Research Article

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Assessing The Effectiveness Of Both The Xpert Mtb/Rif Assay And Nested Pcr For *Mycobacterium Tuberculosis* Identification.

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ABSTRACT

Objective: The Polymerase Chain Reaction (PCR) provides a more sensitive, specific, and efficient approach for identifying Mycobacterium tuberculosis (Mtb) in comparison to traditional (AFB) smear and culture procedures. This research seeks to evaluate the potential benefits of replacing the nested PCR technique with the Xpert MTB/rifampicin (RIF) test for the detection of Mycobacterium tuberculosis in an area with a moderate incidence of tuberculosis (TB).
Methodology: This research retrospectively evaluated the diagnostic accuracy of Xpert MTB/RIF and nested PCR for tuberculosis, examining sensitivity, specificity, and predictive values using SPSS. A cost-effectiveness study was performed. Data was collected from 350 individuals (210 men, 140 females) at Ibn Sina institutions and hospitals in Dhaka City from July 2021 to June 2023, using standardized questionnaires to obtain demographic and clinical information.
Result: An examination including 350 individuals (210 men, 140 women) revealed that 28% of samples tested positive for Mycobacterium tuberculosis (Mtb), with 72.44% of the cases occurring in males. Pulmonary specimens, especially sputum, had the greatest detection rates. Molecular techniques including as GeneXpert and Nested PCR exceeded AFB smear and culture, with Nested PCR demonstrating 100% sensitivity and specificity across all specimen types.
Conclusion: In conclusion, the Xpert MTB/RIF assay offers faster, more sensitive detection of rifampicin resistance than nested PCR, making it key for rapid TB diagnosis. However, nested PCR remains superior in low-bacterial-load cases. High costs and specialized equipment limit Xpert's use in resource-poor settings, emphasizing the need for affordable, accessible diagnostics.

Keywords : *Mycobacterium tuberculosis (MTB), Molecular detection, polymerase chain reaction (PCR), Pulmonary tuberculosis, Xpert MTB/RIF.*

INTRODUCTION

Tuberculosis (TB) is an important global health issue due to its high transmissibility among individuals [1]. Tuberculosis is a significant public health concern in Korea, with the disease's prevalence being at a moderate level. In 2012, Korea recorded 49,532 cases of TB, with an estimated annual incidence rate of 108 per 100,000 inhabitants [2]. Timely and precise identification of the condition, followed by the prompt start of anti-TB treatment, is essential for attaining positive patient results. However, traditional diagnostic procedures do have some limits [3]. The (AFB) direct test has a restricted capacity to identify the existence of AFB, whereas the mycobacterial culture method is known for its protracted procedure, often taking 2-6 weeks to provide a conclusive result [4]. Advancements in (NAA) techniques have recently facilitated the quick identification and detection of (MTB) in clinical samples [5]. These approaches are appealing because they enable the direct identification of small quantities of MTB

