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Population-Based Tuberculosis Disease Prevalence Survey in Ghana: The Role and Lessons Learnt from the Laboratory

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Abstract

Background: Bacteriologically-confirmed tuberculosis (TB) cases used in calculating TB prevalence in a country are obtained through laboratory examination of sputum specimens. **Objective:** This article describes laboratory processing of specimens, results overview, conclusions and key lessons learnt from the perspective of laboratory personnel involved in the conduct of TB disease prevalence survey in Ghana in 2013. **Methods:** Symptoms screening and Chest X-ray suggestive of TB were used to select participants who produced sputum to confirm TB cases using microscopy, culture and Xpert® MTB/RIF assay (GeneXpert). **Results:** A total of 15,935 single and paired sputum specimens were received from eligible participants. About half of Ziehl-Nielsen (129/263) and Auramine O (122/246) stained smear positives were scanty positive. Culture positivity rate for *Mycobacterium tuberculosis* complex was 266/14,994 (1.7%) and 100/15,179 (0.7%) in Mycobacterial Growth Indicator Tube (MGIT) and Lowenstein-Jensen (LJ) media respectively; while non-tuberculous mycobacterium was 294/14,994 (1.96%) and 167/15,179 (1.1%). Total contamination rates in MGIT (5.4%) were higher than in LJ (1.7%). Prevalence of smear positive TB and bacteriologically confirmed TB among adult population (≥15 years) was estimated at 111 (95% CI: 76 - 145) and 356 (95% CI: 288 - 425) per 100,000 population respectively. **Conclusions and Lessons Learnt:** Direct supervision of specimen collection by well-trained laboratory personnel, timely transportation of specimens from field to laboratory, prompt specimen processing and use of electronic data management systems are essential for a reliable TB disease prevalence

survey data. More importantly, strengthening human and logistical capacity of the laboratory must be of utmost priority.

Keywords

Prevalence Survey, Tuberculosis Laboratory, Lessons Learnt, Ghana

1. Introduction

Despite being declared a global health emergency in 1993 by the World Health Organization (WHO), tuberculosis (TB) remains a disease of public health importance globally infecting about one third of the world's population with about two million deaths occurring annually [1]. In Ghana, an estimated 14,668 (Pulmonary, bacteriologically confirmed—7682; Pulmonary, clinically diagnosed—5364; Extra-pulmonary—1181; Relapsed—441) cases were reported in 2014 [2]. While trend analysis of programmatic data provides useful information to indirectly assess progress, there are limitations. Firstly, we are unable to entirely rely on the routine surveillance system owing to its coverage, completeness and accuracy. Secondly, the current WHO estimates may be unrealistic; since the last well conducted prevalence survey based on TB disease in Ghana was in 1957. In the year 2000, TB burden measurement was done, but was based on TB infection, which is less reliable and not very accurate. As of 2012, TB case detection rate in Ghana was very low at 33% with estimated prevalence of more than 70 per 100,000 population [3]. With these aforementioned reasons, it was imperative for a prevalence survey to be conducted. Therefore, in 2013, a population-based TB disease prevalence survey was conducted with a general objective to obtain a direct measurement of the absolute burden of disease caused by TB in Ghana. A combination of symptom screening using standardized questionnaire and chest X-ray was used in order to selectively target the survey participants who provided sputum for bacteriological tests. We report here the general overview of the prevalence survey process with emphasis on the laboratory activities including sample collection and processing, data obtained, analysis and interpretation, as well as lessons learnt.

2. Methods

2.1. Study Design

The study was cross sectional and population based carried out on a random sample of the general adult population (15 years and above) in Ghana, in which the number of people with TB disease was measured. There were 98 survey clusters in two strata (urban—53, rural—45).

2.2. Sample Size

The overall estimated sample size was 63,905 with an estimated cluster size of

650 people.

The parameters used to calculate the sample size and underlying assumptions are described as follows:

Smear-positive prevalence for ≥15 years (270 per 100,000 population);

Precision (0.2);

Design effect (1.44);

k^2 (0.5); where k is the coefficient of between-cluster variation

Response rate (85%).

2.3. Selection of Eligible Participants

All inhabitants including visitors in a cluster were enumerated by households prior to the survey. After enumeration, the survey team selected eligible participants from each household based on study inclusion criteria and provided invitation cards to participate in the survey.

