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Phenotypic and Molecular Identification of Dermatophytes from Clinical Specimens at a Teaching Hospital in Bangladesh

Zannatul Ferdous Tania[1], Afreen Sultana[2], Pompy Dey[3], Ayesha Ahmed Khan[4], Mahmood Al Farabi[5], Ripon Barua[6]

[1]Lecturer, Department of Virology, Chittagong Medical College, Chattogram, Bangladesh; [2]Assistant Professor, Department of Microbiology, Chittagong Medical College, Chattogram, Bangladesh; [3]Lecturer, Dept of Microbiology, Rangamati Medical College, Rangamati, Bangladesh; [4]Assistant Professor, Department of Microbiology, Institute of Applied Health Sciences (IAHS), Chattogram, Bangladesh; [5]Consultant, Department of Paediatrics, Chittagong Medical College Hospital, Chattogram, Bangladesh; [6]Professor & Head, Department of Microbiology, Chittagong Medical College, Chattogram, Bangladesh

Abstract

Background: Clinical diagnosis can be challenging as dermatophytoses often mimic other dermatoses, underscoring the need for accurate laboratory methods. **Objectives:** The purpose of the present study was to isolate and identify dermatophyte species using both phenotypic and molecular approaches. **Methodology:** This cross-sectional study was conducted over one year in the Department of Microbiology, Chittagong Medical College, Chattogram, Bangladesh enrolling 300 patients with clinically suspected dermatophytosis from the Department of Dermatology and Venereology at Chittagong Medical College Hospital, Chattogram, Bangladesh. The clinical samples (skin, hair, and nails) were subjected to direct microscopy and culture on Dermatophyte Test Medium and Sabouraud's Dextrose Agar. Identification was based on colony morphology, lactophenol cotton blue staining, and biochemical tests, including urease and hair perforation. Molecular confirmation was done via PCR using pan-dermatophyte and species-specific primers. **Results:** Out of 300 samples, 103(34.3%) were culture-positive, with microscopy positivity in 31.7% and concordant results in 29.0%. Most cases were in the 21 to 30 age group, with a female predominance (67.0%). *Tinea corporis* was the most common clinical presentation, and *Trichophyton mentagrophyte*s was the *predominant species*. PCR confirmed 95.14% of culture-positive isolates; 88 were identified at the species level, while 10 showed no amplification. **Conclusion:** Traditional methods remain valuable; PCR offers improved accuracy in species identification and supports better management of dermatophytic infections.

Keywords: Dermatophytes; LPCB; Trichophyton; PCR

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Introduction

Fungi, although traditionally categorized in the plant kingdom, are more closely related to humans and animals. They are all around us, and when they come into contact with our skin, they can lead to a variety of skin illnesses. Fungal infections on the skin are primarily caused by dermatophytes. These are closely related filamentous fungus damaging and using keratin present in the skin, hair, and nails[1].

Dermatophyte infections, often known as tinea or ringworm, are limited to the nonliving stratum corneum of the epidermis. Trichophyton, Microsporum, and Epidermophyton are the most common causes of human infections[2]. Trichophyton species are the primary cause of dermatophytosis, with a prevalence of 70.0% to 90.0% for onychomycosis and 53-86% for various tinea infections[3]. The clinical lesion of dermatophytosis is named after its ability to

Correspondence: Dr. Zannatul Ferdous Tania, Lecturer, Department of Virology, Chittagong Medical College, Chattogram, Bangladesh; Email: taniaullah123@gmail.com; Cell: 01673600379;
ORCID: https://orcid.org/0009-0001-5909-4617

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attack various bodily parts, such as Tinea capitis (scalp), Tinea corporis (body), Tinea cruris (groin), Tinea pedis (foot), Tinea unguium (nail), Tinea facie (face), Tinea barbae (beard), and Tinea manuum (hand)[4].

Dermatophytosis is not a fatal illness, but its high morbidity and cosmetic effects make it a major public health concern. Dermatophytic infections are mainly detected clinically, but because steroid ointments and creams are applied topically, they are frequently misdiagnosed and mistreated as other skin infections[5].

Traditionally, clinical samples are directly analyzed under a microscope to identify dermatophytes using potassium hydroxide (KOH). The diagnosis is supported by biochemical testing such as urease and hair perforation tests[6]. The shortcomings of traditional approaches have been resolved by contemporary molecular tests like polymerase chain reaction which offer faster and higher sensitivity in dermatophyte identification[7].