genetic material in samples. (PCR) is a method which employs (NAA) technology to quickly identify (TB) [6]. The university currently utilizes two commercially accessible standardized PCR methods: the Xpert MTB/rifampicin test and MTB nested PCR [7]. The MTB nested PCR was designed to specifically identify and detect certain elements of the MTB complex by targeting either the *IS6110* insertion region or the *mtp40* gene [8]. The Xpert MTB/RIF assay is an automated diagnostic test using real-time PCR to detect Mycobacterium tuberculosis (MTB) and determine its resistance to rifampin. This approach operates via the use of cartridges and is capable of doing many tasks concurrently by selectively targeting the rpoB gene linked to TB [9]. An innovative tuberculosis detection test has been introduced in Korea [10]. This research conducted a comparative analysis to evaluate the clinical effectiveness of the Xpert MTB/RIF test and nested PCR in detecting MTB in patients with active tuberculosis at a recently established university hospital [11]. Tuberculosis (TB) is a significant global health issue, becoming the second leading cause of mortality worldwide in 2022, behind COVID-19 [12]. It results in about double the number of fatalities each year compared to HIV/AIDS [13], with over 10 million new cases documented each year [14]. Tuberculosis (TB) is common in countries with low and middle incomes, where healthcare systems may encounter challenges in efficiently controlling the illness [15]. Bangladesh, a country severely affected by tuberculosis (TB), has an annual increase of over 360,000 new cases [16], resulting in more than 73,000 fatalities [17]. This highlights the substantial influence of TB on the overall health of individuals in the area, resulting in a notable rise in the prevalence of sickness and death [18]. Tuberculosis (TB) propagates by the inhalation of airborne droplets released when an infected person coughs, sneezes, speaks, or spits [19]. The causing agent of tuberculosis (TB), Mycobacterium tuberculosis (MTB), is a nonmotile bacterium characterized by a rod-shaped appearance. It exhibits a modest level of growth and can act as a parasite inside host cells, able to survive both within and outside of the cells. (MTB) demonstrates the ability to multiply and survive inside the host's cells, even under challenging situations. Tuberculosis typically affects the respiratory system, but it may also spread to several other organs, including lymph nodes, the belly, the urinary tract, the central nervous system, skeletal structures, joints, and skin. At times, this might result in the emergence of disseminated tuberculosis [20]. Progress in tuberculosis (TB) detection has resulted in significant improvements in patient care and outcomes. Several nucleic acid amplification techniques have been developed to rapidly identify the presence of (MTB). PCR, an abbreviation for polymerase chain reaction, is a widely used molecular method for rapid identification of tuberculosis (TB) [21]. The inclusion of the Xpert MTB/RIF test in the WHO recommendations in early 2011 is a noteworthy technical accomplishment. The real-time PCR test is fully automated and provides a high degree of diagnostic accuracy, capable of diagnosing TB and rifampin resistance in less than 2 hours. The Xpert MTB/RIF test has revolutionized tuberculosis diagnostics by delivering expedited and accurate results, which are essential for effective patient treatment and timely initiation of medicine. This study emphasizes the persistent importance of tuberculosis (TB) as a significant public health concern, primarily due to its high likelihood of transmission between individuals and the challenges it poses in terms of both diagnosis and treatment.

METHODOLOGY

Study Design

The study employed a retrospective observational design to assess the diagnostic accuracy of the Xpert Mtb/RIF test and nested PCR, focusing on sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV). Data collection was blinded to mitigate bias and ensure impartial evaluation. Conducted at Ibn Sina diagnostic institutes and hospitals in Dhaka City from July 2021 to June 2023, the study involved 350 participants (210 male, 140 female). Information on demographics, TB history, and smoking habits was gathered using a standardized questionnaire.

Specimens Collection

Clinical specimens were meticulously collected from patients displaying symptoms suggestive of TB, encompassing respiratory samples such as morning sputum and non-respiratory samples including cerebrospinal fluid (CSF), lymph node aspirates, urine, tissue biopsies, pericardial fluid, pus, wound swabs, and tracheal aspirates. Highly skilled phlebotomists-maintained specimen integrity through rigorous adherence to contamination-minimizing protocols during both collection and subsequent processing stages. This meticulous approach to study design and specimen handling facilitated a thorough evaluation of the diagnostic tools under investigation, providing valuable insights into their efficacy in diagnosing TB across diverse clinical scenarios.

Laboratory Procedure

After obtaining samples, half were heat-treated at 80°C for 10 minutes to deactivate *M. tuberculosis*. Sputum samples were pre-processed with a 2.5% N-acetyl-L-cysteine-NaOH solution (Kirchner method) to deactivate any live M. tuberculosis. They were centrifuged at 3,000 g for 15 minutes, and the concentrate was preserved for nucleic acid extraction and Xpert MTB/RIF analysis. The remaining samples were sent to the laboratory for tuberculosis culture and acid-fast bacilli (AFB) smear testing using the BACT/ALERT TB method with MP bottles containing 7H9 Middlebrook medium and MB/

BacT supplement. Samples were incubated in the BacT/ALERT 3D system for six weeks. AFB smears were confirmed using auramine-rhodamine fluorescence staining, validated by Ziehl-Neelsen staining. Molecular techniques involved liquefaction with a buffer, treatment with 4% NaOH, DNA extraction using the QIAamp DNA Mini Kit, and subsequent PCR amplification.

