

RESEARCH

Open Access



Comparison of microscopic and xpert MTB diagnoses of presumptive mycobacteria tuberculosis infection: retrospective analysis of routine diagnosis at Cape Coast Teaching Hospital

Kwame Kumi Asare^{1,4*}, Daniel Edem Azumah², Czarina Owusua Adu-Gyamfi¹, Yeboah Kwaku Opoku³, Edward Morkporkpor Adela², Philip Afful¹, Godwin Kwami Abotsi¹, Ernest Awuakye Abban², Paul Ekow Duntu^{1,5}, Akwasi Anyamful^{1,6}, Alberta Bedford Moses², Emmanuel Botchway², Philimon Mwintige², Samuel Kyei^{1,7}, Linda Eva Amoah⁸ and Emmanuel Owusu Ekuman²

Abstract

Introduction *Tuberculosis* is a global health problem that causes 1.4 million deaths every year. It has been estimated that sputum smear-negative diagnosis but culture-positive pulmonary TB diagnosis contribute to 12.6% of pulmonary TB transmission. TB diagnosis by smear microscopy smear has a minimum detection limit (LOD) of 5,000 to 10,000 bacilli per milliliter (CFU/ml) of sputum result in missed cases and false positives. However, GeneXpert technology, with a LOD of 131–250 CFU/ml in sputum samples and its implementation is believed to facilitate early detection TB and drug-resistant TB case. Since 2013, Ghana Health Service (GHS) introduced GeneXpert MTB/RIF diagnostic in all regional hospitals in Ghana, however no assessment of performance between microscopy and GeneXpert TB diagnosis across the health facilities has been reported. The study compared the results of routine diagnoses of TB by microscopy and Xpert MTB from 2016 to 2020 at the Cape Coast Teaching Hospital (CCTH).

Methods The study compared routine microscopic and GeneXpert TB diagnosis results at the Cape Coast Teaching Hospital (CCTH) from 2016 to 2020 retrospectively. Briefly, sputum specimens were collected into 20 mL sterile screw-capped containers for each case of suspected TB infection and processed within 24 h. The samples were decontaminated using the NALC-NaOH method with the final NaOH concentration of 1%. The supernatants were discarded after centrifugation and the remaining pellets dissolved in 1–1.5 ml of phosphate buffer saline (PBS) and used for diagnosis. A fixed smear was Ziehl-Neelsen acid-fast stain and observed under microscope and the remainings were used for GeneXpert MTB/RIF diagnosis. The data were analyzed using GraphPad Prism.

*Correspondence:
Kwame Kumi Asare
kwame.asare@ucc.edu.gh

Full list of author information is available at the end of the article



© The Author(s) 2024. **Open Access** This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>. The Creative Commons Public Domain Dedication waiver (<http://creativecommons.org/publicdomain/zero/1.0/>) applies to the data made available in this article, unless otherwise stated in a credit line to the data.

Results 50.11% (48.48–51.38%) were females with an odd ratio (95% CI) of 1.004 (0.944–1.069) more likely to report to the TB clinic for suspected TB diagnosis. The smear-positive cases for the first sputum were 6.6% (5.98–7.25%), and the second sputum was 6.07% (5.45–6.73%). The Xpert MTB-RIF diagnosis detected 2.93% (10/341) (1.42–5.33%) in the first and 5.44% (16/294) (3.14–8.69%) in the second smear-negative TB samples. The prevalence of Xpert MTB-RIF across smear positive showed that males had 56.87% (178/313) and 56.15% (137/244) and females had 43.13% (135/313) and 43.85% (107/244) for the first and second sputum. Also, false negative smears were 0.18% (10/5607) for smear 1 and 0.31% (16/5126) for smear 2.

Conclusion In conclusion, the study highlights the higher sensitivity of the GeneXpert assay compared to traditional smear microscopy for detecting MTB. The GeneXpert assay identified 10 and 16 positive MTB from smear 1 and smear 2 samples which were microscopic negative.

Keywords *Mycobacterium tuberculosis* (MTB), TB diagnosis, GeneXpert MTB/RIF, Cape Coast Teaching Hospital (CCTH), Ghana

Introduction

Mycobacterium tuberculosis (MTB) is estimated to infect 8.7 million new people and causes approximately 1.4 million deaths yearly [1, 2]. The rate of MTB infections and death is alarming and remains a public health problem globally [3]. The rapid diagnosis of MTB is a challenge, especially among low and middle-income countries, where over 90% of TB cases reside [4, 5]. In addition, there is the extensive development of drug resistance in the ongoing transmission of TB disease [6].

TB diagnosis in developing countries is mainly based on smear microscopy for acid-fast bacillus or by a culture, and in some clinical scenarios and chest X-ray findings [7, 8]. Although Culture method remains the gold standard for TB diagnoses, the method requires cumbersome laboratory procedures and sophisticated biological safety equipment that are very expensive to operate in resource-limited countries [9, 10]. Sputum smear microscopy is the cheapest TB diagnostic tool but has the lowest sensitivity [11]. There are technical challenges in TB slide preparation including generation of droplet nuclei, inhalation risks, and transmissibility [12, 13].

The transmissibility of missed TB cases from sputum smear-negative diagnosis resulting from lower sensitivity is a global health concern [14, 15]. Previous studies have estimated that sputum smear-negative diagnosis but culture-positive pulmonary TB diagnosis contribute to about 12.6% of pulmonary TB transmission [16–18]. This suggests that more sensitive and specific diagnostic tools with rapid turnaround time are required to provide timely diagnosis and prevent delayed case detection, suffering, death, and disease transmission. Prompt and efficient diagnosis is essential for TB control, prevention, and eradication and also provides a timely intervention to prevent TB drug resistance, especially the frontline anti-TB drugs isoniazid (isonicotinic acid hydrazine), pyrazinamide, ethambutol and rifampicin (RIF).

GeneXpert MTB/RIF assay is a molecular-based diagnostic tool that detects MTB and RIF resistance [10, 14,

18]. The World Health Organization (WHO) has recommended the Xpert MTB/RIF diagnostic tool for national tuberculosis programs in developing countries due to its high sensitivity and specificity for TB detection, easy use, automation, and very rapid turnaround time of 2 h [19, 20]. The Xpert MTB/RIF diagnosis operates based on nested real-time PCR [21, 22]. The Xpert MTB/RIF also has the advantage of requiring minimal biosafety facilities, and it is not prone to cross-contamination and is very efficient for TB diagnosis [23–25].

Microscopy-based tuberculosis (TB) diagnosis offers various obstacles, including low sensitivity and specificity, with a minimum detection limit (LOD) of 5,000 to 10,000 bacilli per milliliter (CFU/ml) of sputum, which can result in missed cases and false positives [26]. Accurate results necessitate qualified technicians and high-quality samples, and maintaining the necessary laboratory equipment and supplies can be challenging in resource-constrained environments [27]. In early 2013, light-emitting diode (LED) microscopes were installed at 156 high-burden sites to minimize workload and speed up TB detection. Additionally, GeneXpert technology, with a LOD of 131–250 CFU/ml in sputum samples, was implemented in selected locations to improve TB diagnosis, particularly among people living with HIV (PLHIV) and children, and to facilitate the early detection of drug-resistant TB by the Ghana Health Service (GHS) [28]. This project has been extremely successful, resulting in the installation of additional machines in all Regional Hospitals and Teaching Hospitals across the country. Currently, 134 GeneXpert machines have been bought and disseminated around the country to improve TB diagnosis among PLHIV and ensure early detection of drug-resistant cases. However, no study has been conducted to examine the pattern of TB diagnosis between microscopy and GeneXpert. The study compared the results of routine diagnoses of TB by microscopy and Xpert MTB from 2016 to 2020 at the Cape Coast Teaching Hospital (CCTH).

