

Association Between Delayed Sputum Specimen Receipt and *Mycobacterium tuberculosis* Complex Culture Recovery at the National Tuberculosis Reference Laboratory, Zambia: A Retrospective Laboratory-Based Study, 2025

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Abstract

Background

Mycobacterial culture remains an important method for recovery of viable *Mycobacterium tuberculosis* complex (MTBC), particularly for confirmation of tuberculosis and drug susceptibility testing. The quality of culture results may be affected by delays between sputum collection and receipt at the culture laboratory. In Zambia, sputum specimens referred for culture are expected to reach the National Tuberculosis Reference Laboratory (NTRL) within seven days of collection. However, the relationship between specimen receipt delay and MTBC culture recovery has not been systematically assessed using routine laboratory data. This study assessed the association between collection-to-laboratory receipt delay and culture outcomes among sputum specimens received at the NTRL.

Methods

A retrospective laboratory-based observational study was conducted using Laboratory Information System (LIS) data from the NTRL for specimens received from January to December 2025. Patient and specimen characteristics, collection and receipt dates, accessioning dates, and culture outcomes were analysed. Primary culture-outcome analyses were restricted to baseline diagnostic specimens. Specimens received within seven days of collection were classified as on-time, while those received after seven days were classified as delayed. Contaminated cultures were retained as a separate outcome and excluded from the binary MTBC recovery analysis. Logistic regression was used to estimate crude and adjusted odds ratios (ORs) with 95% confidence intervals (CIs) for MTBC recovery. The adjusted model included receipt delay, age group, sex, and HIV status, with robust standard errors clustered at the patient level to account for potential correlation among specimens contributed by the same patient. Receipt-to-accessioning intervals were compared across receipt-delay categories using the Kruskal–Wallis test.

Results

A total of 1,150 specimens from 712 patients were included. The median patient age was 40 years (IQR: 30–49), and 512 (71.91%) patients were male. Overall, 182 (15.83%) specimens yielded MTBC, 159 (13.83%) yielded non-tuberculous mycobacteria, 697 (60.61%) were negative, and 112 (9.74%) were contaminated. The median collection-to-receipt interval was 4 days (IQR: 2–6), and 999 (86.87%) specimens were received within seven days. Among 573 baseline specimens, 513 (89.53%) were received within seven days and 60 (10.47%) after seven days. After excluding contaminated specimens, MTBC was recovered from 114/457 (24.95%) specimens received within seven days and 8/51 (15.69%) received after seven days. Delayed receipt was associated with lower odds of MTBC recovery in both crude (OR 0.56, 95% CI: 0.26–1.23) and adjusted analyses (aOR 0.51, 95% CI: 0.20–1.26), but neither association was statistically significant. In the adjusted model, MTBC recovery was significantly

associated with age group and HIV status, while sex was not statistically significantly associated with recovery. The median receipt-to-accessioning interval was 3 days (IQR: 1–5), with no significant difference across receipt-delay categories ($P = 0.695$).

Conclusion

Most sputum specimens reached the NTRL within the recommended seven-day interval. Although MTBC recovery was lower among specimens received after seven days, delayed receipt was not statistically significantly associated with MTBC recovery after adjustment for age, sex, and HIV status and accounting for within-patient correlation. Age and HIV status were associated with MTBC culture recovery, while sex was not statistically significantly associated with recovery. Continued strengthening of timely specimen referral and transportation is warranted, alongside further studies incorporating transport conditions, storage temperature, specimen quality, and other pre-analytical factors.

1 Introduction

Tuberculosis (TB) remains a major public health problem, with laboratory diagnosis playing a central role in the identification and management of affected individuals [1]. Although rapid molecular tests have substantially improved the speed of TB diagnosis, TB remains a major global health burden, affecting more than 10.7 million people and causing approximately 1.23 million deaths in 2024 [2]. Globally, TB remains the world's leading cause of death from a single infectious agent and is among the top 10 causes of death [2]. Despite improvements in TB diagnostic services, many people with TB remain undiagnosed or are diagnosed after substantial delays [2–4].

Mycobacterial culture remains an important method for TB diagnosis because it enables recovery of viable bacilli and provides material for further testing, including drug susceptibility testing (DST) and confirmation of drug-resistant TB [4–6]. Culture is particularly important for the laboratory investigation of rifampicin-resistant TB, where confirmation and additional drug resistance testing are required to guide treatment [7]. However, successful culture recovery depends not only on laboratory procedures but also on the quality and condition of specimens received by the culture laboratory [8, 9]. Sputum is a biological specimen in which delays between collection and laboratory processing may affect the viability of mycobacteria and, consequently, the likelihood of successful culture recovery [10]. Prolonged transportation and delays before specimens reach the culture laboratory may therefore contribute to reduced recovery of viable MTBC or increased culture contamination [11].

In Zambia, Tuberculosis culture and DST are provided through specialized TB culture laboratories within the national TB diagnostic network [12]. According to the Zambia National TB and Leprosy Programme (NTLP) Consolidated TB Guidelines, specimens from patients with rifampicin resistance detected by Xpert® MTB/RIF are referred to designated culture laboratories for confirmatory testing and drug susceptibility testing [13, 14]. The NTRL, based in Lusaka, is responsible for TB culture testing specifically for Central, Southern, Eastern, Northern and Muchinga provinces. It also supervises the entire

TB laboratory network in the country [12]. Other provinces are supported by the University Teaching Hospital (UTH) TB Laboratory in Lusaka and the National Health Research and Training Institute (NHRTI) in the Copperbelt. These laboratories have capacity for mycobacterial culture and first- and second-line phenotypic and genotypic drug susceptibility testing [12]. In addition to specimens from patients with rifampicin resistance detected by Xpert® MTB/RIF; specimens from patients with relapse, treatment failure, loss to follow-up, and other patients considered appropriate for culture testing may also be referred for culture and DST [13, 15, 16].