Xpert Mtb/RIF Assay

The Xpert MTB/RIF test employed real-time PCR for diagnosing M. tuberculosis within a two-hour timeframe. This system comprised the GeneXpert machine and the Xpert MTB/RIF cartridge, utilizing molecular beacon technology and hemi-nested PCR. Five probes, each with distinct fluorophores, targeted the rpoB gene of rifampicin-susceptible Mtb. Detection required positive signals from at least two probes with a cycle threshold (CT) ≤38 cycles. Resistance was indicated by a ΔCT exceeding 3.5 cycles between the earliest and latest signals. Bacilli concentration was categorized based on CT ranges: high (<16), medium (16–22), low (22–28), and extremely low (>28). Data collection and interpretation were automated by the GeneXpert machine.

Nested PCR

Nested PCR involved two consecutive amplification reactions using different primer sets. The Seeplex® Mtb Nested ACE Detection test, developed by Seegene Inc., was utilized to identify tuberculosis (TB) by detecting IS6110 and mpb64 sequences in the Mtb genome, enhancing accuracy and reducing false negatives. The first PCR round used outer primers for 15 cycles, followed by a second round with inner primers for 45 cycles. The PCR mixture included Tris-HCL, KCL, MgCl2, dNTPs, and Platinum Taq DNA polymerase. Amplified products were separated by agarose gel electrophoresis, treated with ethidium bromide, and visualized under UV light. Controls included Tris-EDTA (negative), and M. tuberculosis strain H37Rv DNA (positive).

Ethical consideration

Ethical clearance was obtained from the institutional review boards of the Ibn Sina Trust in Dhaka. A formal permission letter to conduct the study and publish its findings was secured from the Ibn Sina Diagnostic and Imaging Centre in Dhanmondi. The confidentiality of the collected information was strictly maintained, ensuring all patient information remained confidential.

Statistical analysis

SPSS version 26 was used to conduct the statistical analyses. The median test, which generated a chi-square statistic from nonparametric data, was used to compare medians. Using MS Excel, replicates' means, standard errors, and standard deviations were computed. With the use of statistical analytical software, the data was also examined for (ANOVA).

RESULTS

Demographic analysis

The research included a sample size of 350 individuals, consisting of 210 men and 140 females. The cohort's gender distribution is skewed towards men, which could influence the generalizability of the results to the wider population and perhaps compromise the accuracy of the tests being reviewed. The gender imbalance could be attributed to several factors, such as differences in healthcare-seeking behaviour or changes in the frequency of illnesses among women. When evaluating diagnostic performance metrics such has become the (NPV) and positive predictive value, sensitivity, and specificity, it is crucial to include gender disparities.

Microscopic analysis

Among the 350 samples that were analyzed, 28% (98 out of 350) were found to be positive for *Mycobacterium tuberculosis* (Mtb) by microscopic examination (Figure 2). Out of the total number of identified instances of *Mycobacterium tuberculosis* Mtb, 72.44% (71 out of 98) were male, while 27.55% (27 out of 98) were female. Mtbwas detected in 38.50% of sputum samples (67 out of 174) and 11.11% of pleural fluid samples (2 out of 18) obtained from respiratory specimens. Mtb was identified in 19.33% (29 out of 150) of patients in samples obtained from beyond the lungs. The frequencies of detecting certain extra-pulmonary sample types were as follows: 36.73% (18 out of 49) for pus, 16.67% (3 out of 18) for cerebrospinal fluid (CSF), 20.68% (6 out of 29) for tissue biopsy, 10% (1 out of 10) for wound swab, and 12.5% (1 out of 8) for tracheal aspirate.

Comparative Analysis of *Mycobacterium Tuberculosis* Detection: Non-Molecular Methods vs. Nested PCR and GeneXpert Assay

