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Isolation of Mycobacterium tuberculosis and Determining Their Susceptibility Patterns to Rifampicin among Patients Attending Al-Kuwayfiya Teaching Hospital for Chest Diseases

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Abstract:

Among 627 sputum samples analyzed from 2021 to 2023, 322 (51.3%) were confirmed as pulmonary tuberculosis (TB). This study, conducted at Al-Kuwaifiya Specialized Teaching Hospital in Benghazi, Libya, identified 2022 as the year with the highest number of cases (38.5%). A significant majority of patients were male (76%), and most were Libyan (68.6%), followed by Sudanese nationals (16.1%). Rifampicin-resistant TB (RR-TB) was detected in 37 cases, representing 11.5% of all confirmed TB patients. This resistance was more prevalent in males and was most common in the 34–43-year age group. Semi-quantitative analysis via the GeneXpert MTB/RIF assay indicated that most positive samples contained a high bacterial load. Furthermore, the incidence of TB was higher during the dry season compared to the rainy season. In conclusion, while the overall TB prevalence was high, the rate of rifampicin resistance was relatively low in this patient population. The findings underscore the critical role of the GeneXpert MTB/RIF

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assay as a rapid and reliable tool for the simultaneous detection of M. tuberculosis and rifampicin resistance, which is essential for effective treatment and controlling the spread of drug-resistant TB.
Keywords: Benghazi, drug resistance, GeneXpert MTB/RIF, Mycobacterium tuberculosis, rifampicin resistance.

عزل المتفطرة السُّلية *Mycobacterium tuberculosis* أنماط حساسيتها تجاه الريفامبيسين بين المرضى المترددون على مستشفى الكويفية التعليمي للأمراض الصدرية

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الملخص:

من بين 627 عينة بلغم تم تحليلها خلال الفترة من عام 2021 إلى عام 2023، تم تأكيد إصابة 322 حالة (بنسبة 51.3%) بمرض السل الرئوي. أُجريت هذه الدراسة في مستشفى الكويفية التعليمي التخصصي في بنغازي، ليبيا، حيث تبيّن أن عام 2022 كان العام الذي سُجل فيه أعلى عدد من الحالات (38.5%). كان معظم المرضى من الذكور بنسبة (76%)، وغالبية الحالات من الجنسية الليبية (68.6%)، تليها الجنسية السودانية (16.1%). تم الكشف عن السلالات المقاومة للريفامبيسين (RR–TB) في 37 حالة، أي ما يمثل (11.5%) من إجمالي حالات السل المؤكدة. وقد كانت المقاومة أكثر شيوعاً بين الذكور، وسُجلت غالباً ضمن الفئة العمرية 34–43 سنة. أظهرت التحاليل شبه الكمية باستخدام فحص GeneXpert MTB/RIF أن أغلب العينات الإيجابية احتوت على حمولة بكتيرية عالية. علاوة على ذلك، تبين أن معدل الإصابة بالسل كان أعلى خلال موسم الجفاف مقارنة بموسم الأمطار. في الختام، على الرغم من أن معدل انتشار

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السل كان مرتفعاً، إلا أن معدل المقاومة للريفامبيسين كان منخفضاً نسبياً بين هذه الفئة من المرضى. وتؤكد النتائج على الدور الحيوي لاختبار GeneXpert MTB/RIF كأداة سريعة وموثوقة للكشف المتزامن عن المتفطرة السلية ومقاومتها للريفامبيسين، وهو أمر ضروري للعلاج الفعّال والحد من انتشار السلالات المقاومة للأدوية.

الكلمات المفتاحية: بنغازي، مقاومة الأدوية، GeneXpert MTB/RIF، المتفطرة السلية، مقاومة الريفامبيسين.

