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Original Article

Tuberculosis trends in eastern Libya, Benghazi region, from 2021 to 2023

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ABSTRACT

Background: Tuberculosis (TB) is a global public health problem, particularly in developing countries. TB is the 13th leading cause of death worldwide and the top cause from a single infectious agent in 2019. Drug-resistant (DR-TB) is another area of concern, particularly Multidrug resistant (MDR) or Rifampicin Risistant (RR-TB), which is caused by *Mycobacterium tuberculosis* complex (MTC) with resistance to RIF and Isoniazid (INH) or only to RIF.**Objective:** Determine the prevalence of tuberculosis and Rifampicin resistant in Benghazi, Libya.**Materials and Method**s: A retrospective study included 1192 TB-suspected cases within three years from 2021 to 2023 registered in Al-Kuwaifeya Specialized Teaching Hospital for Chest and Tuberculosis in Benghazi, Libya. Pulmonary or extrapulmonary samples were collected from cases based on infection type. GeneXpert MTB/RIF and acid-fast stain (AFS) assays were utilized for the rapid diagnosis of *M. tuberculosis* infection and DR-TB. **Results:** A total of 27.1% (323/1192) tested positive for MTB, 11.5% were resistant to Rifampicin. The majority of TB cases were pulmonary TB (97.2%). The highest infection rates were in males (75.9%), while females were (24.1%). An increase in the infection rate was observed for the age category 25-34 (30.7%), and a decrease rate was observed in the age category 0-14 (4.0%). Libyans had the maximum infection rate (68.1%), and the highest rates of TB cases among non-Libyans were Sudanese (15.8%) and Chadian (8.7%). **Conclusion:** This study reported the increase in the infection rate of tuberculosis and rifampicin resistance compared with previous studies. Further studies is recommended to determine the risk factors related to TB infection among the population. Rapid and accurate methods of diagnosing *Mycobacterium tuberculosis* are essential for early initiation of treatment, prevention, and improvement of patient outcomes.

Keywords: *Mycobacterium tuberculosis*, Pulmonary TB, Extrapulmonary TB, GeneXpert MTB/RIF, AFB

Introduction

Tuberculosis (TB) is the 13th leading cause of death worldwide and the top cause of a single infectious agent in 2019. The reported global number of people newly diagnosed with TB was 7.5 million in 2022, and globally TB caused an estimated 1.30 million deaths. Worldwide, an estimated 10.6 million people will develop TB in 2022 [1]. In Libya, there was a change in estimated TB incidence in 2022 compared with 2015 (an increase of > 5%), and the number of deaths caused by TB was more than 5% above the level of 2015 [1]. In 2010, a retrospective study estimated the incidence of pulmonary TB cases among the total population (Libyans, non-Libyans) calculated as 0.09 cases/100.000 and estimated the prevalence of total pulmonary TB cases (Libyans, non-Libyans) calculated as 14 cases/100.000 [2].

TB is a potentially severe infectious disease caused by *Mycobacterium tuberculosis* (Mtb), which mainly affects the lungs by spreading from one person to another through the air. TB usually attacks the lungs (pulmonary TB), but it can affect other sites such as the kidney, spine, and brain (extra-pulmonary TB) [3]. The pathogens that cause TB in humans and animals are the *Mycobacterium tuberculosis complex* (MTC), which are phylogenetically very similar. The MTC includes *M. tuberculosis*, *M. bovis, M. africanum, M. microti, M. caprae, and M. pinnipedii* [4].

The risk factors favoring infection and progression to active TB disease include a compromised immune system (as patients with human immunodeficiency virus / acquired immunodeficiency syndrome (HIV/AIDS) and the incidence of multidrug-resistant *M. tuberculosis* strains, as well as malnutrition, diabetes mellitus, tobacco smoking, poverty, undocumented migration [5], ignorance, overcrowding, and poor hygiene, especially during the war and economic depression [6]. TB is curable and preventable, with most people (85%) who develop TB disease able to be successfully treated [3]. Without treatment, the death rate from TB is high [7].

