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Prevalence and risk factors of rifampicin-resistant tuberculosis among patients attending the Kumba district hospital – Southwest region of Cameroon

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ABSTRACT:

— ***Objective:*** Rifampicin-resistant tuberculosis (RR-TB) is an emerging challenge for tuberculosis (TB) control programs globally. Updated knowledge of the increase in RR-TB is critical to allocate resources, address prevention, and control. This study aimed to assess the prevalence and risk factors of RR amongst suspected pulmonary TB patients attending the Kumba District Hospital in the Southwest region of Cameroon.

— ***Patients and Methods:*** A cross-sectional study including 153 suspected TB participants was conducted between April 12 and June 30, 2022. Data on socio-demographics and risk factors were collected using a semi-structured questionnaire. Microscopy using the Ziehl-Neelsen staining technique and GeneXpert MTB/RIF Ultra was used to detect TB disease and RR.

— ***Results:*** Of the 153 participants included in the study, 32 (20.9%) and 37 (24.18%) were positive for Acid Fast Bacilli (AFB) using microscopy and *M. tuberculosis* using GeneXpert MTB/RIF Ultra, respectively [86.49% sensitivity and 100% specificity]. RR was detected in 3/153 (1.96%) patients. RR-TB was significantly associated with a history of contact with a TB patient (p = 0.038), previously diagnosed TB patients (p = 0.045; OR = 14.67; 95% CI: 1.27-170.05), as well as family size (p = 0.002).

— ***Conclusions:*** Despite the low prevalence of RR-TB in the study area, systematic monitoring of TB contacts is advocated to prevent the dissemination of drug resistance to rifampicin. Upgrading diagnostic performance is necessary to avoid missing cases in the region, as well as the country, which remains largely reliant on sputum smear microscopy.

— ***Keywords:*** Tuberculosis, Rifampicin resistance, TB contacts, GeneXpert, Kumba, Cameroon.

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INTRODUCTION

Tuberculosis (TB) is a communicable disease caused by the *Mycobacterium tuberculosis* complex and is principally transmitted through the respiratory route[1]. Though curable, TB continues to ravage the world, leading to tremendous morbidity and mortality[1]. In 2023, TB returned as the world's leading cause of death after 3 years of coronavirus (COVID-19) pandemic predominance, leading to approximately twice the number of deaths caused by Human Immunodeficiency Virus/Acquired Immune Deficiency Syndrome (HIV/AIDS)[1]. More than 10 million cases of TB are reported annually and have been increasing since 2021[1].

The impact of risk factors varies at the population level, depending on the local prevalence of health events such as HIV/AIDS, diabetes, alcohol consumption, tobacco smoking, and indoor air pollution[2]. These factors impact a larger section of the population and accelerate the progression of TB disease worldwide, but mostly in developing countries such as Cameroon[2,3]. Besides several risk factors leading to TB disease, it has been shown in the country that late presentation as well as limited rapid molecular TB diagnostic facilities at the peripheral level of care may lead to missed opportunities for diagnosis[4]. It was reported in 2021 that Cameroon is among the 30 countries with the highest TB/HIV burden in the world, with an incidence of 164 cases per 100,000 population[1,5]. It was estimated that 43% and 77% TB and RR-TB patients, respectively, were not diagnosed[6]. A recent study[4] has reported a 53% and 50% of TB and RR-TB diagnostic gaps, respectively.

Fortunately, there is a combination of drugs to treat TB[7]. Early diagnosis and treatment are the most effective ways to prevent the dissemination of TB. A person with infectious TB can infect up to 10-15 other people per year[8]. Once diagnosed with TB and started on effective treatment, the infectiousness of most patients diminishes after 5 days of taking medication[9].

