Student's Journal of Health Research Africa
e-ISSN: 2709-9997, p-ISSN: 3006-1059
Vol.6 No. 9 (2025): September 2025 Issue
https://doi.org/10.51168/sjhrafrica.v6i9.1891
Original Article

The prevalence of multidrug-resistant Mycobacterium tuberculosis in patients diagnosed with pulmonary TB at Ngwelezana Hospital. A quantitative cross-sectional retrospective.

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Abstract

Introduction

Multidrug-resistant tuberculosis (MDR-TB), which is TB resistant to critical anti-TB drugs such as isoniazid and rifampicin, presents a major challenge to tuberculosis treatment, leading to significant public health concerns. The study objective is to evaluate the patterns of antibiotic drug resistance of MTB in patients taking treatment for TB and determine the demographic profile of patients in whom drug-resistant TB is the most prevalent.

Aim

This research aims to determine the prevalence of drug resistance in patients with *Mycobacterium tuberculosis* using retrospective data.

Methodology

This study aimed to evaluate the prevalence of multidrug-resistant tuberculosis by analyzing retrospective data from the National Health Laboratory Services for patients diagnosed at Ngwelezana Hospital, KwaZulu-Natal. A sample of 695 participants was included in the study, with 267 (38.4%) males and 428 (61.6%) females. Among these participant's demographic characteristics of patients tested for TB were evaluated, and an analysis was done to explore the rifampicin susceptibility.

Findings

The study correlation analysis revealed a weak but statistically significant positive correlation between age and gender (ρ = 0.077, p = 0.022). Rifampicin resistance was detected in 5.3% of the tested participants, while 94.7% were found to be sensitive to the drug. The relatively low prevalence of rifampicin resistance is a positive finding, suggesting that first-line TB treatment with rifampicin remains largely effective in this population. However, the presence of any resistance is still a cause for concern, as rifampicin-resistant TB can lead to more complex and expensive treatment regimens, with higher morbidity and mortality rates.

Conclusion

The study's findings indicate the low prevalence of rifampicin resistance, but continuous monitoring is necessary to prevent the spread of resistant strains. Future research should include exploring track changes in rifampicin resistance over time and investigating potential contributors to its resistance.

Keywords: *Multidrug-resistant tuberculosis, TB prevalence, drug resistance, epidemiology of TB.*
Submitted: *2025-06-26* ***Accepted:*** *2025-07-24* ***Published:*** *2025-09-08*

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Background

Tuberculosis (TB) remains a significant contributor to illness and death worldwide, particularly in regions with limited access to healthcare resources. It's caused by the bacterium *Mycobacterium tuberculosis* (MTB). TB can affect various parts of the body, with pulmonary TB being the most common form.

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Epidemiology and pathogenesis

TB is an airborne disease transmitted through droplets released when an infected person coughs or sneezes. The global burden of TB is substantial, with approximately 10 million individuals developing the disease annually. In South Africa, TB is a major public health concern, exacerbated by factors such as poverty, overcrowding, and limited access to healthcare.

Multidrug-resistant tuberculosis: worldwide relevance

The emergence of drug-resistant strains, including multidrug-resistant TB (MDR-TB) and extensively drug-resistant TB(XDR-TB), poses a significant challenge to global health security. MDR-TB is resistant to at least rifampicin and isoniazid, making treatment more complex and expensive. This highlights the need for improved diagnosis, treatment protocols, and intensified efforts to prevent its emergence and spread.

Impact of TB and MDR-TB

The impact of TB and MDR-TB extends beyond physical health, with significant economic, social, and psychological dimensions. Patients with MDR-TB often experience prolonged periods of illness, financial struggle, and social stigma, which can lead to feelings of isolation and hopelessness. The economic burden on individuals, families, and communities is substantial.

Current efforts and challenges

South Africa has made notable advancements in improving TB control, including the implementation of effective interventions and a comprehensive TB screening program. However, challenges persist, particularly in addressing the needs of the underprivileged communities with limited access to healthcare services.

The approach for ending MDR-TB

To combat TB and MDR-TB effectively, a comprehensive approach is necessary. This includes investing in healthcare infrastructure, enhancing community engagement, promoting awareness about TB, and improving the health and well-being of affected individuals and communities.

