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A Descriptive Epidemiological Study of Tuberculosis (in Wasit province): Retrospective Analysis of Laboratory and Temporal Data Over a One-Year

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Abstract

This study presents a descriptive analysis of the distribution of tuberculosis in Wasit Governorate in 2024, based on data collected over a full year, divided into quarterly sections. The study tracked epidemiological changes in the disease throughout the year, laboratory diagnostic methods, reliance on traditional methods such as microscopic examination and bacterial culture, as well as modern molecular diagnostic methods such as GeneXpert.

Routine microscopic examination demonstrated very low sensitivity, with a positive result rate of less than 2%, and no statistically significant quarterly differences were observed. In contrast, bacterial culture showed high detection efficiency, with a positive rate of 30.3%, and exhibited statistically significant differences between quarters ($P \leq 0.01$), peaking in quarters 3 and 4. Molecular diagnosis identified 11.29% of cases as positive, with the highest rate in quarter 3 (30%), revealing a statistically significant variability ($P = 0.0078$).

The study identified one case of rifampicin resistance that emerged in the third quarter, while 92.86% were sensitive, this discrepancy was confirmed by a p-value of (0.0495) as statistically significant.

In conclusion, the study showed that microscopic examination results have limited accuracy and demonstrated the superiority of bacterial culture diagnosis. However, it requires more time, and this delay is not in the patient's best interest. Furthermore, molecular diagnosis using GeneXpert offers greater accuracy and a faster diagnostic time, making its use essential.

Keywords: Mycobacterium tuberculosis, Tuberculosis (TB), Retrospective Study, Epidemiological study, Diagnostic Methods, Annual Review

Research Problem:

Tuberculosis constitutes a significant challenge to public health and infectious diseases. Problems arise in understanding the differences between bacteria, especially those that are definitively resistant to drugs, and the various methods used to detect them. Understanding the varying distribution of infections throughout the year and analyzing the data is crucial to identifying the seasons with the highest incidence.

Research Objective:

- A study of the prevalence of tuberculosis bacteria throughout the year, divided into quarters.
- Determining the variance in the number of cases and identifying the peak in any quarter
- Compare the results of different tests (such as smear, GeneXpert, culture) to determine which contributed most to diagnoses during this period.

Conclusion:

This meta-analysis highlights the significant variability in the performance of tuberculosis diagnostic methods, with bacteriological testing being superior in sensitivity and molecular testing unique in providing rapid drug resistance information, while microscopic testing exhibits limited sensitivity. The results support the global trend towards incorporating rapid molecular tests into diagnostic algorithms, while maintaining culture for difficult cases and monitoring treatment, and accurate detection of drug resistance to meet the challenge of resistant tuberculosis.

Introduction

Tuberculosis (TB) is one of the most dangerous infectious diseases worldwide, affecting millions of people annually. It is the leading cause of death from a single infectious agent, despite being a preventable and effectively treatable disease (1).

TB is an infectious disease caused by Mycobacterium tuberculosis.

It is primarily transmitted through the air when an infected person coughs or sneezes. Historically, it was also known by names such as "consumption" or "the white plague" due to its widespread prevalence and deadly impact in the past.(2) (Jonathan P. Smith∗, 2022)

In 2024, approximately 10.7 million people worldwide contracted TB, and 1.23 million died from it, including 150,000 people living with HIV (1)(3) (Beth Gilmour1, 2024).

TB is the leading cause of death among people living with HIV and is also a major cause of death related to antibiotic resistance. Since 2000, global efforts have contributed to saving more than 83 million lives through early diagnosis and treatment(3) (Beth Gilmour1, 2024).

This study is significant for several vital reasons that contribute to the development of the health and research system, including:

Supporting health decision-making: The study provides public health officials with accurate data on the temporal patterns of tuberculosis (TB) spread, enabling more effective allocation of medical and human resources during peak infection quarters.

Improving diagnostic efficiency: By comparing different tests (laboratory, radiological, and molecular), the study provides scientific insight into the most efficient and reliable tests for routine diagnosis, helping to optimize laboratory spending and expedite patient placement on treatment protocols.