Methodology

Study Settings and Population: This cross-sectional study was conducted in the Department of Microbiology, Chittagong Medical College for one year. Using purposive sampling, 300 patients with clinically suspected dermatophytosis from the Department of Dermatology and Venereology, CMCH, were included based on specific clinical features, excluding other skin conditions and recent antifungal use.

Laboratory Procedure: Specimens (skin scrapings, nail clippings, hair root plucking) were collected after cleaning suspected lesions with 70% ethyl alcohol to eliminate surface contaminants[8]. The samples were divided into two portions: one for microscopic examination and one for culture[9].

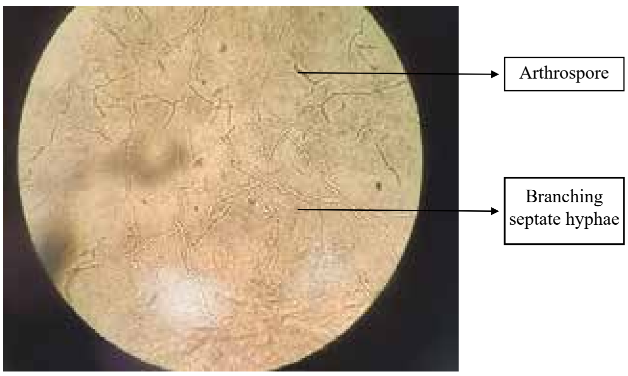

Figure I: Direct microscopic examination of specimen in KOH mount (40X) showing branching septate hyphae with arthrospore

Isolation of Dermatophytes: For direct microscopy, a small specimen was placed in 20% KOH on a slide, covered, and cleared with time or gentle heat as needed. Sabouraud's dextrose agar media with antibiotics and antifungal (Mycobio media) and Dermatophyte test medium (DTM) with antibiotics (HiMedia©, India) were used for the primary isolation of the fungus[10].

All plates containing SDA media and DTM with antibiotics were incubated at room temperature (25ºC) for 4 weeks[11]. The inoculated petri dishes were examined every alternate day from the day of inoculation. SDA and DTM plates were observed for 4 and 2 weeks, respectively. The evidence of growth was considered positive, and subculture was done in PDA media.

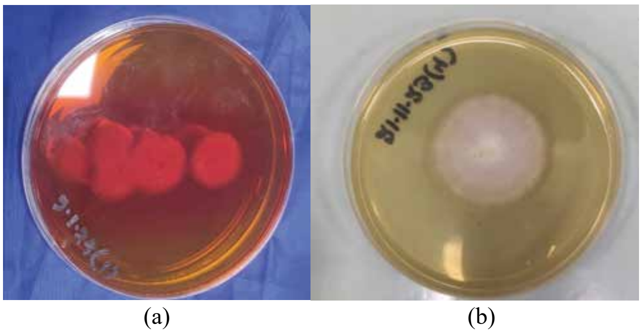

Figure II: Fungal growth on DTM and SDA media; II(a) showing the colour change of DTM from straw colour to red colour after fungal growth and Figure II(b) showing cottony growth of dermatophytes.

Microscopic Examination (Lactophenol Cotton Blue Stain): Fungal growth from culture media was picked with a sterile wire loop, placed in a drop of lactophenol cotton blue on a slide, teased gently with a needle, and covered with a coverslip. The slide was examined under low and high power to observe hyphae, macroconidia, microconidia, and other distinctive structures[12].

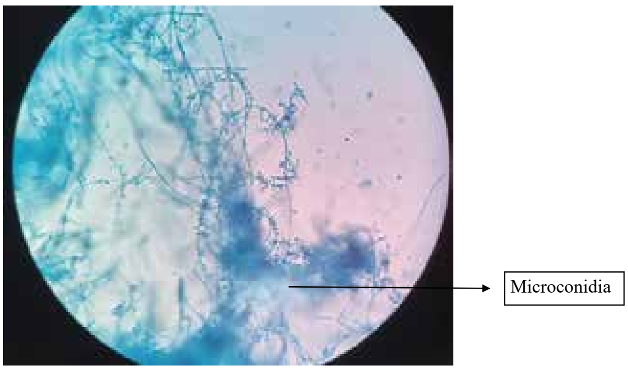

Figure III: Microscopic Findings of Trichophyton rubrum in LPCB mount(40X) showing Microconidia

