Xpert Ultra Assay on Stool to Diagnose Pulmonary Tuberculosis in Children

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(See the Editorial Commentary by Gaensbauer on pages 235–6.)

Background. The World Health Organization recommends the Xpert MTB/RIF Ultra assay for diagnosing pulmonary tuberculosis (PTB) in children. Though stool is a potential alternative to respiratory specimens among children, the diagnostic performance of Xpert Ultra on stool is unknown. Thus, we assessed the diagnostic performance of Xpert Ultra on stool to diagnose PTB in children.

Methods. We conducted a cross-sectional study among consecutively recruited children (<15 years of age) with presumptive PTB admitted in 4 tertiary care hospitals in Dhaka, Bangladesh, between January 2018 and April 2019. Single induced sputum and stool specimens were subjected to culture, Xpert, and Xpert Ultra. We considered children as microbiologically confirmed on induced sputum if any test performed on induced sputum was positive for Mycobacterium tuberculosis and microbiologically confirmed if $M. \text{tuberculosis}$ was detected on either induced sputum or stool.

Results. Of 447 children, 29 (6.5%) were microbiologically confirmed on induced sputum and 72 (16.1%) were microbiologically confirmed. With "bacteriologically confirmed on induced sputum" as a reference, the sensitivity and specificity of Xpert Ultra on stool were 58.6% and 88.1%, respectively. Xpert on stool had sensitivity and specificity of 37.9% and 100.0%, respectively. Among microbiologically confirmed children, Xpert Ultra on stool was positive in 60 (83.3%), of whom 48 (80.0%) had "trace call."

Conclusions. In children, Xpert Ultra on stool has better sensitivity but lesser specificity than Xpert. A high proportion of Xpert Ultra assays positive on stool had trace call. Future longitudinal studies on clinical evolution are required to provide insight on the management of children with trace call.

Keywords. Xpert; Xpert Ultra; stool; childhood TB; SORT IT.

Globally in 2018, an estimated 1 million children (<15 years of age) developed tuberculosis (TB), and 0.17 million died due to TB [1]. More than half of the estimated children with TB remained undetected or were detected but not notified to national TB programs [1]. Diagnosis of TB among children is challenging due to nonspecific symptoms and the paucibacillary nature of the disease [2].

Bacteriological confirmation of pulmonary TB (PTB) in children is done with microscopy, culture, and Xpert MTB/RIF assay (Xpert, Cepheid, Sunnyvale, California) on sputum specimens. However, the performances of these tests are dependent on the volume and quality of sputum. Children fail to produce quality sputum and thus require invasive procedures to collect induced sputum and gastric aspiration. Such invasive procedures have a low diagnostic yield and may be inaccessible in low-resource settings [3]. Thus, clinicians depend on clinical signs and symptoms and non-sputum-based tests such as tuberculin skin test and chest radiography for diagnosis [4]. In regions where diseases (human immunodeficiency virus [HIV], systemic infections, parasitic infections, or atypical pneumonia) with overlapping clinical features are endemic, the specificity of non-sputum-based diagnosis is low [5].

Because children swallow sputum, stool specimen is a potential alternative for bacteriological confirmation of PTB. Mycobacteria present in sputum may pass through the gastrointestinal tract. This may allow the detection of mycobacterial DNA in stool specimens with molecular techniques like Xpert assay [3]. Around 30%–68% of children with bacteriologically
confirmed PTB can be detected using Xpert on stool specimen. Also, Xpert on stool specimen can detect PTB in 2%–8% of bacteriologically negative (in sputum) children [2, 6]. Systematic review reported the estimated pooled sensitivity and specificity of Xpert on stool specimen as 67% and 99%, respectively [7]. However, the limit of detection (LOD) for Xpert is 131 colony-forming units (CFU)/mL, and specimens with load less than the LOD are missed [8]. Stool specimens have a low load of Mycobacterium tuberculosis (MTB) due to challenges in its processing and lack of standard procedures [7]. Thus, molecular tests with lower LOD could be beneficial [9].