Research Methodology

Study Design

A quantitative, cross-sectional retrospective study was conducted to determine the prevalence of drug-resistant *Mycobacterium tuberculosis* in patients diagnosed with pulmonary TB at Ngwelezana Hospital.

Study setting and population

A retrospective study was carried out to assess the prevalence of multidrug-resistant *Mycobacterium tuberculosis* among patients diagnosed with pulmonary TB at Ngwelezana Hospital, which is a regional referral hospital located in Empangeni, in the northern region of KwaZulu-Natal province, South Africa. Covering the period from January to June 2021. The study population in this research project comprised both males and females whose sputum samples tested positive for *Mycobacterium tuberculosis* and showed resistance to at least isoniazid and rifampicin. The resistance of *Mycobacterium tuberculosis* to this set of drugs is categorized as MDR.

Inclusion criteria

This study included male and female patients diagnosed with pulmonary tuberculosis at Ngwelezana Hospital between January and June 2021, whose sputum samples tested positive for *Mycobacterium tuberculosis* and showed resistance to at least isoniazid and rifampicin, meeting the definition of multidrug-resistant tuberculosis (MDR-TB).

Exclusion criteria

Patients were excluded if they tested negative for *M. tuberculosis*, had drug-susceptible TB, were diagnosed outside the study period, or had incomplete laboratory data in the NHLS(AARMS) database.

Effort to address a potential source of bias

To ensure the reliability and validity of the study, several measures were implemented to reduce bias:

A diverse and representative sample of patients with pulmonary TB was selected.

Standard operating procedures and tests were used to ensure consistent and reliable results.

Other factors such as age, sex, and previous TB treatment should be considered to ensure that the results accurately reflect the relationship between the MDR-TB and the variable of interest.

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Sampling and sample size

The study population involved 695 =N patients, both males and females, who were tested for *Mycobacterium tuberculosis* within 6 6-month period. The sample size included all patients diagnosed with MDR-TB, and the data obtained were analyzed using descriptive statistics**.**

Data collection

Patients' age, gender, and drug (rifampicin) susceptibility were considered, while data were requested from the National Health Laboratory Services (AARMS)
Patient sputum samples were collected and tested for Mycobacterium tuberculosis as per Standard Operating Procedures outlined below**:**

Sample Preparation

The sputum samples collected from the patient were assessed macroscopically, ensuring they were mucoid and at least 1ml.
If all samples have low volume in terms of laboratory protocol, a second sputum sample should be requested.
A Sample reagent (SR) buffer was added to the sputum specimen in a 2:1 ratio (sample reagent: specimen).
The lid of the container should be closed tightly and mixed vigorously 10-20 times or vortexed for at least 10 seconds. The sputum sample should then be incubated at room temperature for at least 5 minutes.
Each specimen should be prepared separately to prevent cross-contamination.

Adding Specimen to Xpert MTB/RIF Ultra Cartridge

After 15 minutes, the Xpert cartridge should be labelled with the sample ID,
Using a pipette, the liquefied specimen (>2ml) should be aspirated and transferred into the cartridge port. The test should be initiated within 4 hours of adding the sample. Each cartridge should be prepared separately to avoid cross-contamination.

Running Assay on GeneXpert analyser

The GeneXpert instrument should be initiated by logging onto the computer and opening GeneXpert Dx software. Tests should then be created by scanning the sample, cartridge barcodes, filling in the necessary information, and adding optional notes. The cartridges should then be loaded, and the test should be initiated.

Quality Control (QC)

The GeneXpert should automatically conduct the Internal Quality Control (IQC) for each sample. During the testing, the system should utilize one or more controls. The sample processing control (SPC) should consist of non-infectious spores included in each cartridge. Its purpose is to verify the adequate processing of MTB, ensure lysis of the MTB organism if present, confirm sufficient specimen processing, and monitor the presence of PCR inhibitors.
In a negative sample, the SPC should yield positive results. In a positive sample, the SPC should yield either positive or negative results. If the SPC is not detected in a negative test, the results should be deemed invalid. The SPC should pass if it meets the specified criteria. Additionally, the Probe Check Control (PCC) is conducted before starting the PCR reaction, and if it meets the assigned criteria.