1. Introduction:

Tuberculosis (TB) is a chronic infectious disease caused by Mycobacterium tuberculosis (MTB), primarily transmitted through airborne droplets expelled when individuals with pulmonary TB cough, sneeze, or speak. TB remains one of the leading causes of morbidity and mortality worldwide (World Health Organization [WHO], 2021). The introduction of streptomycin marked the first effective antibiotic treatment for TB, demonstrating strong bactericidal activity. However, the emergence of drug-resistant strains, largely due to streptomycin monotherapy, underscored the necessity for combination therapy. Over time, multidrug-resistant TB (MDR-TB) has evolved into a significant global health challenge. The WHO has identified additional categories of resistance, including extensively drug-resistant TB (XDR-TB), characterized by resistance to at least four core anti-TB drugs, and totally drug-resistant TB (TDR-TB), which is resistant to all first-line and many second-line drugs (Prasad, 2010). Resistance can be classified as primary, occurring when patients are initially infected with resistant strains, or acquired, developing as a result of inadequate or incomplete treatment (Damtie *et al*., 2014). Standard treatment for drug-susceptible TB typically involves a six-month regimen of four antibiotics, notably rifampicin and isoniazid. Resistance to these first-line drugs complicates clinical management, necessitating longer, more toxic, and less effective treatment regimens (Sotgiu *et al*., 2015). In Libya, TB has been endemic for centuries. In 2021 alone, approximately 4,000 cases were reported, with an incidence rate of 59 per 100,000 populations (Gallant *et al*., 2017). Notifications of new TB cases declined from 2,209 in 2019 to 1,744 in 2020 before rising again to 1,932 in 2021. The disease affects both local residents and migrant populations, including refugees (WHO, 2020, 2021).

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1.1 Innate Mechanisms of Drug Resistance in Mycobacterium tuberculosis

1.1.1 Impermeable Cell Envelope

A key intrinsic resistance mechanism of M. tuberculosis is its unique cell wall composition, which is rich in mycolic acids, cord factor, and wax-D, forming a robust barrier that limits antibiotic penetration. Consequently, drugs accumulate slowly around the bacterium and are often degraded or neutralized by bacterial enzymes (Chiaradia *et al*., 2017; Maitra *et al*., 2019).

1.1.2 Reduced Metabolic Activity

The inherently slow growth rate of MTB also contributes to drug resistance. Many antibiotics, such as carbapenems, degrade more rapidly than the bacteria replicate, thereby reducing their efficacy over time (Baek *et al*., 2011; Hett & Rubin, 2008).

1.1.3 Efflux Pump Systems

Efflux pumps further enhance resistance. These membrane-spanning proteins actively expel antibiotics from the bacterial cell, reducing intracellular drug concentrations and diminishing therapeutic impact (Zhang *et al*., 2016).

1.1.4 Genetic and Molecular Resistance Mechanisms

Resistance in MTB primarily arises from spontaneous chromosomal mutations that alter the interaction between anti-TB drugs and their molecular targets.

Rifampicin is commonly used alongside isoniazid and functions by inhibiting bacterial RNA synthesis. Resistance is predominantly associated with mutations in the rpoB gene, which encodes the β-subunit of RNA polymerase. These mutations impair rifampicin binding, leading to therapeutic failure (Campbell *et al*., 2011; Caws *et al*., 2006).

Pyrazinamide is essential in TB therapy, particularly in cases resistant to other first-line drugs. The drug is converted into its active form, pyrazinoic acid, by the enzyme pyrazinamidase, encoded by the pncA gene. Mutations in pncA are the most frequent cause of pyrazinamide resistance, as they disrupt this activation process (Huy *et al*., 2017; Njire *et al*., 2016).

Isoniazid, a prodrug, is activated by the catalase–peroxidase enzyme KatG, encoded by the katG gene. Once activated, it disrupts mycolic acid synthesis, which is vital for maintaining cell wall integrity. Resistance often results from mutations in katG, particularly at codon Ser315 (Ando *et al*., 2014; Johnsson *et al*., 1997).

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Ethambutol inhibits cell wall biosynthesis and is typically included in combination regimens to prevent resistance, especially in isoniazid-resistant cases. Resistance has been associated with mutations in several genes, including those co-occurring with katG mutations (Ahmad *et al*., 2007; Parsons *et al*., 2005).

Fluoroquinolones, such as ciprofloxacin, target bacterial DNA gyrase and topoisomerase IV. Resistance is usually caused by mutations in the gyrA and gyrB genes and may also involve efflux pumps or protective proteins such as MfpA (Zhang *et al*., 2016).

Ethionamide also inhibits mycolic acid synthesis. Although the exact mechanisms of resistance are not fully elucidated, recent studies suggest that mutations in the inhA promoter region and related genes (e.g., orf1) contribute to reduced susceptibility (Brossier *et al*., 2011; Carette *et al*., 2012).

Second-line injectables, including kanamycin, amikacin, viomycin, and capreomycin, inhibit protein synthesis. Amikacin and kanamycin belong to the aminoglycoside class, while viomycin and capreomycin are cyclic peptides. The latter two act as bacteriostatic agents by binding to the 50S ribosomal subunit, thereby disrupting translation (Zaunbrecher *et al*., 2009).

