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Original Article

Xpert Ultra Outperforms Xpert MTB/RIF in Diagnosing Tuberculous Meningitis in a Paediatric Cohort from a High-Burden Setting

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ABSTRACT

Background: Tuberculous meningitis is the most devastating form of childhood tuberculosis and delayed diagnosis is strongly linked with death and disability. Xpert MTB/RIF is rapid but insufficiently sensitive in this paucibacillary disease. Xpert Ultra was engineered to overcome this limitation, but paired paediatric comparisons from high-burden settings remain scarce. **Objective:** To compare the diagnostic performance of Xpert Ultra with Xpert MTB/RIF against culture for diagnosing tuberculous meningitis in children. **Methods & Materials:** This prospective, cross-sectional study was conducted in the Department of Paediatrics, Dhaka Medical College Hospital, Bangladesh, over one year. Eighty children aged one month to twelve years with presumptive tuberculous meningitis were enrolled by purposive sampling. Cerebrospinal fluid was tested by acid fast staining, Xpert MTB/RIF, Xpert Ultra and culture. Sensitivity, specificity, predictive values and accuracy were calculated with 95% confidence intervals using SPSS version 26, significance $p<0.05$. **Results:** Culture, acid fast staining, Xpert MTB/RIF and Xpert Ultra were positive in 12.5, 10.0, 17.5 and 32.5 percent of specimens. Against culture, Xpert Ultra had sensitivity of 100.0 percent versus 40.0 percent for Xpert MTB/RIF; specificity was 77.1 percent versus 85.7 percent. Negative predictive value for Xpert Ultra was 100.0 percent and it detected rifampicin resistance in 7.7 percent of positive specimens, versus none by Xpert MTB/RIF. **Conclusion:** Xpert Ultra markedly outperformed Xpert MTB/RIF in sensitivity for diagnosing paediatric tuberculous meningitis, with an acceptable specificity trade off, supporting its adoption as the preferred initial test in high burden settings.

Keywords: *Tuberculous meningitis; Xpert Ultra; Xpert MTB/RIF; cerebrospinal fluid; diagnostic accuracy.*

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INTRODUCTION

Tuberculosis remains among the leading causes of death from a single infectious pathogen worldwide, with close to ten million new cases arising each year and almost ninety percent of them concentrated in thirty high tuberculosis burden countries [1]. Extrapulmonary disease constitutes a substantial share of this burden and tuberculous meningitis represents its most lethal manifestation, occurring when Mycobacterium tuberculosis breaches the blood brain and blood cerebrospinal fluid barriers to invade the meninges and the fluid surrounding the brain and spinal cord [2]. The pathogenic sequence is thought to begin with the formation of a caseating subpial or subependymal focus that may rupture into the subarachnoid space months to years after primary infection, triggering the intense inflammatory cascade responsible for the severe neurological sequelae of the disease [3].

Children bear a disproportionate share of this burden. Childhood tuberculosis accounts for roughly ten to twenty percent of all tuberculosis cases in South Asia and contributes eight to twenty percent of tuberculosis related deaths in the region, with tuberculous meningitis responsible for the largest share of this mortality [4]. The natural history of tuberculosis differs in children, who show a greater propensity toward disseminated disease and more rapid progression to severe forms than adults [5]. Even with treatment, tuberculous meningitis results in death or permanent disability in more than half of affected patients and delayed diagnosis is consistently linked with the poorest outcomes [6,7].

This diagnostic delay is driven largely by the paucibacillary nature of tuberculous meningitis and the small volume of cerebrospinal fluid available for testing in young children [8]. Cerebrospinal fluid smear microscopy for acid fast bacilli, although inexpensive and widely available, is insensitive, detecting fewer than fifteen percent of culture confirmed cases in routine practice [9]. Mycobacterial culture, though more sensitive, requires two to six weeks to yield a result and is therefore too slow to guide initial treatment decisions in a disease where early therapy determines outcome [6].

The Xpert MTB/RIF assay, endorsed by the World Health Organization as the preferred initial test for tuberculous meningitis, shortened turnaround time to under two hours, yet pooled sensitivity against culture in cerebrospinal fluid has remained modest and a negative result cannot reliably exclude the diagnosis [10].