2.3.1. Inclusion Criteria

All inhabitants of selected clusters who were 15 years old and above; residents and visitors who have lived in the household for the most of the last two (2) weeks preceding survey census (which means at least 7 days or more in the last 14 days); permanent residents who have lived in the household few days (less than 7 days) in the last 2 weeks before the census (i.e. residents who were away for some time, but that just came back home a couple of days before the census).

2.3.2. Exclusion Criteria

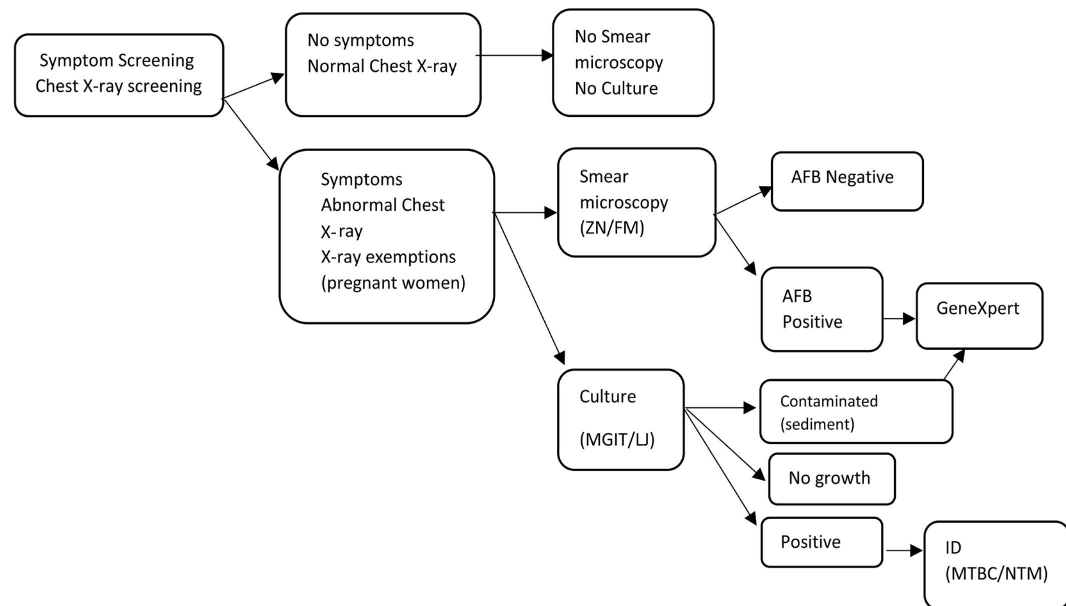
Eligible residents who are incapable of consenting (mentally incapacitated, people between 15 - 17 years who cannot secure consent etc.); visiting residents who have lived in the enumeration area for less than 7 days in the last 2 weeks prior to census day; permanent resident who have not lived in the household in the last 2 weeks before the census (i.e. people who travelled since more than 2 weeks before the census); hotel guests; prisoners; students in hostels; residents of student dormitories in boarding school premises; residents of seminaries, sisters convents and monastries; diplomatic compounds; military facilities; street dwellers (with no fixed address).

2.4. Screening Strategy

Symptom screening and/or chest X-ray and/or chest X-ray exemption were used to identify individuals eligible to produce sputum for bacteriological examinations as recommended by WHO [4]. The overall algorithm for screening eligible participants and specimen processing is shown in **Figure 1**.

2.5. Laboratory Technical Staff and Sample Processing Sites

Two groups of well-trained laboratory technicians and biomedical scientists were fully engaged for the study. The first group consisted of one technician attached to each of the four field survey teams to supervise the collection of sputum



AFB: Acid Fast Bacilli; FM: Fluorescence Microscopy; GeneXpert: Xpert® MTB/RIF assay; ID: Identification; LJ: Lowenstein-Jensen; MGIT: Mycobacterial Growth Indicator Tube; MTBC: *Mycobacterium tuberculosis* complex; NTM: Non-tuberculous mycobacteria; ZN: Ziehl-Nielsen.

