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WHOLE GENOME SEQUENCING OF ENDOMETRIAL TISSUE BIOPSY TO DETECT DRUG RESISTANCE IN CASES OF GENITAL TB IN FEMALES

Respiratory Medicine

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ABSTRACT

Background: Female genital tuberculosis (FGTB) is an underdiagnosed form of extrapulmonary tuberculosis, often leading to infertility and reproductive complications. Drug-resistant strains of Mycobacterium tuberculosis (MTB), particularly multidrug-resistant tuberculosis (MDR-TB), pose significant challenges in FGTB management. Traditional diagnostic tools have limited efficacy due to the low bacterial load in genital tissues, highlighting the need for more advanced diagnostic methods such as whole genome sequencing (WGS). **Aims & Objectives:** This study aims to evaluate drug resistance in FGTB cases using WGS of endometrial tissue biopsies and to assess the clinical outcomes following anti-tuberculosis treatment (ATT). Additionally, we seek to analyze the prevalence of rifampicin resistance and its correlation with treatment response. **Methods:** A total of 60 FGTB cases were analyzed. Specimens were processed using the NALC-NaOH method and cultured with the BD BBL MGIT kit. DNA was extracted and prepared using the Illumina Nextera system for WGS analysis. The bioinformatics analysis was conducted using ΩTB software to detect drug resistance. Statistical analysis was performed using SPSS 22.0, with significance determined at $p < 0.05$. **Results:** MTB was detected in 4% of cases, and no cases showed rifampicin resistance. Post-ATT, 95% of cases improved, with only 5% showing no improvement. MDR-TB suspicion was low, with only 5% of cases presenting potential drug resistance based on clinical response. **Conclusions:** FGTB primarily affects women of reproductive age, with no significant prevalence of MDR-TB. WGS proved effective in detecting drug resistance, supporting its use for precise diagnostics and management of FGTB, especially in regions with high TB prevalence.

KEYWORDS

FGTB, multidrug-resistant tuberculosis, whole genome sequencing, rifampicin resistance, endometrial biopsy.

INTRODUCTION

Female genital tuberculosis (FGTB) remains a critical yet underdiagnosed form of extrapulmonary tuberculosis, particularly in regions with high TB prevalence.[1] Despite being less common than pulmonary TB, FGTB significantly impacts women's health, often leading to infertility, chronic pelvic pain, and other debilitating reproductive issues.[2] The disease predominantly affects women of reproductive age, with manifestations including tubal blockages, menstrual irregularities, and complications like hydrosalpinx and tubo-ovarian masses.[3] Diagnosing FGTB is often challenging due to subtle symptoms and the low bacterial load in affected tissues, making traditional diagnostic tools like microscopy and culture less effective.[4]

Drug resistance has further complicated FGTB management, with rising cases of multidrug-resistant tuberculosis (MDR-TB) that show resistance to first-line drugs like isoniazid and rifampicin.[5] The global spread of drug-resistant TB has made it imperative to develop more accurate diagnostic methods.[6] Recent advancements, such as whole genome sequencing (WGS), offer a transformative solution. WGS provides detailed genetic insights into Mycobacterium tuberculosis, enabling the detection of specific mutations linked to drug resistance. This cutting-edge technology allows clinicians to rapidly identify resistant strains, thereby facilitating timely and targeted treatment regimens.[7]

Method	Drug	Minimum (days)	Maximum (days)	Mean (SD) (days)	Median (IQR) (days)
BACTEC	INH	22.4	71.7	35.5 (15.3)	30.5 (25–34)
BACTEC	RIF	14.7	63.7	29.6 (7.4)	28.7 (23–34)
BACTEC	PZA	14.7	71.7	30.2 (8.8)	28.7 (24–33)
BACTEC	AMI	14.7	71.7	29.0 (6.8)	28.6 (24–33)
BACTEC	CAP	14.7	71.7	29.3 (7.7)	28.6 (24–33)
BACTEC	MFX	14.7	71.7	29.0 (6.8)	28.6 (24–33)
BACTEC	LEV	14.7	71.7	29.2 (7.1)	28.6 (24–33)
BACTEC	BDQ	27.7	118.7	53.2 (26.3)	52.6 (28–69)
BACTEC	LZD	26.7	118.7	48.9 (25.9)	43.6 (28–61)
BACTEC	CFZ	26.7	118.7	46.8 (26.4)	31.7 (28–60)
BACTEC	DLM	26.7	118.7	46.8 (26.4)	31.7 (28–60)
WGS	All drugs*	9.5	18.5	12.0 (2.2)	11.5 (9–13)

In this study, we leverage WGS of endometrial tissue biopsies to detect drug resistance in FGTB cases. By offering a comprehensive analysis of genetic markers associated with drug resistance, WGS promises to enhance diagnostic accuracy and guide more effective clinical management. As drug-resistant TB continues to pose a global threat, implementing WGS in diagnosing FGTB could be a crucial step forward in improving patient outcomes and curbing the spread of resistant strains.