Statistical analysis

Descriptive statistical and inferential methods were employed to generate summary statistics for the data obtained. The pie charts were used to determine demographics in terms of age and gender, in which MDR-TB is most prevalent. Spearman's rho correlation analysis was done to determine the statistical significance of drug resistance in patients with *Mycobacterium tuberculosis* by calculating a p-value. Percentages, frequencies for prevalence occurrences were summarized using a bar graph and a table. The prevalence was determined by calculating the percentage of individuals affected by MDR TB using the following calculation:

$$\text{The prevalence of MDR (\%)} = \frac{\text{Number of all patients diagnosed with MDR-TB}}{\text{Number of all patients tested}} \times 100\%$$

Ethical considerations

Approval to conduct this study was granted by the Research Ethics Committee of Mangosuthu University of Technology on 15 December 2023. Approval to gather the data was secured from Academic Affairs and the Research Management System (AARMS) of the National Health Laboratory Services (NHLS). All the data of patients had been gathered and did not present any risks to patients. Patient data was protected with a password, and all patient-identifying information was removed to keep the patient's identity anonymous.

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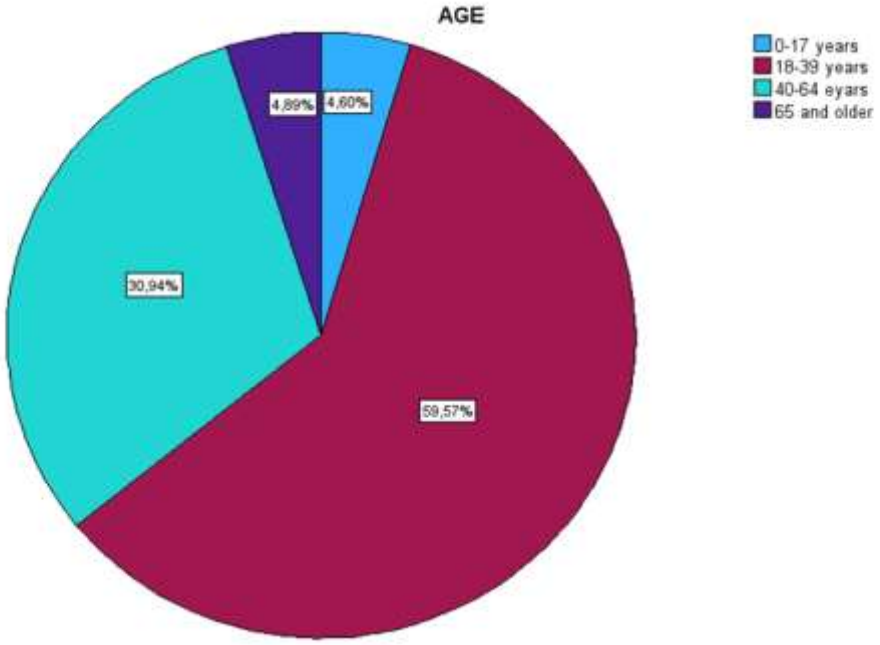
Research Results/ findings

Frequency of Tuberculosis in tested patients by age and gender

Figure 1: Pie chart showing the distribution of TB in tested patients by age.



The age distribution of the study sample consisted of 695 participants. Of these, 32 individuals (4.6%) were aged 0-17 years. The largest age group, representing 414 participants (59.57%), fell within the range of 18-39 years, accounting for a cumulative total of 64.2% when combined with the younger group. The 40- 64 years' age group included 215 participants, making up 30.94% of the sample, bringing the cumulative total to 95.1%. Finally, 34 participants (4.89%) were aged 65 and older. This distribution provided a comprehensive overview of the age composition of the participants, with all age groups represented.