1.2 Study Context

This study aimed to assess the prevalence of tuberculosis and its resistance to rifampicin over a three-year period (2021–2023) among patients at Al-Kuwayfiya Specialized Teaching Hospital for Chest and Tuberculosis in Benghazi, Libya.

2. Material and Method

2.1 Sample Collection

Sputum specimens were obtained from patients with suspected pulmonary tuberculosis admitted to the Department of Chest and Tuberculosis at Al-Kuwayfiya Specialized Teaching Hospital in Benghazi, Libya. Samples were collected in sterile, leak-proof, screw-capped containers to ensure proper biosafety and maintain sample integrity.

2.2 Study Design

This retrospective study included a total of 627 sputum samples collected from patients with clinical suspicion of pulmonary tuberculosis between January 2021 and December 2023 at Al-Kuwayfiya Specialized Teaching Hospital. The study focused on the identification of Mycobacterium tuberculosis and determination of its resistance to rifampicin.

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2.3 Sample Processing

Laboratory analysis was performed using the GeneXpert MTB/RIF assay (version 5.0, Cepheid, USA), following the manufacturer's instructions. Sputum specimens of adequate volume were transferred into sterile 15 mL Falcon tubes, and a sample reagent buffer was added at a 2:1 buffer-to-sample ratio. This buffer solution facilitated liquefaction of the sputum and lysis of bacterial cells. Samples were incubated at 37°C for 15 minutes with intermittent gentle mixing. Subsequently, 2 mL of the processed sample was loaded into the GeneXpert cartridge using the provided disposable pipettes. The cartridge was then inserted into the GeneXpert instrument for automated processing. The platform performs sample filtration, washing, ultrasonic lysis for DNA extraction, mixing with dry PCR reagents, and semi-nested real-time PCR amplification. Test results, including M. tuberculosis detection and rifampicin resistance status, were available approximately two hours after initiation.

2.4 Composition of Sample Reagent Buffer (SR Buffer)

2.4.1 The SR buffer consisted of:

- Sodium hydroxide (NaOH): Facilitates breakdown of the bacterial cell wall, enabling DNA release.
- Isopropanol: Assists in the inactivation of non-essential sample components and enhances extraction of target compounds.
- Sterile water: Serves as a solvent, promoting the activation of key catalytic agents during processing.

2.5 Data Collection

Patient demographics and diagnostic results were extracted from the microbiology unit records of the hospital. Data including age, gender, nationality, year of diagnosis, and TB test outcomes were entered into Microsoft Excel 2019 for organization and analysis. The dataset covered cases from January 2021 to December 2023.

2.6 Statistical Analysis

Collected data were analyzed using IBM SPSS Statistics (version 23) to evaluate the prevalence of tuberculosis and associated factors over the study period.

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3. Results:

Out of the total 627 samples analyzed over the three-year period, 322 (51.3%) were confirmed positive for Mycobacterium tuberculosis, while 305 samples (48.6%) were negative.

3.1 Prevalence of Tuberculosis among Suspected Cases.

Among the 627 sputum samples collected from suspected TB patients between 2021 and 2023, tuberculosis was confirmed in 322 cases (51.3%), whereas 305 cases (48.6%) tested negative. The majority of TB-positive patients were of Libyan nationality, followed by Sudanese and Chadian nationals.

Table 1: Prevalence of Tuberculosis among Suspected Cases at Al-Kuwayfiya Hospital.

COUNT	NUMBER	TOTAL
No TB DETECTED	TB DETECTED	
305	322	627
(48.6%)	(51.3%)	(100%)

3.2 Gender-Based Distribution of Tuberculosis Cases and Rifampicin Resistance Patterns.

Among the 322 confirmed tuberculosis cases during the study period, males constituted the majority, accounting for 245 cases (76%). The annual distribution of male TB cases was 78 in 2021, 94 in 2022, and 73 in 2023. Correspondingly, the number of male patients exhibiting rifampicin resistance during these years was 6, 13, and 9, respectively.

Table 2: Gender-Based Distribution of Tuberculosis Cases and Rifampicin Resistance Patterns.