Understanding epidemiological dynamics: The study helps bridge the information gap regarding the relationship between seasonal variations and the emergence of TB cases in the studied region, which is essential for predicting and controlling future epidemics.

A reference for future research: This study provides a valuable epidemiological database that researchers can use to conduct cohort studies or intervention studies in the future, thereby evaluating national TB control programs.

Methods

Collection of samples:

A sputum sample was taken from the patient into a disposable container labeled with the relevant data. Patient data, such as age and sex, are also recorded in a dedicated institutional statistical record.

The samples are then sent to the tuberculosis testing laboratory for routine examination, beginning with microscopic examination. Positive samples are sent to the bacteriology laboratory for inoculation on standard culture media.

If the results are positive, they are confirmed through molecular testing using DNA amplification technology (GeneExpert).

Microscopic diagnosis:

The microscopic examination of sputum samples suspected of being infected with tuberculosis was carried out according to the following steps:

1. Sample Collection

- Sputum samples were collected from patients according to WHO standards to ensure sample quality.
- The samples were stored in sterile, sealed containers and transported directly to the laboratory.

2. Slide Preparation

1. A portion of the sample was taken and placed on a clean glass slide.
2. The sample was fixed by direct heat (flame fixation).
3. The slides were stained with Ziehl-Neelsen stain for acid-resistant bacilli,

3. Microscopic Examination

1. The slides were examined under a light microscope using an oil immersion lens (×100).
2. A specific number of optical fields were examined according to the standard protocol (usually 100 fields).

4. Interpretation of Results

Based on the number of bacilli stained with carmine (Zell's-Neelsen stain), which is considered a positive result, the results were recorded according to the AFB smear grading (Negative, Scanty, 1+, 2+, etc.)

5.Quality Control

Positive and negative control slides were used with each batch of tests to ensure the accuracy of the results. The examination was performed by specialists trained in the microscopic diagnosis of tuberculosis.

Inoculation on Culture Media

1. **Medium Preparation**

Lowenstein–Jensen (LJ) medium used as a selective medium for the detection of Mycobacterium tuberculosis (Mymicrolab Manufacture).

The medium was prepared according to standard instructions and stored at an appropriate temperature until use.

2. **Sample Inoculation**

After treating the sputum with NaOH to remove contaminants, a portion of the sediment was taken and cultured directly onto the surface of LJ medium. Sterile instruments were used to avoid cross-contamination.) Sumona Datta 1(2017 ،

3. **Incubation Conditions**

Inoculated tubes were placed in an incubator at 37°C. Samples were incubated for 6–8 weeks with weekly monitoring for growth.

4**. Interpreting the Results**

A result was considered positive when coarse, dry, creamy-yellow colonies, characteristic of tuberculosis bacteria, were observed. The results were accurately recorded, including the time of growth.

5. **Quality Control**

Positive control samples containing a known strain of M. tuberculosis were used to ensure the medium's suitability. Negative control samples were added to ensure there was no contamination.

Molecular diagnostic method using GeneXpert

1. **Principle**

The GeneXpert MTB/RIF device relies on Real-Time Polymerase Chain Reaction (RT-PCR) technology to detect the presence of Mycobacterium tuberculosis and determine bacterial resistance to the antibacterial rifampicin directly from clinical samples.

2. **Sample Preparation**

A portion of the processed sample was inserted into the GeneXpert . The cartridge was placed in the device, where all extraction, amplification, and detection steps were performed automatically. The analysis took approximately 120 minutes to obtain the results (Sumona Datta 1, 2017).

3. **Interpreting Results**

- The instrument displayed the result directly on the screen:
- Positive/Negative for M. tuberculosis.
- Susceptibility/Resistance to rifampicin.

The results were recorded electronically and entered into the study database.