Xpert MTB/RIF Ultra assay (Xpert Ultra, Cepheid) was developed with an LOD of 16 CFU/mL and improved rifampin resistance determination [10]. Incorporation of probes for IS1081 along with the IS6110 region has improved the MTB detection from unprocessed specimens [10]. Compared to Xpert, the Xpert Ultra has an additional semiquantitative category called “trace call” corresponding to the lowest bacillary burden. The sensitivity of Xpert Ultra on sputum varies from 64% to 74%, while that of Xpert varies from 53% to 72% [9]. The Xpert Ultra has better sensitivity compared to Xpert in detecting paucibacillary TB [11, 12]. The trace call (on sputum specimen) improved the sensitivity, and anti-TB treatment is recommended on trace calls in childhood TB, extrapulmonary TB, and TB in people living with HIV [13]. However, no such recommendation has been made on treatment initiation with trace call on other alternative specimens [13].

Still, there is no literature on diagnostic validity of Xpert Ultra on stool specimens to detect PTB in children. Thus, we aimed to assess the diagnostic validity and yield of Xpert Ultra on stool specimens in detecting bacteriologically confirmed PTB among children.

MATERIALS AND METHODS

Study Design and Study Setting
Bangladesh is a low-middle-income country in Southeast Asia with 161 million inhabitants. Bangladesh is a high-TB-burden country with an estimated 357,000 new patients in 2018, and among all forms of TB, only 4% were children compared to the estimated 11% globally [1]. We conducted a facility-based cross-sectional study involving primary data collection.

Study Sites
We conducted the study in 4 tertiary care hospitals of Dhaka: Sir Salimullah Medical College and Mitford Hospital; Shaheed Suhrawardy Medical College and Hospital; Dhaka Medical College Hospital; and icddr,b Dhaka Hospital. The first 3 hospitals are tertiary public health facilities with a high bed occupancy rate. The icddr,b Dhaka Hospital is a private not-for-profit hospital for diarrheal diseases. All hospitals have separate inpatient wards for children, and the services are provided free of cost.

Study Population
We enrolled all admitted children with presumptive PTB from the 4 selected tertiary care hospitals between January 2018 and April 2019. The criteria for presumptive PTB in children included (1) persistent, nonremitting cough for >2 weeks not responding to conventional antibiotics, and/or (2) persistent documented fever (>38°C/100.4°F) for >2 weeks, and/or (3) documented weight loss or not gaining weight adequately during the past 3 months, and/or fatigue, reduced playfulness, and decreased activity [14].

Children with other serious comorbid conditions who were admitted to the intensive care unit, already initiated on anti-TB treatment and suspected clinically of intestinal TB, were excluded.

Procedures
The in-house research staff interviewed the caregivers of enrolled children on demographic and clinical profiles and measured the weight and length/height of children.

Specimen Collection, Transportation, and Investigations
The treating physicians collected single induced sputum specimens (Supplementary Figure 1). We provided the caregivers with a sterile stool container and requested them to keep the portion of stool specimen in the container (half of the container: approximately 10 g). We labeled the specimens with a unique study identifier, stored in a cold box (specimen carrier box with 2 ice packs to maintain cold chain) and transported them to the icddr,b mycobacteriology laboratory within the same day of collecting the specimen.

We conducted laboratory investigations at the icddr,b mycobacteriology laboratory. Both induced sputum and stool specimens underwent culture, Xpert, and Xpert Ultra assays. Culture was done in Lowenstein-Jensen medium, and drug susceptibility was tested in the same media using the proportion method. Drug susceptibility testing was done against first-line anti-TB drugs (rifampin, isoniazid, ethambutol, and streptomycin) with culture-positive isolates. Xpert and Xpert Ultra were performed according to the manufacturer’s instructions. Stool specimens were processed with an indigenously developed method described elsewhere [15]. Supplementary File 1 has details related to stool processing, induced sputum decontamination and processing, smear microscopy, culture and drug susceptibility testing, and Xpert and Xpert Ultra assays [13, 16, 17]. All of the laboratory test reports were delivered to the respective physicians when those were available.

Diagnosis and Treatment
All the study hospitals implemented a standard procedure for diagnosis and treatment of childhood PTB. All children with detection of MTB in any test on induced sputum or stool specimens were considered to have PTB. In the rest, the physicians
clinically diagnosed PTB based on tuberculin skin test, chest radiography, and clinical signs and symptoms. The physicians initiated anti-TB treatment in the same health facilities where the children were admitted.