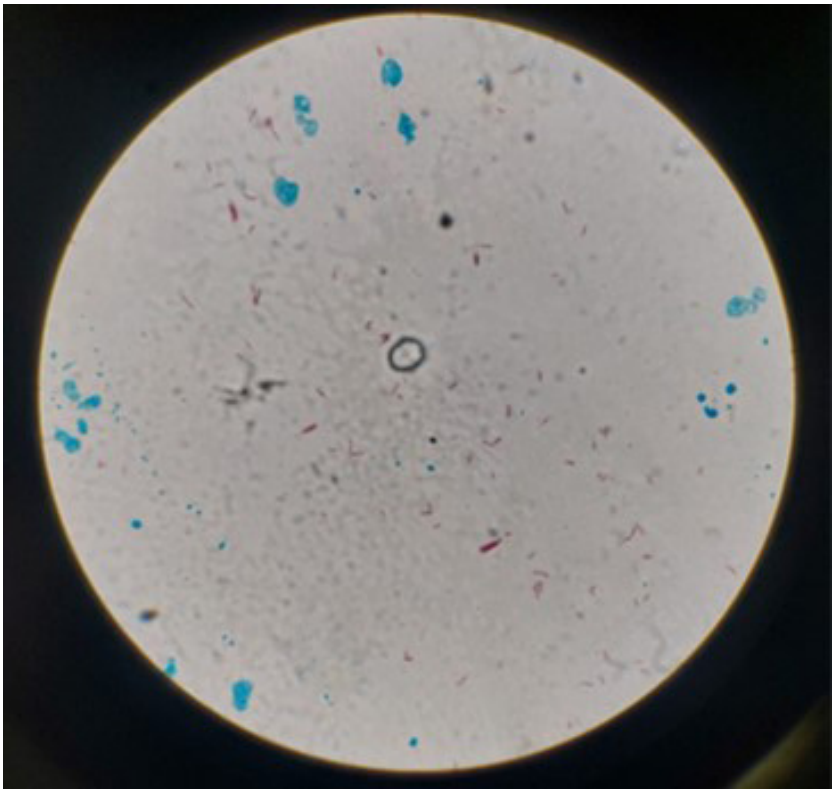
Table 1 displays a comparative comparison of several diagnostic techniques used to diagnose Mycobacterium tuberculosis. Out of the 204 samples that were shown to be positive by Nested PCR, 62.3% (127/204) were also confirmed to be positive through both Mtb Culture (TBC) and Acid-Fast Bacilli (AFB) tests (**Fig-1**). On the contrary, GeneXpert accurately detected 85.4% (174 out of 204) of the instances that were positive according to the Nested PCR test. When evaluating the combination of AFB and TBC, the detection rate, as shown by the Nested PCR data, was 62.3% (127/204), demonstrating the differing sensitivity of both approaches.

Journal of Respiratory Medicine and Research

Table 1. Comparison of detection methods for Mycobacterium tuberculosis, including Mtb Culture (TBC), Acid Fast Bacilli (AFB), Nested PCR, and GeneXpert assays.

Methods	Mtb Culture (TBC)	Acid Fast Bacilli (AFB)	Nested PCR Positive (204)	GeneXpert Positive (174)	Combination of AFB and TBC
Positive	127	98	127	102	127
Negative	223	106	77	62	223
Nested PCR Positive	127	77	204	146	127
Nested PCR Negative	0	146	0	0	146
GeneXpert Positive	102	97	146	174	97
GeneXpert Negative	112	161	0	176	161

Figure 1. Microscopic Analysis of Mycobacterium tuberculosis.



Positivity Rate Analysis of Mtb Based on Pulmonary and Extra-Pulmonary Sample

Table 2 illustrates the effectiveness of different diagnostic methods for detecting *Mycobacterium tuberculosis* (Mtb) across various sample types. Sputum samples yielded the highest positivity rates for GeneXpert and Nested PCR (67% each), while AFB Smear and Mtb Culture had lower detection rates. In extra-pulmonary samples, Nested PCR showed superior sensitivity with a positivity rate of 53.33%, compared to GeneXpert (33.33%). Notably, AFB Smear and Mtb Culture had lower detection rates, highlighting the higher diagnostic performance of molecular methods in diverse sample types.

Journal of Respiratory Medicine and Research

Table 2. Positivity Rates of Mtb Based on Sample Type and Diagnostic Method.

Sample Type	Total Samples (N)	GeneXpert	Nested PCR	AFB Smear	Mtb Culture
Pulmonary (N=200)					
Sputum (n=174)	174	117 (67%)	117 (67%)	67 (38%)	94 (54%)
Branchial Aspirate (n=8)	8	2 (25%)	2 (25%)	-	-
Pleural Fluid (n=18)	18	5 (27.78%)	5 (27.78%)	2 (11.11%)	5 (27.78%)
Extra-Pulmonary (N=150)					
CSF (n=18)	18	3 (16.67%)	9 (50%)	3 (16.67%)	3 (16.67%)
Lymph Node Aspirate (n=11)	11	1 (9.09%)	5 (45.45%)	-	-
Urine (n=10)	10	3 (30%)	3 (30%)	-	3 (30%)
Tissue Biopsy (n=29)	29	9 (31.03%)	19 (65.51%)	6 (20.68%)	6 (20.68%)
Pericardial Fluid (n=5)	5	1 (20%)	1 (20%)	-	-
Pus (n=49)	49	30 (61.22%)	37 (75.51%)	18 (36.73%)	16 (32.65%)
Wound Swab (n=10)	10	1 (10%)	4 (40%)	1 (10%)	-
Tracheal Aspirate (n=8)	8	1 (12.50%)	1 (12.50%)	1 (12.50%)	-
Ascitic Fluid (n=10)	10	1 (10%)	1 (10%)	-	-

