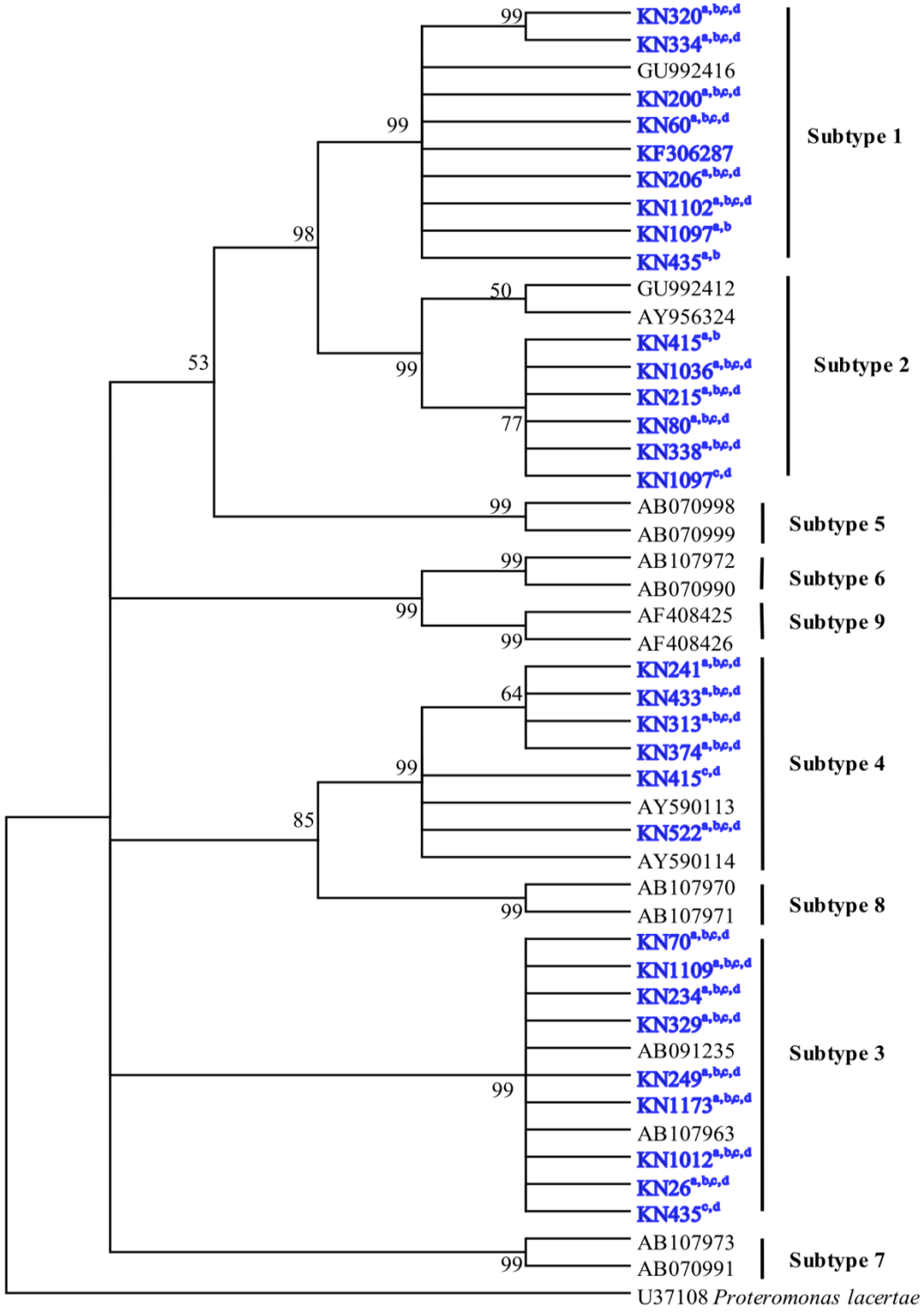

Figure 3: Phylogenetic tree of the SSU rDNA gene sequences of *Blastocystis* isolates as inferred using the neighbour-joining method. Reference isolates and accession numbers are available in GenBank. Sequences generated in this study (designated as 'KN—' followed by clones 'a,b,c,d'). *Proteromonas lacertae* (U37108) served as the out-group. Bootstrap values (%) are indicated at the internal nodes (1,000 replicates). Bootstrap values of less than 50% are not shown.