Data Variables and Sources of Data
The research staff used a tablet-based data capture application to collect the demographic and clinical profiles. The supervisors regularly checked the data and synced it to the cloud after verification with strict confidentiality. We also collected the microbiological data from the laboratory registers and single-entered into the SPSS software (version 20). We merged the data extracted from tablet-based database with the laboratory database using the unique study identifier.

Data Analysis and Statistics
Sample Size
We calculated a minimum sample size of 435 assuming sensitivity of 80% and specificity of 95% for Xpert Ultra on stool specimens (when compared to “bacteriologically confirmed on induced sputum”) at 10% level of precision when TB prevalence was expected to be 15% among the study population considering 5% loss to follow-up/incomplete data.

Statistical Analysis
We analyzed data using SPSS version 20 software. We used proportions to summarize the sociodemographic details, nutritional status, and symptom profile.

The children who were bacteriologically positive in any of the tests performed (culture/Xpert/ Xpert Ultra) on either induced sputum or stool specimens were considered bacteriologically confirmed. The children who were positive on induced sputum were considered as bacteriologically confirmed on induced sputum, and those who were positive on stool specimens were considered as bacteriologically confirmed on stool specimen. We used a Venn diagram to depict the bacteriological confirmation using culture, Xpert, and Xpert Ultra on induced sputum and stool specimens.

We calculated the sensitivity and specificity with 95% confidence intervals (CIs) for detecting PTB for Xpert and Xpert Ultra using stool specimen with “bacteriologically confirmed on induced sputum” as reference [18]. This was done since culture positivity on induced sputum might be very low in children due to paucibacillary nature of TB and also use of solid culture, which has relatively lower yield than the liquid culture [9]. We also calculated the sensitivity and specificity of Xpert Ultra on stool specimens considering trace call on Xpert Ultra as negative. We have also presented the above results using “bacteriologically confirmed on induced sputum culture” as the reference test.

We calculated the proportion of children with presumptive PTB positive for individual and combination of tests against “bacteriologically confirmed,” “diagnosed as TB,” and “all enrolled.”

Ethical Considerations
We obtained ethics approval from the icddr,b Ethical Review Committee (PR-17072, 28 August 2017) and the Ethics Advisory Group of the International Union Against Tuberculosis and Lung Disease, Paris, France (23/19, 01-04-2019). We obtained written informed consent from parents or caregivers of all children and assent from children aged > 11 years.

RESULTS
Baseline Characteristics
Of 454 children with presumptive PTB, 7 (1.4%) could not provide a stool specimen (Figure 1). Of 447 included, 296 (66.2%) were aged < 5 years, 254 (56.8%) were male, 321 (71.8%) had cough for > 2 weeks, 306 (68.5%) had fever for > 2 weeks, 381 (85.2%) had significant weight loss, and 114 (25.5%) had contact with a TB patient in their family within the last year (Table 1).

Results of Investigations, Diagnosis of TB, and Treatment
With induced sputum, 9 (2.0%) were positive on culture, 12 (2.7%) by Xpert, and 28 (6.3%) by Xpert Ultra (Figure 1). Of the 28 positive on Xpert Ultra, 11 (39.3%) had trace call (Table 2). In total, 29 (6.5%) were bacteriologically confirmed on induced sputum, of which 15 (51.7%) were exclusively diagnosed with Xpert Ultra (Figure 2).

With stool specimens, 2 (0.4%) were positive by culture, 11 (2.5%) by Xpert, and 60 (13.4%) by Xpert Ultra (Figure 1). Of the 60 positive on Xpert Ultra, 48 (80.0%) had trace call (Table 2). In total, 60 (13.4%) were MTB detected on stool specimen, of which 49 (81.7%) were exclusively detected with Xpert Ultra (Figure 2). The demographic and clinical characteristics of children with trace call on stool specimen are depicted in Supplementary Table 1. In total, 72 (16.1%) were bacteriologically confirmed, of which 43 (59.7%) were exclusively detected through Xpert Ultra on stool specimen (Table 3).

A total of 39 (8.7%) children were clinically diagnosed. Of 111 (72 plus 39) children diagnosed with TB, 105 (94.6%) were initiated on anti-TB treatment (Figure 1). Six children (5.4%) positive by Xpert Ultra on stool specimen did not receive anti-TB treatment. Five of them were not treated based on physicians’ suggestion, and parents of another child refused treatment. All 5 children in whom treatment was not started based on physician suggestion were followed up by telephone for 6 months, and they did not develop active TB.