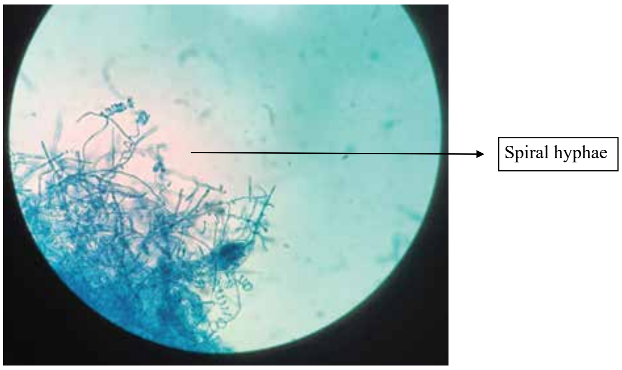

Figure IV: Microscopic findings of Trichophyton mentagrophytes in LPCB mount(40X) showing spiral hyphae.

Biochemical Tests: Christensen's urea agar slants were inoculated and incubated at 25°C for 7 days. Straw-to-pink colour change indicated urease activity[12].

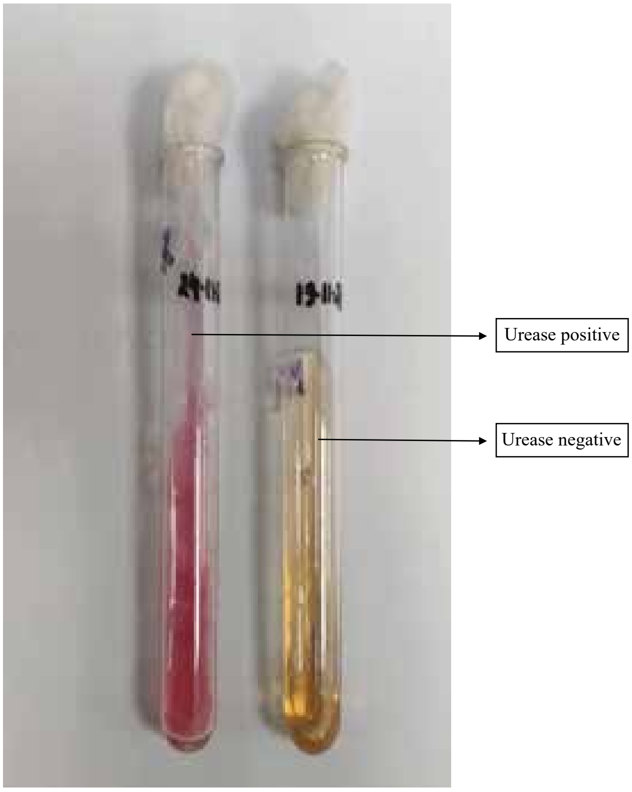

Figure V: Urease Test in Christensen's Urea Agar Media showing the colour change of media from straw to red colour after fungal growth

Hair Perforation Test: Sterilized hair fragments were placed in sterile water with yeast extract, inoculated with fungi, and incubated at 25^{0}C. After 10 days, lactophenol cotton blue mounts showed wedge-shaped hair perforations in positive cases[12]. So phenotypic identification of the organisms was done by observing hyphae and conidia under a microscope, colony morphology, microscopic characteristics of the colony on LPCB mount and relevant biochemical tests (Hair perforation test and urease test).

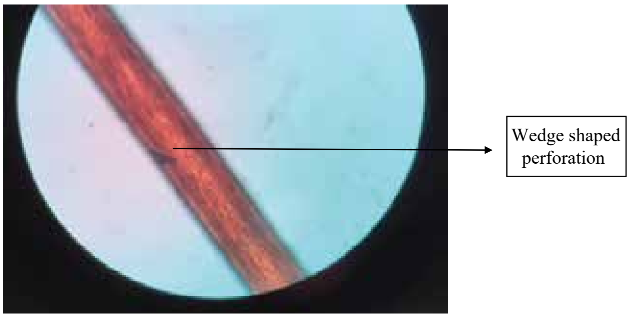

Figure VI: Wedge-shaped perforation in Hair-by-Hair perforation test.