Discussion

Blastocystis sp. is currently the most common unicellular eukaryote in human faecal samples (Osman et al., 2015; El Safadi et.al., 2014). Prevalence of *Blastocystis* sp. in humans may exceed 50% in developing countries and up to 20% in industrialised countries (El Safadi et al., 2014; Scanlan et al., 2014). The overall prevalence of *Blastocystis* infection was 29.2%. The prevalence of *Blastocystis* infection is remarkably high in many African cohorts, sometimes reaching up to 100% in specific communities (El Safadi et al., 2014), primarily linked to poor sanitation and close contact with animals.

Despite the debate over the involvement of *Blastocystis* sp. in clinical manifestations (Kurt et al., 2016), in Nigeria, the detection of *Blastocystis* sp. is not routinely performed. Few studies reported on *Blastocystis* sp., including two which employed the direct faecal smear technique, with a prevalence of 2.83% and 0.94% among HIV-positive and HIV-negative patients, respectively (Ochigbo et al., 2011), while Adekunle (2002) found *Blastocystis* sp. in only one child out of 1,273 subjects. Alfellani et al. (2013a) found a higher prevalence (49%) of the infection among clinical samples from Lagos, Nigeria, as determined by PCR. Poulsen and colleagues (2016) found that 84% of children in Alero were infected. It has been speculated that the disparity between prevalence data is mainly dependent on the methods used for detection (Poirier et al., 2012).

In other parts of Africa, Abdulsalam et al. (2013a) reported the prevalence of *Blastocystis* sp. as 21.2% among outpatients in Libya. Correspondingly, Alfellani et al. (2013a) found that 28% of samples from Sebha, Libya, and 70% of Liberian samples were infected with *Blastocystis* sp. In Egypt, the prevalence of *Blastocystis* sp. has been reported as 22% (El-Shazly et al., 2006). El Safadi et al. (2014) reported a 100% prevalence in a study conducted on 6- to 10-year-old children in Northern Senegal, while Khaled and colleagues (2020) reported an 80.4% infection rate among school children from the same country. Furthermore, similar prevalence was reported in Iran (28.2%), Malaysia (25.6%), and Colombia (22.4%) (Abdulsalam et al., 2012; Boeke et al., 2010; Daryani et al., 2006). Higher prevalence exceeding 40% was reported from communities in Venezuela (Velasco et al., 2011), Peru (Machicado et al., 2012), Colombia (Ramirez et al., 2013), rural Laos (Sanpool et al., 2017) and Senegal (El Safadi et al., 2014). Likewise, a high prevalence was reported among immigrants in Naples, Italy (Gualdieri et al., 2016), as well as in Qatar and the UAE, where the majority were from African and Asian countries (AbuOdeh et al., 2016; Abu-Madi et al., 2015). Besides, lower prevalence has been reported among school-aged children from Thailand, Turkey, Indonesia and India (Idris et al., 2010; Rayan et al., 2010; Aksoy et al., 2007; Yaicharoen et al., 2005). Thus, *Blastocystis* sp. is cosmopolitan in the human population.

