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# Higher prevalence of pulmonary tuberculosis revealed by Xpert MTB/RIF ultra among drug users in Kinshasa, Democratic Republic of Congo

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## Abstract

**Introduction** The Democratic Republic of Congo (DRC) is one of the eight countries with the highest burden of tuberculosis (TB) in the world. The public health system is inadequate and the screening for TB in the key and vulnerable population (KVP), including drug users (DU) is not currently done. The present study aimed to determine the prevalence of pulmonary TB among DU in Kinshasa by comparing molecular tests with microscopic techniques.

**Methods** A cross-sectional study covering 22 townships (out of 24) of Kinshasa was conducted from October to December 2023. Sputum samples were collected from DUs aged  $\geq 18$  years, clinically suspected of TB, and attending drug consumption sites. The samples were analyzed by both the Acid-fast bacilli (AFB)-Nelsen hot staining and Xpert MTB/RIF Ultra for TB and rifampin-resistance diagnosis.

**Results** For 399 DUs included in the study, the age range was from 18 to 77 years old, with a median of 31 (IQR: 25–39). Among these DUs, 359 (89%; 95% CI: 86.64–92.55%) were male. TB prevalence was 3.5% (95% CI: 1.9–5.8%) when the AFB-Nelsen hot staining was used for diagnosis. However, the prevalence was significantly higher at 13.8% (95% CI: 10.6–17.6%) with the Xpert MTB/RIF test ( $p=0.000$ ). Xpert MTB-RIF Ultra contributed with an added value of 82% (95% IC: 79.25–86.47%) to the diagnosis of TB in DUs. The KAPPA test showed a low concordance at 25%. Alcohol, diazepam and tobacco consumption have been identified as practical risks associated with the onset of pulmonary TB ( $p < 0.05$ ).

**Conclusion** DUs are a population at risk of TB that should not be neglected among all KVPs in Kinshasa. In this specific population, the determination of TB prevalence was significantly improved with the use of Xpert MTB/RIF Ultra compared to hot AFB-Neelsen staining. Among the DU included in the present study, those who habitually consumed alcohol in its different forms, diazepam for non-medical purposes, and tobacco, were significantly more infected than the others. DUs should be considered for systemic screening for pulmonary TB alongside other key

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populations such as people living with HIV and Xpert should be maintained as a first-line test instead of microscopy. Further studies also including asymptomatic participants are needed to assess the burden due to pulmonary TB in DUs as well as in their living environment.

**Keywords** Tuberculosis, Drug users, Xpert MTB, Acid-fast bacilli-nelseen hot staining, Kinshasa

## Introduction

Tuberculosis (TB), is a disease associated with significant morbidity and mortality, and remains a major and persistent threat to public health [1–3]. It is the tenth leading cause of death worldwide from a single infectious agent, behind COVID-19 and before HIV/AIDS [4].

The Democratic Republic of Congo (DRC) is one of eight countries with the highest burden of TB in the world. It accounted for nearly 3,0% of TB cases in the world in 2023. In the same year, 258,069 incident cases of susceptible TB out of the 334,000 expected cases were diagnosed, i.e. 243 incident cases out of 316 expected cases per 100,000 population. In addition, 13,096 drug-resistant TB cases were reported with increasing trends in the same report [4]. Despite the efforts made by the National Tuberculosis Program (NTP), at least 27% of patients escape the healthcare system. To remedy this problem, the strategy of systematic TB screening among key and vulnerable population (KVP), notably drug users (DU), people living with HIV (PLHIV), and children under 15 years has been adopted and implemented [5, 6]. KVPs are at greater risk of developing TB due to crowding, behavioral risk factors, limited access to health services in general, and biological factors. This situation is amplified by stigmatization, social and economic difficulties, and HIV infection [7, 8].