Diagnostic Sensitivity and Accuracy of GeneXpert and Nested PCR Assays in Respiratory Specimens and Non-Respiratory samples

Table 3 presents a comprehensive evaluation of the effectiveness of several approaches for detecting pulmonary TB in both respiratory and non-respiratory samples. The sensitivity and specificity of nested PCR were both 100% in both specimen types, which was substantially better than other approaches ($p < 0.001$). GeneXpert Mtb/RIF had a high level of sensitivity (96.4%) and specificity (99.0%), although it was shown to be less productive in comparison to Nested PCR. The (Acid-fast bacillus) AFB Smear and Culture tests showed reduced sensitivity, with the AFB Smear test having a sensitivity of 80.6% for pulmonary specimens. The Culture test identically possessed a sensitivity of 100% but a lower specificity of 96.1%. The findings demonstrate that Nested PCR outperforms other approaches in accurately identifying *M. tuberculosis* in a range of specimens.

Table 3. Efficiency Comparison of Pulmonary Tuberculosis Detection by Nested PCR and Xpert Mtb/RIF Assay.

Respiratory Specimens.							
Test Method	**Positive (n=28)**	**Negative (n=122)**	**Sensitivity (%)**	**Specificity (%)**	**PPV (%)**	**NPV (%)**	**P-Value**
GeneXpert Mtb/RIF	27	1	96.4 (96.2-96.6)	99.0 (98.9-99.1)	96.4	99.0	0.313
Nested PCR	28	0	100.0 (100.0-100.0)	100.0 (100.0-100.0)	100.0	100.0	<0.001
AFB Smear	25	1	80.6 (80.4-80.9)	96.1 (95.9-96.4)	81.1	57.4	<0.001
Culture	26	0	100.0 (100.0-100.0)	96.1 (95.9-96.4)	80.6	56.5	0.313
Non-Respiratory Samples							
GeneXpert Mtb/RIF	27	1	96.4 (96.2-96.6)	99.0 (98.9-99.1)	96.4	99.0	0.313
Nested PCR	28	0	100.0 (100.0-100.0)	100.0 (100.0-100.0)	100.0	100.0	<0.001
AFB Smear	25	1	80.6 (80.4-80.9)	96.1 (95.9-96.4)	81.1	57.4	<0.001
Culture	26	0	100.0 (100.0-100.0)	96.1 (95.9-96.4)	80.6	56.5	0.313