Figure 1. Algorithm for participant screening and specimen processing.

from eligible participants. The other group was based at two laboratories—a biosafety level 3 (BSL3) TB laboratory of Noguchi Memorial Institute for Medical Research (NMIMR) and the Chest Clinic laboratory of the Korle-Bu Teaching Hospital (KBTH) to examine the sputum specimens by microscopy, culture and Xpert® MTB/RIF assay (GeneXpert) (Cepheid, Sunnyvale, CA, USA). NMIMR is a biomedical research facility at the University of Ghana that conducts research, mainly into infectious diseases. The Chest Clinic laboratory is a specialist diagnostic centre at KBTH that receives and process clinical specimens from suspected cases of TB and other chest diseases. These laboratories serve as national reference laboratories for TB in Ghana and subscribe to proficiency testing scheme for TB microscopy and culture from the World Health Organization/National Institute for Communicable Diseases (WHO/NICD) in South Africa and WHO Supranational TB reference Laboratory in Borstel, Germany respectively.

2.6. Bacteriological Investigations

2.6.1. Sputum Sample Collection and Processing

Two sputum specimen (spot and morning) were collected from each eligible participant according to symptoms screening or abnormal chest radiograph, and from all pregnant women who were exempted from chest X-ray. All the sputum specimens collected from participants in a particular survey cluster were transported in cold chain within 24 - 48 hours to NMIMR and KBTH Chest Clinic laboratories for concentrated Ziehl Nielsen (ZN) and Auramine O (AO) stained smear microscopy, Mycobacterial Growth Indicator Tube (MGIT) and Lowenstein-Jensen (LJ) culture as well as GeneXpert. All specimens were processed

within one week from the date of collection in a Biosafety cabinet class II.

2.6.2. Sputum Digestion and Decontamination

Sputum specimen were first processed using Mycoprep™ (BD Diagnostic System, Sparks, MD, USA) which is a commercially available Sodium hydroxide-N-acetyl L-cysteine (NaOH-NALC) formulation for sputum digestion and decontamination. This initial process was done according to manufacturer's instructions. In brief, equal volumes of specimen (3 - 5 ml) and NaOH-NALC solution were mixed together and the preparation allowed to stand for 15 minutes at room temperature (25˚C). Next, about 40 - 45 ml of phosphate buffer saline (PBS) solution with pH 6.8 was added to the decontaminated specimen and allowed to stand for 20 minutes to neutralize the reaction. Further, the whole preparation was centrifuged at 3000×g for 15 minutes to concentrate the specimen and also wash the NaOH solution. Then the supernatant was discarded to obtain sediment. Finally, a small volume (2 ml) of PBS (pH 6.8) was added to the sediment to make the inoculum for smears and cultures.

2.6.3. Smear Preparation and Microscopy

Two smears were prepared from each inoculum and stained for microscopy using ZN and Auramine O staining methods. The smears were examined after staining for the presence or absence of acid fast bacilli (AFB). The ZN stained smear was examined using Olympus™ light microscope (Olympus Corporation, Shinjuku, Tokyo, Japan) with oil immersion at 1000× and the Auramine O stained smear examined with Primo Star iLED™ microscope (Carl Zeiss Microimaging, Oberkochen, Germany), without oil immersion at 400×. Smears were graded using the World Health Organization and the International Union against Tuberculosis and Lung Diseases (WHO/IUALTD) standards. All smear positive specimens and contaminated culture (where sediments were available) were run on GeneXpert for *Mycobacterium tuberculosis* confirmation as well as rifampicin resistance.

2.6.4. Culture and Identification of Mycobacterial Species

Two tubes each of commercially available MGIT medium and LJ slants (BD Diagnostic System, Sparks, MD, USA) were inoculated with 0.5 ml of decontaminated sputum specimen per tube and incubated at 37˚C (maximum of 8 weeks) and BACTEC MGIT 960 system (6 weeks) respectively. Growth of mycobacteria and other bacteria in the MGIT was indicated by increasing fluorescence. Once a tube flagged positive, smear was prepared, stained by ZN method and examined microscopically for presence or absence of AFBs. In addition, a portion of the positive culture was streaked onto blood agar plates. Growth on the plates indicates contamination. LJ growth was indicated by visible colonies on the slants.