According to WHO estimations, in 2021, over 296 million people used psychoactive substances in the world and, almost half a million lost their lives prematurely. The diseases are set to rise by 11% worldwide and 40% in Africa by 2030 [9, 10]. The consumption of psychoactive substances, such as tobacco, alcohol and drugs of various nature (cannabis, cocaine, heroin, etc.) has a deleterious effect on the immune response and neuronal activity. Having different physicochemical properties, these substances weaken immunity on the one hand by significantly reducing mucociliary clearance and inhibiting macrophage activity and on the other by inducing the production of cytokines or tumor necrosis factor (TNF) responsible for a permanent inflammatory reaction (case of alcohol and tobacco via nicotine). Other substances, such as cannabis (hemp), have the effect of preferentially activating lymphocytes, increasing the production of B lymphocytes to the detriment of T lymphocytes, transforming the cellular immunity needed to eradicate micro-organisms that have penetrated the cell [2].

DRC is a transit country for heroin and cocaine traffic, and a producer of cannabis. Its internal instability

and porous borders facilitate the illicit marketing and consumption of drugs. The national toxicology report (in 2023 five-year plan) shown that there are around 2,360 drug consumption and traffic sites and networks in Kinshasa. The main users of these sites are unemployed young people, students, construction workers, drivers of large vehicles and musicians. According to testimonies, they use drugs in the hope of improving their intellectual and/or physical performance [11]. Among the many drugs and psychoactive substances consumed, cannabis, which contains the harmful hallucinogen Tetra Hydro Cannabinol (THC), comes in the first position, followed by cocaine, an alkaloid extracted from the coca leaf. Consumption of Tobacco, known for its high content of the addictive chemical nicotine, is noted. In recent years, an indigenous substance cocktail known as “Bombe”, composed of carbon monoxide, nitrogen monoxide and sulfur oxide extracted from automobile catalytic exhaust residues has been widely consumed by the unemployed and discouraged youth in Kinshasa [12, 13]. These different practices have become very common in Kinshasa and are no longer a source of discomfort, increasing the number of DUs. The use of these substances constitutes a serious public health problem, especially as it does not only promote non-communicable diseases (cancer, hypertension, stroke, and diabetes), but also constitutes a factor in the spread of communicable diseases such as TB, and may also hinder the elimination of TB in an endemic country such as the DRC [14].

To date, information on the prevalence of TB and specially the contribution of the Xpert test to TB diagnosis in DUs is insufficiently documented. To fill this gap, the present study aims to determine the prevalence of TB among DUs in Kinshasa and the contribution of RIF/Ultra Xpert versus microscopy techniques in the diagnosis of pulmonary TB, and to identify risk factors associated with the progress of TB. The study findings may help the NTP in developing strategies and actions based on endogenous data, to effectively fight TB in the DRC.

## Materials and methods

### Study design and sites

A cross-sectional study was conducted from October 1 to December 31, 2023. The City of Kinshasa is comprised of 24 townships, 22 of which were the sites of the present study. Two townships, Nsele and Maluku, were excluded from the study due to insecurity at the time of study implementation. In each selected township, all the drug

**Table 1** Quantification scale of Acid-fast bacilli Neelsen staining

Number of detected AFB	Examined fields	Results
No AFB*	100 fields	0
1 to 9 AFB on one length	100 fields = 1 length	Exact number
10 to 99 AFB/100 fields	100 fields	+
1 to 9 AFB/Field	50 fields	++
More than 10 AFB/Field	20 fields	+++

\* Acid-fast bacilli

consumption sites commonly known as “NGANDA” and listed by the DRC’s National HIV/AIDS Control Program, were visited one after the other until the number of DUs required for the study was reached for each township.

### Study participants

DUs aged  $\geq 18$  with suspected pulmonary TB and living in Kinshasa for at least 3 months were enrolled in the present study. Pulmonary TB was suspected in participants presenting with any of the following signs: cough lasting more than a week, low-grade fever, weight loss, fatigue, etc. A sample of 418 DUs was calculated using the “Survey Monkey” online sampling program, taking into account 50% of Kinshasa population (6 862 502 (INS) [15], a confidence level of 95% and a standard error of 5%. A total of 19 participants were registered per administrative township.