The Xpert MTB/RIF Ultra assay was engineered specifically to overcome this limitation [11]. A larger reaction chamber and incorporation of two multicopy amplification targets, IS6110 and IS1081, lowered the limit of detection roughly seven-fold compared with Xpert MTB/RIF, while a shortened run time improved laboratory throughput [9]. These modifications have translated into meaningfully higher sensitivity for Mycobacterium tuberculosis detection in cerebrospinal fluid in several cohorts, prompting the World Health Organization to recommend replacement of Xpert MTB/RIF with Xpert Ultra across all testing settings [10]. However, direct paired comparisons of the two assays in children, particularly from high tuberculosis burden

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settings in South Asia, remain scarce and most published series have enrolled only small numbers of microbiologically confirmed paediatric cases [8].

Given the exceptionally high burden of paediatric tuberculosis in Bangladesh and the paucity of locally generated diagnostic performance data, a focused, prospective comparison of the diagnostic accuracy of Xpert Ultra against Xpert MTB/RIF in cerebrospinal fluid from children with presumptive tuberculous meningitis is warranted to determine whether the analytical advantages of the newer assay translate into clinically meaningful gains in this vulnerable population.

OBJECTIVE

To compare the diagnostic performance of Xpert Ultra with Xpert MTB/RIF against mycobacterial culture in cerebrospinal fluid for the diagnosis of tuberculous meningitis in children with presumptive disease.

METHODS & MATERIALS

This was a prospective, single center, cross sectional diagnostic performance study conducted in the Department of Paediatrics, Dhaka Medical College Hospital (DMCH), Dhaka, Bangladesh, over a twelve-month period from July 2020 to June 2021. The study population comprised children aged one month to less than twelve years who were admitted with clinical manifestations suggestive of tuberculous meningitis. Of 89 children initially screened, 80 met the eligibility criteria and were enrolled by consecutive, purposive sampling.

Sample selection

Inclusion criteria

- Age between one month and twelve years.
- Clinically diagnosed as a case of presumptive tuberculous meningitis according to standard operational case definitions.

Exclusion criteria

- Stage III tuberculous meningitis, characterized by coma, disorientation, decerebrate posturing, or deterioration of vital signs.
- Children who had already commenced anti-tuberculous treatment prior to enrolment.
- Children whose parents or legal guardians did not provide consent for participation.

Study procedure

Eligible children underwent physical and fundoscopic examination to exclude raised intracranial pressure prior to lumbar puncture. Under aseptic precautions, cerebrospinal fluid was withdrawn via a spinal needle from the third lumbar intervertebral space. One millilitre of cerebrospinal fluid was sent for routine biochemical, cytological and adenosine deaminase analysis, while a further two millilitres was dispatched for acid-fast bacilli staining, Xpert MTB/RIF, Xpert Ultra and mycobacterial culture. Specimens were transported in sealed containers within a cold box maintained between 4°C and 8°C to prevent leakage and cross-contamination. Xpert MTB/RIF and Xpert Ultra assays were performed on the GeneXpert system (model GXI-IV R2, Cepheid, Sunnyvale, California, USA) according to the manufacturer's instructions using assay-specific cartridges. Ziehl-Neelsen staining was used for smear microscopy and culture was performed on Lowenstein-Jensen solid media. Demographic, clinical and laboratory data were recorded on a pre-tested, semi-structured questionnaire by the investigator and independently verified.

Ethical consideration

Ethical approval for the study was obtained from the Ethical Review Committee (ERC), Dhaka Medical College Hospital (memo no. ERC-DMC/ECC/2020/321, dated 21 September 2020). Written informed consent was obtained from the parents or legal guardians of all participating children after the purpose, procedures, risks and benefits of the study were explained in easily understandable local language. Participants were informed of their right to withdraw from the study at any stage without any effect on their clinical care and confidentiality of all patient information was strictly maintained throughout the study, with data used exclusively for research purposes.

Statistical analysis

Data were entered, cleaned and analyzed using Statistical Package for Social Sciences (SPSS) version 26.0. Continuous variables were expressed as mean ± standard deviation and categorical variables were expressed as frequency and percentage. Using mycobacterial culture as the reference standard, sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy of acid-fast bacilli staining, Xpert MTB/RIF and Xpert Ultra were calculated, each with 95% exact binomial confidence intervals. The chi-square test was used to assess associations between categorical variables. A p-value of less than 0.05 was considered statistically significant throughout the analysis.