MATERIALS AND METHODS

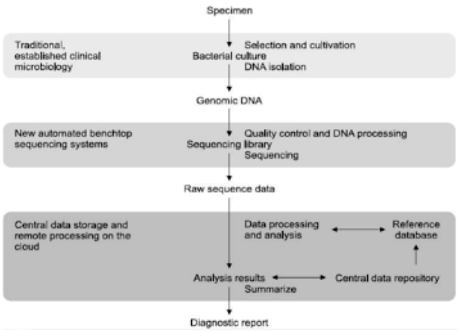
Laboratory Tests and DNA Extraction: Specimens were processed using the NALC-NaOH method followed by enrichment with the BD BBL MGIT kit. To each MGIT tube, 0.1 mL of reconstituted BD BBL MGIT PANTA antibiotic mixture was added aseptically, with 0.5 mL of the concentrated specimen suspension inoculated into the tube. Tubes were incubated at 37°C, and daily readings began on the second day. Fluorescence was assessed visually under UV light, and positive tubes underwent acid-fast bacilli staining, while negative tubes were monitored for up to eight weeks for growth absence.

DNA Library Preparation and Amplification: DNA libraries were prepared using the Illumina Nextera system. Genomic DNA was fragmented and tagged through tagmentation at 55°C for 5 minutes. Amplification of tagmented DNA was done using the NXT PCR program (12 cycles), incorporating Index 1 (i7) and Index 2 (i5) adapters necessary for sequencing. After PCR, purification was performed using Agencourt AMPure XP beads, ensuring removal of contaminants.

Library Cleanup and Dilution: Following amplification, libraries were cleaned using single-sided bead purification and then diluted with RSB solution for uniform representation in the pooled library. Quality control criteria included >100X coverage depth, 99% genome coverage, and Phred scores meeting the threshold, ensuring high-quality libraries.

Bioinformatics Analysis: Sequence alignment and mutation analysis were conducted using ΩTB software, determining drug resistance profiles for 18 tuberculosis antibiotics.

Statistical Analysis: Statistical analysis was done using SPSS 22.0. Qualitative data were analyzed with Yates continuity correction and Fisher's exact tests, with p-values <0.05 considered statistically significant.



RESULTS

Table 1 Distribution According to Age Group

Age Group	No. of Cases	Percentage (%)
20-30 years	25	42%
31-40 years	25	42%
41-50 years	8	13%
51-60 years	2	3%
Total	60	100%

Mean ± S.D.: 33.34 ± 7.01

This table presents the age distribution of cases, with the majority (84%) of cases falling within the 20-40 year age group. The mean age of the patients was 33.34 years, with a standard deviation of 7.01 years.

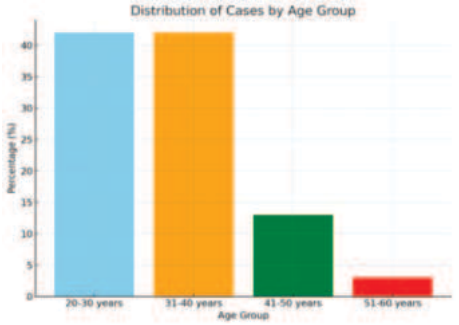


Table 2 Distribution of Clinical Features in Genital Tuberculosis (TB)

Clinical Features/Genital TB	No. of Cases	Percentage (%)
Bleeding	11	44.00%
Menstrual Irregularity	8	32.00%
Unable to Conceive	6	24.00%

This table shows the most common clinical features of genital TB, with bleeding being the most frequent symptom, followed by menstrual irregularities and infertility issues.

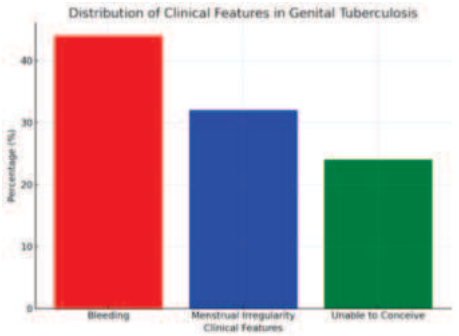


Table 3 Distribution of MTB Detection and Rifampicin Resistance in WGS Reports

Category	Cases	Percentage (%)
MTB Not Detected	58	96.00%
MTB Detected	2	4.00%
Rifampicin Sensitive	2	100.00%
Rifampicin Resistant	0	0.00%

This table summarizes the detection of Mycobacterium tuberculosis (MTB) and its rifampicin resistance status. Out of 60 cases, MTB was detected in 4% of cases, all of which were rifampicin-sensitive, with no cases showing resistance.