Diagnostic Validity of Xpert and Xpert Ultra Assays on Stool Specimens
The sensitivity of Xpert was 37.9% (95% CI, 22.7%–56.0%) and that of Xpert Ultra was 58.6% (95% CI, 40.7%–74.5%) when compared to children who were bacteriologically...
confirmed on induced sputum. The specificity was 100% (95% CI, 99.1%–100.0%) with Xpert and 89.7% (95% CI, 86.4%–92.3%) with Xpert Ultra. On considering trace call as negative on Xpert Ultra, the sensitivity was 37.9% (95% CI, 22.7%–56.0%) (Table 3).

The intention-to-diagnose approach with inclusion of the 7 children who were excluded for the nonavailability of stool specimens improved the specificity of Xpert and Xpert Ultra on stool specimens without affecting the sensitivity (Supplementary Table 2). Similarly, the sensitivity analysis with the composite reference of “bacteriologically confirmed on induced sputum,” excluding results of Xpert Ultra on induced sputum, improved the sensitivity of both Xpert and Xpert Ultra on stool specimens (Supplementary Table 3).
Using “bacteriologically confirmed on induced sputum culture” as the reference test, the sensitivity of Xpert was 77.8% (95% CI, 45.3%–93.7%) and that of Xpert Ultra was 88.9% (95% CI, 56.5%–98.0%). The specificity was 99.1% (95% CI, 84.7%–99.8%) with Xpert and 88.1% (95% CI, 84.7%–90.8%) with Xpert Ultra (Table 4).

**Performance of Xpert and Xpert Ultra Assays on Stool Specimen**

Of the 72 bacteriologically confirmed PTB cases, Xpert Ultra on stool specimen detected 60 (83.3%) as positive. Of 111 diagnosed with TB, Xpert Ultra on stool specimen detected 60 (54.1%) as positive. Of all 447 children with presumptive PTB, the diagnostic yield with Xpert Ultra on stool specimen was 13.4% (Table 5).

**DISCUSSION**

Globally, this is the first study that has assessed the diagnostic performance of Xpert Ultra assay on stool specimens for the diagnosis of PTB among children. Our study has some key findings. First, the Xpert Ultra on stool specimen had higher sensitivity and lower specificity compared to Xpert. Second,
about 8 of 10 cases positive on Xpert Ultra on the stool specimen had trace call as semiquantitative burden. On considering trace call as negative, the sensitivity of Xpert Ultra reduced, but specificity improved. Third, a high proportion of stool specimens positive by Xpert Ultra were negative on induced sputum. Fourth, a high percentage of bacteriologically confirmed PTB was positive with Xpert Ultra on stool specimen.

The study has some limitations. First, we included only children admitted to the hospitals and they could have had a severe degree of symptoms, the spectrum of disease, and a higher pretest probability of having PTB. Therefore, the overall positivity rate in the study sample might be high and it could have affected the sensitivity and specificity [19, 20]. Hence, the study results might not be generalizable to ambulatory presumptive PTB children with a low positivity rate. Second, when there were trace calls on Xpert Ultra, we did not repeat the test to confirm positivity. Thus, we might have overestimated the sensitivity and underestimated the specificity. Third, we had a smaller sample size for calculating sensitivity as the proportion of children with bacteriologically confirmed PTB on induced sputum was less than that anticipated. This could be due to use of solid culture on induced sputum over the liquid culture with better yield. Hence, our estimation of sensitivity is not precise. Fourth, physicians made clinical diagnosis only when there was no bacteriological confirmation. Thus, the proportion with positive Xpert Ultra results among those the physicians otherwise would have diagnosed clinically is unknown, and the utility of Xpert Ultra in ascertaining bacteriological confirmation among children diagnosed clinically cannot be commented on.

There are several key study findings and programmatic implications. First, Xpert Ultra on a stool specimen had higher sensitivity and lower specificity compared to Xpert on stool specimen. Our finding is consistent with the higher sensitivity and lower specificity of Xpert Ultra on sputum specimen in both adults and children [11, 18]. The sensitivity of Xpert Ultra on stool specimens was higher than the reported sensitivity of 33%–59% (with culture on induced sputum as reference) for Xpert on stool specimens in previous studies [21, 22]. The potential reason for such high sensitivity of Xpert Ultra might be due to low LOD and the ability to detect trace call. The specificity of Xpert Ultra on stool specimen was lower than that reported for Xpert previously [21, 22]. However, the specificity of Xpert Ultra on stool specimens cannot be judged due to the low yield of induced sputum.