YEAR			RIFAMPICIN SUSCEPTIBILITY		TOTAL
			S	RR	
2021	GENDER	Male	72	6	78
		Female	22	2	24
	TOTAL		94	8	102
2022	GENDER	Male	81	13	94
		Female	24	6	30
	TOTAL		105	19	124
2023	GENDER	Male	64	9	73
		Female	22	1	23
	TOTAL		86	10	96

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3.3 Distribution of Rifampicin Susceptibility Patterns by Patient Nationality.

In 2021, a total of 102 tuberculosis cases were documented. Among these, Libyan nationals accounted for the majority with 67 cases (65.7%), followed by 19 Sudanese (18.6%) and 11 Chadian patients. In 2022, 124 clinical isolates were obtained, of which 105 were susceptible to rifampicin, while 19 exhibited resistance. Libyans represented the largest proportion (74.2%), followed by Sudanese and Chadians. By 2023, the majority of TB cases (64.6%) were still among Libyan patients, followed by Sudanese (17.7%) and Chadians (6.2%). In total, 96 isolates were recorded in 2023, with 86 showing rifampicin sensitivity and 10 exhibiting resistance.

Table 3: Distribution of Rifampicin Susceptibility Patterns by Patient Nationality.

YEAR			RIFAMPICIN SUSCEPTIBILITY		TOTAL
			S	RR	
2021	NATIONALITY	Libyan	62	5	67
		Sudanese	19	0	19
		Chadian	9	2	11
		Egyptian	0	1	1
		Bangladeshi	2	0	2
		Eritrean	1	0	1
		Ethiopian	1	0	1
	TOTAL		94	8	102
2022	NATIONALITY	Libyan	80	12	92
		Sudanese	13	3	16
		Chadian	8	3	11
		Bangladeshi	2	0	2
		Nigerian	1	1	2
		Filipino	1	0	1
	TOTAL		105	19	124
2023	NATIONALITY	Libyan	56	6	62
		Sudanese	14	3	17
		Chadian	5	1	6
		Egyptian	2	0	2
		Bangladeshi	2	0	2
		Nigerian	3	0	3
		Syrian	1	0	1
		Arthurian	1	0	1
		Malian	1	0	1
		Tunisian	1	0	1
	TOTAL		86	10	96

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3.4 Distribution of Tuberculosis Patients According to Departments.

The majority of TB patients were from the Men's Asthma Department 1 (n = 147), followed by the Outpatient Clinic (n = 127), and the Women's Asthma Department 1 (n = 23). Additionally, 8 cases were reported from the Children's Hospital, while the lowest number of cases (n = 13) was recorded at the Benghazi Medical Center.

Table 4: Distribution of Tuberculosis Patients According to Departments

YEAR		RIFAMPICIN SUSCEPTIBILITY	
		S	RR
WORD	MSI	37	5
	OPD	47	3
	FSI	7	0
	CHB	2	0
	BMC	1	0
TOTAL		94	8
WORD	MSI	49	11
	OPD	40	6
	FSI	5	2
	CHB	6	0
	BMC	5	0
TOTAL		105	19
WORD	MSI	39	6
	OPD	29	2
	Prison	3	0
	FSI	9	0
	BMC	5	2
	Isolation Department	1	0
TOTAL		86	10

Note: MSI: Male Section I (Asthma department); OPD: Out Patient Department; FSI: Female Section I (Asthma department); CHB: Central Child's center; BMC: Benghazi Medical Center.

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3.5 Seasonal Distribution of Tuberculosis Cases and Rifampicin Sensitivity Patterns.

The majority of tuberculosis cases were reported during the summer months, particularly between June and August, with a noticeable increase also observed from March to May. Overall, a higher incidence of TB was associated with the dry season compared to the rainy season.

Table 5: Seasonal Distribution of Tuberculosis Cases and Rifampicin Sensitivity Patterns.

YEAR			RIFAMPICIN SUSCEPTIBILITY		TOTAL
			S	RR	
2021	SEASON	12-2	27	3	30
		3-5	25	3	28
		6-8	30	2	32
		9-11	12	0	12
	TOTAL		94	8	102
2022	SEASON	12-2	21	4	25
		3-5	24	6	30
		6-8	19	6	25
		9-11	41	3	44
	TOTAL		105	19	124
2023	SEASON	12-2	17	2	19
		3-5	19	3	22
		6-8	28	4	32
		9-11	22	1	23
	TOTAL		86	10	96

3.6 Detection Frequency of MTB and Rifampicin Resistance Using the GeneXpert MTB/RIF Assay.

Based on the findings, a total of 322 specimens tested positive for Mycobacterium tuberculosis (MTB) using the GeneXpert MTB/RIF assay. Semi-quantitative analysis of these results revealed that 36 samples (11.1%) had a very low bacterial load, 73 (22.7%) were classified as low, 138 (42.8%) as medium, and 75 samples (23.3%)

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demonstrated a high bacterial load, based on cycle threshold (CT) values.