Molecular Methods: Molecular analysis was done using conventional multiplex PCR. Molecular studies of isolated dermatophytes were carried out to detect Trichophyton mentagrophytes, Trichophyton rubrum, Epidermophyton floccosum, and Microsporum canis by polymerase chain reaction. Four major steps of PCR included fungal pellet formation, DNA extraction, DNA amplification in a thermal cycler, and visualisation/documentation under UV light.

Each PCR run comprised of pre-heat at 94°C for 5 minutes followed by 30 cycles for denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds, extension at 72°C for 30 seconds with the final extension at 72°C for 10 minutes.The amplified DNA was analyzed by 1.5% agarose gel-electrophoresis at 100 volts for 35 minutes, stained with SYBR-green DNA gel stain and visualized under ultraviolet light (Figure VII).

Statistical Analysis: Statistical analysis was performed by Windows based software named as Statistical Package for Social Science (SPSS), versions 22.0 (IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp.). Categorical data were summarized in terms of frequency counts and percentages. Continuous data were expressed as mean, standard deviation, minimum and maximum.

Ethical Consideration: All procedures of the present study were carried out in accordance with the principles for human investigations (i.e., Helsinki Declaration 2013) and also with the ethical guidelines of the Institutional research ethics. Formal ethics approval was granted by the local ethics committee.

Table 1: Primers Used in This study for Species of Dermatophyte

Fungal species		Primer Sequence	Size (bp)	Reference
Dermatophyte	F	GAA GCC TGG AAG AAG ATT GTC G	432	13
	R	CCT TGA TTT CAC CGC AGG CAC		
Trichophyton rubrum	F	CCC CCC ACG ATA GGG ACCG	214	13
	R	GAC TGA CAG CTC TTC AGA GAA		
Trichophyton mentagrophyte	F	GCC CCC CAC GAT AGG NGCC AA	132	13
	R	CTC GCC GAA CGG CTC TCC TG		
Epidermophyton floccosum	F	GAA GCC TGG AAG AAG ATT GTC	313	14
	R	CCT TGA TTT CAC CGC AGG CAC G		
Microsoprum canis	F	GTG TGA TGG ACG ACC GTC CCC CCT	176	14
	R	ATA ATA CAT GGT GCG TTA GGC CAG		

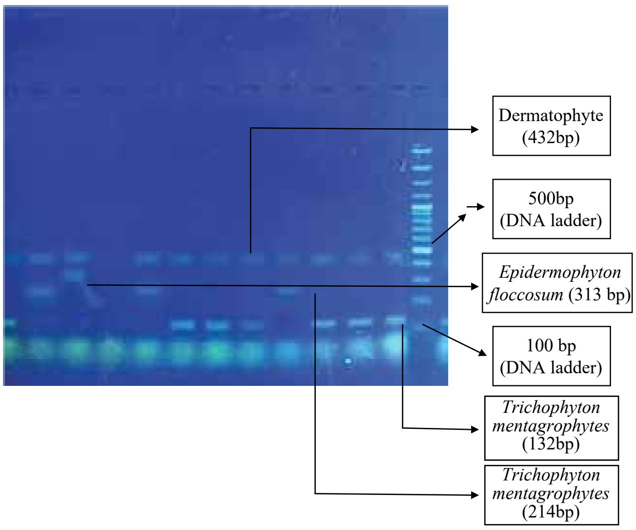

Figure VII: Visualization of amplified DNA products of Pan dermatophyte primer and different species-specific primers in Gel electrophoresis showing 100 bp DNA ladder(lane 13), DNA of 432 bp for pan dermatophyte gene in all lanes (except lane 4), DNA of 313bp for Epidermophyton floccosum gene(lane 3), DNA of 214 bp for Trichophyton rubrum(lane 2,5,9) and DNA of 132 bp for Trichophyton mentagrophytes (lane 1,6,7,8,10,11,12)

Participants in the study were informed about the procedure and purpose of the study and the confidentiality of information provided. All participants consented willingly to be a part of the study during the data collection periods.

Results

A total of 300 samples were collected in the present study where maximum specimens were from skin 254(85.0%), followed by nail 33(11.0%) and hair 13(4.0%). Out of 300 samples, 106 fungi were identified by microscopic examination of 20.0% KOH mount and 194 were negative (Table 2).