Journal of Respiratory Medicine and Research

DISCUSSION

Tuberculosis (TB) counts as the second most fatal infectious illness worldwide, behind COVID-19, according to the World Health Organization (WHO, 2023) [22]. It represents a substantial global health concern. Bangladesh is among the 30 nations having the greatest burden of tuberculosis (TB). In 2021, the World Health organization (WHO) reported that the nation experienced an annual incidence of 362,000 new cases of tuberculosis (TB), with 30,000 cases occurring in children [23]. The issue of TB becomes more severe because of delays in diagnosis, which lead to higher mortality rates, increased transmission among the population, and more frequent incidences of the disease. *Mycobacterium tuberculosis* (Mtb) is the underlying cause of tuberculosis. The challenges in recognizing Mtb arise from the constraints of conventional methods such as mycobacterial culture and acid-fast bacilli smear microscopy [24]. Nucleic acid amplification techniques have greatly improved the ability to diagnose tuberculosis considering recent developments in molecular diagnostics. In this study, the effectiveness of two diagnostic methods, nested PCR and the Xpert Mtb/RIF test, was evaluated. The results demonstrated that the Xpert Mtb/RIF test exhibited greater sensitivity compared to acid-fast bacillus (AFB) microscopy. However, the Xpert Mtb/RIF test did not achieve optimal accuracy, highlighting the need for further advancements in diagnostic techniques to enhance precision and reliability. Similarly, Kim and colleagues (2014) noted variations in the precision and accuracy of PCR-based techniques for detecting *Mycobacterium tuberculosis* (Mtb) in lung and other body tissue samples. Their findings highlighted that, although test reliability could differ, PCR testing could provide more sensitivity than previous methods [25]. The study found that for samples that had negative findings in culture, nested PCR had better sensitivity than the Xpert Mtb/RIF test. By a ratio of 1.24, the nested PCR outperformed the original PCR in terms of sensitivity. For every sample, nested PCR had a sensitivity of 82.2% and a specificity of 72.2%. The Xpert Mtb/RIF test has a 100% sensitivity but a rather poor 65.5% specificity, according to Chang *et al.* (2012). These results align with previous research that has shown the variable effectiveness of molecular diagnostics in various clinical contexts. (Mtb) was more effectively detected in samples obtained from sites other than the lungs using both techniques. The Xpert Mtb/RIF test revealed a sensitivity of 96.4%, whereas the nested polymerase chain reaction (PCR) demonstrated a sensitivity of 100% [26].

In contrast to the three-day turnaround time for nested PCR, the Xpert Mtb/RIF test demonstrated a turnaround time of less than 24 hours. Fast findings from the Xpert Mtb/RIF test allow for the prompt start of anti-TB therapy, particularly in instances of pulmonary tuberculosis. However, Allahyartorkaman *et al*., 2019 evaluated the disparities in effectiveness between nested PCR and the Xpert Mtb/RIF test could be attributed to their unique techniques. The nested PCR nucleic acid purification technique was considerably more sensitive than the direct cartridge technology used in the Xpert Mtb/RIF test when it comes to sensitivity. According to the research, men had greater rates of tuberculosis (TB) detection (63.33%) than females (50.71%). This finding confirms earlier research suggesting a greater incidence of tuberculosis in males, perhaps due to social and occupational variables [27]. Furthermore, Ayala *et al.*, 2023 found that the prevalence of TB was greater in urban areas (71.87%) compared to rural populations (41.77%). The observed disparity in socioeconomic situations and higher population density in urban areas could be the cause of this mismatch [28]. After conducting a thorough examination of data from all around the world, Alavi-Naini *et al.* (2012) found a strong link between smoking and an increased risk of tuberculosis (TB). Their findings underscored the serious public health risk associated with smoking since it increases the likelihood of developing tuberculosis (TB) by weakening immunity and accelerating the spread of TB infection [29]. Although both the Xpert Mtb/RIF test and nested PCR are used to diagnose tuberculosis, their efficacy varies according to the specific clinical situation. The Xpert Mtb/RIF test yields rapid outcomes and has a high sensitivity for detecting pulmonary tuberculosis. Even though it takes more time to manufacture, the nested PCR method is more sensitive in identifying extra-pulmonary tuberculosis. Optimizing the effectiveness of TB medication and lowering rates of illness and death requires tailoring diagnostic protocols to individual patient groups and sample types. In conclusion, the comparison of the Nestled PCR and the Xpert Mtb/RIF assay for the identification of Mycobacterium tuberculosis reveals that the Xpert Mtb/RIF assay provides expedited results with superior sensitivity and specificity, especially in the detection of rifampicin resistance, thereby establishing it as an essential instrument in rapid diagnostics. However, nested PCR remains useful for its higher sensitivity in low-bacterial-load samples. Despite the Xpert Mtb/RIF assay's advantages, its higher cost and reliance on specialized equipment pose challenges for widespread implementation in resource-limited settings. Future research should focus on improving the accessibility and cost-effectiveness of molecular diagnostics while enhancing the sensitivity of rapid assays to improve tuberculosis detection, especially in low-resource regions.

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Complicit of interest

No conflict of interest from the authors regarding the publication of this manuscript.

Authors Contribution

This work was performed in collaboration between all authors. Ahmed Abu Rus'd and Mamun Ahmed designed and supervised the study, and Md. Arifur Rahman performed the research work and performed the statistical analysis. Shafiqul Islam, S.M. Rafiq Bapari, Muhammad Faisal Azim, Masum Parvez and Sadia Akhtar contributed technical assistance in the study.

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