All pure mycobacterial isolates obtained from both MGIT and LJ culture were identified broadly as *Mycobacterium tuberculosis* complex (MTBC) and Non-tuberculosis mycobacterium (NTM) using BD MGIT TBc ID test kit (BD Diag-

nostic System, Sparks, MD, USA). The test was performed according to manufacturer's instruction. Briefly, each TBc ID test kit was inoculated with 100 µl of a positive MGIT culture or in the case of LJ culture, one loopful of colonies suspended in 200 µl of extraction buffer (phosphate buffer with 0.05% Tween 20 and 0.02% sodium azide) and 100 µl of the suspension used in the assay. The results were interpreted 15 min after application of the sample. A positive result was indicated by the development of two pink bands, one in the control zone (C) and another in the test zone (T).

2.6.5. Laboratory Case Definitions

Culture-confirmed TB case: isolation of MTBC from a sputum specimen grown on either LJ, MGIT or both.

Culture confirmed non-TB case: isolation of NTM from a sputum specimen grown on either LJ, MGIT or both.

Sputum smear-positive TB case: acid-fast bacilli (AFB)-positive by ZN or Auramine-O stained sputum smear examination, i.e. at least one AFB in 100 immersion fields.

2.6.6. Quality Assurance

Standard procedures were strictly followed for all laboratory investigations. New batches of all stains used were tested with known positive and negative control slides. Reference TB strains ($H_{37}Rv$) were inoculated with each new batch of MGIT tubes and LJ slants to assess the medium quality. A senior laboratory technical officer at KBTH Chest Clinic re-checked all positive slides and ten percent of negative slides. An external senior microbiologist from the Kumasi Centre for Collaborative Research (KCCR), Ghana rechecked ten percent of all examined slides available as at the end of the first quarter of the survey period. Laboratory and data analysis experts from WHO visited KBTH and NMIMR TB laboratories on three occasions for monitoring and evaluation.

2.7. Data Management and Analysis

All data collected from the field and laboratory were transferred electronically to a central server hosted at the secretariat of the National Tuberculosis Programme (NTP). Data analysis was performed using Stata (v12, Stata Corporation, USA) and SPSS (v20, IBM Corporation, USA).

2.8. Ethical Consideration

The study obtained ethical approval from the Institutional Review Board of Noguchi Memorial Institute for Medical Research [NMIMR-IRB CPN: 031/10-11 *amend* 2012].

3. Results

Among 67,757 eligible adults invited, 61,726 (91.1%) made up of 24,688 males and 37,038 females participated in the study. Majority of the participants,

33,122/61,626 (54%) were urban residents. The major reported occupation of participants was farming (29%) followed by trading (24%). Of the total participants, 48 (0.1%) reported that they were receiving TB treatment at the time of the survey while 331 (0.5%) said they had been treated for TB at least once in their life (Table 1). After symptom screening and/or chest X-ray and/or chest

Table 1. Socio-demographic and clinical characteristics of study participants (N = 61,726).

Characteristic	N	%
Sex		
Male	24,668	40
Female	37,038	60
Age group (years)		
15 - 24	17,089	27.7
25 - 34	13,584	22
35 - 44	11,057	18
45 - 54	8689	14
55 - 64	5442	8.8
≥65	5868	9.5
Place of residence		
Urban	33,122	53.7
Rural	28,604	46.3
Occupation		
Farmer	17,760	28.8
Trader	14,831	24
Student	10,448	16.9
Artisan	6450	10.5
Civil/Public servant	4189	6.8
Unemployed	3262	5.3
Fisherman	3216	5.2
Pensioner	1189	1.9
Miner	375	0.6
Other[a]	6	0
Current anti-TB treatment[b]		
Yes	48	0.1
No	61678	99.9
Previously treated for TB[c]		
Yes	331	0.5
No	61395	99.5

[a]Any other occupation not stated above as used in the study questionnaire. [b]Number of participants who reported that they were receiving TB treatment at the time of the survey. [c]Number of participants who reported that they had been treated for TB at least once in their life.