### Sample collection

DUs showing suggestive signs of TB, attending (daily) drug consumption sites (NGANDA), were asked after consent has been obtained, to fill in a questionnaire to collect socio-demographic data (age, sex, education level, marital status, occupation, residence) and the type of drugs usually consumed (cannabis with Indian hemp, heroin, tobacco, cocaine, alcohol and “Bombé”) [see Additional file 1]. The administration of the survey questionnaire was carried out in Lingala (local language) and informed consent was obtained by trained investigators. A pre-survey was conducted in one site to test the study tools before extension to all other collection sites.

The questionnaire was administered before the collection of sputum samples in a spittoon that was placed in

**Table 2** Results on Xpert MTB/RIF ultra scoring sheet

Results on the screen	Interpretation	Rating
MTB* not detected	Absence of <i>M. tuberculosis</i> DNA	Not
MTB high, medium, low and very low detected, Rif not detected	Presence of MTB and susceptible to rifampicin	T
MTB high, medium, low and very low detected, Rif detected	Presence of MTB and resistance to rifampicin	RR
Trace amounts MTB Detected	Presence of MTB on track	Trace
MTB high, medium, low and very low detected, Rif indetermined	Presence of MTB and resistance to rifampicin not determined	IT

\*Mycobacterium tuberculosis

a suitable transport box at room temperature. DUs who could not submit a sputum specimen were excluded from the study and referred to a closer health center for follow up. All collected samples were daily transferred to the Provincial Tuberculosis Reference Laboratory of Kinshasa for analysis.

### Laboratory analyses

The collected samples were simultaneously analyzed by both the phenotypic method mostly used in the DRC’s NTP, namely microscopic examination after Hot AFB-Neelsen staining, and the genotypic method recommended by the WHO, namely Xpert MTB/RIF Ultra. The phenotypic method was performed according to the procedure described by the DRC’s NTP [6], and results were expressed as shown on Table 1.

The genotypic method used in the present study (Xpert MTB/RIF Ultra Test) consists of an automated real-time PCR integrating DNA extraction and amplification. The amplified segment of interest consists of an 81 bp DNA sequence from the MT *rpoB* gene that carries genetic elements reflecting resistance to rifampicin [16, 17].

The samples were analyzed according to the procedure described by the manufacturer [18]. Briefly, 1 ml of each sample was mixed with 2 ml of the SR reagent (provided in the kit) and shaken vigorously for 15 s. The mixture was then incubated for 10 min at room temperature. Two ml of the treated sample were loaded into the Xpert Rif/Ultra cartridge and the analysis was started for two hours. The results obtained were then interpreted by the DX software and displayed in the window of the instrument (Table 2) [18].

### Ethical considerations

The study protocol as well as the informed consent form of the participants were reviewed and approved by the Kinshasa Public Health School Ethics Committee, University of Kinshasa (Approval No. ESP/CE/143/2023). Participation to the study was voluntary after the participant had signed the informed consent form. This consent

**Table 3** Distribution of DUs per age group and per sex

Age group (years)	Sex		Total n (%; 95% CI)
	F n	M n	
18–20	3	19	22 (5.5; 3.67–8.21)
21–30	23	154	177 (44.4; 39.56–49.27)
31–40	10	114	124 (31.1; 26.74–35.78)
41–50	2	44	46 (11.5; 8.76–15.04)
50 and over	2	28	30 (7.5; 5.32–10.53)
Total	40	359	399 (100.00%)

**Table 4** Prevalence of pulmonary tuberculosis using Xpert versus Acid-fast bacilli-Nelseen staining among drug users by sex