Multidrug-resistant TB (MDR-TB) is defined as TB caused by strains of *Mycobacterium tuberculosis* that are resistant to isoniazid and rifampicin, with or without resistance to other first-line drugs[3]. MDR-TB is a major threat to global TB control. In 2020, there was 71% (2.1/3.0 million) RR among bacteriologically confirmed pulmonary TB cases, showing a gradual increase from 50% (1.7/3.4 million) in 2018 to 61% (2.2/3.6 million) in 2019[10]. Among these reported cases in 2020, 132,222 cases of MDR/RR-TB and 25,681 cases of extensively drug-resistant tuberculosis (XDR-TB) resistant to rifampicin, a fluoroquinolone, plus at least one of the drugs (bedaquiline or linezolid) or pre-XDR-TB (resistant to rifampicin and a fluoroquinolone) were identified, making a total of 157,903 cases[10]. There was a large reduction of 22% from a total of 201,997 drug-resistant TB cases in 2019. This is consistent with similarly large reductions of 18% in the total number of people newly diagnosed with TB and 17% in the total number of people diagnosed with bacteriologically confirmed pulmonary TB between 2019 and 2020[10]. The estimated number of global MDR-TB cases in 2023 was 400,000 (95% Uncertainty Interval: 360,000-440,000)[1]. Several factors, such as the use of immunosuppressive drugs, HIV/AIDS, smoking, alcohol consumption, diabetes mellitus, malnutrition, young age, overcrowding, poor housing conditions, and economic deprivation, have been identified as predictors of drug-resistant tuberculosis[2].

Moreover, 150,359 people with RR/MDR-TB were enrolled in treatment in 2020 worldwide, indicating a 15% drop from the total of 177,100 people in 2019[10]. This level of enrolment was equivalent to about one in three of the people who develop MDR/RR-TB each year[8]. Even though the treatment success rate in Cameroon was estimated at 86% in 2020, the prevalence of adverse treatment failures continues to increase[5]. It has been reported that numerous factors influence successful TB treatment, including sex, age, lifestyle, treatment regimen, HIV status, and history of previous TB treatment[5].

Since 2013, the Cameroon national tuberculosis control program (NTCP) has been using the standardized shorter treatment regimen (STR) of 9-12 months duration on patients infected with RR-TB strains, considering the similar bad prognosis as MDR-TB cases[11]. It has been shown that the rate of 20.2%, higher than the WHO-recommended 10% of unfavorable treatment outcome (death, treatment failure, and loss to follow-up) of RR/MDR-TB has been observed in the country[12], with the major cause being death. This can be attributed to the socio-economic challenges faced by patients, which often delay healthcare seeking and result in presentation at an advanced stage of disease, as evidenced by the involvement of three or more lung zones. In addition, previous drug-resistant TB status, HIV infection, severe anemia, and male sex – likely linked to higher rates of loss to follow-up during treatment – have been identified as independent factors associated with unfavorable treatment outcomes in the country[13,14].

MDR-TB is, therefore, a relevant global health challenge that threatens the progress of TB eradication programs across the world and Africa, particularly in Cameroon[1]. The MDR-TB prevalence in the country was 1.4% among new and 8.3% among retreatment cases in 2022[14].

The roll-out of GeneXpert by the World Health Organization (WHO) as one rapid molecular diagnostic tool for TB and RR-TB[15] has improved the diagnosis of MDR-TB worldwide[16]. In many countries, laboratories are unable to analyze drug resistance and the clinical predictors of MDR-TB[16]. In Cam-

eroon, there are limited data available on the risk factors of MDR-TB[12], a gap this study seeks to address by determining the prevalence and RR among suspected cases of pulmonary TB patients attending the District Hospital of Kumba.

PATIENTS AND METHODS

Study Design and Area

This was a cross-sectional hospital-based study conducted from April 12 to June 30, 2022. All consenting patients (in-patients and out-patients) clinically suspected to have pulmonary TB (cough/hemoptysis, etc.) and who sought care at the District Hospital of Kumba, a tertiary (fourth category) health care facility located in the capital metropolitan city of the Meme department, Southwest Region of Cameroon, were enrolled in this study. Data were collected *via* a semi-structured questionnaire. Authorization was obtained from the Regional Delegation of Public Health of the Southwest Region (R11/MINSANTE/SWR/RDPH/PS/416/421 on 02/4/2022). Patients who withdrew their consent later were thus excluded from the study. The minimum sample size of 45 was obtained based on a prevalence of 3% from a previous study in the southwest region[17] and using the Cochran's formula for a cross-sectional study.