Timely transportation of sputum specimens is an important component of maintaining specimen quality before culture processing [17]. At the NTRL, an operational requirement specifies that sputum specimens intended for culture should be received at the laboratory within seven (7) days of collection (CDL-TB-M-003). However, the extent to which specimens exceed this recommended time frame and whether delayed receipt is associated with MTBC culture recovery or contamination has not been systematically assessed using routine laboratory data in Zambia.

Therefore, this study retrospectively assessed the relationship between the interval from sputum specimen collection to receipt at the NTRL and subsequent mycobacterial culture outcomes among specimens received between January and December 2025. Specifically, the study assessed MTBC culture recovery according to receipt within or beyond seven days, examined MTBC recovery across increasing receipt-delay categories, described the distribution of MTBC, Non-Tuberculous Mycobacteria (NTM), negative culture, and contaminated culture outcomes across these categories, and assessed the relationship between collection-to-receipt delay and the interval from laboratory receipt to accessioning.

2 Methods

2.1 Study design

This was a retrospective laboratory-based observational study using routinely collected Laboratory Information System (LIS) data from the NTRL, Zambia. The study assessed the association between the interval from sputum specimen collection to receipt at the NTRL and subsequent MTBC culture recovery during the period January to December 2025. The primary unit of analysis was the individual sputum specimen. The NTRL recommends the submission of two sputum specimens per patient for mycobacterial testing, with the second specimen providing an alternative specimen when the first is inadequate or otherwise unsuitable for testing. Consequently, some patients contributed two sputum specimens to the dataset, whereas others contributed only one specimen. Patient-level characteristics were therefore summarized using the unique patient identification number, while analyses of specimen receipt and culture outcomes were conducted at the specimen level. Where multiple specimens were linked to the same patient, each specimen was treated as a distinct laboratory record for specimen-level analyses.

For analyses of MTBC culture recovery and other culture outcomes, the dataset was restricted to baseline diagnostic specimens. Baseline specimens were defined as records designated in the LIS as “Diagnosis (0 months),” representing specimens collected for initial diagnostic evaluation. Records designated as follow-up, relapse, treatment-related follow-up, “DR contact,” loss to follow-up, or records containing follow-up month designations were classified as follow-up or other specimens and were excluded from the primary culture-outcome analyses. This restriction was applied to maintain a clinically comparable study population and to minimize potential misclassification arising from specimens collected after initiation of tuberculosis treatment, when the likelihood of recovering viable *M. tuberculosis* organisms may differ from that at initial diagnostic evaluation.

2.2 Study setting

The study was conducted at the NTRL in Lusaka, Zambia. The NTRL is a national-level tuberculosis reference laboratory that receives and processes specimens referred from health facilities and diagnostic laboratories across Zambia for mycobacterial culture, drug susceptibility testing, and other specialized tuberculosis diagnostic investigations. Sputum specimens submitted for mycobacterial culture are received at the NTRL, where they undergo specimen acceptance and quality assessment before laboratory processing and culture. Information relating to specimen collection, receipt, accessioning, processing, and culture outcomes is routinely recorded in the LIS. These routinely captured data provided the basis for assessing the interval between sputum collection and receipt at the NTRL and its association with subsequent culture recovery.

According to the NTRL laboratory operational requirements outlined in the Client Handbook (CDL-TB-M-003), sputum specimens intended for mycobacterial culture should be received at the laboratory within seven days of specimen collection. The seven-day period was therefore considered the recommended maximum interval for specimen referral and receipt. Specimens received after this period were considered to have exceeded the recommended receipt interval and were used to characterize delayed specimen receipt in the study. Upon receipt at the NTRL, sputum specimens undergo routine acceptance and quality checks in accordance with laboratory procedures. These include assessment of specimen volume, container integrity, evidence of leakage, and transport and storage conditions. Specimens are expected to meet established requirements for processing, including provision of an adequate specimen volume (2 mL and above) and appropriate transport and storage conditions. The temperature of specimens on arrival is documented on the individual laboratory request forms. The recommended temperature range for transport and storage of sputum specimens prior to processing is 2–8°C, and specimens requiring storage before processing are maintained under appropriate refrigerated conditions in accordance with laboratory procedures.

2.3 Study period

The study included laboratory records generated from 1 January 2025 to 31 December 2025.

2.4 Study population

The study population comprised patients and sputum specimens recorded in the NTRL LIS from January to December 2025 and submitted for mycobacterial culture. A total of 1,150 specimen records with complete information on the study variables (province, HIV status, category, health facility, age, sex, collection date, accessioning date, receipt date, and culture result) were available, representing 712 unique patients. Of these, 573 (49.8%) were baseline specimens and 577 (50.2%) were follow-up or other specimens. The 573 baseline specimens constituted the analytical population for assessing the association between specimen receipt delay and culture outcomes, including MTBC recovery, NTM isolation, negative culture, and contamination. Follow-up or other specimens were excluded from these analyses.