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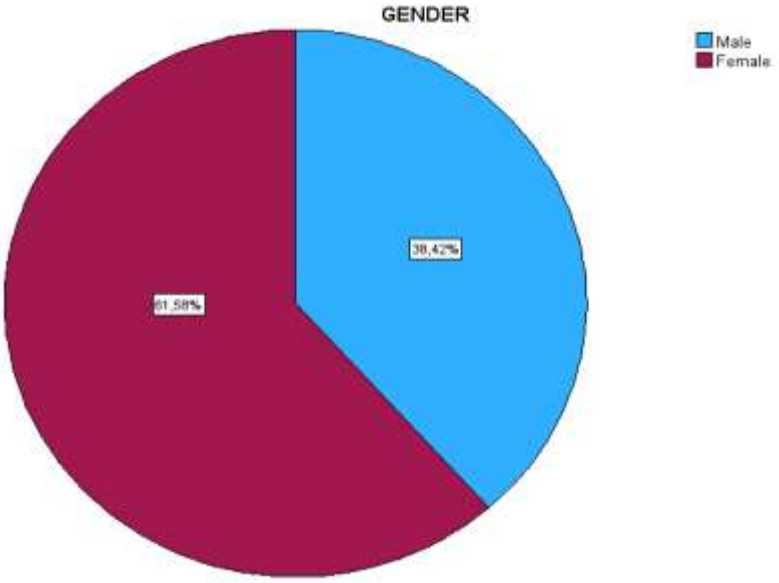
Figure 2: Pie chart showing the distribution of TB in tested patients by gender.



On the other hand, the gender distribution of the study sample showed that out of 695 participants, 267 (38.42%) were male and 428 (61.58%) were female. This indicated that females made up the majority of the participants. The cumulative percent for males was 38.4%, while for females, it reached 100%, meaning that both genders were fully accounted for within the sample. This breakdown provided a clear view of the gender composition within the study.

Sensitivity of Rifampicin in tested patients

The rifampicin susceptibility results for the study sample indicate that out of 695 participants, 37 (5.3%) showed resistance to rifampicin, while the majority, 658 participants (94.7%), were sensitive to the antibiotic. The overall prevalence of MTB (which is resistant to rifampicin) was calculated using the following equation:

$$\text{The prevalence of MDR-TB (\%)} = \frac{\text{Number of all patients diagnosed with MDR}}{\text{Number of all patients tested}} \times 100\%$$

The cumulative percentage for resistance was 5.3%, and for sensitivity, it reached 100%, meaning all participants have been accounted for in this analysis. This data revealed that rifampicin resistance was relatively low among the sample.

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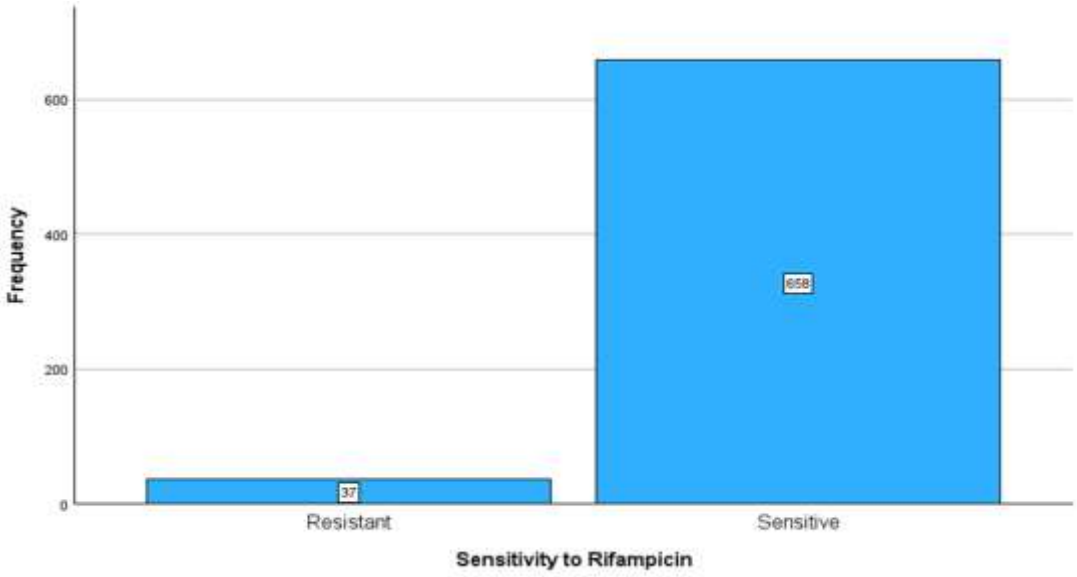
Figure 3: Bar graph showing the sensitivity of rifampicin in tested patients.