Sample Collection and Laboratory Analysis

Sputum samples were collected from enrolled patients and subjected to laboratory analysis, including microscopy using Ziehl-Neelsen staining for Acid Fast Bacilli (AFB) detection and GeneXpert MTB/RIF Ultra (Cepheid, Maurens-Scopont, France). GeneXpert is a molecular test used to diagnose TB by detecting the presence of *Mycobacterium tuberculosis*, as well as RR, in 80 minutes.

Statistical Analysis

Data was copied into Microsoft Excel and analyzed using Statistical Package for Social Sciences (SPSS), version 21.0 (IBM Corp., Armonk, NY, USA). Univariate analysis was performed with Fisher's Exact test. Statistical significance was set at $p < 0.05$ with a 95% confidence interval.

RESULTS

Socio-Demographic Characteristics of Study Participants

One hundred and fifty-three pulmonary TB (no extrapulmonary case) patients aged 4 to 75 years were included in this study. The mean age of the study population was 35.56 years (± 16.38 standard deviation). The participants in this study were categorized according to their socio-demographic characteristics as shown in Table 1. The age ranges of the153 participants were ≤ 20, 21-30, 31-40, 41- 50 and > 50 years, representing 20.9% (n = 32), 24.8% (n = 38), 15.7% (n = 24), 22.2% (n = 34), and 16.3% (n = 25) of the study participants, respectively. The majority (52.3%, n = 80) of the participants were males. Concerning the educational level, a higher proportion (57.5%, 88/153) of the subjects had achieved tertiary education (at the University level), while 7.2% (11/153) of them had achieved primary education. The majority (45.8%, n = 70) of participants were employed.

Also, 50.3% (77/153) of the participants were single, 41.8% (64/153) were married, and 7.8% (12/153) were divorced. The majority of participants knew their HIV status, with 9.8% (15/153) being HIV positive, and 2% (3/153) were diabetic (Table 1).

Prevalence of Pulmonary and RR Tuberculosis

A total of 153 AFB sputum smears were carried out for each of the study participants. 20.9% (32/153) of the subjects were found positive using microscopic analysis, 24.18% (37/53) using GeneXpert MTB/RIF Ultra, and 1.96% (3/153) were positive for RR (Figure 1).

Measure of Validity of Microscopy Compared to Xpert MTB/RIF Ultra

Of the 153 sampled individuals, 20.9% (32/153) were found positive using both microscopy and Xpert MTB/RIF Ultra, while 3.3% (5/153) were only positive using Xpert MTB/RIF Ultra. All 116 (75.8%) negative samples were negative for both microscopy and Xpert MTB/RIF Ultra. The comparison between the microscopy and Xpert MTB/RIF Ultra showed a sensitivity of 86.49% (95% CI: 71.23% - 95.46%) and a specificity of 100% (95% CI: 96.87% - 100%), as shown in Table 2.

Demographic Factors Associated with RR-TB Among Suspected Patients

The highest prevalence rate of RR of 5.3% (2/38) was among patients aged 21-30 years, while the lowest (0%) was recorded in patients aged ≤ 20, 31- 40, and ≥ 50 years. There was no significant association between age group and RR ($p = 0.524$). Based on their HIV status, the highest prevalence rate (2.4%, 3/125) was observed among HIV negative patients, and none (0%) among those who either did not know their HIV status or were HIV positive. However, there was no significant association between HIV status and RR ($p > 0.999$).

Higher prevalence rates were also observed among subjects who lived alone and those with 4 members in their family. While there was a significant association between family size and RR ($p = 0.002$), gender ($p = 0.606$), civil status ($p = 0.680$), level of education ($p = 0.875$), occupation ($p = 0.754$), and diabetic status ($p > 0.999$) did not significantly impact the likelihood of having RR infection. (Table 3).

Table 1. Socio-demographic characteristics of study participants.