2.5 Data source and variables

Data were retrospectively extracted from the NTRL LIS. The primary exposure was the collection-to-receipt interval, calculated in days from specimen collection to receipt at the NTRL. Specimens received within ≤ 7 days were classified as on-time, while those received after > 7 days were classified as delayed. For assessment of increasing delay, the interval was categorized as ≤ 7 , 8–14, 15–21, and > 21 days and was also retained as a continuous variable. The receipt-to-accession interval was calculated as the number of days between specimen receipt and accessioning at the NTRL. This variable was assessed separately to characterize processing time after laboratory receipt and its relationship with collection-to-receipt delay. The primary outcome was MTBC culture recovery, defined as an LIS result of “MTBC isolated.” Secondary outcomes were NTM isolation, negative culture, and culture contamination. All four outcomes were retained separately in the analysis. Patient-level variables included age, sex, and HIV status. Age was categorized as < 15 , 15–24, 25–34, 35–44, 45–54, and ≥ 55 years. HIV status was classified as HIV-positive, HIV-negative, or unknown. Province of origin was assessed at the specimen level. The dataset included specimens from Central, Copperbelt, Eastern, Lusaka, Muchinga, Northern, North-Western, Southern, and Western provinces.

2.6 Data management and cleaning

The LIS dataset was reviewed for duplicate records, inconsistent formats, missing values, and implausible entries before analysis. Patient numbers were used to identify patients contributing multiple specimen records. Age was standardized and converted to years. Sex and HIV status were harmonized into consistent categories, including consolidation of HIV-positive entries recorded with different letter cases. The specimen category variable was cleaned to distinguish baseline from follow-up/other records. Records coded as “Diagnosis” were classified as baseline, while follow-up and other categories were retained separately. Collection-to-receipt and receipt-to-accession intervals were calculated from the corresponding LIS dates, after which receipt-delay categories were derived from the collection-to-receipt interval. Culture results were standardized into four categories: MTBC isolated, NTM isolated, negative, and contaminated. Contaminated records were retained as a separate outcome and were not recoded as negative for MTBC.

2.7 Statistical analysis

Data were analysed using Stata/SE version 14.2. Descriptive analyses were conducted at both the patient and specimen levels. Categorical variables were summarized using frequencies and percentages. Continuous variables were assessed for their distribution and summarized using the median as the measure of central tendency and the interquartile range (IQR) as the measure of dispersion because the distributions were non-normal or right-skewed. Age, specimen collection-to-laboratory receipt delay, and laboratory receipt-to-accessioning interval were therefore summarized using the median and IQR.

The proportion of specimens received within ≤ 7 days and > 7 days of collection was calculated for the complete specimen dataset. Among baseline specimens, culture outcomes were summarized across four collection-to-receipt delay categories (≤ 7 , 8–14, 15–21, and > 21 days) using frequencies and row percentages. The receipt-to-accessioning interval was summarized within each receipt-delay category using the median and IQR, and differences in the distribution of receipt-to-accessioning intervals across delay categories were assessed using the Kruskal–Wallis test.

For the primary analysis, contaminated specimens were excluded because MTBC recovery could not be reliably determined. MTBC recovery was defined as isolation of MTBC, while MTBC not recovered comprised specimens with negative cultures or NTM isolation. Logistic regression was used to assess factors associated with MTBC culture recovery among baseline specimens with interpretable culture outcomes.

The unit of analysis was the individual sputum specimen. Patients could contribute up to two specimens, with each specimen representing a separate laboratory observation with its own collection-to-receipt interval and culture outcome. Because specimens contributed by the same patient may be correlated, logistic regression models accounted for potential within-patient correlation using robust standard errors clustered at the patient level.

Age was initially categorized as < 15 , 15–24, 25–34, 35–44, 45–54, and ≥ 55 years for descriptive analyses. For regression analysis, the < 15 and 15–24-year groups were combined into a < 25 -year category because of the small number of specimens from participants aged < 15 years. The ≥ 55 -year age group was used as the reference category. Crude and adjusted odds ratios (CORs and aORs), with 95% confidence intervals (CIs), were estimated. The adjusted model included specimen receipt delay, age group, sex, and HIV status. All statistical tests were two-sided, and $P < 0.05$ was considered statistically significant.

3 Results

3.1 Characteristics of the study participants and specimens

A total of 712 patients contributed 1,150 sputum specimens to the study. At the patient level, the median age was 40 years (IQR: 30–49). The largest age group was 35–44 years (189, 26.54%), followed by 25–34 years (177, 24.86%) and 45–54 years (144, 20.22%). Patients aged ≥ 55 years accounted for 114 (16.01%), 70 (9.83%) were aged 15–24 years, and 18 (2.53%) were aged < 15 years. Most patients were male (512, 71.91%), while 200 (28.09%) were female. Regarding HIV status, 293 (41.15%) were HIV-positive, 289 (40.59%) were HIV-negative, and 130 (18.26%) had unknown HIV status. At the specimen level, Southern Province contributed the largest proportion of specimens (591, 51.39%), followed by Central Province (292, 25.39%), Eastern Province (118, 10.26%), and Muchinga Province (82, 7.13%). Lusaka, Northern, Copperbelt, Western, and Northwestern provinces contributed 36 (3.13%), 17 (1.48%), 7 (0.61%), 6 (0.52%), and 1 (0.09%) specimens, respectively. The culture results showed that 697 (60.61%) specimens were negative, 182 (15.83%) had MTBC isolated, 159 (13.83%) had NTM isolated, and 112 (9.74%) were contaminated. The median time from specimen collection to laboratory receipt was 4 days (IQR: 2–6). Overall, 999 (86.87%) specimens were received within 7 days of collection, while 151 (13.13%) were received after 7 days. Of the total specimens, 128 (11.13%) were received within 8–14 days, 14 (1.22%) within 15–21 days, and 9 (0.78%) after more than 21 days (Table 1).