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Antituberculosis agents are classified mainly into first- and second-line drugs. First-line agents are the most effective anti-TB drugs, including isoniazid (INH), rifampicin (RIF), ethambutol (EMB), and pyrazinamide (PZA) [8]. Second-line anti-TB drugs include injectable aminoglycosides (kanamycin, amikacin, capreomycin), ethionamide, cycloserine, para-aminosalicylic acid, and fluoroquinolones (FQ) (levofloxacin, moxifloxacin, ofloxacin, ciprofloxacin). Drug-resistant TB (DR-TB), which is a TB disease caused by MTC with resistance to RIF and INH or only to RIF (MDR/RR-TB) [9]. Extensively drug-resistant TB (XDR-TB) is resistant to the same drugs as MDR-TB and that is also resistant to at least one fluoroquinolone (levofloxacin or moxifloxacin) and to at least one other "Group A" drug (bedaquiline or linezolid) [1]. MDR-TB and XDR-TB cannot be treated with the standard 6-month course of first-line agents and can take up to 2 years or more to treat with second-line drugs [10]. In 2022, the estimated proportion of people with TB who had MDR/RR-TB was 3.3% among new cases and 17% among those previously treated. Globally, an estimated 410,000 people developed multidrug-resistant or RIF-resistant TB, but the number of people diagnosed and starting treatment was much lower [1].

In 2021 Libya had an estimated population 6.9 million people. In 2020 the country had an estimated TB incidence of around 4000 cases, corresponding to a rate of 59 cases per 100.000 population. However, it is estimated that only around 50% of TB cases are diagnosed, and of these, just under 63% are bacteriologically confirmed. TB mortality in Libya is considered to be high, as 20% of estimated cases, or 12 people per 100,000 population [11]. The official TB data for 2022 indicate that 2150 cases were notified (31 per 100.000 population) [12]. Given the absence of prevalence studies and limited laboratory capacities in Libya, there are major uncertainties around rates of drug-resistant TB (DR-TB), but it is thought to represent at least 2.6% of new cases and 21% of previously treated cases [11]. The World Health Organization's (WHO's) global strategy for TB prevention, care, and control for 2015-2035, known as the End TB Strategy (WHO), calls for the early diagnosis of TB and universal access to DST [1]. To meet the End TB Strategy targets, molecular WHO-recommended rapid diagnostic tests (mWRDs) should be made available to all individuals with signs or symptoms of TB. Individuals with bacteriologically confirmed TB should receive testing for resistance to RIF; those with RR-TB should receive testing for resistance to FQs, and those with pre-XDR-TB should receive testing for resistance to bedaquiline and linezolid [1]. Comprehensive drug susceptibility testing (DST) (phenotypic and/or genotypic) is needed to inform physicians about the best treatment [13]. The culture have been the method of testing with the greatest degree of sensitivity, and they are able to diagnose over 95% of TB cases [11]. Acid-Fast Bacilli (AFB) smear microscopy is still an important part of the management of MDR-TB in many countries [14]. The GeneXpert is an automated polymerase chain reaction (PCR) diagnostic tool for the simultaneous detection of MTC and RR-TB. In 2010, the WHO endorsed Xpert as a replacement for smear microscopy [15]. The recommendation by the WHO in 2010 for Xpert MTB/RIF's use as the initial diagnostic test was for the detection of HIV-associated TB and where high rates of drug resistance were suspected [16]. The Food and Drug Administration (FDA) subsequently approved the assay in 2013 as a medium complexity test [17].