Materials and methods

Study design and area

The study compared routine TB diagnosis results at the Cape Coast Teaching Hospital (CCTH) from 2016 to 2020 retrospectively. The Xpert MTB-RIF method was compared to the microscopy TB smear method from 2016 to 2020 at CCTH in the Central Region of Ghana. The Central Region of Ghana spans an area of 9,826 square kilometers, comprising about 6.6% of the country's total land area. It is one of Ghana's sixteen administrative regions, bordered by the Gulf of Guinea to the south, the Western and Western North Regions to the west, the Greater Accra Region to the east, the Ashanti Region to the north, and the Eastern Region to the north-east. The region consists of 22 administrative districts, with Cape Coast as the capital. Approximately 63% of the population lived in rural areas as of 2008. Predominantly inhabited by the Akan people, the majority are Fantes. As of 2020, the estimated population was 2,605,490, with a regional growth rate of 3.1% and a population density of about 215 inhabitants per square kilometer. The CCTH is the only teaching hospital in the Region and serves as the referral hospital for the Region. In all, 6019 suspected TB cases visited the Microbiology Laboratory of CCTH between 2016 and 2020 for TB diagnosis. Of the 6019 suspected TB, 2393 (39.8%) cases were referrals from various health facilities across the Region.

Ethical approval

The Biomedical and Clinical Research Centre Research Board, University of Cape Coast, approved the study (BCRC/22/03_0001/01). CCTH permitted the analyses of routine TB data from the hospital. Since this study used retrospective data, consent to participate and or for publication did not apply. All data obtained from laboratory records were anonymous.

Mycobacteria Tuberculosis (TB) diagnostic strategy at CCTH

Two sputum samples were collected from each presumptive TB individual at an interval of three or more hours. The TB smears were prepared from each sputum sample for microscopic diagnosis (Smears from the initial sputum sample were labeled as TB smears 1 and the smear from the second sputum sample was labeled as TB smear 2). Similarly, the Xpert MTB-RIF diagnosis was conducted for all the collected sputum samples. The second sputum was collected three hours or more after the collection of the first sputum sample to increase the chance of capturing the shed TB in the sputum and also to reduce the turnaround time for TB diagnosis and treatment. The Xpert MTB-RIF test was performed for smear-positive (+) and smear-negative (-) TB cases (Fig. 1).

Sample collection

Sputum specimens were collected into 20 mL sterile screw-capped containers for each case of suspected TB infection and processed within 24 h by a laboratory technologist as recommended by the World Health Organisation WHO [29].

Decontamination of the sputum samples

The samples were decontaminated using the NALC-NaOH method with the final NaOH concentration of 1% [30–32]. The supernatants were discarded after the centrifuge and the remaining pellets dissolved in 1–1.5 ml of phosphate buffer saline (PBS) and used for diagnosis.

Microscopy

A fixed sputum smear (smears 1 and 2) was made using the pellets and stained with Ziehl-Neelsen acid-fast stain [33, 34]. Positive slides with acid-fast bacilli (AFB) were reported based on the following criteria at a 400X magnification: no AFB seen was considered a negative, 1 to 9 AFB per 100 fields was considered rare (scanty), 10–99 AFB per 100 fields was considered moderate (positive +), 1–10 AFB per field (check 50 fields) (positive ++), greater than 100 AFB per 100 fields was considered many (positive +++) according to Samuel & Kanna, 2022 [35].

Xpert MTB/RIF Diagnosis

All sputum samples were processed and diagnosed using the Xpert MTB/RIF [25]. The test uses the G4 version of the cartridges. Decontaminated sputum samples were neutralized with an excess of 0.067 M phosphate buffer containing 0.2% phenol red, adjusting the pH to 6.8 ± 0.2 using 4% sodium hydroxide or 1 M hydrochloric acid as necessary. The neutralized samples were then centrifuged at $3000 \times g$ for 15 min, after which the supernatant was carefully discarded. The pellet obtained from centrifugation was used for further tuberculosis diagnostic testing. All the processes followed the manufacturer's instructions (Cepheid, Sunnyvale, CA).

Statistical analysis

The data that was entered into the Excel spreadsheet were cross-checked by two independent researchers to validate the data on Excel to the recordings in the Laboratory Logbooks and analyzed using Excel 2016 (Microsoft Corporation). The results of this study are presented in tables and figures. The data are in frequencies and percentages. The Clopper-Pearson test was used to determine the confidence intervals of proportions of relevant outcome variables under study. The odds ratios and associated 95% confidence intervals were used to assess the odds of stratified variables by sociodemographic and clinical variables. All data analyses were performed using GraphPad version 9.3.1.

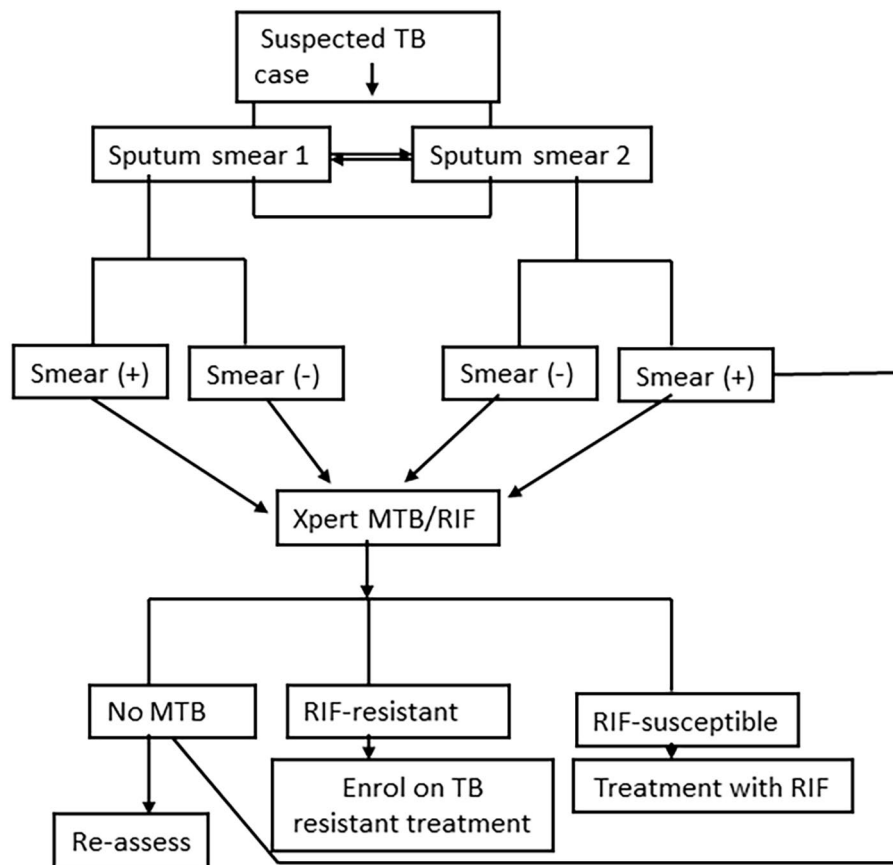


Fig. 1 Diagnostic strategy of Mycobacterium tuberculosis (MTB)