Table 1
Characteristics of patients and specimens included in the study

Characteristic	N	%
Patient-level characteristics (N = 712)		
Age, years		
Median (IQR)	40 (30–49)	—
< 15 years	18	2.53
15–24 years	70	9.83
25–34 years	177	24.86
35–44 years	189	26.54
45–54 years	144	20.22
≥ 55 years	114	16.01
Sex		
Male	512	71.91
Female	200	28.09
HIV status		
HIV-negative	289	40.59
HIV-positive	293	41.15
Unknown	130	18.26
Province of specimen origin		
Southern	316	44.38
Central	241	33.85
Eastern	62	8.71
Muchinga	47	6.60
Lusaka	24	3.37
Northern	11	1.54
Copperbelt	6	0.84
Western	4	0.56
Northwestern	1	0.14

Characteristic	N	%
Patient-level characteristics (N = 712)		
Age, years		
Specimen-level characteristics (N = 1,150)		
Province of specimen origin		
Southern	591	51.39
Central	292	25.39
Eastern	118	10.26
Muchinga	82	7.13
Lusaka	36	3.13
Northern	17	1.48
Copperbelt	7	0.61
Western	6	0.52
Northwestern	1	0.09
Culture result		
MTBC isolated	182	15.83
NTM isolated	159	13.83
Negative	697	60.61
Contaminated	112	9.74
Collection-to-laboratory receipt delay		
≤ 7 days	999	86.87
8–14 days	128	11.13
15–21 days	14	1.22
> 21 days	9	0.78
Median receipt delay (IQR), days	4 (2–6)	

3.2 MTBC culture recovery by specimen receipt delay

Of the 1,150 specimens, 573 (49.83%) were baseline specimens and 577 (50.17%) were follow-up or other specimens. The analysis of MTBC culture recovery was restricted to baseline specimens. Among the 573 baseline specimens, 513 (89.53%) were received within seven days of collection and 60

(10.47%) were received after seven days. Culture outcomes comprised 122 (21.29%) specimens with MTBC recovered, 386 (67.36%) with MTBC not recovered (including negative and NTM-isolated specimens), and 65 (11.34%) contaminated specimens. For comparison of MTBC recovery by receipt delay, contaminated specimens were excluded, leaving 508 specimens with interpretable recovery outcomes. MTBC was recovered from 114 (24.95%) of 457 specimens received within seven days and from 8 (15.69%) of 51 specimens received after seven days (Table 2).

Table 2
MTBC culture recovery according to specimen collection-to-laboratory receipt delay among baseline specimens

Characteristic	N	%
Specimen category, total (N = 1,150)		
Baseline	573	49.83
Follow-up/other	577	50.17
Baseline specimens by receipt delay (N = 573)		
On-time (≤ 7 days)	513	89.53
Delayed (> 7 days)	60	10.47
Culture outcome among baseline specimens (N = 573)		
MTBC recovered	122	21.29
MTBC not recovered	386	67.36
Contaminated	65	11.34
MTBC recovery by receipt delay		
On-time (≤ 7 days)	457	100.00
MTBC recovered	114	24.95
MTBC not recovered	343	75.05
Delayed (> 7 days)	51	100.00
MTBC recovered	8	15.69
MTBC not recovered	43	84.31

3.3 MTBC culture recovery by specimen receipt delay and patient characteristics

In the multivariable logistic regression model, delayed receipt (> 7 days) had an adjusted odds ratio (aOR) of 0.51 (95% CI: 0.20–1.26; $P = 0.144$) for MTBC culture recovery. Compared with participants aged ≥ 55 years, the aORs were 4.04 (95% CI: 1.13–14.46; $P = 0.032$) for those aged < 25 years, 11.92

(95% CI: 4.06–34.95; $P < 0.001$) for those aged 25–34 years, 6.45 (95% CI: 2.21–18.84; $P = 0.001$) for those aged 35–44 years, and 3.63 (95% CI: 1.17–11.28; $P = 0.025$) for those aged 45–54 years. Male participants had an aOR of 1.64 (95% CI: 0.86–3.12; $P = 0.132$) compared with females. Compared with HIV-negative participants, the aOR was 0.47 (95% CI: 0.25–0.89; $P = 0.020$) among HIV-positive participants and 0.81 (95% CI: 0.38–1.71; $P = 0.578$) among those with unknown HIV status (Table 3).