Characteristics	Total (n = 153) Participants (n)	Percentage (%)
Age-group	Mean ± SD (35.56 ± 16.38)	
≤ 20	32	20.9
21-30	38	24.8
31-40	24	15.7
41-50	34	22.2
> 50	25	16.3
Gender		
Male	80	52.3
Female	73	47.7
Civil status		
Divorced	12	7.8
Married	64	41.8
Single	77	50.3
Level of education		
Primary	11	7.2
Secondary	54	35.3
University	88	57.5
Occupation		
Business	23	15.0
Employed	70	45.8
Pupil	9	5.9
Retired	4	2.6
Student	36	23.5
Unemployed	11	7.2
HIV status		
Negative	125	81.7
Positive	15	9.8
Unknown	13	8.5
Diabetic status		
Negative	103	67.3
Positive	3	2.0
Unknown	47	30.7
Family size		
1	1	0.6
2	18	11.8
3	48	31.4
4	34	22.2
5	35	22.9
> 5	17	11.1

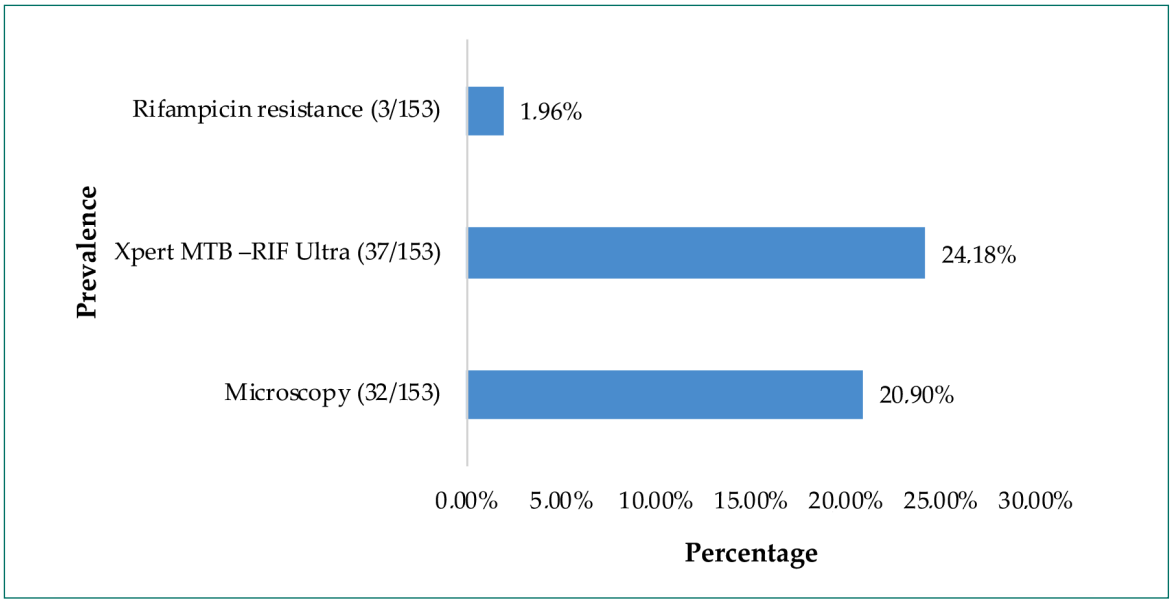

Figure 1. Prevalence of *Mycobacterium tuberculosis* and RR in suspected TB individuals in the District Hospital of Kumba.

Table 2. Sensitivity and specificity of microscopy compared to Xpert MTB/RIF Ultra.

		XPERT MTB-RIF Ultra		
		POS	**NEG**	**Total**
Microscopy	POS	32	0	32
	NEG	5	116	121
Total		37	116	153

POS. Positive; NEG. Negative.

Risk Factors Associated with RR Infection in TB-Suspected Patients

Among the 153 study participants, 133 were newly diagnosed with TB, 97 had less than 3 rooms in their house, 52 had a history of contact with a TB patient, 27 had a history of mental illness, 20 were smokers, 19 had history of alcohol consumption, 11 had no knowledge about TB, 5 had previously been treated for TB, 2 had a history of imprisonment, and one had a history of drug resistance.