Results

Characteristics of the presumptive TB cases

The CCTH TB clinic tested 6019 suspected TB cases from 2016 to 2020. A total 39.9% (95% CI; 38.67–41.16%) of the presumptive TB cases were referrals from other health facilities across the Central region of Ghana. Of the cases, 50.11% (48.48–51.38%) were females with an odd ratio (95% CI) of 1.004 (0.944–1.069) more likely to report to the TB clinic for presumptive TB diagnosis. Of the presumptive TB cases, 52.69% (51.41–53.97%), 23.98% (22.89–25.09%), and 17.8% (16.84–18.8%) were among the age categories 31–60 years, 16–30 years and greater than 60 years age categories respectively. The smear-positive cases for the first sputum were 6.6% (5.98–7.25%), and the second sputum was 6.07% (5.45–6.73%). The positive Xpert MTB-RIF diagnosis among smear-positive samples was 83.59% (79.56–87.1%) and 83.9% (79.58–87.7%) for the first and second sputum. Also, the smear-negative samples tested positive by the Xpert MTB-RIF diagnosis for the first and second sputum were 2.93% (1.42–5.33%) and 5.44% (3.14–8.69%) (Table 1).

Prevalence of Xpert MTB-RIF across smear classification of TB infection

The TB smears classification as no infection (negative) 93.4% (5607/6003), scanty 1.2% (74/6003), Positive (+) 2.4% (143/6003), positive (++) 1.5% (92/6003), and positive (+++) 1.4 (87/6003) for the first sputum. A similar pattern of diagnosis was observed for the second sputum. Interestingly, 2.9% (10/341) and 5.4% (16/331) of the Xpert MTB-RIF positive diagnosis were smear-negative for the first and second sputum. There were more positives in the Xpert MTB-RIF diagnosis for the negative and scanty smears in the second TB sputum compared to the first sputum. The scanty samples had very high false positive results of 78.38% (58/74) for smear 1 and 57.89% (44/76) for smear 2. However, the positive samples had low false positive results ranging from 1.09% (1/92) in positive (++) of smear 1 to 2.8% (4/143) in positive (+) of smear 1. Also, false negative smears were 0.18% (10/5607) for smear 1 and 0.31% (16/5126) for smear 2 (Table 2). The Xpert MTB-RIF prevalence for negative smears was 0.18% (10/5607) and 0.31% (16/5126) (Fig. 2 a), scanty smears were 20.27% (15/74) & 40.79% (31/76) (Fig. 2 b), positive (+) were 97.2% (139/143) & 96.77% (90/93),

Table 1 Demographic characteristics of the study subjects with suspected Mycobacteria Tuberculosis infections

Characteristics	n/N (%)	95% CI	OR (95% CI)
Age categories/years			
< 5 y	34/5914 (0.57)	0.4–0.8	0.006 (0.004–0.01)
5–15 y	29/5914 (0.49)	0.33–0.7	0.005 (0.003–0.01)
16–30 y	1418/5914 (23.98)	22.89–25.09	0.315 (0.294–0.34)
31–60 y	3116/5914 (52.69)	51.41–53.97	1.114 (1.046–1.19)
> 60 y	1053/5914 (17.80)	16.84–18.8	0.217 (0.201–0.23)
Sex			
Male	3003/6019 (49.89)	48.62–51.16	0.996 (0.936–1.06)
Female	3016/6019 (50.11)	48.84–51.38	1.004 (0.944–1.07)
Health facility			
CCTH	3603/5996 (60.09)	58.84–61.33	1.506 (1.414–1.60)
Referral	2393/5996 (39.91)	38.67–41.16	0.664 (0.624–0.71)
Diagnosis			
Smear 1			
Pos	396/6003 (6.60)	5.98–7.25	0.071 (0.063–0.08)
Neg	5607/6003 (93.40)	92.75–94.02	14.14 (12.7–15.75)
Smear 2			
Pos	331/5457 (6.07)	5.45–6.73	0.065 (0.06–0.07)
Neg	5126/5457 (93.93)	93.27–94.55	15.46 (13.75–17.39)
S1-Xpert MTB-RIF			
Pos	341/6003 (5.68)	5.11–6.3	0.060 (0.05–0.07)
Neg	5662/6003 (94.32)	9.37–94.89	16.58 (14.78–18.60)
S1-Xpert +/S1+	331/396 (83.59)	79.56–87.1	14.71 (12.28–17.66)
S1-Xpert+/S1-	10/341 (2.93)	1.42–5.33	+infinity (3.46- +infinity)
S2-Xpert MTB-RIF			
Pos	294/5457 (5.39)	4.8–6.02	0.057 (0.050–0.06)
Neg	5160/5457 (94.56)	93.92–95.14	17.52 (15.49–19.82)
S2-Xpert +/S2+	278/331 (83.99)	79.58–87.7	15.59 (12.78–18.94)
S2-Xpert+/S2-	16/294 (5.44)	3.14–8.69	+infinity (4.38- +infinity)

Table 2 Prevalence of Mycobacteria Tuberculosis diagnosed with smear TB Microscopy and GeneXpert MTB-RIF

MTB Test	Classification, n/N (%)				
	Neg	Scanty	Pos (+)	Pos (++)	Pos (+++)
Microscopy					
Smear 1	5607/6003 (93.4)	74/6003 (1.2)	143/6003 (2.4)	92/6003 (1.5)	87/6003 (1.4)
Smear 2	5126/5456 (94.0)	76/5456 (1.4)	93/5456 (1.7)	79/5456 (1.4)	82/5456 (1.5)
Xpert MTB test					
Sm1_Xpert MTB-RIF (+)	10/341 (2.9)	16/341 (4.7)	139/341 (40.8)	91/341 (26.7)	86/341 (25.2)
Sm2_Xpert MTB-RIF (+)	16/297 (5.4)	32/297 (10.8)	91/297 (30.6)	78/297 (26.3)	80/297 (26.9)
False positive Smear					
Smear 1	-	58/74 (78.38)	4/143 (2.8)	1/92 (1.09)	1/87 (1.15)
Smear 2	-	44/76 (57.89)	2/93 (2.15)	1/79 (1.27)	2/86 (2.33)
False negative Smear					
Smear 1	10/5607 (0.18)	-	-	-	-
Smear 2	16/5126 (0.31)	-	-	-	-