Table 3
Multivariable logistic regression analysis of factors associated with MTBC culture recovery among baseline diagnostic sputum specimens, Zambia, 2025

Variable	aOR	95% CI	P-value
Receipt delay > 7 days	0.51	0.20–1.26	0.144
Age < 25 years	4.04	1.13–14.46	0.032
Age 25–34 years	11.92	4.06–34.95	< 0.001
Age 35–44 years	6.45	2.21–18.84	0.001
Age 45–54 years	3.63	1.17–11.28	0.025
Male	1.64	0.86–3.12	0.132
HIV positive	0.47	0.25–0.89	0.020
HIV unknown	0.81	0.38–1.71	0.578

aOR, adjusted odds ratio; CI, confidence interval. Reference categories were receipt within ≤ 7 days, age ≥ 55 years, female sex, and HIV-negative status. Robust standard errors were clustered at the patient level to account for multiple specimens contributed by the same patient.

3.4 Distribution of culture outcomes by specimen receipt-delay category

Among the 573 baseline specimens, 513 (89.53%) were received within 7 days, 49 (8.55%) within 8–14 days, 9 (1.57%) within 15–21 days, and 2 (0.35%) after more than 21 days. Overall, 122 (21.29%) specimens had MTBC isolated, 70 (12.22%) had NTM isolated, 316 (55.15%) were negative, and 65 (11.34%) were contaminated. Among the 513 specimens received within 7 days, 114 (22.22%) had MTBC isolated, 58 (11.31%) had NTM isolated, 285 (55.56%) were negative, and 56 (10.92%) were contaminated. Among the 49 specimens received within 8–14 days, 6 (12.24%) had MTBC isolated, 10 (20.41%) had NTM isolated, 25 (51.02%) were negative, and 8 (16.33%) were contaminated. Among the 9 specimens received within 15–21 days, 1 (11.11%) had MTBC isolated, 1 (11.11%) had NTM isolated, 6 (66.67%) were negative, and 1 (11.11%) was contaminated. Among the 2 specimens received > 21 days, 1 (50.00%) had MTBC isolated and 1 (50.00%) had NTM isolated; none were negative or contaminated (Table 4).

Table 4

Distribution of mycobacterial culture outcomes according to specimen collection-to-laboratory receipt delay among baseline specimens

Collection-to-receipt delay	MTBC isolated, n (%)	NTM isolated, n (%)	Negative, n (%)	Contaminated, n (%)	Total, n (%)
≤ 7 days	114 (22.22)	58 (11.31)	285 (55.56)	56 (10.92)	513 (100.00)
8–14 days	6 (12.24)	10 (20.41)	25 (51.02)	8 (16.33)	49 (100.00)
15–21 days	1 (11.11)	1 (11.11)	6 (66.67)	1 (11.11)	9 (100.00)
> 21 days	1 (50.00)	1 (50.00)	0 (0.00)	0 (0.00)	2 (100.00)
Total	122 (21.29)	70 (12.22)	316 (55.15)	65 (11.34)	573 (100.00)

3.5 Interval between laboratory receipt and accessioning by specimen receipt delay

Among the 573 baseline specimens, the median interval between laboratory receipt and accessioning was 3 days (IQR 1–5). The median interval was 3 days (IQR 1–5) among specimens received within 7 days, 3 days (IQR 1–4) among those received within 8–14 days, 3 days (IQR 1–6) among those received within 15–21 days, and 1.5 days (IQR 0–3) among specimens received after more than 21 days. The Kruskal–Wallis test showed no statistically significant difference in the receipt-to-accessioning interval across the four-specimen receipt-delay categories (χ^2 (3) = 1.44, P = 0.695) (Table 5).

Table 5

Interval between laboratory receipt and accessioning according to specimen collection-to-laboratory receipt delay among baseline specimens

Collection-to-receipt delay	N	Accessioning delay, median (IQR), days
≤ 7 days	513	3 (1–5)
8–14 days	49	3 (1–4)
15–21 days	9	3 (1–6)
> 21 days	2	1.5 (0–3)
Total	573	3 (1–5)
χ^2 (3) = 1.44, P = 0.695		

4 Discussion

4.1 Principal findings

This study assessed the relationship between sputum specimen receipt delay and mycobacterial culture outcomes at the NTRL in Zambia from January to December 2025. A total of 1,150 sputum specimens from 712 patients were included. The median patient age was 40 years (IQR: 30–49), and most patients were male (71.91%). Similar proportions of patients were HIV-positive (41.15%) and HIV-negative (40.59%), while HIV status was unknown for 18.26%. The predominance of adults aged 25–54 years is consistent with the distribution of tuberculosis among economically productive age groups [18]. The high proportion of males is also consistent with the higher occurrence of tuberculosis among men reported in many settings, including sub-Saharan Africa [19].

The main finding was that specimens received more than seven days after collection had lower odds of MTBC culture recovery than those received within seven days, although this association was not statistically significant after adjustment for age, sex and HIV status and accounting for within-patient correlation (aOR 0.51, 95% CI: 0.20–1.26; $P = 0.144$). The direction of the association was consistent with the hypothesis that delayed specimen receipt may adversely affect recovery of viable MTBC; however, the study did not provide sufficient statistical evidence to conclude that receipt after seven days was associated with reduced MTBC recovery. The crude estimate showed a similar pattern (COR 0.56, 95% CI: 0.26–1.23; $P = 0.147$), suggesting that adjustment for the measured patient characteristics did not materially alter the observed association.