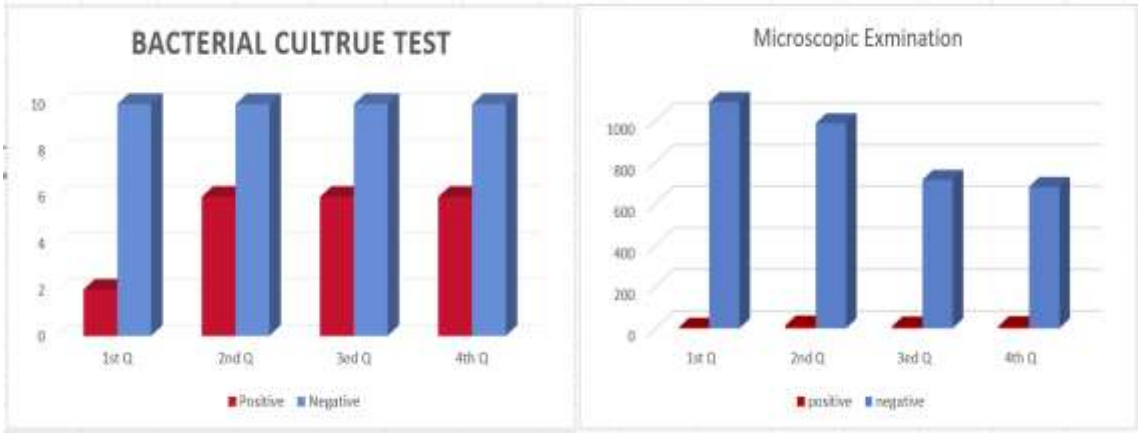
4. **Quality Control**

Positive and negative control cartridges were used to verify the instrument's accuracy.

Results

Evaluation of Microscopic Examination versus Culture Outcomes: A Comparative Study of Positive and Negative Cases

The results of the three tests (microscopic, culture, and molecular) showed a clear variation in the detection rates of tuberculosis cases across the different quadrants, with culture recording the highest positive rates, while microscopic examination was the least sensitive, and molecular examination demonstrated a remarkable ability to detect antibiotic resistance.



Quarter	Patients number	Positive (+ve)	Negative	Positive %
1st Q	1093	3	1090	0.28
2nd Q	1001	14	987	1.40
3rd Q	724	9	715	1.24
4th Q	692	11	681	1.59
Total	3510	37	2473	1.05
P-value	---	---	---	0.0944 NS
NS: Non-Significant.				

Figure:1 Quarterly Distribution of Tuberculosis Cases by Bacterial Culture Test and Microscopic Examination

Comparative Evaluation of Microscopy and Culture Outcomes in Quarterly Pulmonary Tuberculosis Samples

Among 3510 patients tested microscopically, only 37 (1.05%) were positive. The positivity rate ranged from 0.28% in the first quarter to 1.59% in the fourth quarter. However, the differences between quarters were not statistically significant (P = 0.0944, NS).

Table 1: Results of Microscopic test in Pulmonary Tuberculosis-TB sample study

Out of 66 patients examined, 20 (30.3%) were positive for pulmonary tuberculosis in the culture. The positivity rate varied across quarters, with the lowest in the first quarter (16.67%) and the highest in the third and fourth quarters (37.5%). Statistical analysis revealed a significant difference between quarters ($P = 0.0091$, $P \leq 0.01$).

Quarter	Patients number	Positive (+ve)	Negative	Positive %
1st Q	12	2	10	16.67
2nd Q	22	6	16	27.27
3rd Q	16	6	10	37.50

Table	4th Q	16	6	10	37.50
	Total	66	20	46	30.30
	P-value	---	---	---	0.0091 **
	** (P≤0.01).				

2: Results of Bacterial test in Pulmonary Tuberculosis-TB sample study

A comparative assessment of tuberculosis-positive cases identified through molecular diagnostic methods

Of 124 patients screened using GeneXpert technology for pulmonary tuberculosis, 14 were positive (11.29%), compared to 110 negative cases. Distributing the results across the four quarters was in the first quarter, 54 patients were screened, with 4 positive cases (7.41%) and in the second quarter, 10 patients were screened, with 1 positive case (10%). While in the third quarter, 20 patients were screened, with 6 positive cases (30%), the highest positivity rate among the quarters. Also in the fourth quarter, 40 patients were screened, with 3 positive cases (7.5%).