Table 6: Detection Frequency of MTB and Rifampicin Resistance Using the GeneXpert MTB/RIF Assay

YEAR			RIFAMPICIN SUSCEPTIBILITY		TOTAL
			S	RR	
2021	MTB	LOW DETECTED	18	2	20
		MEDIUM DETECTED	35	4	39
		VERY LOW DETECTED	6	1	7
		HIGH DETECTED	35	1	36
	TOTAL		94	8	102
2022	MTB	LOW DETECTED	28	3	31
		MEDIUM DETECTED	45	11	56
		VERY LOW DETECTED	11	1	12
		HIGH DETECTED	21	4	25
	TOTAL		105	19	124
2023	MTB	LOW DETECTED	21	1	22
		MEDIUM DETECTED	38	5	43
		VERY LOW DETECTED	17	0	17
		HIGH DETECTED	10	4	14
	TOTAL		86	10	96

3.7 Rifampicin Susceptibility Patterns of Tuberculosis by Age Group

Over the three-year study period, the age group 24–33 years recorded the highest number of tuberculosis cases, with 28 isolates sensitive to rifampicin and 3 exhibiting resistance. However, rifampicin-resistant TB was most frequently observed in individuals aged 34–43 years.

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Table 7: Rifampicin Susceptibility Patterns of Tuberculosis by Age Group.

YEAR			RIFAMPICIN SUSCEPTIBILITY		TOTAL
			S	RR	
2021	AGE	4-13	2	0	2
		14-23	17	2	19
		24-33	28	3	31
		34-43	20	1	21
		44-53	10	1	11
		54-63	7	0	7
		64-73	2	0	2
		74-83	8	1	9
	TOTAL		94	8	102
2022	AGE	4-13	7	0	7
		14-23	14	3	17
		24-33	33	5	38
		34-43	26	8	34
		44-53	9	1	10
		54-63	7	1	8
		64-73	8	0	8
		74-83	0	1	1
		84-93	1	0	1
	TOTAL		105	19	124
2023	AGE	4-13	2	0	2
		14-23	15	2	17
		24-33	32	3	35
		34-43	18	2	20
		44-53	7	2	9
		54-63	5	0	5
		64-73	6	0	6
		74-83	1	1	2
	TOTAL		86	10	96

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3.8 Regional Distribution and Comorbidities.

Out of the total sample, 37 cases (11.5%) were identified as rifampicin-resistant tuberculosis. The highest number of resistant cases was documented in 2022, followed by 2021, while the lowest incidence occurred in 2023. Geographically, the majority of rifampicin-resistant cases originated from Benghazi, accounting for 19 cases (5.9%), followed by 5 cases (1.5%) from the Al-Kuwayfiya area and 3 cases (0.9%) from Al-Kuwayfiya prison. Furthermore, the occurrence of culture-confirmed tuberculosis was more frequent among individuals who tested negative for HIV, hepatitis C virus (HCV), and COVID-19, compared to those who tested positive for these infections.