The lack of a statistically significant association should be interpreted in the context of the distribution of the data. Most baseline specimens were received within seven days, and only 51 interpretable specimens were received after seven days. The numbers were particularly small at longer delays, with nine baseline specimens received 15–21 days after collection and two received after more than 21 days. Consequently, the study had limited precision to detect differences associated with prolonged delays, particularly at the extreme ends of the delay distribution. The findings therefore do not exclude a clinically relevant effect of delayed transport; rather, they indicate that such an effect could not be demonstrated conclusively in this dataset.

4.2 Specimen referral and transportation patterns

Southern Province contributed more than half of all specimens (51.39%), followed by Central Province (25.39%) and Eastern Province (10.26%). This distribution indicates that the NTRL receives specimens through a geographically broad referral network, with substantial contributions from provinces outside Lusaka. The concentration of specimens from Southern and Central provinces may reflect differences in the number of submitting facilities, patient volume, referral arrangements, and geographical access to culture services rather than differences in tuberculosis occurrence alone [20].

The relatively low numbers of specimens from some provinces require additional consideration. During the study period, withdrawal of United States-supported funding for specimen courier services affected some COAG-supported provinces. This affected the availability of riders responsible for transporting

sputum specimens to referral laboratories and may have contributed to the low numbers of specimens received from some provinces. Changes in external financing and disruptions to specimen transportation systems can affect the continuity and geographic coverage of laboratory specimen referral networks, particularly where transport systems depend substantially on donor-supported mechanisms [21]. This is important when interpreting the geographic distribution of specimens in the present study because low representation from a province may reflect limitations in specimen transportation rather than a low burden of presumptive or confirmed tuberculosis.

The overall median interval from sputum collection to laboratory receipt was four days (IQR: 2–6). Most specimens (999, 86.87%) reached the NTRL within seven days, while 151 (13.13%) were received after seven days. Among the delayed specimens, 128 (11.13%) were received 8–14 days after collection, 14 (1.22%) were received 15–21 days after collection, and nine (0.78%) were received after more than 21 days. Thus, although the majority of specimens met the seven-day laboratory receipt recommendation, a measurable proportion experienced delays beyond the recommended interval.

Timely transportation is particularly important for specimens intended for mycobacterial culture because recovery depends on the survival of viable organisms between collection and processing [22]. Prolonged storage and transportation, particularly under suboptimal temperature conditions, may reduce the viability of *M. tuberculosis* and thereby affect culture recovery [22, 23]. The Zambia TB manual recommends rapid transportation of specimens for culture, with refrigerated storage when immediate testing is not possible [12]. WHO guidance similarly emphasizes prompt transportation and appropriate temperature control for specimens intended for culture [24]. However, information on temperature and storage conditions during transportation was not available in the present dataset. Consequently, receipt delay should be interpreted as a proxy for the duration of specimen transit rather than a direct measure of specimen quality or organism viability.

4.3 Mycobacterial culture outcomes, contamination and NTM isolation

Overall, 182 (15.83%) specimens had MTBC isolated, 159 (13.83%) had NTM isolated, 697 (60.61%) were negative, and 112 (9.74%) were contaminated. Among baseline specimens, MTBC was recovered from 122 (21.29%), while 70 (12.22%) yielded NTM, 316 (55.15%) were negative, and 65 (11.34%) were contaminated. The proportion of NTM isolation was notable and is consistent with previous evidence from Zambia showing that NTM can be frequently recovered from respiratory specimens submitted for tuberculosis investigation [25]. A national Zambian TB prevalence survey reported NTM in approximately 15% of individuals with presumptive TB [25]. Earlier Zambian studies have also demonstrated NTM isolation among both HIV-positive and HIV-negative individuals [24]. In the present study, NTM results were therefore retained as a distinct culture outcome rather than being treated as equivalent to a culture-negative result.

For the primary binary analysis, NTM-isolated specimens were classified as MTBC not recovered because the outcome of interest was specifically recovery of MTBC. This distinction is important

because isolation of an NTM organism confirms mycobacterial growth but does not constitute evidence of MTBC recovery. At the same time, NTM isolation from a respiratory specimen does not by itself establish NTM pulmonary disease [26, 27]. Clinical interpretation of NTM recovered from respiratory specimens requires consideration of clinical, radiological and microbiological criteria because NTM may represent colonisation or transient environmental contamination rather than disease [28–30].

The overall contamination rate among baseline specimens was 11.34%, which was higher than the approximately 5% reported in the Zambian national TB prevalence survey [25]. Differences in contamination rates between studies may arise from variation in specimen collection quality, transportation conditions, storage duration, referral pathways, laboratory procedures and workload [31]. The higher contamination observed in this study is therefore likely to reflect a combination of pre-analytical and laboratory factors rather than transportation delay alone.

Contamination is important in the context of the primary analysis because a contaminated culture cannot reliably establish whether viable MTBC was present in the original specimen. For this reason, contaminated specimens were excluded from the binary MTBC recovery analysis. Excluding contaminated specimens from culture-yield analyses is methodologically appropriate where contamination prevents definitive classification of the target organism's recovery status [32].

There was some descriptive variation in culture outcomes across receipt-delay categories. MTBC isolation decreased from 22.22% among specimens received within seven days to 12.24% among those received 8–14 days and 11.11% among those received 15–21 days. However, the > 21-day category consisted of only two specimens, one of which yielded MTBC and the other NTM. The apparent 50% MTBC recovery in this category is therefore not interpretable as evidence of improved recovery with very prolonged delay. Similarly, the higher NTM proportion in the 8–14-day category should be regarded as descriptive rather than evidence of a causal relationship between delay and NTM isolation.