Table 2: Detection Rate of Dermatophytes by Microscopy and Culture (N=300)

Diagnostic Methods	Positive	Negative	Total
Microscopic Examination	106(35.3%)	194(64.7%)	300
Culture	122(40.7%)	178(59.3%)	300

Among 300 samples,122 fungi were isolated by culture and 178 could not be isolated. Out of 122 culture-positive cases, Dermatophytes are the commonest fungus 103(84.0%) followed by non-dermatophytic mould 12(10.0%) and Candida 7(6.0%) (Figure VIII).

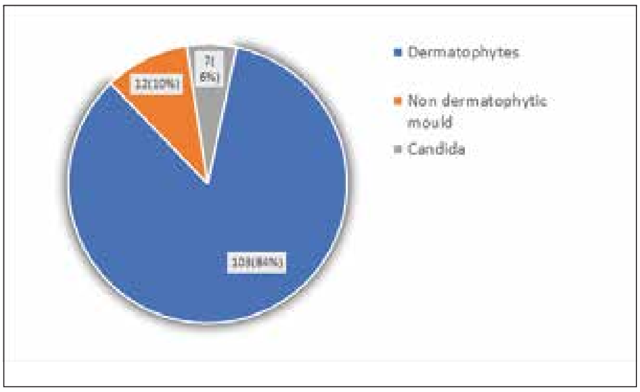

Figure VIII: The Isolation Rate of Different Fungi from Culture Media

Out of 300 specimens, skin samples (n=254) showed microscopy positivity of 33.07% and culture positivity of 33.85%, while nail samples (n=33) showed 30.30% and 45.45%, respectively. Hair samples (n=13) had the lowest positivity, with 7.7% by microscopy and 15.4% by culture, giving overall positivity rates of 31.7% (microscopy) and 34.3% (culture) (Table 3).
The distribution of dermatophyte species isolated from culture from different clinical specimens showed that the highest number of isolation of dermatophytes was

obtained from Tinea corporis. Among the 48 cases of Tinea corporis, 38(79.2%) cases were caused by Trichophyton mentagrophytes. Trichophyton rubrum was found as main causative organism for Tinea cruris in 20(62.5%) cases and Tinea unguium in 6(54.5%) cases. Biochemical tests on 103 culture-positive dermatophyte isolates showed that all 58 isolates of Trichophyton mentagrophytes were urease-positive (100%), while 5 (8.62%) showed hair perforation activity (Table 4).

Table 3: Comparison of Microscopy and Culture Positivity for Skin, Nail, And Hair Specimens (N=300)

Specimen Type	Total	Microscopy Positive	Culture Positive
Skin	254	84 (33.1%)	86 (33.9%)
Nail	33	10 (30.3%)	15 (45.5%)
Hair	13	1 (7.7%)	2 (15.4%)
Total	**300**	**95(31.7%)**	**103 (34.3%)**

Table 3: Comparison of Microscopy and Culture Positivity for Skin, Nail, And Hair Specimens (N=300)

Suspected species	Biochemical test	
	Urease Test	Hair Perforation Test
Trichophyton mentagrophyte(n=58)	58(100.0%)	5(8.6%)
Trichophyton Rubrum (n=36)	0(0.0%)	0(0.0%)
Trichophyton tonsurans (n=4)	4(100.0%)	0(0.0%)
Epidermophyton floccosum (n=5)	0(0.0%)	0(0.0%)

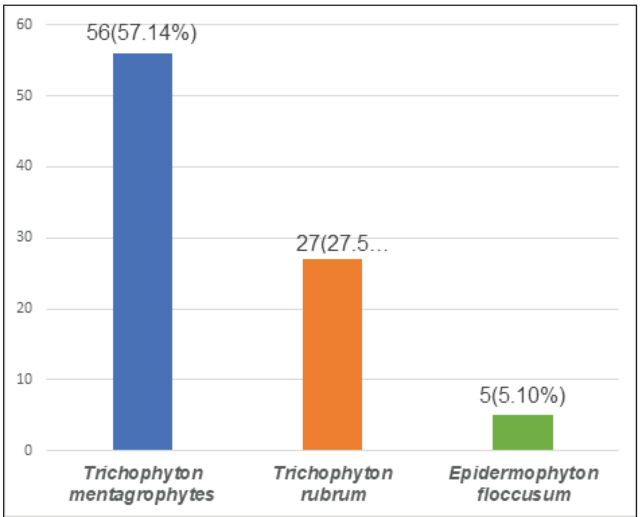

Figure IX: Detection of Dermatophyte Species by PCR (n= 88)