In relation to knowledge about TB, the highest prevalence rate of RR of 2.1% (3/142) was among patients having knowledge about TB, while the lowest (0%) was recorded in subjects with no knowledge about the disease. There was no significant association between knowledge about TB and RR ($p = 0.626$). Based on their history of contact with a TB patient, a higher prevalence rate of 5.8% (3/52) was observed among patients who were in contact with a TB patient, while those with no contact with TB patients had the lowest (0%). A positive association was observed between the history of contact with a TB patient and RR ($p = 0.038$).

There was a significant association between previously diagnosed TB patients (10%, 2/20) and RR ($p = 0.045$; OR = 14.67; 95% CI: 1.27 - 170.05) compared to newly diagnosed ones (0.8%, 1/133). The number of rooms in the house ($p = 0.393$), history of drug resistance ($p > 0.999$), history of alcohol consumption ($p > 0.999$), history of imprisonment ($p > 0.999$), history of previous TB treatment ($p > 0.999$), and history of mental illness ($p = 0.444$; OR = 2.38; 95% CI: 0.21 - 27.29) on the other hand, did not significantly impact the likelihood of having RR infection (Table 4).

DISCUSSION

Although TB remains one of the most difficult infections to treat, the current Direct Observed Treatment Short regimen, which usually takes 6 to 9 months of continuous chemotherapy, is quite effective in the treatment of drug-susceptible TB[18]. Mutations in genes targeted by antibiotics have contributed to the evolution of *M. tuberculosis* strains that are resistant to the currently available first-line antimycobacterial drugs used against TB[18]. This study aimed to assess the prevalence and risk factors associated with RR-TB among suspected cases of pulmonary TB attending the district hospital in Kumba.

In this study, 20.9% of the participants had a positive acid-fast bacilli smear, which is higher than that found by Amare et al[19] in Ethiopia (6.2%) and Zaman et al[20] in Bangladesh (1.5%). This may be due to differences in the study population, study design, or sample size.

The prevalence of RR using the GeneXpert Ultra test in our study population was 1.96%. Since the endorsement of GeneXpert by the WHO, several studies[21] using this diagnostic technology have been conducted in various WHO TB-endemic areas. Our finding was similar to that reported by Noeske et al[22] in the Littoral region of Cameroon (1.3%), but lower than that of Kouemo et al[23] (6.7% in 2021) and Pokam et al[24] (8.8% in 2020) in Cameroon, and Ossoga[25] in Chad (12.88% in 2015). Other recent studies in the Adamawa (2025)[26] and Littoral (2021)[27] regions of Cameroon in similar hospital settings to ours have shown a RR prevalence of 4.7% and 4.4%, respectively. This discrepancy is perceivable since it has been demonstrated that Xpert MTB/RIF Ultra enabled more TB patients to be detected and treated earlier as compared to smear microscopy[28]. In other words, Xpert MTB/RIF Ultra has a higher sensitivity than smear microscopy[29]. In our current context, the precise and early detection of TB is necessary to enhance case management and prevent TB transmission[30].

The prevalence of RR was the highest (5.3%) among patients aged 21-30 years. This could be explained by the fact that: (i) there is a higher TB risk related to their occupation, exposing them to mycobacterial particles since individuals in this age group are much more active, and (ii) they are more likely to move from another area, due to their occupation including business and other types of employment, as well as tourism or studies, resulting in a higher risk of treatment interruption[31]. However, Kouemo et al[23] (2020) reported a higher prevalence (20%) of RR among patients aged 21-30 years old. This is due to the poor TB management during the onset of the COVID-19 epidemic in Cameroon[1].

Although the prevalence of RR was higher in females than in males, the difference was not statistically significant. This finding is in contrast

Table 3. Demographic factors and RR infection in suspected TB patients.