4.4 Factors associated with MTBC culture recovery

Among the 508 baseline specimens with interpretable culture outcomes, age group and HIV status were associated with MTBC culture recovery in the adjusted logistic regression model, whereas sex and receipt delay were not statistically significant. The analysis accounted for potential correlation between specimens contributed by the same patient by using robust standard errors clustered at the patient level. Age showed the strongest association with MTBC culture recovery. Using the ≥ 55 years age group as the reference, the adjusted odds of MTBC recovery were approximately four-fold higher among specimens from patients aged < 25 years (aOR 4.04, 95% CI: 1.13–14.46; $P = 0.032$), nearly twelve-fold higher among those aged 25–34 years (aOR 11.92, 95% CI: 4.06–34.95; $P < 0.001$), more than six-fold higher among those aged 35–44 years (aOR 6.45, 95% CI: 2.21–18.84; $P = 0.001$), and more than three-fold higher among those aged 45–54 years (aOR 3.63, 95% CI: 1.17–11.28; $P = 0.025$). The higher likelihood of bacteriologically confirmed pulmonary TB among younger and middle-aged adults compared with older adults has been reported in population-based and laboratory-based studies and

may reflect differences in TB epidemiology, disease presentation, comorbidity and healthcare-seeking patterns across age groups [33, 34].

The particularly high odds observed among the 25–34-year age group are consistent with the age distribution of the study population and may reflect a greater underlying burden of active pulmonary TB in this age range. Younger and middle-aged adults often constitute an important proportion of the population affected by transmissible pulmonary TB because of greater social contact and exposure opportunities [35]. However, the present study was laboratory-based and did not measure individual-level exposure history, disease severity or clinical symptoms; therefore, the observed age associations should not be interpreted as evidence that age itself causes increased culture recovery.

The association between sex and MTBC recovery was attenuated after adjustment. Male specimens had higher odds of MTBC recovery in the crude analysis (COR 2.09, 95% CI: 1.28–3.40), but the association was not statistically significant after adjustment (aOR 1.64, 95% CI: 0.86–3.12; $P = 0.132$). The higher crude prevalence of bacteriologically confirmed TB among men has been widely documented and may reflect differences in biological susceptibility, occupational and social exposure, health-seeking behaviour and access to diagnostic services [19, 24, 36]. In the present analysis, the adjusted association was less precise after accounting for age, HIV status, receipt delay and within-patient clustering. Because the confidence interval for the adjusted estimate included one, the study did not demonstrate a statistically significant association between sex and MTBC culture recovery.

HIV status remained associated with MTBC culture recovery after adjustment. Compared with HIV-negative specimens, HIV-positive specimens had approximately half the odds of MTBC recovery (aOR 0.47, 95% CI: 0.25–0.89; $P = 0.020$). The finding is consistent with the diagnostic challenges associated with pulmonary TB among people living with HIV. HIV-associated immunosuppression can result in paucibacillary disease, atypical clinical and radiological presentations, and a greater likelihood of smear-negative disease, all of which may reduce the probability of recovering MTBC from respiratory specimens [2, 37–39].

The lower odds of culture recovery among HIV-positive specimens should nevertheless be interpreted carefully. Culture recovery is influenced by the bacillary burden in the specimen, disease site, specimen quality, timing of specimen collection, previous treatment and other clinical factors that were not fully available in the LIS dataset. The finding therefore indicates an association between recorded HIV status and MTBC culture recovery rather than a direct effect of HIV infection on culture viability.

Importantly, adjustment for age, sex and HIV status did not substantially alter the direction or magnitude of the association between delayed receipt and MTBC recovery. Specimens received after seven days had approximately half the odds of MTBC recovery compared with specimens received within seven days (aOR 0.51, 95% CI: 0.20–1.26; $P = 0.144$). Experimental and observational evidence suggests that prolonged transport or storage under inappropriate conditions can compromise recovery of viable mycobacteria, providing biological plausibility for the direction of the observed association [22–24].

However, the present analysis did not demonstrate a statistically significant association between delayed receipt and MTBC culture recovery.

The confidence interval around the adjusted estimate indicates uncertainty in the magnitude of the association. This may be related, at least in part, to the relatively small number of delayed specimens with interpretable culture outcomes. In addition, the analysis could not account for potentially important transport-related factors such as duration of storage before dispatch, use of appropriate transport containers, the specific transport route. These factors may influence culture recovery independently of the recorded collection-to-receipt interval. Although patient-level clustering was accounted for in the regression analysis, residual confounding by clinical, specimen-quality and transport-related factors cannot be excluded.

4.5 Receipt-to-accessioning interval and the diagnostic pathway

The median interval from laboratory receipt to accessioning was three days (IQR: 1–5) among the 573 baseline specimens. The median interval was similar across the collection-to-receipt delay categories: three days (IQR: 1–5) among specimens received within seven days, three days (IQR: 1–4) among those received 8–14 days safter collection, three days (IQR: 1–6) among those received 15–21 days after collection, and 1.5 days (IQR: 0–3) among the two specimens received after more than 21 days.