Inferential Analysis

Table 1: showing the distribution of rifampicin sensitivity by age and gender.

GENDER			Rifampicin		
			Resistant	Sensitive	Total
Male	*AGE*	0-17 years	0	15	15
		18-39 years	10	160	170
		40-64 years	1	65	66
		65 and older	0	16	16
	Total		11	256	267
Female	*AGE*	0-17 years	1	16	17
		18-39 years	13	231	244
		40-64 years	11	138	149
		65 and older	1	17	18
	Total		26	402	428

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Total	*AGE*	0-17 years	1	31	32
		18-39 years	23	391	414
		40-64 years	12	203	215
		65 and older	1	33	34
	Total		37	658	695

The table summarizes rifampicin resistance across different age and gender groups. Among males, 267 cases were analyzed, with 11 showing resistance to rifampicin. Age-wise, no resistance was observed in males under 18 years (0 out of 15), while the highest resistance was noted for those aged 18–39 (10 out of 170 cases). For males aged 40–64, only 1 out of 66 cases was resistant, and none in the 65 and older category showed resistance.

For females, there were 428 cases, with 26 showing resistance. Resistance was observed in 1 out of 17 cases among those aged 0–17 years. In the 18–39 age group, 13 out of 244 cases showed resistance, followed by 11 resistant cases out of 149 in the 40–64 age group, and 1 out of 18 cases in those 65 and older.

Overall, research found that 37 out of 695 were resistant to rifampicin. The 18-39 age group showed the highest resistance with (23 out of 414 cases), followed by the 40–64 age group (12 out of 215 cases), and minimal resistance occurred among those aged 0–17 and those aged 65 and older.

This pattern suggests higher rifampicin resistance in younger adult populations, particularly among females in the 18–39 and 40–64 age brackets. Thus, the main objective of the study was to establish the prevalence of MDR-TB in patients from January 2021 to June 2021 in Ngwelezana Hospital. The findings revealed that the resistance rate was 5.3%, indicating that rifampicin resistance was relatively low within the sample.

Table 2: Showing the correlation between rifampicin sensitivity and demographics (age and gender).

			AGE	GENDER	RMP
Spearman's rho	AGE	Correlation Coefficient	1,000	,077*	,001
		Sig. (1-tailed)	.	,022	,491
		N	695	695	695
	GENDER	Correlation Coefficient	,077*	1,000	-,042
		Sig. (1-tailed)	,022	.	,132
		N	695	695	695
	RIFAMPICIN	Correlation Coefficient	,001	-,042	1,000
		Sig. (1-tailed)	,491	,132	.
		N	695	695	695

The correlation between age and rifampicin was virtually non-existent (correlation coefficient of 0.001). The p-value was 0.491 (greater than 0.05), meaning this correlation was not statistically significant.

There was a very weak negative correlation between gender and rifampicin (correlation coefficient of -0.042). The p-value was 0.132 (greater than 0.05), indicating that this correlation was not statistically significant.

Discussion and analysis of findings

Demographics of the study population

The demographic analysis found that the majority of participants (59.6%) were between the ages of 18 and 39, indicating that TB is more common among younger and middle-aged people in the research population. This conclusion is consistent with worldwide tuberculosis epidemiology, which shows that working-age individuals are more likely to be afflicted due to increased exposure risks and mobility (Rickman et al., 2022). The remaining age

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Original Article

groups, 0 to 17 years (4.6%), 40 to 64 years (30.9%), and 65 and older (4.9%), provided important insights into the TB burden across all ages, but their lower representation suggests that the younger and older extremes are less affected in this population, possibly due to differences in risk factors or healthcare access.