Factor	Number of examined	No resistance n = 150 (%)	RR n = 3 (%)	*p*-value
Age-group				
≤ 20	32	32 (100)	0 (0)	
21-30	38	36 (94.7)	2 (5.3)	
31-40	24	24 (100)	0 (0)	0.524
41-50	34	33 (97.1)	1 (2.9)	
> 50	25	25 (100)	0 (0)	
Gender				
Male	80	79 (98.8)	1 (1.3)	0.606
Female	73	72 (97.3)	2 (2.7)	
Civil status				
Divorced	12	12 (100)	0 (0)	0.680
Married	64	62 (96.9)	2 (3.1)	
Single	77	76 (98.7)	1 (1.3)	
Level of education				
Primary	11	11 (100)	0 (0)	
Secondary	54	53 (98.1)	1 (1.9)	0.875
University	88	86 (97.7)	2 (2.3)	
Occupation				
Business	23	22 (95.7)	1 (4.3)	
Employed	70	68 (97.1)	2 (2.9)	
Pupil	9	9 (100)	0 (0)	0.754
Retired	4	4 (100)	0 (0)	
Student	36	36 (100)	0 (0)	
Unemployed	11	11 (100)	0 (0)	
Family size				
1	1	0 (0)	1 (100)	
2	18	18 (100)	0 (0)	**0.002**
3	48	48 (100)	0 (0)	
4	34	32 (94.1)	2 (5.9)	
5	35	35 (100)	0 (0)	
> 5	17	17 (100)	0 (0)	
HIV status				
Negative	125	122 (97.6)	3 (2.4)	
Positive	15	15 (100)	0 (0)	> 0.999
Unknown	13	13 (100)	0 (0)	
Diabetes status				
Negative	103	101 (98.1)	2 (1.9)	
Positive	3	3 (100)	0 (0)	> 0.999
Unknown	47	46 (97.9)	1 (2.1)	

to the results of Masenga et al[32] (2017) in Zambia and Kouemo et al[23] (2020) in Cameroon. Patients' previous status (being previously diagnosed and treated for TB) significantly impacted their likelihood of having RR. This is particularly due to poor adherence to the drugs consumed during their previous treatment of TB. This finding is similar to an earlier report in the country by Kouemo et al[23] in 2021.

Although few studies[33] have reported an association between HIV infection and the development of drug resistance, our study did not find any. This lack of association between HIV infection status and RR is consistent with the results of earlier studies in Addis Ababa[34,35] and other sub-Saharan African countries[36]. Although Cameroon is among the top 30 TB/HIV high-burden countries[5], the lack of an association between TB and HIV could be due to the low number of known HIV positive cases included in the study, which reduces the power of statistical tests.

RR was significantly associated with having contact with a confirmed TB patient. This finding is similar to that of Meriki et al[37] in the Northwest and Southwest regions of Cameroon, and that of Mbuh et al[27] in the Littoral region of Cameroon. Due to the consistency of this latter association, it should be seriously considered during interventions for the mitigation of resistant TB among close contacts of TB patients.

Factors like history of smoking, drug resistance, alcohol consumption, being in prison, and mental illness were not significantly associated with RR infection. These could be explained by the fact that a limited number of patients were exposed to these factors. Even though the District Hospital of Kumba is a referral hospital, not every suspected case of TB is followed in this healthcare facility, leading to false negative associations. For example, this may have led to the underestimation of RR-TB among participants consuming alcohol.

Table 4. Risk factors associated with RR infection in suspected TB patients.

Factor	Number examined	No resistance n = 150 (%)	RR n = 3 (%)	OR (95% CI)	*p*-value
Knowledge about TB					
No	11	11 (100)	0 (0)		0.626
Yes	142	139 (97.9)	3 (2.1)		
Number of rooms in the house					
1	26	25 (96.2)	1 (3.8)		
2	71	70 (98a.6)	1 (1.4)		0.393
3	36	36 (100)	0 (0)		
4	13	12 (92.3)	1 (7.7)		
>4	7	7 (100)	0 (0.0)		
History of contact with TB					
No	101	101 (0)	0 (0)		**0.038**
Yes	52	49 (94.2)	3 (5.8)		
Newly diagnosed TB				14.67 (1.27-170.05)	
No	20	18 (90.0)	2 (10.0)		**0.045**
Yes	133	132 (99.2)	1 (0.8)		
History of smoking					
No	133	130 (97.7)	3 (2.3)		> 0.999
Yes	20	20 (100)	0 (0)		
History of drug resistance					
No	152	149 (98.0)	3 (2.0)		> 0.999
Yes	1	1 (100)	0 (0)		
History of alcohol consumption					
No	134	131 (97.8)	3 (2.2)		> 0.999
Yes	19	19 (100.0)	0 (0)		
History of imprisonment					
No	151	148 (98.0)	3 (2.0)		> 0.999
Yes	2	2 (100)	0 (0)		
History of previous treatment of TB					
No	148	145 (98.0)	3 (2.0)		> 0.999
Yes	5	5 (100)	0 (0)		
History of mental illness				2.38 (0.21-27.29)	
No	126	124 (98.4)	2 (1.6)		0.444
Yes	27	26 (96.3)	1 (3.7)		