X-ray exemption, a total of 8298 participants were eligible for sputum examination. Out of this number, 8126 (98%) submitted at least one specimen (either spot, morning or both). About half of the smear positives were of the scanty positive grade (Table 2). Of the 126 spot ZN positive and 137 morning ZN positive, 36/126 (28.6%) and 45/137 (32.8%) as well as 48/126 (38.1%) and 52/137 (38.0%) were also positive for MGIT and GeneXpert respectively. Overall, 266/14,994 (1.7%) and 100/15,179 (0.65%) specimens were MGIT and LJ culture positive for MTBC respectively. On the other hand, 294/14,994 (1.96%) and 167/15,179 (1.1%) NTM were isolated from MGIT and LJ cultures respectively. Contamination rates for specimen per tube were: [MGIT Spot—993/7282 (13.6%); Morning—1307/7712 (16.9%)] and LJ [Spot—423/7289 (5.8%); Morning—654/7720 (8.5%)]. The rate of total contamination (both spot and morning tubes contaminated) was 417/7706 (5.4%) and 134/7706 (1.7%) in MGIT and LJ culture respectively (Table 3). A total of 1034 specimens (both single and paired)

Table 2. Acid-fast Bacilli (AFB) detection using Ziehl-Nielsen (ZN) and Auramine O (AO) staining methods.

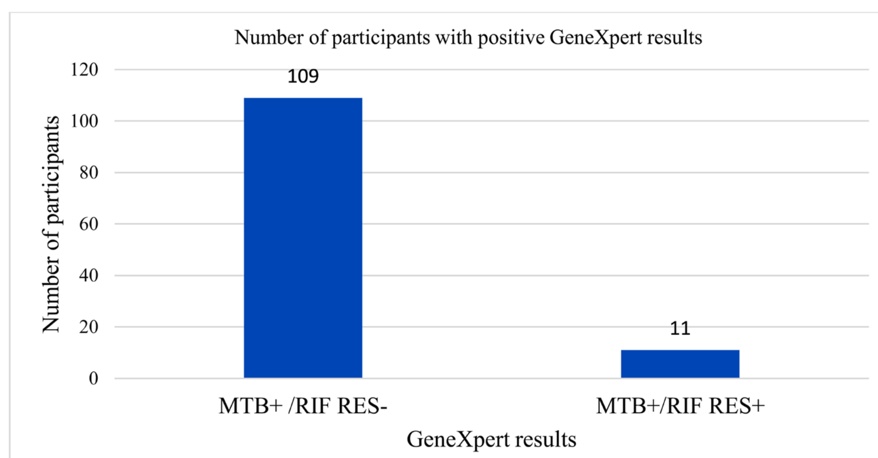
Sputum Grade[a]	Sputum type/staining method			
	Spot ZN	Morning ZN	Spot AO	Morning AO
Negative	8033	7591	8037	7603
Scanty	63	66	68	63
1+	24	21	16	17
2+	20	17	12	10
3+	19	33	26	34

[a]World Health organization and the International Union against Tuberculosis and Lung Diseases (WHO/IUALTD) standards. Bright field microscopy-ZN stained (1000×, 1 length = 100 High Power Field {HPF}). Negative = No AFBs/1 length; Scanty = 1 - 9 AFB/1 length; 1+ = 10 - 99 AFB/1 length; 2+ = 1 - 10 AFB/1 HPF on average; 3+ ≥ 10 AFB/1 HPF on average. iLED Fluorescence microscopy-AO stained (400×, 1 length = 40 fields = 200 HPF). Negative = No AFBs/1 length; Scanty = 1 - 19 AFB/1 length; 1+ = 20 - 199 AFB/1 length; 2+ = 5 - 50 AFB/1 field on average; 3+ ≥ 50 AFB/1 field on average. AO: Auramine O; ZN: Ziehl-Nielsen.

Table 3. Growth outcomes from specimen types and culture methods.

	MGIT		LJ	
	Spot[a], N (%)	Morning[b], N (%)	Spot[a], N (%)	Morning[b], N (%)
MTBC	117 (1.6)	149 (1.9)	47 (0.6)	53 (0.7)
NTM	294 (4.1)	312 (4.1)	80 (1.1)	87 (1.1)
Negative[c]	5878 (80.7)	5944 (76.7)	6868 (94.2)	6966 (90.2)
Contaminated[d]	993 (13.6)	1307 (16.9)	424 (5.7)	654 (8.4)
Totally Contaminated[e]	417 (5.4)		134 (1.7)	

[a]Specimen taken on the spot; [b]Specimen taken early morning; [c]No growth after eight weeks of incubation; [d]Tubes that have either spot only or morning only contaminated; [e]Tubes that have both spot and morning contaminated; LJ: Lowenstein-Jensen; MGIT: Mycobacterial Growth Indicator Tube; MTBC: *Mycobacterium tuberculosis* complex; NTM: Non-tuberculous mycobacteria.