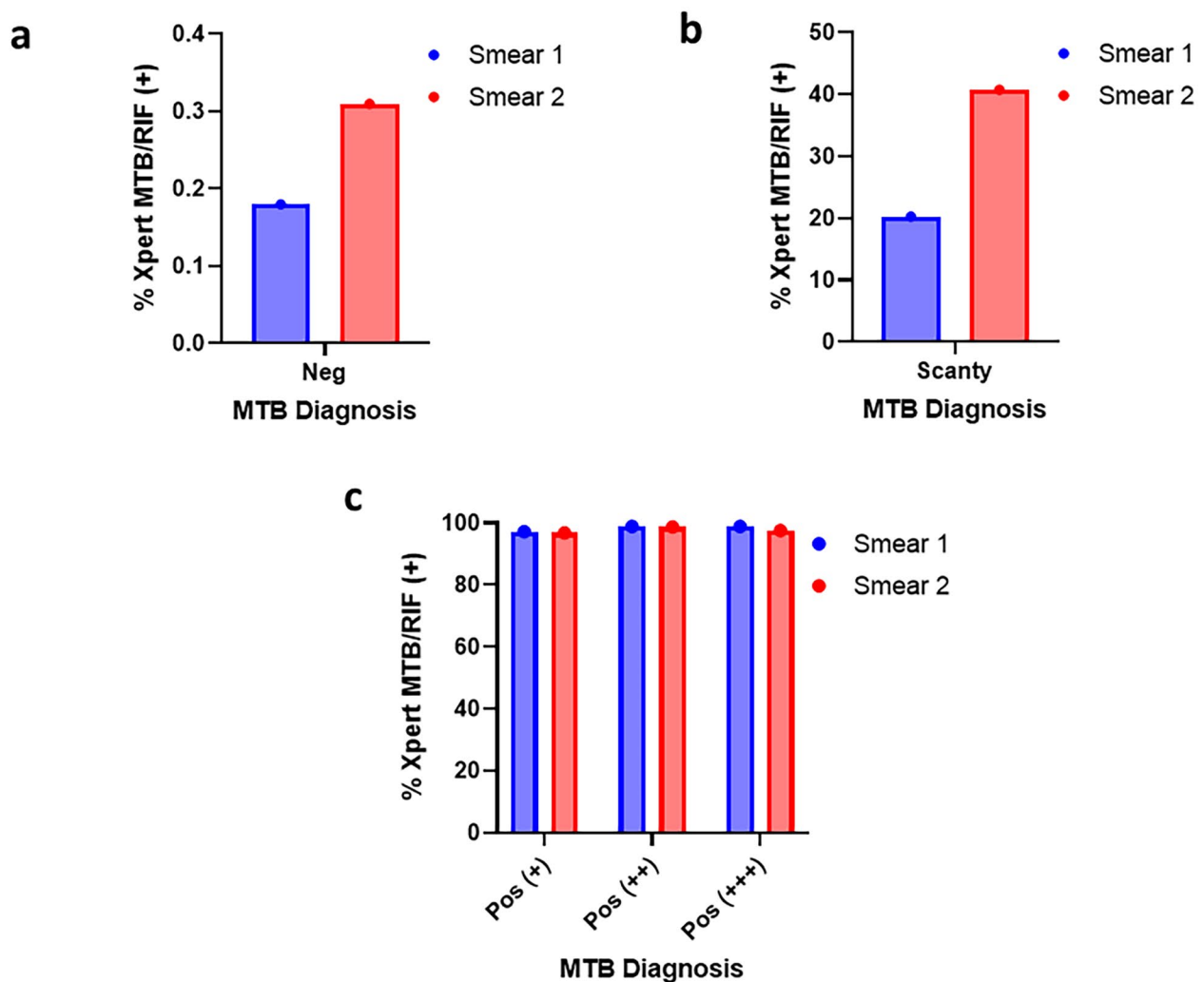


Fig. 2 Percentage of GeneXpert MTB/RIF positive cases across smear-classified AFB Microscopic diagnosis. **a.** The overall GeneXpert TB prevalence among smear negative. **b.** The overall GeneXpert TB prevalence among scanty smear. **c.** The overall GeneXpert TB prevalence among smear positive cases. MTB- Mycobacteria tuberculosis. RIF-Rifampicin. Negative-no AFB seen. 1 to 9 AFB per 100 fields was considered rare (scanty). 10–99 AFB per 100 fields was considered moderate (positive +). 1–10 AFB per field (check 50 fields) (positive ++). greater than 100 AFB per 100 fields was considered many (positive +++)

positive (++) were 98.91% (91/92) & 98.73% (78/79) and positive (+++) were 98.85% (86/87) and 97.56% (80/82) for first and second sputum respectively (Fig. 2 c).

TB prevalence among gender and age categories

The prevalence of Xpert MTB-RIF across smear positive showed that males had 56.87% (178/313) and 56.15% (137/244) and females had 43.13% (135/313) and 43.85% (107/244) for the first and second sputum (Fig. 3). The Xpert MTB-RIF diagnosis showed that TB is more prevalent among age category 16–30 years (53.2% (174/327) and 55.8% (134/240)) followed by 31–60 years (32.7% (107/327) and 32.5% (78/240)) and >60 years (11.3% (37/327) and 8.3% (20/240)) in the first and second

sputum respectively. The age category <5 years were group least infected with TB (Fig. 4).

Discussion

A prompt diagnosis of pulmonary TB plays a significant role in TB disease management, especially in high-endemic TB countries [36]. The impact of accurate and timely TB diagnoses on treatment outcomes and prevention of TB transmission cannot be over-emphasized. Microscopic diagnosis of low TB infections with acid-fast bacilli smear is a challenge for TB management as sub-microscopic TB diagnosis contributes to an estimated 12.6% of TB transmission [37]. The GeneXpert MTB-RIF has superior sensitivity and specificity and a rapid turnaround time for diagnosing TB infection [38–40].

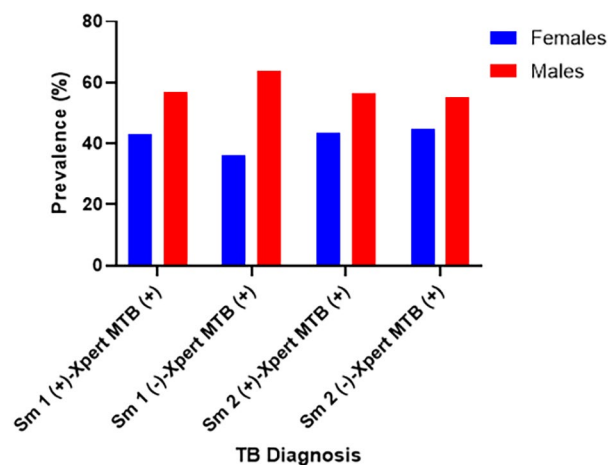


Fig. 3 Prevalence of Mycobacteria tuberculosis (MTB) infections by GeneXpert MTB/RIF among males and females with smear-positive and smear-negative cases

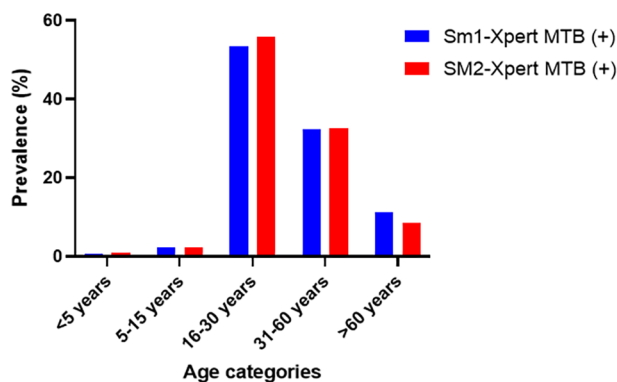


Fig. 4 Prevalence of Mycobacteria tuberculosis (MTB) infections by GeneXpert MTB/RIF across age categories

The study compared the results of routine diagnoses of TB by microscopy and Xpert MTB from 2016 to 2020 at the Cape Coast Teaching Hospital (CCTH).