RESULTS

Table I summarizes the demographic and clinical characteristics of the cohort. Most children (67.5%) were between one month and less than five years of age, with a mean age of 3.82 ± 3.42 years and a modest male preponderance (60.0%) was observed. The majority resided in rural areas (72.5%) and possible tuberculous meningitis (70.0%) was more frequently diagnosed than probable disease (30.0%), based on standard clinical case definitions.

Table I

Demographic and clinical characteristics of children with presumptive TBM (*n*=80).

Characteristic	Frequency (n)	Percentage (%)
Age, 1 month to <5 years	54	67.5
Age, 5–12 years	26	32.5
Mean age ± SD, years	3.82 ± 3.42	
Age range	2 months–11 years	
Male	48	60.0
Female	32	40.0
Rural residence	58	72.5
Urban residence	22	27.5
Possible TBM	56	70.0
Probable TBM	24	30.0

TBM = tuberculous meningitis; SD = standard deviation.

Table II outlines the presenting symptoms and neurological findings at admission. Fever was present in all children (100.0%), followed by irritability (55.0%), altered consciousness (45.0%) and convulsion (42.5%). Only 17.5% of children had a documented history of contact with a tuberculosis case. On neurological assessment, a substantial proportion of children presented with a depressed sensorium, with 47.5% recording a Glasgow Coma Scale of 9–12 and 17.5% recording a score of 3–8. Focal neurological deficits were noted in 40.0% of children and a BCG scar was present in 77.5%.

Table II
Presenting symptoms and neurological findings (*n*=80).

Finding	Frequency (n)	Percentage (%)
Fever	80	100.0
Irritability	44	55.0
Altered consciousness	36	45.0
Convulsion	34	42.5
Vomiting	24	30.0
Headache	18	22.5
Weight loss	18	22.5
TB contact	14	17.5
Night sweats	12	15.0
Neck stiffness	8	10.0
GCS 13–15	28	35.0
GCS 9–12	38	47.5
GCS 3–8	14	17.5
Focal neurological deficits	32	40.0
Neck rigidity	14	17.5
Bulged fontanelle	8	10.0
BCG mark present	62	77.5

TB = tuberculosis; GCS = Glasgow Coma Scale; BCG = Bacillus Calmette–Guérin.

Table III presents the laboratory and cerebrospinal fluid parameters of the cohort. The cerebrospinal fluid profile was characterized by a mildly elevated white cell count with lymphocytic predominance, together with raised protein and reduced glucose concentrations, a pattern consistent with tuberculous meningitis.

Table III
Laboratory and CSF characteristics (*n*=80).

Parameter	Mean ± SD	Range
Haemoglobin, g/dL	10.0 ± 1.7	6.3–13.8
WBC, /mm³	11,756.8 ± 6,210.5	4,680–32,000
Neutrophils, %	65.3 ± 14.8	40.0–94.0
Lymphocytes, %	31.2 ± 26.4	5.0–174.4
CSF WBC, /mm³	49.6 ± 62.1	1–292
CSF lymphocytes, /mm³	29.1 ± 36.5	0–164
CSF protein, mg/dL	87.8 ± 78.1	14–460
CSF glucose, mg/dL	51.2 ± 19.0	14.4–93.6
CSF ADA, U/L	3.87 ± 3.21	0.60–17.40

SD = standard deviation; WBC = white blood cell count; CSF = cerebrospinal fluid; ADA = adenosine deaminase.

Table IV shows the yield of Mycobacterium tuberculosis detection across the four diagnostic modalities evaluated. Xpert Ultra detected the highest proportion of positive specimens (32.5%), nearly double the yield of Xpert MTB/RIF (17.5%) and considerably higher than culture (12.5%) and acid-fast bacilli staining (10.0%).

Table IV
Detection of Mycobacterium tuberculosis by microbiological and molecular methods (*n*=80).