MTB+/RIF RES− = *M. tuberculosis* detected; Rifampicin resistance not detected. MTB+/RIF RES+ = *M. tuberculosis* detected; Rifampicin resistance detected.

Figure 2. Patterns of GeneXpert results.

were run on GeneXpert. *Mycobacterium tuberculosis* was detected in at least one sputum specimen of 109 participants. Eleven out of the 109 participants had their specimens being rifampicin resistant (**Figure 2**). Prevalence of smear positive TB and bacteriologically confirmed TB among adult population (≥15 years) was estimated at 111 (95% CI: 76,145) and 356 (95% CI: 288,425) per 100,000 population respectively.

4. Discussion and Lessons Learnt

The overall goal of the prevalence survey was to gain better understanding of the burden of TB disease and identify ways by which TB control can be improved. The laboratory played a very important role in the successful conduct and outcome of the survey. Sample collection and processing were all performed by the laboratory which generated data for calculating the prevalence of TB disease. The proportion of survey participants eligible for sputum collection was higher (13.4%) than what was reported in other African countries such as Ethiopia (13%) [5] and Nigeria (10.6%) [6], but lower than in Zambia (14.6%) [7] and Gambia (13.8%) [8]. The high sputum collection rate (98% for at least one specimen and 93% for both spot and morning specimen) was comparable to results from surveys in other countries [5] [7] [8]. In the case of Ghana, we can attribute this finding to factors such as effective community sensitization prior to commencement of the survey, professional skill of the survey team, supervision by field laboratory staff whereby in some instances, follow-up to residences of participants for specimen collection and the provision of digital chest X-ray examination. In addition, those who returned the early morning sputum sample were also given a vitamin fortified cereal.

Smear results from majority of the specimens were negative which was much expected in a TB prevalence survey where in addition to the gold standard muco-purulent sputum, salivary specimens are accepted [9]. It was also observed

that smear positivity were of the lower grade (scanty) and this could be due to the paucibacillary load of specimens received. Thus, the quality and bacillary load of sputum specimens collected as well as method used can determine AFB detection rate [10]. The high number of smear negative-culture positive specimens from this survey confirms the general trends observed in surveys from other countries [5] [6] [7] [8]. We could safely assumed that these culture positive cases would have been missed if microscopy was the only tool used for detecting TB in the survey. Both spot and morning specimens for MGIT and LJ culture as used in this survey were similar to what was used in Zambia, Cambodia and Lao [8] [11] [12], although both specimens were used for only one culture method in these instances. On the contrary, the surveys in Pakistan [13] and Vietnam [14] used one specimen one culture method. MGIT culture positivity (isolation of both MTBC and NTM) was higher than LJ. This result is expected results due to the high recovery rate and short detection time of the MGIT system and this was consistent with reports from other studies [15] [16] [17]. Due to the extremely low mycobacterial growth on the LJ medium, only MGIT culture positive as well as ZN stained positive results are considered in the definition of a case of TB and hence in the calculation of the TB prevalence rate in this study. The relatively high recovery rate of NTM underscores the need for culture and subsequent identification of mycobacterial species since microscopy alone cannot distinguish between MTBC and NTM. A significant feature of this survey which was also used during the Zambia survey was the use of GeneXpert to confirm all smear positive cases and contaminated culture provided their decontaminated sediments were available. For instance, *Mycobacterium tuberculosis* was detected in 14 (26.9%) out of 52 available sediments whose cultures were totally contaminated. Of the 14 GeneXpert positive sediments, one was rifampicin resistant. These cases among others would have gone away undetected and might pose a threat to public health. High contamination rates (20% - 30%) were observed during the initial stages of the survey period due to some operational challenges. The most common types of contamination were fungal and non-mycobacterial growth leading to liquefying or discolouration of inoculated LJ media and homogenous turbidity of MGIT culture. As expected, contamination rate of the MGIT were higher than LJ cultures. Also, majority of the contaminated tubes of both media were those inoculated with the morning specimen. This may be due to the fact that the participants were not directly supervised by the field staff during the production of the morning specimens which could result in contamination with food debris in the mouth unlike the spot one whereby cleaned water was used to rinse the mouth of participants before sputum production. However, due to mitigating measures put in place by laboratory staff with support from the survey coordinating team, the contamination rates declined steadily (13% - 17% for MGIT and 5% - 9% for LJ) till the end of the survey period. These rates were still higher than the general recommendation of 5% for all media types although up to 7% - 8% may be accepted for liquid media [18].