No band was found in 10 pan dermatophyte primer-positive samples. The isolation rate of dermatophytes by PCR from culture using pan dermatophyte primer showed that out of 86 culture-positive skin samples, 82(94.3%) was PCR-positive. In the case of the nail specimens,15(100.0%) showed PCR positivity out of 15 culture-positive samples. Hair specimens showed 1(50.0%) PCR positivity. A total of 98(95.1%) were positive in PCR out of 103 culture-positive samples. Species-specific PCR identified T*richophyton mentagrophytes* in 56(57.1%) cases, *Trichophyton rubrum* in 27(27.6%), and *Epidermophyton floccosum* in 5(5.1%) cases. No amplification was seen in 10(9.7%) samples. *Microsporum canis* and *Trichophyton tonsurans* were not detected PCR primers for the latter were not included (Figure IX).

Discussion

This study found a dermatophyte culture positivity rate of 34.3%, aligning with findings by Khan et al[13] (30.9%) and Islam et al16 (38.8%). Higher rates of 36.6% to 78.4% reported in India may be due to climate, population density, hygiene, and healthcare access[17]. A female predominance (67.0%) was noted, similar to Khan et al[13], possibly due to occlusive clothing and sweating. However, other reported male predominance, likely due to outdoor exposure and physical labor[16-17].

Most cases (34.0%) occurred in the 21 to 30 age group, consistent with Khan et al[13] and Kamothi et al[18] reflecting higher exposure, sweating, and physical activity. Tinea corporis was the most common clinical type (30.4%), consistent with Khan et al[13] and Sudha et al[19]. Direct microscopy was positive in 31.7%, comparable to Mohiuddin et al[20]. Culture alone missed 2.7% and microscopy missed 5.3% of infections. This highlights the benefit of combining both methods, though variations in media, temperature, and incubation can affect outcomes.

Culture identified *Trichophyton mentagrophytes* (57.3%) as the *predominant species*, followed by *Trichophyton rubrum* (34.9%), similar to Khan et al[13] and Murya et al[21]. Its dominance may be linked to anthropization, hemolysin production, and host immunity factors. In contrast, other studies like Mohiuddin et al20 and Lee et al[22] found *Trichophyton rubrum* more prevalent, possibly due to its slow-growing and chronic nature.

PCR confirmed dermatophyte presence in 95.1% of culture-positive cases. False negatives (4.8%) may be due to environmental contamination, poor DNA extraction, or PCR inhibitors. The sensitivity and specificity of PCR (95.2% and 100.0%) were comparable to Pontone[23], confirming its diagnostic utility.

Conclusion

This study revealed that among skin, nail, and hair specimens, *Trichophyton mentagrophytes* was the most frequently isolated species, followed by *Trichophyton rubrum*. Dermatophytosis was most common among young adults. Tinea corporis emerged as the predominant clinical type. Among the diagnostic methods, culture proved to be the most sensitive and reliable. Combining microscopy and culture improves detection accuracy, while PCR offers high specificity for confirmation.

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Conflict of Interest
The authors have no conflicts of interest to disclose.

Financial Disclosure
The authors received no specific funding for this work.

Authors' contributions
Zannatul Ferdous Tania conceived and designed the study, analyzed the data, interpreted the results, and wrote up the draft manuscript; Ripon Barua contributed to the analysis of data and critical review of the manuscript; Afreen Sultana contributed to the analysis of the data, interpretation of the results, and critical review of the manuscript; Pompey Dey was involved in the manuscript review and editing. All authors read and approved the final manuscript; Mahmood Al-Farabi analyzed the data and interpreted the results.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. As this was a prospective study the written informed consent was obtained from all study participants. All methods were performed in accordance with the relevant guidelines and regulations.

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ORCID
Zannatul Ferdous Tania: https://orcid.org/0009-0001-5909-4617
Afreen Sultana: https://orcid.org/0009-0003-0902-7840
Pompy Dey: https://orcid.org/0009-0002-3145-9626
Ayesha Ahmed Khan: https://orcid.org/0000-0003-4101-2035
Mahmood Al Farabi: https://orcid.org/0009-0009-1340-9976
Ripon Barua: https://orcid.org/0000-0001-9898-7306

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