Test	Positive, n (%)	Negative, n (%)
AFB staining	8 (10.0)	72 (90.0)
Xpert MTB/RIF	14 (17.5)	66 (82.5)
Xpert Ultra	26 (32.5)	54 (67.5)
CSF culture	10 (12.5)	70 (87.5)

Table V presents the comparative diagnostic performance of Xpert MTB/RIF and Xpert Ultra against culture as the reference standard. Xpert Ultra achieved 100.0% sensitivity, markedly higher than the 40.0% sensitivity of Xpert MTB/RIF, while specificity was somewhat lower for Xpert Ultra (77.14%) than for Xpert MTB/RIF (85.71%). Negative predictive value was correspondingly higher for Xpert Ultra (100.0%) than for Xpert MTB/RIF (90.91%), while both assays showed an identical overall accuracy of 80.0%.

Table V
Comparative diagnostic performance of Xpert MTB/RIF and Xpert Ultra against CSF culture.

Measure	Xpert MTB/RIF	Xpert Ultra
Sensitivity	40.00% (12.16–73.76)	100.00% (69.15–100.00)
Specificity	85.71% (75.29–92.93)	77.14% (65.55–86.33)
PPV	28.57% (13.38–50.88)	38.46% (28.90–49.01)
NPV	90.91% (85.66–94.36)	100.00% (CI not reported)
Accuracy	80.00% (69.56–88.11)	80.00% (69.56–88.11)

Values are percentages with 95% confidence intervals in parentheses.

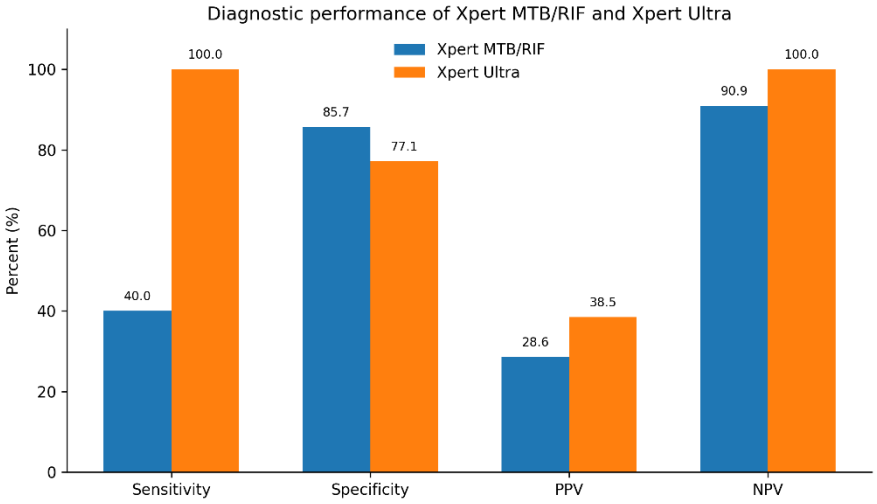


Figure 1 Comparative diagnostic performance of Xpert MTB/RIF and Xpert Ultra.

Figure 1 illustrates this comparative diagnostic performance graphically, highlighting the substantially higher sensitivity and negative predictive value of Xpert Ultra relative to Xpert MTB/RIF, alongside its modestly lower specificity and comparable positive predictive value and accuracy.

Table VI
Rifampicin susceptibility/resistance results among molecular-test-positive specimens.

Molecular test	n	Sensitive, n (%)	Resistant, n (%)	Intermediate, n (%)
Xpert MTB/RIF	14	14 (100.0)	0 (0.0)	0 (0.0)
Xpert Ultra	26	16 (61.5)	2 (7.7)	8 (30.8)

Table VI shows the rifampicin susceptibility results among molecular-test-positive specimens. All 14 Xpert MTB/RIF-positive specimens were categorized as rifampicin sensitive, with no resistant or intermediate results detected. Among the 26 Xpert Ultra-positive specimens, 61.5% were sensitive, 7.7% were resistant and 30.8% returned an intermediate result, reflecting the trace-category positive results characteristic of the assay's higher analytical sensitivity.

DISCUSSION

Rapid and accurate diagnosis of tuberculous meningitis continues to pose a formidable challenge in resource limited, high tuberculosis burden settings, where delay in confirming the diagnosis and initiating treatment remains a major determinant of the disease's characteristically poor outcome [12]. This prospective, single center study was undertaken in the Department of Paediatrics, Dhaka Medical College Hospital, over a twelve month period, to compare the diagnostic performance of Xpert Ultra with that of Xpert MTB/RIF against mycobacterial culture in cerebrospinal fluid obtained from children with presumptive tuberculous meningitis.