The pathogenicity of *Blastocystis* remains contentious, with recent evidence suggesting it may function as both a commensal and a pathogen depending on various factors, including subtype variation, host immune status, and gut microenvironment (Ibrahim et al., 2025; Zaman Wahid et al., 2023). Although many carriers show no symptoms, *Blastocystis* is increasingly associated with gastrointestinal issues and symptoms outside the gut, particularly in immunocompromised people (Alfellani et al., 2013a). Recent studies have identified potential virulence factors, such as cysteine proteases that break down mucin glycoproteins in the gut mucus layer, thereby weakening the intestinal barrier. Furthermore, *Blastocystis* can disrupt tight junction proteins such as claudin-7, leading to greater epithelial permeability and possibly triggering inflammatory responses (Lepczyńska et al., 2016).

The clinical significance of specific *Blastocystis* subtypes is being increasingly elucidated, with recent studies suggesting that specific subtypes may be more likely to cause disease than others.

For instance, ST1 has been associated explicitly with diarrhoea-predominant irritable bowel syndrome (IBS) in some populations. However, the relationship is complex, as a 2025 review highlighted the "dual roles of *Blastocystis* subtypes that can either exacerbate gut inflammation by disrupting the epithelial barrier or confer protection by enhancing barrier integrity and inducing anti-inflammatory immune responses" (Deng & Tan, 2025; Rudzińska & Sikorska, 2023; Deng et al., 2021). Furthermore, a recent study reported a significantly higher prevalence of *Blastocystis* spp. in IBS patients (33.5-71.4%) compared to control groups (12%). Notably, the reduction in IBS symptoms following antiparasitic treatment further supports the potential involvement of *Blastocystis* in IBS pathogenesis (Rudzińska & Sikorska, 2023). The exact nature of this relationship remains unclear—whether IBS-related gut dysfunction facilitates *Blastocystis* colonisation or whether the protozoan directly contributes to intestinal disturbances requires further investigation (Kesuma et al., 2019). Accordingly, in the present study, the prevalence of the subtypes was determined.

From the total of 161 *Blastocystis* positive samples, only 54 (32.3%) were successfully cultured. The remaining were unable to grow; perhaps the parasites lost potency during the course of transportation from Nigeria to Malaysia. However, DNA extraction and amplification were successfully carried out against cultured samples only, and a phylogenetic tree was constructed to demonstrate the genetic diversity of the STs present. The phylogenetic tree revealed four well-defined clades that identified and classified the isolates into four different subtypes. The isolates clustered under ST1, ST2, ST3 and ST4 each with well-supported bootstrap values. Isolates belonging to ST4 were clustered into two groups within a well-supported clade (64-99%), while all others formed an individual clade. Although ST5-ST9 were not discovered in our study, the topology and branch order in the present results showed that ST1 and ST2 are closely related. The tree also revealed that ST4 and ST8, as well as ST6 and ST9, are closely related. Other researchers have obtained comparable tree topology and branch order (Abdulsalam et al., 2013b; Whipps et al., 2010; Noël et al., 2005). The close relationship between ST1 and ST2, ST4 and ST8, as well as ST6 and ST9, has been previously noted in several phylogenetic studies (Abdulsalam et al., 2013b; Santín et al., 2011; Whipps et al., 2010; Stensvold et al., 2009b, 2007).

In this study, *Blastocystis* ST1 was the most prevalent, followed by ST3 and ST4, with ST2 being the least prevalent. ST1–ST4 isolates have been postulated as the most dominant in all human populations worldwide, while ST5-ST9 are sporadically found (Meloni et al., 2011). ST10–ST17 are exclusively reported in animals (Alfellni et al., 2013b). A similar observation of higher ST1 prevalence was reported among children of Ilero, Nigeria (Poulsen et al., 2016). In a study by Alfellni et al. (2013a), the distribution of *Blastocystis* subtypes in four African countries was dominated by ST1. Corroborating these are reports from Libya and Egypt (Abdulsalam et al., 2013a; Hussein et al., 2008). Similarly, among immigrants of African origin in Qatar, ST1 was predominant, whereas the general distribution of *Blastocystis* sp. subtypes in their study was dominated by ST3 (Abu-Madi et al., 2015). Consistent with the latter was the report from Senegal, Egypt, UAE, Turkey, Malaysia and India (Dogan et al., 2017; AbuOdeh et al., 2016; Pandey et al., 2015; El Safadi et al., 2014; Souppart et al., 2010; Souppart et al., 2009; Özyurt et al., 2008). In most of these studies, ST1 was the second most prevalent.