MTB		Xpert MTB/RIF Ultra		Acid fast bacilli Neelsen		p-value
		n	% (95% CI)	n	% (95% CI)	
Positive	M	52	13.0 (9.9–16.7)	13	3.2 (1.7–5.5)	0.0000
	F	3	0.8 (0.2–2.2)	1	0.3 (0.0–1.4)	
	Total	55	13.8 (10.6–17.6)	14	3.5 (1.9–5.8)	
Negative	M	307	76.9 (72.5–81.0)	346	86.7 (83.0–89.9)	
	F	37	9.3 (6.6–12.6)	39	9.8 (7.0–13.1)	
	Total	344	86.2 (82.4–89.2)	385	96.5 (94.2–98.1)	
Total		399	100.00%	399	100.00%	

**Table 5** Prevalence of Xpert-based TB among drug users per age group

Age (years) group	Positive n (%)	Negative n (%)	Total N (%)	p-value
18–20	3 (13.6)	19 (86.3)	22 (100)	0.6518
21–30	21 (11.8)	156 (88.1)	177 (100)	
31–40	19 (15.3)	105 (84.6)	124 (100)	
41–50	6 (13.0)	40 (86.9)	46 (100)	
50 and over	6 (20.0)	24 (80.0)	30 (100)	
Total	55 (13.7)	344 (86.2)	399 (100)	

form was translated into Lingala, as was the administration of the questionnaire. The study was conducted in compliance with ethical principles and good clinical practices. Thus, participating in the study had no harm to the moral and physical integrity of the participant. The examinations provided in the study were free of charge as was the treatment if TB was detected. The data was collected and managed anonymously and stored in a database to which only the investigators had access.

### Statistical analysis

Data were encoded and verified in the 2010 Microsoft Excel database and imported into SPSS Statistics 20.0 for analysis. Categorical variables have been presented as proportions with their 95% confidence intervals. Frequencies were statistically compared using chi-squared tests or Fisher Exact and the *p*-values less than 0.05 were considered significant. Univariate analysis was performed to assess the association between the psychoactive product consumed and pulmonary TB.

## Results

### Demographic characteristics of drugs users

Overall, 399 DUs aged 18–77 years with a median age of 31 years (IQR: 25–39 years) were included in the study. Among them, 359 (89%; 95% CI: 86.64–992.55%) were men and 40 (10%; 95% CI: 7.45–13.36%) were women; 318 (79.7%; 95% CI: 75.3–83.5%) were single versus 81 (20.3%; 95% CI: 16.5–24.7%) married/cohabiting. Details on the distribution of DUs according to age groups and sex are provided in Table 3.

**Table 6** Prevalence of Xpert-diagnosed TB among drug users per sex

	F n (%)	M n (%)	Total n (%)
Positive	3 (7.5)	52 (14.5)	55 (13.8)
Negative	37 (92.5)	307 (84.5)	344 (86.2)
Total	40 (100%)	359 (100%)	399 (100)

**Table 7** Cross tabulation comparison of the Xpert TB and the AFB-Neelsen staining on DU's sputum

		Xpert MTB/RIF Ultra		Total
		Positive	Negative	
Hot AFB-Neelsen staining	Positive	10	4	14
	Negative	45	340	385
	Total	55	344	399

### Prevalence of pulmonary tuberculosis

Out of a total of 399 DUs enrolled based on the cough symptom, 14 (3.5%; 95% CI: 1.9 – 5.8%) were positive for AFB-Neelsen hot stain test while 55 (13.8%; 95% CI: 10.6 – 17.6%) were positive for Xpert MTB/RIF Ultra test (*p* = 0.000). Details of prevalence of TB by sex and by diagnostic technique used are shown in Table 4.

Delving deeper into the high-risk DU population for TB, Table 5 provides information on the frequency of TB by Xpert MTB in the age groups of the included subjects. TB was found to be evenly distributed across all age groups (*p* = 0.6518).

When considering the prevalence in each sex, 3 (7.5%) out of 40 female DUs were positive for TB using Xpert, whereas 52 (14.5%) out of 359 male DUs were positive using the same test (*p*-value = 0.224) (Table 6).