The Xpert MTB-RIF diagnosis showed that 2.9% and 5.4% of smears negative TB samples for the first and second sputum harbors *M. tuberculosis*. Similarly, the false positive TB diagnoses with scanty diagnoses have up to 78.38% diagnosed as missed by the Xpert MTB-RIF. The suboptimal microscopic diagnosis of TB smear has explicit or implicit consequences on the National TB Control Program (NTCP) and elimination strategies. The missed diagnosis of pulmonary TB prolongs morbidity and mortality in tuberculosis [41, 42]. Since Microscopy is the mainstay of TB diagnosis in Ghana, an estimated 4.15% missed TB smear diagnosis is evidence of the challenges associated with mapping TB infections, diagnosis, treatment, improving quality of care for patients, and preventing TB transmissions [41, 43, 44].

A previous study reported 2.6% smear-negative but culture positive in Nigeria; the authors recommended TB culture as a confirmatory diagnostic test [45]. The challenge with TB culture is the turnaround time for the results, poor primary healthcare infrastructure, stable electricity, and the skills of laboratory personnel [19, 46]. Also, the GeneXpert MTB/RIF test has reported 16.4% increase in TB detection among cases that were negative in ZN smear microscopy [47, 48]. The study shows that the GeneXpert MTB/RIF test efficiently detects missed ZN TB smear microscopy, similar to the previous studies conducted at Pakistan Institute of Medical Sciences which showed that GeneXpert could detect 15.3% (50/326) MTB DNA among the patients while ZN smear microscopy could only detect 9.2% (30/326) of AFB among the same patients [49]. The AFB smear microscopy has a higher detection threshold and lower sensitivity than culture or GeneXpert [50, 51]. Also, microscopic has a high limit of TB detection and can detect TB cells while GeneXpert has low limit of detection and detect the TB DNA. Thus, increasing the sensitivity and specificity of TB diagnosis by GeneXpert when compared with TB smear by microscopic diagnosis [28].

The GeneXpert assay identified 10 and 16 positive MTB from smear 1 and smear 2 samples which were microscopic negative. The result highlights the higher sensitivity of the GeneXpert assay compared to traditional smear microscopy for detecting MTB [52]. The smear microscopy relies on visualizing bacteria under a microscope, which may miss cases with low bacterial load or non-visible bacilli [53]. In contrast, the GeneXpert can detect MTB DNA even at low concentrations, offering higher sensitivity and the ability to detect cases missed by smear microscopy [54–56]. This makes GeneXpert crucial for accurate diagnosis, especially in cases where traditional microscopic methods yield negative results but clinical suspicion remains high.

However, due to the high sensitivity of the GeneXpert assay, it can detect *Mycobacterium tuberculosis* (MTB) DNA without distinguishing between live and dead bacteria [53, 57]. Since the GeneXpert assay detects dead bacteria with intact DNA, a positive result does not distinguish between viable and non-viable bacteria [58]. The detection of the dead MTB DNA in a sample could still be clinically relevant, as it may indicate a previous or ongoing infection, even if the bacteria are no longer actively replicating [59, 60]. However, in making treatment decisions, it is important to distinguish between live and dead bacteria to avoid misuse of drugs and induction of resistant MTB bacteria.

The GeneXpert MTB is estimated to have a detection limit of 136 bacilli/ml of the specimen and provides detection and assessment of the TB resistance to rifampicin, a first-line anti-TB drug [61, 62]. The GeneXpert

MTB/RIF provides rapid detection of rifampicin resistance and could prevent transmission from potent smear-negative pulmonary TB [63, 64].

The high male-to-female predominance in TB infections is well known [65, 66]. In low and middle-income countries like Ghana, the high TB prevalence among men is attributed to poor health-seeking behaviour and accessing TB care [67–70]. Men are less likely to have timely TB diagnosis since male TB patients often delay care-seeking longer than female TB patients [70]. The study showed a high prevalence of TB infections among males compared to females in both smear-positive-Xpert positive and smear-negative-Xpert positive cases. The findings are in agreement with the previous studies [66, 67, 70]. Thus, men have a relatively high risk of TB, and disadvantages in accessing TB diagnoses and treatments suggest higher undiagnosed cases of TB in males [71, 72]. Other factors attributed to a high prevalence of TB in men include males being sole breadwinners, lack of disease awareness, working in unorganized sectors, and higher probability of default treatment [71, 72].

The study showed high TB prevalence among the 16–30 years and 31–60 years. The findings suggest that young and older people have a higher risk of TB disease than those below 16 years in this study. Similar studies in Argentina and India have reported slightly higher risks in young adults [73, 74]. In another study, there were significantly lower risks among people under 15 years, and the risks were higher in all groups over 15 years old [75, 76]. Air pollution, smoking, other determinants, continuous TB transmission within the community, staying in cluster environments such as schools, frequent social activities, or disease comorbidities are associated with increased risk exposure to TB disease among specific age groups [76–80].

In conclusion, the study highlights the higher sensitivity of the GeneXpert assay compared to traditional smear microscopy for detecting MTB. The GeneXpert assay identified 10 and 16 positive MTB from smear 1 and smear 2 samples which were microscopic negative. Males are more at risk of TB infections than females. Also, the ages 16–30 years and 31–60 years are more prone to TB infections than the age below 15 years. The sex and age-specific results underscore the importance of understanding demographic characteristics to effectively target high-risk TB populations. Tailored interventions that consider these demographic factors are critical for enhancing TB prevention, early detection, and treatment efforts, ultimately reducing the burden of the disease in vulnerable populations. This demographic insight supports the development of more effective TB control strategies that align with the epidemiological realities of different regions and communities.

Acknowledgements

We thank the Laboratory Department of Cape Coast Teaching Hospital CCTH and the staff of the Biomedical and Clinical Research Centre for supporting the study.

Author contributions

Conceptualization, AKK; Formal analysis, KKA, LEA, YKO, EMA, JA, EAA; Methodology, PED, AA, ABM, EB, COA-G, GKA, PA, PM; Supervision, LEA, SK, AKK; Validation, KKA, DEA, AA, JA, YKO; Visualization LEA, KKA, SK; Writing – original draft, KKA, YKO, LEA, JA, AA, COA-G, GKA, PA, SK, EMA; Writing – review & editing; KKA, LEA, YKO, EMA, JA, EAA, PED, AA, ABM, EB, PM, SK.

Funding

AKK was partly supported with the 2022/2023 Ghana government books and research allowance BRA2022/2023.

Data availability

All the data are available in the manuscript and supplementary data.

Declarations

Ethics approval and consent to participate

The Cape Coast Teaching Hospital Ethical Review Committee (CCTHERC), approved the study (CCTHERC/EC/2023/248). CCTH Laboratory permitted the analyze the TB data.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Consent to participate

Not applicable.