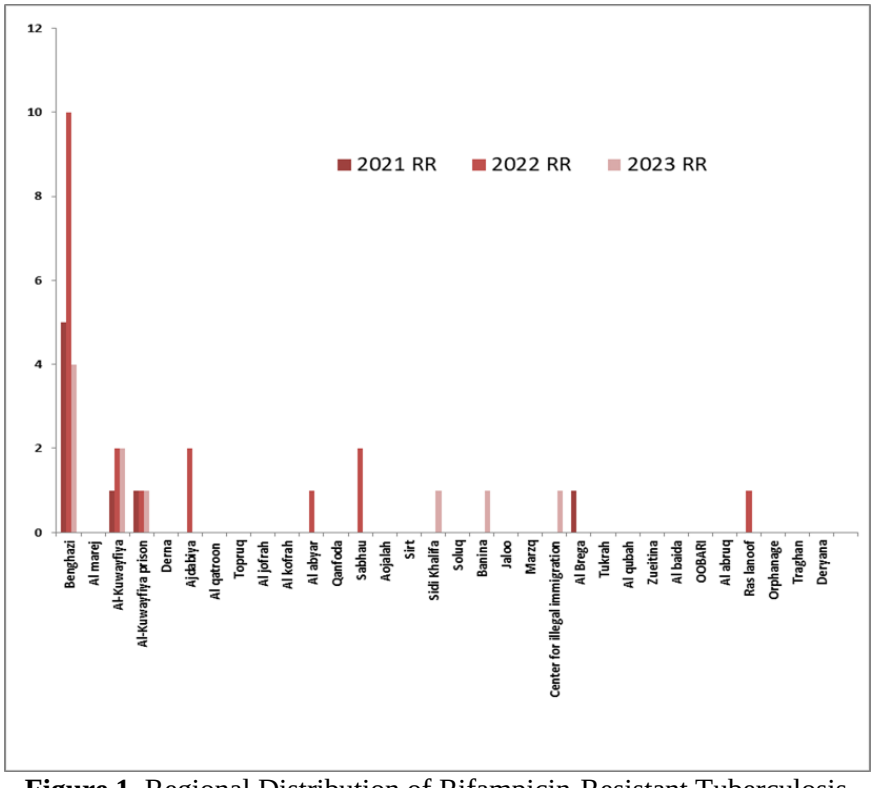

Figure 1. Regional Distribution of Rifampicin-Resistant Tuberculosis Cases in Libya**.**

3.9 Distribution of Tuberculosis Cases in Relation to Associated Diseases.

The occurrence of culture-confirmed tuberculosis was more frequent among individuals who tested negative for HIV, hepatitis C virus (HCV), and COVID-19, compared to those who tested

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positive for these infections. The highest number of TB cases was documented in 2022, with a total of 124 cases, while the lowest number was recorded in 2023, with 96 cases.

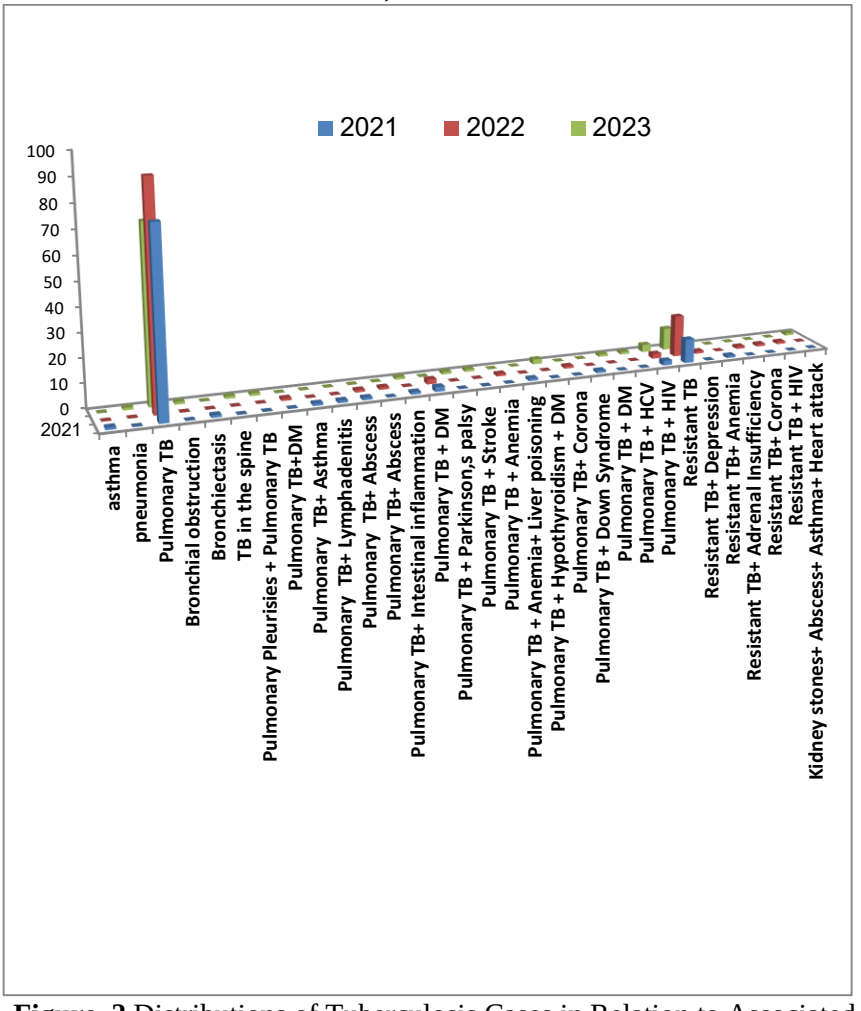

Figure. 2 Distributions of Tuberculosis Cases in Relation to Associated Diseases**.**