The results presented in Table 7 show a poor concordance between microscopy and Xpert with a Kappa coefficient of 25%, defining a sensitivity of AFB-Neelsen staining at 18.18% (95% CI: 10.19–30.33) and a specificity at 98.84% (95% CI: 97.05–99.55). The likelihood ratio of the negative test is therefore calculated at 0.8278 (95% CI: 0.7925–0.8647), which reflects a considerable added value of the Xpert MTB/RIF Ultra test.

### Association between psychoactive products and pulmonary tuberculosis

Considering the risk of developing TB in relation to the consumption of psychoactive substances among 55 DUs with Xpert-diagnosed TB, only consumers of alcohol (OR 2.1637; 95% CI 1.1598–4.0365;  $p=0.00707328$ ), diazepam (OR 2.6932; 95% CI 1.0009–7.2470;  $p=0.035$ ) and tobacco (OR 1.672; 95% CI 0.9126–3.0631;  $p=0.04851373$ ) were significantly more likely to develop TB than those who did not consume these substances (Table 8). Among the participants, 214/399 (53.6%) consumed more than one substance.

### Discussion

The present study aimed firstly to determine the prevalence of TB among DUs. Pulmonary TB was found in 13.8% and 3.5% of the 399 DUs clinically suspected of TB, respectively with Xpert MTB/RIF Ultra and AFB-Neelsen staining tests. These numbers correspond approximately to 13,800 and 3,500 incident cases per 100,000 DUs respectively, which are higher than the 243 per 100,000 populations reported in the general population [4]. The high prevalence of pulmonary TB found in this cohort was similar to the results of other studies carried out in Ivory Coast in 2018, in Canada in 2017, in Malaysia in 2013 and the report of the United Nations Office on Drugs and Crime (UNODC) in 2017 [8, 19–21]. As reported by Djegbeton et al. (2020), TB in its contagious form, is more common among DUs than in other KVPs [22]. This situation has been suggested by studies that reported values lower than those obtained in the present study, particularly in children under 15 years (11%), people living with HIV (7%), diabetics (11.4%) and prisoners (2.4%) [23–25]. The positivity of the diagnostic test was significantly higher with Xpert MTB/ Ultra compared to the hot AFB-Neelsen staining, which showed a low sensitivity of 18%. These results are different from those reported in the literature, especially studies conducted in the general population, which showed a sensitivity of around 50% (Maugein J. et Bébéar C.2003) [26]. Key populations such as DUs and PLHIV are generally paucibacillary for TB, which can reduce the sensitivity of AFB-Neelsen staining. The frequency of TB determined by the AFB staining technique was 3.5%, which looked like an underestimation compared to the prevalence expressed by Xpert MTB/RIF Ultra, a technique known for its higher sensitivity and specificity compared to microscopy [27]. Estimation of the agreement between microscopy and molecular testing using the Kappa coefficient analysis showed that the discordance between the two tests was high at 75% and the added value to the TB diagnosis by Xpert testing was calculated at 82%. These results are in agreement with those reported by Ahmad M et al.. in 2017 [27].

**Table 8** Risk factors related to psychoactive products associated with tuberculosis

Variable		MTB+ N (%)	OR	95% CI	p-value
Alcohol	Yes	33 (17.3)	2.1637	1.1598–4.0365	0.0071
	No	22 (8.8)			
Cannabis	Yes	44 (13.3)	1.1669	0.4982–2.7332	0.3753
	No	11 (11.6)			
Cocaine	Yes	9 (16.0)	1.3311	0.6073–2.9174	0.2363
	No	46 (12.5)			
Bombé	Yes	10 (19.2)	1.7262	0.8034–3.7088	0.0878
	No	45 (12.1)			
Diazepam	Yes	6 (27.2)	2.6932	1.0009–7.2470	0.0350
	No	49 (12.2)			
Heroin	Yes	5 (15.1)	1.2063	0.443–3.2850	0.3453
	No	50 (12.8)			
Tobacco	Yes	30 (16.0)	1.672	0.9126–3.0631	0.0485
	No	25 (10.2)			
Three Six Birds	Yes	4 (14.8)	1.1682	0.3865–3.5309	0.3759
	No	51 (12.9)			