Author details

¹Biomedical and Clinical Research Centre, College of Allied Health Sciences, University of Cape Coast, Cape Coast, Ghana

²Laboratory Departments, Cape Coast Teaching Hospital, Cape Coast, Ghana

³Department of Biology Education, Faculty of Science Education, University of Education, Winneba, Ghana

⁴Department of Biomedical Sciences, School of Allied Health Sciences, College of Allied Health Sciences, University of Cape Coast, Cape Coast, Ghana

⁵Department of Medical Laboratory Science, School of Allied Health Sciences, College of Allied Health Sciences, University of Cape Coast, Cape Coast, Ghana

⁶Department of Medical Biochemistry, School of Medical Sciences, College of Health and Allied Sciences, University of Cape Coast, Cape Coast, Ghana

⁷Department of Optometry and Vision Science, University of Cape Coast, Cape Coast, Ghana

⁸Department of Immunology, Noguchi Memorial Institute for Medical Research, University of Ghana, Accra, Ghana

Received: 9 January 2024 / Accepted: 25 June 2024

Published online: 02 July 2024

References

1. Keri RS, Chand K, Ramakrishnappa T, Nagaraja BM. Recent progress on pyrazole scaffold-based antimycobacterial agents. *Arch Pharm*. 2015;348(5):299–314.
2. Soni NK. Bioactivity, Molecular Docking, and Pharmacophore Modeling of Mycobacterium tuberculosis: A Study Targeting the Microarray Data of the Microbe. *Asian Journal of Pharmaceutics (AJP)*. 2017;11(04).
3. Dhingra S, Rahman NA, Peile E, Rahman M, Sartelli M, Hassali MA, Islam T, Islam S, Haque M. Microbial resistance movements: an overview of global

- public health threats posed by antimicrobial resistance, and how best to counter. *Front Public Health*. 2020;8:535668.
- Meghji J, Mortimer K, Agusti A, Allwood BW, Asher I, Bateman ED, Bissell K, Bolton CE, Bush A, Celli B, Chiang CY. Improving lung health in low-income and middle-income countries: from challenges to solutions. *Lancet*. 2021;397(10277):928–40.
 - Harries AD, Kumar AM. Challenges and progress with diagnosing pulmonary tuberculosis in low-and middle-income countries. *Diagnostics*. 2018;8(4):78.
 - Dheda K, Limberis JD, Pietersen E, Phelan J, Esmail A, Lesosky M, Fennelly KP, Te Riele J, Mastrapa B, Streicher EM, Dolby T. Outcomes, infectiousness, and transmission dynamics of patients with extensively drug-resistant tuberculosis and home-discharged patients with programmatically incurable tuberculosis: a prospective cohort study. *Lancet Respiratory Med*. 2017;5(4):269–81.
 - Paul DC, Ngeow YF, Yap SF, Dony JF, Avoi R, Mohammad R, Ng HF. Concentration specimen smear microscopy utilising a polymer membrane sandwich filtration vessel for the detection of acid-fast bacilli in health facilities in Sabah, East Malaysia. *Tuberculosis*. 2022;133:102183.
 - Ang M, Vasconcelos-Santos DV, Sharma K, Accorinti M, Sharma A, Gupta A, Rao NA, Chee SP. Diagnosis of ocular tuberculosis. *Ocul Immunol Inflamm*. 2018;26(2):208–16.
 - Yalley AK, Ahiatrogah S, Kafintu-Kwashie AA, Amegatcher G, Prah D, Botwe AK, Adusei-Poku MA, Obodai E, Nii-Trebi NI. A systematic review on suitability of molecular techniques for diagnosis and research into infectious diseases of concern in resource-limited settings. *Curr Issues Mol Biol*. 2022;44(10):4367–85.
 - Weyer K, Mirzayev F, Migliori GB, Van Gemert W, D'Ambrosio L, Zignol M, Floyd K, Centis R, Cirillo DM, Tortoli E, Gilpin C. Rapid molecular TB diagnosis: evidence, policy making and global implementation of Xpert MTB/RIF. *Eur Respir J*. 2013;42(1):252–71.
 - Gelaw B, Shiferaw Y, Alemayehu M, Bashaw AA. Comparison of loop-mediated isothermal amplification assay and smear microscopy with culture for the diagnostic accuracy of tuberculosis. *BMC Infect Dis*. 2017;17:1–6.
 - Randall K, Ewing ET, Marr LC, Jimenez JL, Bourouiba L. How did we get here: what are droplets and aerosols and how far do they go? A historical perspective on the transmission of respiratory infectious diseases. *Interface Focus*. 2021;11(6):20210049.
 - Stadnytskiy V, Anfirrud P, Bax A. Breathing, speaking, coughing or sneezing: what drives transmission of SARS-CoV-2? *J Intern Med*. 2021;290(5):1010–27.
 - Opota O, Senn L, Prod'homme G, Mazza-Stalder J, Tissot F, Greub G, Jaton K. Added value of molecular assay Xpert MTB/RIF compared to sputum smear microscopy to assess the risk of tuberculosis transmission in a low-prevalence country. *Clin Microbiol Infect*. 2016;22(7):613–9.
 - Keflie TS, Ameni G. Microscopic examination and smear negative pulmonary tuberculosis in Ethiopia. *Pan Afr Med J*. 2014;19(1).
 - Ahmad M, Ibrahim WH, Al Sarafandi S, Shahzada KS, Ahmed S, Haq IU, Raza T, Hameed MA, Thomas M, Swehli HA, Sattar HA. Diagnostic value of bronchoalveolar lavage in the subset of patients with negative sputum/smear and mycobacterial culture and a suspicion of pulmonary tuberculosis. *Int J Infect Dis*. 2019;82:96–101.
 - Zhang ZX, Sng LH, Yong Y, Lin LM, Cheng TW, Seong NH, Yong FK. Delays in diagnosis and treatment of pulmonary tuberculosis in AFB smear-negative patients with pneumonia. *Int J Tuberc Lung Dis*. 2017;21(5):544–9.
 - Kabir S, Parash MT, Emran NA, Hossain AT, Shimmi SC. Diagnostic challenges and Gene-Xpert utility in detecting Mycobacterium tuberculosis among suspected cases of Pulmonary Tuberculosis. *PLoS ONE*. 2021;16(5):e0251858.
 - World Health Organization. Rapid implementation of the Xpert MTB/RIF diagnostic test: technical and operational 'How-to'; practical considerations. No. WHO/HTM/TB/2011.2. World Health Organization; 2011.