In terms of gender, females made up the bulk of the sample (61.6%). This contrasts with worldwide TB trends, which frequently disproportionately afflict men (Wong, 2021). The increased female representation in this study may reflect disparities in healthcare-seeking behavior, with women being more likely to seek tests or having better access to health facilities. It might also reflect a distinct epidemiological pattern in the studied location, requiring more inquiry into gender-specific risk factors or socioeconomic determinants of health that may contribute to this distribution.

Rifampicin resistance

Rifampicin resistance was detected in 5.3% of the tested participants, while 94.7% were found to be sensitive to the drug. The relatively low prevalence of rifampicin resistance is a positive finding, suggesting that first-line TB treatment with rifampicin remains largely effective in this population. However, the presence of any resistance is still a cause for concern, as rifampicin-resistant TB can lead to more complex and expensive treatment regimens, with higher morbidity and mortality rates. Given the growing threat of drug-resistant TB globally, continuous surveillance of antibiotic resistance patterns is crucial to ensuring effective TB control in the region (Farhat, 2024).

Correlation analysis

The correlation analysis using Spearman's rho demonstrated a weak but statistically significant positive correlation between age and gender ($\rho = 0.077$, $p = 0.022$). This suggests a slight demographic difference between males and females across age groups, which aligns with results from previous studies that have identified gender-specific age patterns in the TB prevalence.

No significant association with age and rifampicin resistance ($\rho = 0.001$, $p = 0.491$), aligning with other research that shows rifampicin resistance does not appear to be age dependent. Similarly, a study by Zurcher et al. (2019) found no significant correlation between age and drug resistance in their cohort, suggesting that age is not a reliable predictor of rifampicin susceptibility. The lack of a significant link between gender and rifampicin resistance ($\rho = -0.042$, $p = 0.132$) is consistent with findings from studies conducted in other high TB-burden settings. For instance, a review by Araya et al. (2020) reported that gender did not significantly influence the prevalence of drug-resistant TB, indicating that both males and females are equally affected by rifampicin resistance. These results suggest that rifampicin resistance in this population is independent of both age and gender, underscoring the need for universal treatment protocols. Hence, one of the objectives of the study is to evaluate the patterns of antibiotic drug resistance in MTB patients undergoing treatment for TB.

Generalizability of the study

The results of this study on rifampicin resistance in *Mycobacterium tuberculosis* at Ngwelezana Hospital can be applied to similar settings, but with some caution. The study has a good sample size of 695 patients, and the standardized, reliable laboratory procedures, such as GeneXpert MTB/RIF ultra assay, were used.

Limitations

The study was done at one hospital, which may limit the generalizability of the findings to other settings. However, the hospital's status as a reference laboratory for culture and susceptibility testing in KwaZulu-Natal lends credibility to the results.

Limited geographic scope: the study was conducted in a specific region of South Africa, which may not be representative of other regions or countries. However, the high prevalence of HIV and TB co-infections in KwaZulu-Natal makes the findings relevant to similar settings.

Potential for variation in rifampicin resistance: other studies have shown different rates of rifampicin resistance in different groups of people

Conclusion and recommendations

The primary aim of this research was to evaluate the prevalence of drug resistance in patients with *Mycobacterium tuberculosis* in a population from Ngwelezana Hospital from January to June 2021. This research aimed to evaluate the patterns of antibiotic drug resistance of MTB in patients taking treatment for TB and evaluate such patterns among demographic profiles. Rifampicin resistance was detected in 5.3% of the tested participants, while 94.7% were found to be sensitive to the drug. The relatively low prevalence of rifampicin resistance is a positive finding, suggesting that first-line TB treatment with rifampicin remains largely effective in this population. The correlation analysis revealed a weak but statistically

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significant positive correlation between age and gender ($\rho = 0.077$, $p = 0.022$). However, no significant relationship was found between age and rifampicin resistance ($\rho = 0.001$, $p = 0.491$). These results suggest that rifampicin resistance in this population is independent of both age and gender, underscoring the need for universal treatment protocols. In conclusion, the results of this study demonstrated the prevalence of MDR-TB amongst different demographics. In this study, a low rate of rifampicin resistance was found, which is suggestive that MDR-TB is not widespread in some areas. This underscores the need to continue with early monitoring and detection of MDR-TB, as well as taking into consideration that effective treatment strategies and recommendations can play a role in decreasing the distribution of MDR-TB.