Statistical analysis showed a p-value of 0.0078, which is less than 0.01, indicating a highly significant difference between the quarters. This confirms that the variation in positivity rates reflects a real difference in detection patterns or disease prevalence across the studied time periods.

Table 3: Results of Gene Xpert in Pulmonary Tuberculosis-TB sample study

Quarter	Patients number	Positive (+ve)	Negative	Positive %
1st Q	54	4	50	7.41
2nd Q	10	1	9	10.00
3rd Q	20	6	14	30.00
4th Q	40	3	37	7.50
Total	124	14	110	11.29
P-value	---	---	---	0.0078 **
** (P≤0.01).				

Rifadin Resistance Results in Pulmonary Tuberculosis by Gene Xpert

The results of molecular testing using GeneXpert technology to determine rifadin sensitivity in pulmonary tuberculosis patients across four quarters of the year showed the During the first quarte, all positive cases (4) were 100% sensitive to rifadin. Subsequently, in the second quarter one positive case was recorded, which was also 100% sensitive. By the third quarter, six positive cases were detected, five of which were sensitive and one resistant, with a sensitivity rate of 83.33%. In contrast, the fourth quarter revealed, three positive cases appeared, all of which were 100% sensitive.

Thus, the total number of positive cases across the year was 14, of which 13 were sensitive and one was resistant, with an overall sensitivity rate of 92.86%.

Statistically, the p-value was 0.0495, which is less than 0.05, indicating a significant difference in the sensitivity distribution across the quarters and confirming that the results are statistically significant.

Discussion

The results presented indicate a clear variability in the efficacy of the three diagnostic methods (bacterial culture, rapid microscopic examination (staining), and the GeneXpert molecular assay) in detecting positive tuberculosis cases over the four quarters. This variability reflects the diagnostic characteristics of each test in terms of sensitivity and specificity, and its ability to provide additional information such as drug resistance.

Evaluating the Diagnostic Performance of Microscopic Examination compared Gold Standard Culture

When comparing the results of microscopic examination and culture media, we observe that the number of positive cases in microscopic examination was almost negligible compared to the number of negative instances. This is insignificant because it is a preliminary examination, and

Chi-square analysis revealed significant differences between microscopic examination and bacterial culture results across all four quadrants. In the first, third, and fourth quadrants, the differences were highly significant ($p < 0.0001$), reflecting the superiority of culture in detecting positive cases. In the second quadrant, a marginally significant difference was observed ($p \approx 0.048$), indicating an advantage of culture over microscopic examination. These results support the adoption of culture as the gold standard for diagnosis, while microscopic examination remains a rapid primary tool with limited statistical value.

Table 4: Distributing the value of P-Value and the chi-square test across the quarters of the year

Quarter	X^2 value	p- value
Quarter 1	**71.4**	**< 0.0001**
Quarter 2	**3.9**	**≈ 0.048**
Quarter 3	**104.6**	**< 0.0001**
Quarter 4	**86.6**	**< 0.0001**

(Anjana Gopi, 2018) Their study demonstrated that microscopic examination is less accurate than media, yielding the same results, indicating that cultures are usually more precise.

Despite scientific advancements, bacterial culture remains the most accurate diagnostic method for tuberculosis compared to microscopic examination. However, the main challenge of laboratory work is the time required for detection. Therefore, it has become essential to reinforce the use of modern diagnostic techniques, such as the GeneXpert molecular diagnostic test, which is almost always definitive unless there is a technical error in sample collection methods. This gives it the most important role in diagnosing the disease promptly and with reliable findings.This is what (Rakshit, 2025) presented in his study.