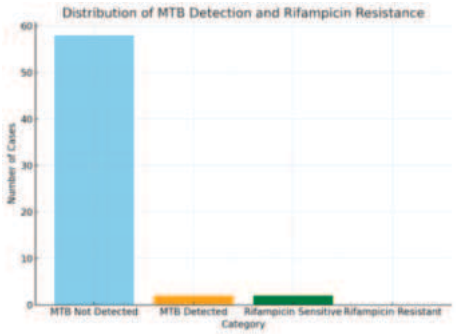
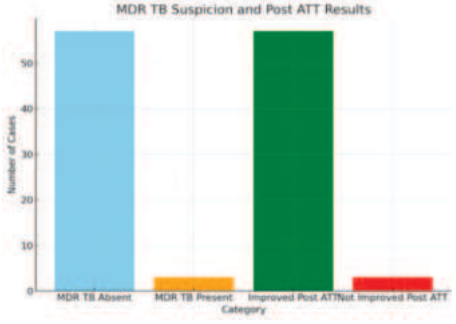


Table 4 Distribution of MDR TB Suspicion and Post-ATT Results

Category	Cases	Percentage (%)
MDR TB Absent	57	95.00%
MDR TB Present	3	5.00%
Improved Post ATT	57	95.00%
Not Improved Post ATT	3	5.00%

This table summarizes the suspicion of MDR TB based on ATT response and the post-treatment outcomes. The majority (95%) showed no suspicion of MDR TB and demonstrated improvement after ATT treatment. Only 5% had suspected MDR TB and did not show improvement.



DISCUSSION

The present study found that 84% of female genital tuberculosis (FGTB) cases occurred in women aged 20 to 40 years, with a mean age of 33.34 years ± 7.01. Sharma et al.[8] (2018) reported similar findings, with 75% of cases in the same age group and a mean age of 32.6 years. Garg et al.[9] (2018) found that 67.9% of FGTB cases were in women aged 20 to 35, focusing on infertility. Naaz et al.[10] (2021) also highlighted the predominance of FGTB in reproductive-aged women, further supporting the age-related findings of this study.

The present study found that the most common clinical feature of female genital tuberculosis (FGTB) was bleeding (44%), followed by menstrual irregularities (32%) and infertility (24%). Similarly, Sharma et al.[8] (2018) reported abnormal uterine bleeding in 38% of cases, with menstrual irregularities in 29% and infertility in 21%, aligning with the current study's findings. Garg et al.[9] (2018) also found menstrual irregularities in 35% of cases, matching the present study, though they reported a higher infertility rate (67.9%) due to their focus on infertility cases. Naaz et al.[10] (2021), while not reporting specific symptoms, highlighted infertility as a major concern, consistent with the current study's findings.

In the present study, Mycobacterium tuberculosis (MTB) was detected in 4% of cases, with no rifampicin resistance, as all detected cases were rifampicin-sensitive. Similarly, Saxena et al.[11] (2022) found MTB in 1.6% of cases, all rifampicin-sensitive, aligning with the current study's findings. Tiwari et al.[12] (2020) reported a 3% positivity rate using GeneXpert, with no MDR-TB, consistent with our study. Naaz et al.[10] (2021) reported a higher detection rate (8.05%), but like the present study, found no rifampicin resistance. These studies confirm that rifampicin resistance is rare in FGTB cases.

In the present study, 5% of cases were suspected of MDR-TB based on ATT response, while 95% showed no suspicion. Post-ATT, 95% improved. Tiwari et al.[12] (2020) found no rifampicin resistance among their 3% MTB-positive cases, aligning with the current study's findings that MDR-TB is rare. Naaz et al.[10] (2021) also reported no rifampicin resistance in their 8.05% MTB-positive cases, supporting

the low MDR-TB prevalence. Garg et al.[9] (2018) similarly found no MDR-TB and reported positive responses to ATT, further confirming the effectiveness of ATT in FGTB cases.

CONCLUSION

The present study demonstrates that female genital tuberculosis (FGTB) predominantly affects women of reproductive age, with the most common clinical features being bleeding (44%), menstrual irregularities (32%), and infertility (24%). The detection rate of Mycobacterium tuberculosis (MTB) was 4%, and all detected cases were rifampicin-sensitive, with no evidence of multidrug-resistant tuberculosis (MDR-TB). These findings align with previous studies, confirming that rifampicin resistance is rare in FGTB. The effectiveness of anti-tuberculosis treatment (ATT) was apparent, with 95% of cases showing clinical improvement post-ATT. This study supports the use of ATT in managing FGTB, particularly in regions with high TB prevalence.

Limitations

This study is limited by its relatively small sample size, which may affect the generalizability of the findings. Additionally, diagnosing FGTB remains challenging due to the often subtle symptoms and low bacterial load in affected tissues, making traditional diagnostic tools less effective. The reliance on conventional methods, despite the introduction of WGS, limits the comprehensive detection of drug resistance in some cases.

Recommendations

Future research should include larger cohorts to validate the findings and explore the role of advanced diagnostics, such as whole genome sequencing (WGS), in detecting drug resistance in FGTB. It is recommended that WGS be integrated into the diagnostic process for FGTB to enhance detection accuracy and facilitate targeted treatments, especially in regions with high rates of drug-resistant tuberculosis. Regular monitoring and updating of treatment protocols for FGTB should also be prioritized to ensure effective management and improved patient outcomes.

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