The Limitations of the Study

The study included the data collected within six months. This allowed assessment of the MDR-TB prevalence during the period of data collection, which made it difficult to establish trends over time. The study may be limited by not considering all underlying mechanisms of drug resistance, possibly missing other factors that contribute to the prevalence of MDR-TB that may require special attention.

Recommendations

1. Improve Infection Control Programs:
Improve monitoring mechanisms to track rifampicin resistance frequently and stop resistant TB strains from spreading throughout the population.
2. Targeted Awareness Campaigns:
Create efforts to raise awareness and prevent TB among young people, as they make up the bulk of TB cases in the study group
3. Gender-Specific TB Interventions:
Examine if there are any gender-specific TB risk factors, and make sure that men and women have equal access to healthcare, treatment, and prevention.
4. Longitudinal Studies:
Perform longitudinal studies to track changes in rifampicin resistance and assess potential contributing factors, including treatment adherence, co-infection with HIV, and socioeconomic characteristics.
5. Expand Sample Diversity:
Future research should include more elderly and pediatric patients to fully understand the epidemiology of tuberculosis in all age groups.

Conflict of interest

The author declares no conflict of interest.

Source of funding

This study was conducted without any source of funding.

Author biography

Nonduduzo Magubane holds a Bachelor of Health Sciences degree in Medical Laboratory Science from Mangosuthu University of Technology. Her research interests lie in infectious diseases and antimicrobial resistance, with a particular focus on tuberculosis. Her current study investigates the prevalence of multidrug-resistant *Mycobacterium tuberculosis* (MDR-TB) among patients diagnosed with pulmonary TB at Ngwelezana Hospital.

Author's contributions

Nonduduzo Magubane: conceptualization and design of the study, collection of data, data analysis, interpretation of findings, and drafting manuscripts.
Mr. Smangaliso Shangase (Supervisor): provide feedback, guidance, and assistance in the revision of the manuscript.

Data availability

The data that support the findings of this study consist of confidential patient records and are stored securely in an Excel file. In accordance with ethical and privacy considerations, these data are not publicly available. Access to the data is limited to the principal investigator and supervisors.

Acronyms and abbreviations

1. AIDS-acquired immune deficiency syndrome
2. HIV- Human Immune Deficiency Virus
3. INH- Isoniazid
4. IPC- Infection Prevention and Control
5. KZN- KwaZulu-Natal
6. MDR-Multidrug resistant
7. MTB- Mycobacterium tuberculosis
8. RFP/RMP-Rifampicin
9. TB-Tuberculosis
10. WHO-World Health Organization
11. XDR- Extensively Drug Resistant

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Acknowledgements

1. I am truly grateful to my supervisor (Mr. S Shangase) for his unwavering patience and guidance throughout this journey.
2. I am grateful to Dr S. I Ndlovu(*PhD*) for his valuable advice and encouragement.
3. Lastly, I would like to acknowledge National Health Laboratory Services (AARMS) for their data, which enabled the completion of this project.

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e-ISSN: 2709-9997, p-ISSN: 3006-1059
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e-ISSN: 2709-9997, p-ISSN: 3006-1059
Vol.6 No. 9 (2025): September 2025 Issue
https://doi.org/10.51168/sjhrafrica.v6i9.1891
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Student's Journal of Health Research Africa
e-ISSN: 2709-9997, p-ISSN: 3006-1059
Vol.6 No. 9 (2025): September 2025 Issue
https://doi.org/10.51168/sjhrafrica.v6i9.1891
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PUBLISHER DETAILS:

Student's Journal of Health Research (SJHR)
(ISSN 2709-9997) Online
(ISSN 3006-1059) Print
Category: Non-Governmental & Non-profit Organization
Email: studentsjournal2020@gmail.com
WhatsApp: +256 775 434 261
Location: Scholar's Summit Nakigalala, P. O. Box 701432, Entebbe Uganda, East Africa