5. Strengths and Limitations

We highlight here some of the strengths of the laboratory that contributed to the success of the survey. Firstly, the use of two specimens (both spot and morning) as well as two methods for both microscopy and culture accounted for the availability of over 90% of results. Next, specimens were readily processed anytime they arrived from the field due to a 24-hour shift system the laboratory staff run. Thus, except in very rare cases there were little delays for inoculation from time of sputum collection (i.e. within 5 days). Furthermore, the use of fully electronic data collection and entry barcode systems ensured minimal human error. By this, some health facilities have shifted from the fully manual system of data collection and management to semi-automated systems. Generally, through the survey, the capacity to conduct laboratory examinations such as microscopy (ZN/AO), culture (MGIT/LJ) and GeneXpert have been enhanced. Despite these strengths, some challenges were also encountered. Key among these were the relatively high contamination rates of specimens in the initial stages of the survey which could have underestimated the TB prevalence. Furthermore, due to the high numbers and frequency of specimens received in the laboratory, there could be some possible cases of cross-contamination. However, during the analysis these challenges were treated by imputation analysis. Another notable challenge was the sudden breakdown of the biosafety cabinet in which the samples were being processed at one of the two laboratories. This necessitated temporary suspension of laboratory work for maintenance, and thus delayed inoculation of some of the collected specimens around that period.

6. Implication for TB Control by NTP

The experiences gained by the laboratory staff have contributed immensely to the improvement of some activities of the NTP. Among these are installation of GeneXpert and hands on training at some regional and district hospitals by some laboratory staff who were involved in the prevalence survey.

7. Conclusion and Recommendation

In this study, most of the specimens were smear negative but culture positive. Also, significant number of smear positive specimens was found to be NTM which indicates that relying on smear microscopy alone may lead to misdiagnosis and unnecessary treatment leading to development of drug resistance. Based on these findings and from the laboratory staff point of view we recommend that a wide roll out of novel technologies such as GeneXpert be implemented nationwide. This may increase case detection and minimize false-positive smear and false-negative culture results. Finally, the capacity and skills of the laboratory staff must be enhanced through regular in-service training and other refresher courses.

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Competing Interest

The authors declare that they have no financial or personal relationship(s) that may have inappropriately influenced them in writing this article.

Authors' Contributions

KKA and FAB came out with the study design. KKA supervised the laboratory work and contributed to the writing and editing of the manuscript. SOA, CB, EM, SE, PD, MAO, SK, HG, SKA performed the laboratory work. SOA contributed to the manuscript writing and data analysis. FAB contributed to the study implementation by providing reagents and equipment. All authors read and approved the final manuscript before submission.

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Disclaimer

The views expressed in the submitted article are that of the authors and not an official position of their respective institution or funders.

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List of Abbreviations

AFB: Acid Fast Bacilli
AO: Auramine O
BSL3: Biosafety Level 3
FM: Fluorescence Microscopy
GeneXpert: Xpert® MTB/RIF assay
HPF: High Power Field
ID: Identification
KBTH: Korle-Bu Teaching Hospital
KCCR: Kumasi Centre for Collaborative Research
LJ: Lowenstein-Jensen
MGIT: Mycobacterial Growth Indicator Tube
MTBC: *Mycobacterium tuberculosis* complex
NaOH-NALC: Sodium hydroxide-N-acetyl L-cysteine
NMIMR: Noguchi Memorial Institute for Medical Research
NTP: National Tuberculosis Programme
NTM: Non-tuberculous mycobacteria
PBS: Phosphate buffer saline
WHO: World Health Organization
WHO/IUATLD: World Health organization/International Union against Tuberculosis and Lung Diseases
WHO/NICD: World Health Organization/National Institute for Communicable Diseases
ZN: Ziehl-Nielsen