OR. Odds Ratio; CI. Confidence Interval.

Limitations

This study has several limitations. The study was conducted among TB patients seeking treatment at only one hospital (health centers and private clinics were not included); therefore, the data obtained may not be representative of all TB patients in Kumba. Moreover, since only patients seeking care at a tertiary hospital were included, results may over-represent individuals with more severe disease or with access to referral care, underestimating or overestimating prevalence compared to the community. Considering the cross-sectional nature of our study, several outcomes, such as treatment completion, failure, relapse, or mortality, could not be assessed, nor can we establish temporality between risk factors and resistance. The associations between family size as well as previous TB history of patients with RR observed in this study must be interpreted with caution, considering the small numbers of RR cases. Data on HIV status, diabetes, smoking, alcohol use, and other risk factors were partly based on patient records or self-reported questionnaire data, which may be incomplete or inaccurate. Other predictors of resistance [e.g., CD4 count, viral load, anti-retroviral therapy (ART) adherence in HIV-infected patients, or detailed prior treatment history] were not available and could not be analyzed. This reduces the ability to fully explore associations with resistance. The small sample size might have equally influenced the estimation of the anti-TB drug resistance proportion in the study site. Despite the lack of isoniazid resistance testing in our study to comply with the MDR definition, it has been shown that up to 90% of RR-TB cases are isoniazid resistant, and RR-TB is often used as a surrogate marker for MDR-TB in countries with limited resources for MDR-TB detection[38]. The current pilot study, however, provides important data regarding RR in an urban hospital setting and could be used by local authorities to initiate appropriate interventions. Lastly, the use of Fisher's exact test for comparison reduces the power of the calculations, since it is more subject to type 2 random error (failing to observe an association, when an association exists). This can be overcome by increasing the size of the study population.

CONCLUSIONS

The overall RR-TB prevalence in this study population was 1.6%, which is comparable to a previous study carried out in another region of Cameroon. However, despite the low prevalence, this study demonstrates the danger of increasing resistance in this area, as shown by the high rate of positive TB smears. It also showed that previous household contact and the previous status of patients were independently associated with the occurrence of RR. However, these associations should be interpreted with caution, considering the low number of RR cases. Monitoring TB contacts in the study area is critical to prevent the dissemination of drug resistance. Prompt management of RR-TB with the 9-11 month standardized shorter treatment regimen is advocated to prevent the dissemination of these resistant strains in their respective communities. Further operational and extensive studies should be conducted in this region and other parts of the country over a longer period to provide more insight into the contribution of different risk factors to the development of drug-resistant TB.

Conflicts of Interest:
The authors declare that they have no conflicts of interest to disclose.

Ethics Approval:
This study was conducted following the Declaration of Helsinki of 1975 (as revised in 2013), and the protocol was reviewed and approved by the Institutional Review Board of the Regional Delegation of Public Health of the Southwest Region (R11/MINSANTE/SWR/RDPH/PS/416/421 on 02/4/2022).

Informed Consent:
All subjects provided written informed consent for inclusion before they participated in the study and for permission to publish this report. Also, participant confidentiality was observed.

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Data Availability:
The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

AI Disclosure:
Authors declare that no form of generative artificial intelligence was used in the writing of this article.

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