4. Discussion:

Mycobacterium tuberculosis (MTB) detection can be effectively achieved using the automated real-time polymerase chain reaction assay, GeneXpert MTB/RIF, which quantifies cycle threshold (Ct) values targeting the rpoB gene to estimate mycobacterial load (Fradejas *et al*., 2018; Najjingo *et al*., 2019). The diagnostic performance of this assay allows for rapid identification of rifampicin-resistant Mycobacterium tuberculosis, which is crucial for timely intervention. Consistent with previous findings (e.g.,

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Aljanaby *et al*., 2022; Liu *et al*., 2024), the incidence of TB was significantly higher in males than in females in our study cohort. While Ziehl–Neelsen (ZN) staining remains a conventional and cost-effective diagnostic method for TB, offering reliable and rapid detection of infection (Munir *et al*., 2015), and culture on Lowenstein–Jensen (LJ) medium is valuable for identifying viable bacilli (Wang *et al*., 2020), the GeneXpert MTB/RIF assay offers distinct advantages. These include a reduced risk of cross-contamination and a faster turnaround time. It exhibits higher sensitivity than ZN smear microscopy; detecting MTB even in samples with low bacterial loads (Geleta *et al*., 2015). The World Health Organization recommends GeneXpert for sputum testing in suspected TB cases because it can detect both TB and rifampicin resistance within two hours, whereas LJ culture requires several weeks. However, a limitation of GeneXpert is its inability to distinguish between live and dead bacilli, unlike culture methods that grow only viable bacteria (Caulfield & Wengenack, 2016).

This retrospective study at Al-Kuwayfiya Specialized Teaching Hospital evaluated TB prevalence and rifampicin resistance over three years. Males represented 76% of confirmed cases, a finding consistent with other studies reporting higher TB incidence in males (Aljanaby *et al*., 2022; Alawkally *et al*., 2022 & Okoro *et al*., 2019). The most affected age groups were 24–33 and 34–43 years, predominantly males, which may reflect factors such as occupational exposure, health-seeking behaviors, and healthcare access. The prevalence of rifampicin resistance in our study (11.5%) is comparable to reports from other regions, such as Baghdad, which noted rates of approximately 11.1–11.6% (Aljanaby *et al*., 2022).

GeneXpert quantifies bacterial load using cycle threshold values, with results categorized as high (Ct <16), medium (Ct 16–22), low (Ct 22–28), and very low (Ct >28) (Fradejas *et al*., 2018). In our study, most cases were detected in the summer months (June–August), aligning with previous seasonal trends (Habte *et al*., 2016). Additionally, culture-positive TB was more prevalent among individuals negative for HIV, HCV, and COVID-19, consistent with findings from other studies (Okoro *et al*., 2019).

5. Conclusion:

In conclusion, this study found a high prevalence of pulmonary tuberculosis among suspected patients attending Al-Kuwayfiya Teaching Hospital, with a relatively low but significant rate of

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rifampicin resistance. The GeneXpert MTB/RIF assay proved to be an indispensable tool for the rapid and accurate diagnosis of TB and detection of drug resistance, facilitating prompt treatment and potentially curbing transmission. The higher incidence among specific demographic groups (males, certain age groups, and nationalities) highlights the need for targeted public health interventions. Continuous surveillance of drug resistance patterns is essential for effective TB control in the region.

6. Recommendations:

4.2.1 Pharmacological Treatment:

- Active TB: Initiate the standard four-drug regimen including Isoniazid (H), Rifampicin (R), Pyrazinamide (Z), and Ethambutol (E) for 2 months, followed by Isoniazid and Rifampicin for 4 months.
- Latent TB Infection (LTBI): Treat with Isoniazid for 6–9 months or a Rifampicin-based regimen for 3–4 months.

4.2.2 Infection Control:

- Isolate infectious patients until sputum smears become negative.
- Promote respiratory hygiene and cough etiquette.
- Notify public health authorities promptly.
- Conduct screening for close contacts.

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