 - World Health Organization. WHO meeting report of a technical expert consultation: non-inferiority analysis of Xpert MTB/RIF Ultra compared to Xpert MTB/RIF. No. WHO/HTM/TB/2017.04. World Health Organization; 2017.
 - Sun L, Yao L, Fu G, Lin L, Zhu E, Huang J. A comparison of the accuracy of the CapitalBio Mycobacterium real-time polymerase chain reaction and the Xpert MTB/RIF assay for the diagnosis of tuberculous meningitis. *Int J Infect Dis*. 2021;104:92–6.
 - Kolia-Diafouka P, Carrère-Kremer S, Lounnas M, Bourdin A, Kremer L, Van de Perre P, Godreuil S, Tuailon E. Detection of Mycobacterium tuberculosis in paucibacillary sputum: performances of the Xpert MTB/RIF ultra compared to the Xpert MTB/RIF, and IS6110 PCR. *Diagn Microbiol Infect Dis*. 2019;94(4):365–70.
 - Rasool G, Khan AM, Mohy-Ud-Din R, Riaz M. Detection of Mycobacterium tuberculosis in AFB smear-negative sputum specimens through MTB culture and GeneXpert® MTB/RIF assay. *Int J ImmunoPathol Pharmacol*. 2019;33:2058738419827174.
 - Iram S, Zeenat A, Hussain S, Yusuf NW, Aslam M. Rapid diagnosis of tuberculosis using Xpert MTB/RIF assay-report from a developing country. *Pakistan J Med Sci*. 2015;31(1):105.
 - Lawn SD, Mwaba P, Bates M, Piatek A, Alexander H, Marais BJ, Cuevas LE, McHugh TD, Zijenah L, Kapata N, Abubakar I. Advances in Tuberculosis diagnostics: the Xpert MTB/RIF assay and future prospects for a point-of-care test. *Lancet Infect Dis*. 2013;13(4):349–61.
 - Gordillo-Marroquín C, Gómez-Velasco A, Sánchez-Pérez HJ, Pryg K, Shinnors J, Murray N, Muñoz-Jiménez SG, Bencomo-Alerm A, Gómez-Bustamante A, Jonapá-Gómez L, Enriquez-Ríos N. Magnetic nanoparticle-based biosensing assay quantitatively enhances acid-fast bacilli count in paucibacillary pulmonary tuberculosis. *Biosensors*. 2018;8(4):128.
 - Latif Khan H, Boothroyd C, Chang TA, Novero V, Chan DY, Chen CH, Chung MK, Ezoe K, Liow S, Malhotra K, Mantravadi K. ASPIRE guidelines for assisted Reproductive Technology (ART) laboratory practice in low and medium resource settings. *Fertility Reprod*. 2023;5(03):115–33.
 - Sorvor FK, Ewusie EA. The impact of Genexpert MTB/RIF Technology on the minimization of tuberculosis: a review of literature. *Asian J Med Health*. 2024;22(1):1–2.
 - Puri MM, Mumwalia N, Sharma A, Myneedu VP, Khayyam KU, Verma A, Sharma PP. Fluorescein Diacetate vital staining for detecting viability of acid-fast bacilli in sputum of pulmonary tuberculosis patients. *Indian J Tuberculosis*. 2022;69(4):626–34.
 - Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J, Banada PP, Deshpande S, Shenai S, Gall A, Glass J. The new Xpert MTB/RIF Ultra: improving detection of Mycobacterium tuberculosis and resistance to rifampin in an assay suitable for point-of-care testing. *MBio*. 2017;8(4):e00812–17.
 - World Health Organization. Technical expert group meeting report: commercial products for preserving clinical specimens for the diagnosis of tuberculosis. No. WHO/HTM/TB/2017.19. World Health Organization; 2017.
 - Peres RL, Maciel EL, Morais CG, Ribeiro FC, Vinhas SA, Pinheiro C, Dietze R, Johnson JL, Eisenach K, Palaci M. Comparison of two concentrations of NALC-NaOH for decontamination of sputum for mycobacterial culture. *Int J Tuberc Lung Dis*. 2009;13(12):1572–5.
 - World Health Organization. Same-day diagnosis of tuberculosis: policy statement. No. WHO/HTM/TB/2011.7. World Health Organization; 2011.
 - Putri FA, Burhan E, Nawas A, Soepandi PZ, Sutoyo DK, Agustin H, Isbaniyah F, Dowdy DW. Body mass index predictive of sputum culture conversion among MDR-TB patients in Indonesia. *Int J Tuberc Lung Dis*. 2014;18(5):564–70.
 - Samuel RD, Kanna BR. A fuzzy strategy to eliminate uncertainty in Grading positive tuberculosis. *Int J Comput Intell Appl*. 2022;21(01):2250006.
 - Li S, Liu B, Peng M, Chen M, Yin W, Tang H, Luo Y, Hu P, Ren H. Diagnostic accuracy of Xpert MTB/RIF for Tuberculosis detection in different regions with different endemic burden: a systematic review and meta-analysis. *PLoS ONE*. 2017;12(7):e0180725.
 - McCreesh N, Karat AS, Govender I, Baisley K, Diaconu K, Yates TA, Houben RM, Kiehlmann K, Grant AD, White RG. Estimating the contribution of transmission in primary healthcare clinics to community-wide TB disease incidence, and the impact of infection prevention and control interventions, in KwaZulu-Natal, South Africa. *medRxiv*. 2021 Aug 4:2021–08.
 - Reechaipichitkul W, Suleesathira T, Chaimanee P. Comparison of GeneXpert MTB/RIF assay with conventional AFB smear for diagnosis of pulmonary tuberculosis in northeastern Thailand. *Southeast Asian J Trop Med Public Health*. 2017;48(2):313–21.
 - Chen P, Sun W, He Y. Comparison of metagenomic next-generation sequencing technology, culture and GeneXpert MTB/RIF assay in the diagnosis of tuberculosis. *J Thorac Disease*. 2020;12(8):4014.
 - Meawed TE, Shaker A. Assessment of diagnostic accuracy of Gene Xpert MTB/RIF in diagnosis of suspected retreatment pulmonary tuberculosis patients. *Egypt J Chest Dis Tuberculosis*. 2016;65(3):637–41.
 - Sy KT, Haw NJ, Uy J. Previous and active tuberculosis increases risk of death and prolongs recovery in patients with COVID-19. *Infect Dis*. 2020;52(12):902–7.
 - Tsai TC, Hung MS, Chen IC, Chew G, Lee WH. Delayed diagnosis of active pulmonary tuberculosis in emergency department. *Am J Emerg Med*. 2008;26(8):888–92.
 - Medrano BA, Salinas G, Sanchez C, Miramontes R, Restrepo BI, Haddad MB, Lambert LA. A missed tuberculosis diagnosis resulting in hospital transmission. *Infect Control Hosp Epidemiol*. 2014;35(5):534–7.