Second, a high proportion of stool specimens positive with Xpert Ultra had trace call. The proportion of trace call was higher in stool specimens compared to induced sputum. Also, considering trace call as negative reduced the sensitivity of Xpert Ultra. The trace call could be due to the presence of nonreplicating...
TB bacilli in specimens of people with recent anti-TB treatment or due to self-cured (incipient TB resolved without treatment) TB or due to laboratory cross-contamination [13]. In our study, none of the children with trace call had previous history of TB, and incipient TB is less likely as all the children had symptoms suggestive of active TB [23]. Acknowledging these reasons, we believe that the detected MTB could be less likely due to anything other than active TB. However, despite adhering to standard operating procedures to reduce cross-contamination in the laboratory, the possibility of cross-contamination during stool processing cannot be ruled out.

Third, a high proportion of stool specimens positive by Xpert Ultra (including trace call) were negative by Xpert Ultra on induced sputum. This can be due either to ability of Xpert Ultra on stool specimen to detect true positives over and above tests on induced sputum, or to false-positive results because of the high percentage of trace calls. Also, of the 5 children with trace call on sputum specimen with Xpert Ultra and not initiated on treatment, none showed signs of active TB during 6 months of follow-up. The speculation of false positivity of Xpert Ultra with trace call cannot be ruled out with the findings of these 5 untreated children. We strongly recommend future research to

### Table 3. Diagnostic Validity of Xpert MTB/RIF and Xpert MTB/RIF Ultra Assays on Stool Specimen Compared With Bacteriological Confirmation With Induced Sputum Specimen Among Children (<15 Years of Age) With Presumptive Pulmonary Tuberculosis Enrolled From Selected 4 Tertiary Care Hospitals in Dhaka, Bangladesh, January 2018–April 2019 (N = 447)

| Tests on Stool Specimen | Bacteriological Confirmation With Induced Sputum |
|-------------------------|-----------------------------------------------|
|                         | Positive | No. (%)<sup>a</sup> | Negative | No. (%)<sup>a</sup> | Sensitivity, % (95% CI) | Specificity, % (95% CI) |
| Total                   |          | 29 (100.0)          | 418 (100.0) | ...            | ...                        |
| Xpert MTB/RIF           |          |                      |           |                |                            |
| Positive                | 11 (37.9)| 0 (0.0)             | 37.9 (22.7–56.0) | 100.0 (99.1–100.0) |
| Negative                | 18 (62.1)| 418 (100.0)         | ...        | ...            |                            |
| Xpert MTB/RIF Ultra assay<sup>b</sup> |          |                      |           |                |                            |
| Positive                | 17 (58.6)| 43 (10.3)           | 58.6 (40.7–74.5) | 89.7 (86.4–92.3) |
| Negative                | 12 (41.4)| 374 (89.7)          | ...        | ...            |                            |
| Xpert MTB/RIF Ultra (trace call as negative)<sup>b</sup> |          |                      |           |                |                            |
| Positive                | 11 (37.9)| 1 (0.2)             | 37.9 (22.7–56.0) | 99.8 (98.7–99.9) |
| Negative                | 18 (62.1)| 416 (99.8)          | ...        | ...            |                            |

Abbreviation: CI, confidence interval.

<sup>a</sup>Column percentage.

<sup>b</sup>One stool specimen showed invalid result on Xpert Ultra assay.

### Table 4. Diagnostic Validity of Xpert MTB/RIF and Xpert MTB/RIF Ultra Assays on Stool Specimen Compared With Culture Results of Induced Sputum Specimen Among Children (<15 Years of Age) With Presumptive Pulmonary Tuberculosis Enrolled From Selected 4 Tertiary Care Hospitals in Dhaka, Bangladesh, January 2018–April 2019 (N = 447)