Bacterial culture recorded the highest positive detection rates, particularly in the third and fourth quarters (37.5%), surpassing the molecular assay. This result is expected and consistent with research confirming that Mycobacterium tuberculosis in culture is the most sensitive method for confirming infection, as it can detect fewer than 10 microorganisms per milliliter of sample (Guilherme Bartolomeu-Gonçalves, 2024) (Linda M Parsons 1, 2011). However, the main drawback of this method, as documented in the protocol, is its lengthy processing time (which can reach several weeks) and its inability to directly differentiate between drug-susceptible and drug-resistant strains without additional drug susceptibility testing (Natalia Zaporojan, 2024).The results here reflect the ability of culture to detect a larger number of cases, but they do not provide the vital information on resistance that the GeneXpert test offersMicroscopic examination

(sputum smear) showed very low positive rates (less than 2%) across all quartiles. The low sensitivity of the rapid test means that it fails to detect a large number of cases (smear-negative tuberculosis), thus reinforcing the text's assertion that it needs to be supplemented with more accurate tests (Emily A Kendall, 2025).These results support the global recommendations to shift from reliance on microscopic examination as the primary diagnostic tool to the use of rapid molecular tests as the first line of diagnosis (WHO, WHO consolidated guidelines on tuberculosis, 2021-2024).

Comparative Analysis of Microscopy and Culture Results

In this study, comparing the results of microscopic examination with those of bacterial culture, the latter proved superior, with a detection rate of 30.3% compared to 1.05% for microscopic examination. This is because culture requires a high bacterial load for visibility under the microscope, making it more accurate, especially in the early stages of symptoms when the bacterial load is at its peak.

Given environmental variations, such as seasonal climate differences and population behavioral variations, particularly regarding health, the variability indicates a statistically significant difference in bacterial culture between seasons ($P \leq 0.01$). Conversely, the low accuracy of microscopic examination confirms its failure in detecting epidemiological cases.

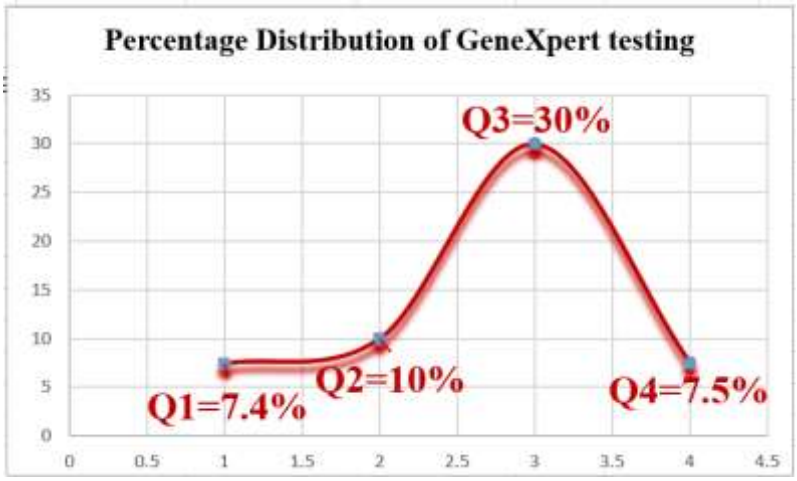
Upon initial observation, the number of individuals who underwent microscopic examination appears high compared to the rest of the examination. This suggests that not all those examined were suspected of having pulmonary tuberculosis. It could be that these were routine examinations for workers in enclosed and high-risk environments, leading to an unrealistic comparison of results with bacterial culture tests. In such cases, individuals clinically diagnosed with pulmonary tuberculosis would have a higher percentage of positive results.

From an epidemiological perspective, current data indicate that relying on bacteriological methods instead of microscopic examination enhances the effectiveness of tuberculosis detection protocols. The gap in diagnostic sensitivity of microscopic examination often leads to statistical misrepresentation of the true disease prevalence. Therefore, it is recommended to integrate more accurate bacteriological tests into routine medical settings as a mechanism to ensure comprehensive and vital surveillance of infected individuals.