44. Heffernan C, Paulsen C, Asadi L, Egedahl ML, Rowe BH, Barrie J, Long R. Individual and public health consequences associated with a missed diagnosis of pulmonary tuberculosis in the emergency department: a retrospective cohort study. *PLoS ONE*. 2021;16(3):e0248493.
45. Kehinde AO, Dada-Adegbola H. Epidemiology of smear-negative tuberculosis in Ibadan, Nigeria. *Afr J Infect Dis*. 2013;7(1):14–7.
46. World Health Organization. Operations manual for delivery of HIV prevention, care and treatment at primary health centres in high-prevalence, resource-constrained settings: Edition 1 for fieldtesting and country adaptation. (2008).
47. Umair M, Siddiqui SA, Farooq MA. Diagnostic accuracy of Sputum Microscopy in Comparison with GeneXpert in Pulmonary Tuberculosis. *Cureus*. 2020;12(11).
48. Varania MN, Jani MV, Kulshrestha A. Evaluation of diagnostic accuracy of Ziehl Neelsen staining of sputum samples in comparison with CBNAAT for the diagnosis of Pulmonary Tuberculosis at the tertiary care hospital from South Rajasthan. *Eur J Mol Clin Med*. 2023;10(4):166–77.
49. Rimal R, Shrestha D, Pyakurel S, Poudel R, Shrestha P, Rai KR, Ghimire GR, Rai G, Rai SK. Diagnostic performance of GeneXpert MTB/RIF in detecting MTB in smear-negative presumptive TB patients. *BMC Infect Dis*. 2022;22(1):321.
50. Malik MI, Ejaz T, Ahmed J, Arshad K, Jamal Y, Zohfree Z. Diagnostic accuracy of GeneXpert assay and comparison with smear AFB on bronchial washings in sputum negative suspected pulmonary tuberculosis. *Pakistan Armed Forces Med J*. 2019;69(4):857–62.
51. Byanyima P, Kaswabuli S, Musisi E, Nabakiibi C, Zawedde J, Sanyu I, Sessolo A, Andama A, Worodria W, Huang L, Davis JL. Feasibility and sensitivity of saliva GeneXpert MTB/RIF Ultra for Tuberculosis diagnosis in adults in Uganda. *Microbiol Spectr*. 2022;10(5):e00860–22.
52. Elbrolosy AM, El Helbawy RH, Mansour OM, Latif RA. Diagnostic utility of GeneXpert MTB/RIF assay versus conventional methods for diagnosis of pulmonary and extra-pulmonary tuberculosis. *BMC Microbiol*. 2021;21:1–0.
53. Borham M, Oreiby A, El-Gedawy A, Hegazy Y, Khalifa HO, Al-Gaabary M, Matsumoto T. Review on bovine tuberculosis: an emerging disease associated with multidrug-resistant *Mycobacterium* species. *Pathogens*. 2022;11(7):715.
54. Prakash AK, Datta B, Tripathy JP, Kumar N, Chatterjee P, Jaiswal A. The clinical utility of cycle of threshold value of GeneXpert MTB/RIF (CBNAAT) and its diagnostic accuracy in pulmonary and extra-pulmonary samples at a tertiary care center in India. *Indian J Tuberculosis*. 2018;65(4):296–302.
55. Habous M, Elimam MA, Kumar R, Deesi ZA. Evaluation of GeneXpert *Mycobacterium tuberculosis*/Rifampin for the detection of *Mycobacterium tuberculosis* complex and rifampicin resistance in nonrespiratory clinical specimens. *Int J Mycobacteriology*. 2019;8(2):132–7.
56. Stevens WS, Scott L, Noble L, Gous N, Dheda K. Impact of the GeneXpert MTB/RIF technology on tuberculosis control. *Tuberculosis Tuber Bacillus*. 2017 Sep;1:389–410.
57. Lu J, Zheng H, Chu P, Han S, Yang H, Wang Z, Shi J, Yang Z. Direct detection from clinical sputum samples to differentiate live and dead *Mycobacterium tuberculosis*. *J Clin Lab Anal*. 2019;33(3):e22716.
58. Nikolayevskyy V, Miotto P, Pimkina E, Balabanova Y, Kontsevaya I, Ignatyeva O, Ambrosi A, Skenders G, Ambrozaitis A, Kovalyov A, Sadykhova A. Utility of propidium monoazide viability assay as a biomarker for a tuberculosis disease. *Tuberculosis*. 2015;95(2):179–85.
59. Alebouyeh S, Weinrick B, Achkar JM, García MJ, Prados-Rosales R. Feasibility of novel approaches to detect viable *Mycobacterium tuberculosis* within the spectrum of the tuberculosis disease. *Front Med*. 2022;9:965359.
60. Alvarez AH. Revisiting tuberculosis screening: an insight to complementary diagnosis and prospective molecular approaches for the recognition of the dormant TB infection in human and cattle hosts. *Microbiol Res*. 2021;252:126853.
61. Vilchèze C, Jacobs WR Jr. The isoniazid paradigm of killing, resistance, and persistence in *Mycobacterium tuberculosis*. *J Mol Biol*. 2019;431(18):3450–61.
62. Caño-Muñoz S, Anthony R, Niemann S, Alffenaar JW. New approaches and therapeutic options for *Mycobacterium tuberculosis* in a dormant state. *Clin Microbiol Rev*. 2018;31(1):10–128.
63. Nguyen TN, Anton-Le Berre V, Bañuls AL, Nguyen TV. Molecular diagnosis of drug-resistant tuberculosis; a literature review. *Front Microbiol*. 2019;10:794.
64. Palomino JC, Martin A. Challenges associated with diagnostics, drug resistance, and pathogenesis of *Mycobacterium tuberculosis*. *Human Emerging and re-emerging infections: viral and parasitic infections*. Dec. 2015;29:863–76.
65. Wu Z, Rueda ZV, Li T, Zhang Z, Jiang Y, Sha W, Yu F, Chen J, Pan Q, Shen X, Yuan ZA. Effect of the Xpert MTB/RIF on the detection of pulmonary tuberculosis cases and rifampicin resistance in Shanghai, China. *BMC Infect Dis*. 2020;20(1):1–0.
66. Fouda ME, Abdel Gwad ER, Fayed SM, Kamel MH, Ahmed SA. A study of the added value of Xpert MTB/RIF assay for assessment of pulmonary tuberculosis transmission risk. *Egypt J Med Microbiol*. 2019;28(3):141–8.
67. Rajwanshi A, Bhambhani S, Das DK. Fine-needle aspiration cytology diagnosis of tuberculosis. *Diagn Cytopathol*. 1987;3(1):13–6.
68. Lee CH, Lee MC, Lin HH, Shu CC, Wang JY, Lee LN, Chao KM. Pulmonary tuberculosis and delay in anti-tuberculous treatment are important risk factors for chronic obstructive pulmonary disease. *PLoS ONE*. 2012;7(5):e37978.
69. Gyimah FT, Dako-Gyeke P. Perspectives on TB patients' care and support: a qualitative study conducted in Accra Metropolis, Ghana. *Globalization Health*. 2019;15(1):1–9.
70. Salifu RS, Hlongwana KW. Barriers and facilitators to bidirectional screening of TB-DM in Ghana: healthcare workers' perspectives. *PLoS ONE*. 2020;15(7):e0235914.
71. Adepoku VA, Oladimeji O, Horsburgh CR. Rethinking public private mix (PPM) performance in the tuberculosis program: how is care seeking impacting this model in high TB burden countries?. *InHealthcare* 2022 Jul 12 (Vol. 10, No. 7, p. 1285). MDPI.
72. Boah M, Kpordoxah MR, Adokiya MN. Self-reported gender differentials in the knowledge of tuberculosis transmission and curative possibility using national representative data in Ghana. *PLoS ONE*. 2021;16(7):e0254499.
73. Horton KC, MacPherson P, Houben RM, White RG, Corbett EL. Sex differences in tuberculosis burden and notifications in low-and middle-income countries: a systematic review and meta-analysis. *PLoS Med*. 2016;13(9):e1002119.
74. Yang WT, Gounder CR, Akande T, De Neve JW, McIntire KN, Chandrasekhar A, de Lima Pereira A, Gummadi N, Samanta S, Gupta A. Barriers and delays in tuberculosis diagnosis and treatment services: does gender matter? *Tuberculosis research and treatment*. 2014;2014.
75. Dong Z, Wang QQ, Yu SC, Huang F, Liu JJ, Yao HY, Zhao YL. Age-period-cohort analysis of pulmonary tuberculosis reported incidence, China, 2006–2020. *Infect Dis Poverty*. 2022;11(04):62–71.
76. Barber MR, Drenkard C, Falasinnu T, Hoi A, Mak A, Kow NY, Svenungsson E, Peterson J, Clarke AE, Ramsey-Goldman R. Global epidemiology of systemic lupus erythematosus. *Nat Rev Rheumatol*. 2021;17(9):515–32.
77. Dowdy DW, Behr MA. Are we underestimating the annual risk of infection with *Mycobacterium tuberculosis* in high-burden settings? *Lancet Infect Dis*. 2022 May 5.
78. Asare KK. Buruli Ulcer: what are the future perspectives in dealing with the extensive necrotizing skin disease. *Global J Dermatology Venereol*. 2020;8:21–31.
79. Marais BJ, Lönnroth K, Lawn SD, Migliori GB, Mwaba P, Glaziou P, Bates M, Colagiuri R, Zijenah L, Swaminathan S, Memish ZA. Tuberculosis comorbidity with communicable and non-communicable diseases: integrating health services and control efforts. *Lancet Infect Dis*. 2013;13(5):436–48.
80. Rylance S, Masekela R, Banda NP, Mortimer K. Determinants of lung health across the life course in sub-saharan Africa. *Int J Tuberc Lung Dis*. 2020;24(9):892–901.

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.