| Investigation | Culture on Induced Sputum |
|---------------|---------------------------|
|               | Positive | Negative | Sensitivity, % (95% CI) | Specificity, % (95% CI) |
|               | No. (%)<sup>a</sup> | No. (%)<sup>a</sup> |                |                            |
| Total         |          | 9 (100.0) | 438 (100.0) | ...        | ...                        |
| Xpert MTB/RIF |          |            |            |            |                            |
| Positive      | 7 (77.8) | 4 (0.9)   | 77.8 (45.3–93.7) | 99.1 (97.7–99.6) |
| Negative      | 2 (22.2) | 434 (99.1) | ...        | ...            |                            |
| Xpert MTB/RIF Ultra<sup>b</sup> |          |            |            |            |                            |
| Positive      | 8 (88.9) | 52 (11.9) | 88.9 (56.5–98.0) | 88.1 (84.7–90.8) |
| Negative      | 1 (11.1) | 385 (88.1) | ...        | ...            |                            |
| Xpert MTB/RIF Ultra (trace call as negative)<sup>b</sup> |          |            |            |            |                            |
| Positive      | 7 (77.8) | 5 (1.1)   | 77.8 (45.3–93.7) | 98.9 (97.4–99.5) |
| Negative      | 2 (22.2) | 432 (98.9) | ...        | ...            |                            |

Abbreviation: CI, confidence interval.

<sup>a</sup>Column percentage.

<sup>b</sup>One stool specimen showed invalid result on Xpert Ultra assay.
systematically assess clinical progression and response to treatment among children with and without trace call. This assessment on clinical evolution could provide a better insight into the utility of trace call of Xpert Ultra on stool specimen for diagnosis of PTB in children.

Fourth, a high percentage of bacteriologically confirmed PTB was positive with Xpert Ultra on stool specimen. Also, the majority were exclusively diagnosed through Xpert Ultra. Xpert Ultra on stool specimen has potential to be adopted as the first-line screening tool in children with presumptive TB subject to (1) consistent findings of high sensitivity from studies similar to this among ambulatory patients with low pretest probability and (2) follow-up of children with trace call demonstrating improvement in clinical status with anti-TB treatment. If future studies show consistent results, the Xpert Ultra on stool specimen would be beneficial to reduce delays in diagnosis and invasive procedures, especially among ambulatory children referred from peripheral health facilities. The feasibility of Xpert Ultra on stool specimen for diagnosis of children with presumptive PTB from peripheral health facilities and resource-poor settings must be explored. These explorations could help to provide insight on challenges in collecting and processing stool specimens in such settings. Moreover, the current stool processing method applied in this study is resource intensive and might not be feasible where optimum resources are not available. Furthermore, studies could be conducted to simplify and optimize the collection, processing, and transportation of samples to facilities with Xpert platforms for testing.

In children, Xpert Ultra on stool has better sensitivity but lesser specificity than Xpert. A high proportion of Xpert Ultra positive on stool had trace call. Future longitudinal studies on clinical evolution are required to provide insight on the management of children with trace call.

Supplementary Data
Supplementary materials are available at Clinical Infectious Diseases online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes
Author contributions. S. K. supervised overall study activities, analyzed data, and developed the manuscript. S. M. M. R. contributed to design methodology, laboratory test performance, and manuscript development and review. S. A. (Shakil Ahmed) contributed to field implementation, data analysis, and manuscript development and review. M. S. I. and S. A. (Shakil Ahmed) contributed to laboratory test performance and manuscript review. S. C. contributed to data collection, data analysis, and manuscript review. S. A. (Shahariar Ahmed) contributed to data analysis and manuscript review. K. K. P. designed the concept and methodology, and contributed to manuscript review. R. K. contributed to data analysis and manuscript review. M. J. C. contributed to field implementation, data collection, and manuscript review. H. D. S. and R. N. contributed to field site preparation, dealing with necessary approvals and data collection, inferring data and analysis, and manuscript review. H. D. S. and P. T. analyzed data, and contributed to manuscript development and review. S. Anwar and N. A. B. contributed to field implementation, manuscript development, and review. R. N. and M. K. M. U. contributed to laboratory test performance and manuscript review. S. C. contributed to data collection, data analysis, and manuscript review. S. A. (Shahariar Ahmed) contributed to data analysis and manuscript review. K. K. P. designed the concept and methodology, and contributed to manuscript review. R. K. contributed to data analysis and manuscript review. M. J. C. contributed to field implementation and manuscript review. S. B. (corresponding and senior author) designed concept and methodology, led full research activities, contributed to the draft manuscript, reviewed the manuscript, and provided feedback.

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