Interpretation of GeneXpert Results in Pulmonary Tuberculosis

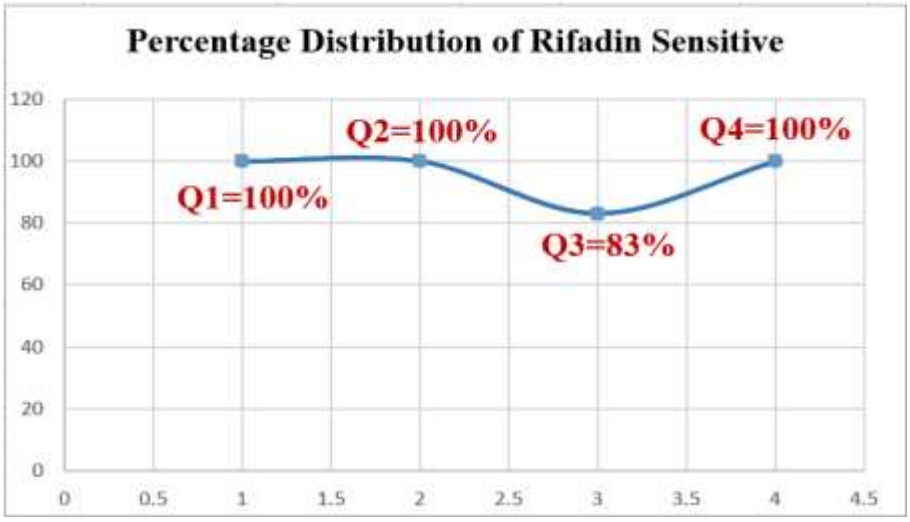
Molecular testing using the GeneXpert system revealed significant variation in the detection rates of positive pulmonary tuberculosis infections among different groups, with the third group exhibiting the highest statistically significant value. This marked variation underscores the strategic importance of integrating molecular testing as a reference tool in evaluating the effectiveness of diagnostic systems and developing more accurate mechanisms for monitoring infection outbreaks.

The GeneXpert microscopic examination showed its highest positivity rate in the third quarter (30%), a lower percentage than that recorded by the bacterial examination in the same period (37.5%). This is consistent with studies indicating that the sensitivity of GeneXpert is slightly lower than that of culture, which is considered the gold standard, especially in paucibacillary samples) MonaMansourAhmed(2024 ،.



Discussion of Rifampicin Resistance Pattern

These results show that the vast majority of pulmonary tuberculosis cases diagnosed by GeneXpert were highly sensitive to rifadine, with only one case of resistance recorded in the third quarter. This reflects the efficacy of rifadine as a primary treatment option and highlights the importance of molecular screening in the early detection of drug resistance to ensure the success of treatment programs and disease control.



However, the major added value of this examination lies in its ability to detect rifampicin resistance, recording a resistance rate of 16.7% in the third quarter. This result is consistent with a World Health Organization (WHO) study on the effectiveness of GeneXpert in the rapid detection of drug-resistant tuberculosis, enabling immediate initiation of appropriate treatment and limiting the spread of resistant strains) Jin Shi(2025 ،. And (Linda M Parsons 1, 2011)The absence of resistance in the remaining quarters confirms the sporadic epidemiological nature of the spread of resistant strains, necessitating continuous surveillance (Viktoria M Brunner, 2025).

The current findings demonstrate the inadequacy of microscopic examination alone in diagnosing infections, as exclusive reliance on it leads to the omission of a large proportion of cases. In contrast, the integration of bacteriological tests (to achieve maximum sensitivity) and molecular techniques (to ensure speed and detect drug resistance) provides a comprehensive diagnostic perspective. The detection of rifampicin resistance (16.7%) represents a critical epidemiological indicator, exceeding the (WHO, 2022).

global estimates of 3–4% in new cases. This significant increase can be attributed to the nature of the sample under study, which may include high-risk cases or those with prior treatment failures, necessitating intensified drug surveillance programs and the adoption of immediate, evidence-based treatment strategies.

Conclusion

The study concludes that there is a significant disparity in the efficacy of tuberculosis diagnostic techniques; bacteriological tests excel in sensitivity, while molecular techniques are faster at detecting drug resistance. Therefore, we recommend integrating rapid molecular tests into diagnostic algorithms, while maintaining laboratory culture as a reference tool for managing complex cases and monitoring treatment effectiveness.

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