Eighty children were enrolled during the study period. The majority, 67.5 percent, were between one month and less than five years of age and the mean age of the cohort was 3.82 ± 3.42 years, a figure closely comparable with the mean age of 36 months reported by Solomons et al. in a comparable paediatric series [8]. Sixty percent of participants were male, giving a male to female ratio of 1.5 to 1, consistent with the male preponderance of 60 to 66 percent described by Donovan et al. across their Xpert Ultra and Xpert comparison groups [10]. Most children, 72.5 percent, were from rural areas, reflecting the largely rural pattern of tuberculosis transmission in this setting.

Fever was universal and was followed in frequency by irritability, altered consciousness and convulsion, with only 17.5 percent of children reporting a documented contact history. These findings broadly mirror those of Shao et al., who described fever, vomiting and night sweats as the dominant presenting complaints and are consistent with the contact history reported by Miftode et al. [13,14]. Nearly half of the children presented with a Glasgow coma scale of 9 to 12 and a further 17.5 percent presented severely obtunded with a score of 8 or below, underscoring the advanced stage at which many children in this setting still present, in keeping with the near universal impairment of consciousness described by Cresswell et al. [15].

Cerebrospinal fluid analysis showed a modestly elevated white cell count with lymphocytic predominance, alongside raised protein and reduced glucose concentrations, a biochemical pattern characteristic of tuberculous meningitis that closely parallels the pattern described by Chin et al. and, for the glucose and protein values specifically [16], by Solomons et al. [8]. Considerable variability in cerebrospinal fluid findings has previously

been reported across cohorts, from the acellular profiles described by Cresswell et al. to the higher cell counts [15] and protein levels reported by Donovan et al. and Shao et al. and the present findings fall within this spectrum [10,13].

Acid fast bacilli microscopy, Xpert MTB/RIF, Xpert Ultra and culture yielded positivity rates of 10.0, 17.5, 32.5 and 12.5 percent respectively, comparable with the culture positivity of 22 percent described by Solomons et al. [8]. Against culture as the reference standard, Xpert Ultra achieved a sensitivity of 100 percent, substantially exceeding the 40 percent sensitivity of Xpert MTB/RIF and the 60 percent sensitivity of acid-fast microscopy, a magnitude of improvement echoed by and Shao et al. and Chin et al. [13,16], although the absolute sensitivity observed here exceeded even the figures reported by Donovan et al. [10].

The specificity of Xpert Ultra, at 77.1 percent, was lower than that of Xpert MTB/RIF and acid-fast microscopy in this cohort, a trade off also described by Dorman et al. in sputum specimens and attributable, in part, to the substantial proportion of Xpert Ultra positive results, nearly a third, that fell into the trace category, below the detection threshold of Xpert MTB/RIF and potentially of culture itself [11]. The use of culture, an imperfect reference standard in paucibacillary extrapulmonary disease as noted by Hoel et al., together with the ability of molecular assays to detect non-viable organisms in children who had frequently received prior antibiotic courses, offers further explanation for this pattern [17]. Positive and negative predictive values for Xpert Ultra were 38.5 and 100 percent respectively, broadly consistent with the high negative predictive value reported by Cresswell et al., while rifampicin resistance was identified in 7.6 percent of Xpert Ultra positive specimens, a detection capability not matched by Xpert MTB/RIF in this cohort and one similarly absent in the series [15]. Taken together, these findings affirm that Xpert Ultra offers a marked sensitivity advantage over Xpert MTB/RIF for the diagnosis of tuberculous meningitis in children, at a modest and explicable cost to specificity, supporting its preferential use in high burden paediatric settings such as this one.

CONCLUSION

In this prospective paediatric cohort from a high tuberculosis burden setting, Xpert Ultra substantially outperformed Xpert MTB/RIF in the diagnosis of tuberculous meningitis from cerebrospinal fluid, achieving complete sensitivity against culture compared with only moderate sensitivity for Xpert MTB/RIF, albeit with a modest reduction in specificity attributable to trace category results. Xpert Ultra also identified rifampicin resistance in specimens that Xpert MTB/RIF failed to characterize. These findings support the preferential adoption of Xpert Ultra as the initial molecular test for children with presumptive tuberculous meningitis in high burden, resource limited settings, where early and accurate diagnosis remains critical to survival.

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