There was no statistically significant difference in the receipt-to-accessioning interval across the four collection-to-receipt delay categories (Kruskal–Wallis $\chi^2=1.44$, $P = 0.695$). This suggests that the delay occurring before specimens reached the NTRL was not accompanied by a corresponding increase in the time taken to accession specimens once they arrived. The two intervals therefore represent distinct components of the diagnostic pathway: transportation from the submitting facility to the NTRL and internal laboratory processing after receipt.

From a programme perspective, the finding suggests that interventions aimed at improving specimen turnaround should consider the pre-laboratory transport component separately from internal laboratory processes. Strengthening courier availability, transport scheduling, route coordination and monitoring of specimen transit times may be particularly important in geographically dispersed referral networks [40]. The low specimen contribution from some provinces during periods of disrupted courier support further highlights the importance of sustainable specimen referral systems.

4.6 Strengths and limitations

This study has several strengths. It used routinely collected data from the national reference laboratory and included specimens referred from multiple provinces, providing a broad view of sputum referral patterns and culture outcomes within the NTRL network. The study also distinguished baseline diagnostic specimens from follow-up and other specimens, thereby improving clinical comparability for the primary analysis. In addition, contaminated cultures were excluded from the binary MTBC recovery analysis rather than being incorrectly classified as MTBC-negative. The analysis further examined both

crude and adjusted associations between receipt delay and MTBC culture recovery, while adjusting for age, sex and HIV status. Importantly, because some patients contributed more than one specimen, the adjusted logistic regression model accounted for potential within-patient correlation using robust standard errors clustered at the patient level.

Several limitations should be considered. First, the retrospective design limited the analysis to variables routinely recorded in the LIS. Important factors that may influence culture recovery, including the time between specimen collection and dispatch, transport conditions, transport route, specimen quality, previous anti-TB treatment and clinical severity, were not consistently available. Second, the number of specimens received after seven days was relatively small, and numbers were particularly limited in the 15–21-day and > 21-day categories. This reduced the precision of estimates and limited the ability to reliably assess a dose-response relationship across increasing delays. Third, the study was conducted at a national reference laboratory and included specimens referred for culture rather than a population-based sample of all individuals evaluated for TB. The findings may therefore not be directly generalisable to all TB diagnostic services in Zambia.

Fourth, the unit of analysis was the individual sputum specimen, and some patients contributed two specimens. Although each specimen had its own collection-to-receipt interval and culture outcome, specimens from the same patient may be correlated. This potential non-independence was addressed by clustering the regression standard errors at the patient level; however, clustering does not eliminate potential bias arising from other patient-level or specimen-level factors that were not measured. Fifth, HIV status was unknown for a proportion of specimens, which may have introduced uncertainty into the adjusted estimates. Finally, because contaminated specimens were excluded from the binary MTBC recovery analysis, the findings apply specifically to specimens with interpretable culture outcomes and should not be extrapolated to contamination as an outcome.

5 Conclusion

Most sputum specimens reached the NTRL within seven days of collection. Although specimens received after seven days had lower odds of MTBC culture recovery, delayed receipt was not statistically significantly associated with MTBC recovery after adjustment for age, sex and HIV status and accounting for within-patient correlation. Age and HIV status were associated with MTBC culture recovery, while sex was not statistically significantly associated with recovery in the adjusted analysis. These findings support continued emphasis on timely specimen referral and transportation within the NTRL network, while highlighting that culture recovery is influenced by multiple patient-, specimen- and transport-related factors.

Abbreviations

aOR Adjusted Odds Ratio

CI: Confidence Interval

DST: Drug Susceptibility Testing

HIV: Human Immunodeficiency Virus

IQR: Interquartile Range

LIS: Laboratory Information System

MOH: Ministry of Health

MTBC: *Mycobacterium tuberculosis* Complex

MTB/RIF: *Mycobacterium tuberculosis*/Rifampicin

NHRTI: National Health Research and Training Institute

NTLP: National Tuberculosis and Leprosy Programme

NTM: Non-Tuberculous Mycobacteria

NTRL: National Tuberculosis Reference Laboratory

TB: Tuberculosis

UTH: University Teaching Hospital

WHO: World Health Organization

Declarations

Ethics approval

The study used routinely collected, de-identified LIS data and involved no direct contact with patients. Ethical approval was obtained from the Livingstone Center for Prevention and Translational Science Research Ethics Committee (Ref: LCPTS/REC/012). The study was conducted in accordance with the principles of the Declaration of Helsinki. The requirement for informed consent was waived by the Livingstone Center for Prevention and Translational Science Research Ethics Committee Research Ethics Committee because the study involved retrospective analysis of de-identified routine laboratory data and did not pose any risk to participants.

Clinical trial number

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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Author Contribution

F.P. conceived and designed the study, developed the methodology, coordinated data collection and data management, performed the statistical analysis, interpreted the findings, and drafted the manuscript. M.W., B.M.M., A.M., W.N., and D.K.M. contributed to data collection, data curation, interpretation of findings, and critical review of the manuscript. F.P. led the writing and revision of the manuscript. All authors critically reviewed the manuscript for important intellectual content, read and approved the final version, and agreed to be accountable for all aspects of the work.

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Not applicable.

Data Availability

The data used in this study is not publicly available because they were obtained from the routine laboratory information system of the National Tuberculosis Reference Laboratory, Zambia, and are subject to institutional data access and confidentiality restrictions. De-identified data will be available from the corresponding author upon reasonable request and with permission from the National Tuberculosis Reference Laboratory and relevant institutional authorities.

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