The third most prevalent subtype, ST4, among the Nigerian samples agrees with the comparative study by Alfellani et al. (2013a), although they did not detect ST2 among Nigerians; in our findings, it has the least prevalence. Subtype ST2 was the least occurring, and ST4 was not found among immigrants in the UAE (AbuOdeh et al., 2016). Discrepancies in the specific and relative proportions of STs observed

between countries and within the same country reflect actual differences between communities. Thus, these denote the divergent epidemiological milieus that abet transmission, most likely related to local living conditions and customs, rather than differences in the susceptibility to infection (Dogan et al., 2017; Souppart et al., 2010; Stensvold et al., 2009a).

As previously suggested, ST1 and ST2 are zoonotic infections originating from domestic animals, and rodents are the primary animal reservoir for ST4 (Alfellni et al., 2013a; Stensvold et al., 2009b, 2012). Hence, the high prevalence of ST1 in this study implies that contact with animal faeces could be a significant source of infection. Several families in Nigeria, especially in rural areas, keep animals at home, including cows, goats, sheep, poultry, cats, dogs, donkeys and horses, while rodents are an unavoidable nuisance. However, studies on the distribution of *Blastocystis* subtypes in animals in Nigeria are needed to provide further evidence. ST3 is relatively highly host-specific, and its infections are primarily transmitted from person to person (Abdulsalam et al., 2013a; Yoshikawa et al., 2000). The prevalence of ST3 in the present study may be attributed to the low living standards and poor personal hygiene prevalent in Nigeria, particularly in rural areas. Circumstances allow for such tranquil human-to-human transmission with ease.

In our findings, the majority of the infections were single-subtype infections, and only 5.6% were mixed infections. The prevalence of mixed infections observed in the present study (5.6%, 3/54) was roughly similar to that described in other countries such as Senegal (8.6%; El Safadi et al., 2014), Libya (6%; Abdulsalam et al., 2013a), Egypt (5%; Souppart et al., 2010), Turkey (4.3%; Dogruman- Al et al., 2008: and 4.3%; Dogan et al., 2017), France (7.5%; Souppart et al., 2009), Italy (13.3%; Meloni et al., 2011) and China (2.6%; Yan et al., 2006). However, Khaled et al. (2020) reported a much higher mixed infection rate of 28% in Senegal. Research has established that most *Blastocystis* infections were of single subtypes; however, Scanlan et al. (2015) have recently demonstrated that mixed infections in healthy individuals are under-estimated. Using another sub-typing assay, they confirmed that 22% of previously classified single-subtype infections were actually mixed-subtype infections.

Conclusion

In conclusion, the prevalence of *Blastocystis* in the Nigerian community was high, indicating the importance of educating health practitioners on the need to incorporate *Blastocystis* diagnosis in relevant infections. Among the apparently healthy population, ST1 and ST3 were the most prevalent subtypes of the four subtypes recovered. Regarding the zoonotic potential of *Blastocystis* infection, studies on the distribution of *Blastocystis* subtypes among animals, particularly domestic animals, are crucial for understanding the epidemiology of *Blastocystis* in Nigeria.

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Conflict of Interest

The authors declare that there is no conflict of interest regarding the publication of the paper.

Author Contribution

The authors confirm contribution to the paper as follows: study conception and design: S.S.D., I.I. and A.H.M.; collection: S.S.D.; analysis and interpretation of results: A.A.M., and S.S.D.; draft manuscript preparation: S.S.D., A.A.M. All authors reviewed the results and approved the final version of the manuscript.

The author confirms sole responsibility for the following: study conception and design, data

collection, analysis and interpretation of results, and manuscript preparation.

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