This lower sensitivity of AFB-Neelsen smear microscopy is explained by the lower detection threshold compared to the Xpert MTB/RIF Ultra test, i.e. 5,000 bacilli/ml for Ziehl microscopy versus 136 bacilli/ml of the sample for Xpert, as explained by Masab et al.. in 2020 [28]. Of the samples tested positive by microscopy, 4 were negative by the molecular technique, which is really unusual. This could be explained by the fact that not all AFB-positive are of the MTB complex. In addition, the Xpert MTB/RIF Ultra test is specific by the amplification of the IS6110 sequence, which is a specific sequence for MTB and not for non-tuberculous mycobacteria [27, 29–31].

TB was found to be evenly distributed across all age groups ( $p=0.6518$ ). However, the 20–40 age group, which corresponds to the most active and productive group of the general population [32] and therefore the group most concerned by the use of psychoactive substances, as reported by the 2023 WHO TB report [4], was the most represented among the participants. There was also no significant difference in TB prevalence between male and female DUs ( $p=0.224$ ). However, there were more male than female DUs. Being the head of the family, males are the most stressed and always on the lookout for ways to satisfy vital needs of the family. For DUs, the use of psychoactive substances is a mean of finding mental and psychological relief. People who abuse alcohol, diazepam and tobacco are described here as being more likely to develop pulmonary TB than others. These data are similar to the results of the studies on risk factors for TB [33].



### Study limitations

Other risk factors for TB, such as malnutrition and HIV infection, should be studied to better control the confounding factors through multivariate analysis. The prevalence of pulmonary TB could be higher if screening had also included asymptomatic DUs, given that almost 30% of TB patients have no clinical signs. The present study referred to the national policy, which recommends symptom-based screening for pulmonary TB using biological tests. Chest X-ray may be requested in some cases according to the clinician's judgment.

### Conclusion

DUs are a population at risk of TB that should not be neglected among all KVPs in Kinshasa. In this specific population, the TB prevalence was significantly improved with the use of Xpert compared to hot AFB staining. Among the DU included in this study, those who habitually consumed alcohol in its various forms, diazepam for non-medical purposes and tobacco, were significantly more infected than the others. DUs should be considered for systemic screening for pulmonary TB alongside other key populations such as PLHIV and Xpert should be maintained as a first-line test instead of microscopy. Further studies also including asymptomatic participants are needed to assess the burden due to pulmonary TB in DUs as well as in their living environment.

### Abbreviations

DRC	Democratic republic of congo
TB	Tuberculosis
KVP	Key vulnerable population
DU	Drug users
TNF	Tumor necrosis factor
THC	Tetrahydrocannabinol
NTP	National tuberculosis program
DNA	Deoxyribonucleic acid
PCR	Polymerase chain reaction
AFB	Acid-fast bacilli
MTB	Mycobacterium tuberculosis
PRL	Provincial reference laboratory
WHO	World health organization
AFB	Acid fast bacilli

### Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12879-025-10853-2>.

**Supplementary Material 1: Additional file 1:** Questionnaire

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### Author contributions

GBN, DMY, SFB initiated the study and wrote study protocol. GBN, SFB carried out the data and sample collection, performed lab analysis and data interpretation. DYM, SFB carried out the statistical analysis. GBN, DMY, BZB, PZK, TMM and SFB drafted the manuscript and were major contributors in

writing the manuscript. All authors read and approved the final version of manuscript.

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The research reported was not funded.

### Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

### Declarations

#### Ethics approval and consent to participate

The protocol of this study as well as the informed consent form were approved by the Ethics Committee of the School of Public Health of Kinshasa, University of Kinshasa (Approval No. ESP/CE/143/2023). Although all the included subjects signed an informed consent for their inclusion, data collected were completely anonymized. The present study was conducted in accordance with the principles of the Declaration of Helsinki.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

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