Materials and Methods

Study Location and Data Collection

A three-year retrospective descriptive analysis to assess the tuberculosis cases and risk factors of 323 TB patients registered from 2021 to 2023 was carried out in Al-Kuwaifeya Specialized Teaching Hospital for Chest and Tuberculosis. Demographic, clinical, radiological, and laboratory data findings of 1192 consecutive probable TB patients (pulmonary and extrapulmonary) were retrieved retrospectively from the patient's medical and laboratory charts during the study period, including age, sex, residence, nationality of patients, and type of TB as well as the associated comorbidities. In addition to the results of the Ziehle-Neelsen (ZN) smear, and GeneXpert findings.

Laboratory Investigations

At the Mycobacterial department of Al-Kuwaifeya Hospital lab, all specimens (sputum, CSF, pleural fluid, gastric lavage aspirate, or other tissue samples) were collected according to their sites. Each sample was divided into 2 parts: one for AFB microscopy by ZN stain and the other part for GeneXpert analysis. If the presence of MTB was detected, GeneXpert was also used to look for evidence of rifampicin resistance MTB/RIF.

In the examination by ZN stain, the sputum smear was prepared, and staining was used for acid-fast bacilli (AFB) detection. The reading of sputum smear direct microscopy was based upon the quantitative presence of AFB in samples reporting according to WHO manual sputum grading [18][19]. The GeneXpert ultra (Cepheid Inc., USA) is a rapid assay using improved assay chemistry and cartridge design and relies on the amplification of two multicopy genes, IS6110 and IS1081, as well as that of the RIF resistance-determining region (RRDR) of the rpoB gene [20]. The GeneXpert system integrates and automates

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sample processing, nucleic acid amplification, and detection of the target sequences in samples using nested real-time PCR. Five rpoB RRDR-specific molecular beacons are then used to detect both the presence of *M. tuberculosis* and mutations responsible for approximately 95% of RIF Resistance [21]. Results on *M. tuberculosis* detection were classified as invalid, not detected, and detected, while those of RIF resistance were classified as detected, not detected, or indeterminate. A semi-quantitative scale (very low, low, medium, or high) was used to determine the concentration of *M. tuberculosis* among GeneXpert ultra-positive samples [20].

Data will be analyzed by using a Statistical Package of the Social Sciences, SPSS Statistics for Windows, Version 26.0 software program (IBM Corporation, Armonk, NY, USA). The following statistics will apply: (a) Descriptive statistics as numbers and percentages will be used for categorical variables. (b) Chi-square (x2) was used, significance was assumed at when p value ≤ 0.05.

Results

In the current study, microscopic examination and GeneXpert testing were performed on a total of 1192 patients who were clinically suspected of having tuberculosis. Among three years (2021, 2022 and 2023). Atotal of (27.1%, 323 cases) tested positive for *M. tuberculosis* (MTB) on GeneXpert assay. The largest number of TB cases was recorded in 2022 (38.4 %, 124 cases), and the lowest was recorded in 2023 (30.0%, 97 cases). Among the GeneXpert MTB positive cases, (11.5%, 37 cases) were resistant to Rifampicin. From 323 cases of TB, (97.2%, 314 cases) were pulmonary, and (2.8%, 9 cases) were extrapulmonary. Our study showed that males constitute the highest of TB infections, (75.9%, 245 cases), while female cases were the lowest, (24.1%, 78 cases). An increase in the infection rate was observed for the age category 25-34 (30.7%, 99 cases) and 35-44 (22.7%, 73 cases), while the decrease in the infection rate was observed in the age category 0-14 (4.0%, 13 cases). The several nationalities living in the Benghazi region were infected with tuberculosis; the Libyan showed the maximum infection rate (68.1%, 220 cases). The highest number of TB cases among others besides Libyan were Sudanese (15.8%, 51 cases), followed by Chadian (8.7%, 28 cases). Most of the TB cases showed medium, high, and low MTB bacterial burden (42.7%, 138 cases), (23.2 %, 75 cases), and (22.6%, 73 cases), respectively. According to the time of the detected TB infection, the highest TB cases were detected in months from July to September (30.3%, 98 cases) and lowest in January to March (21.1%, 68 cases), (p=0.582).

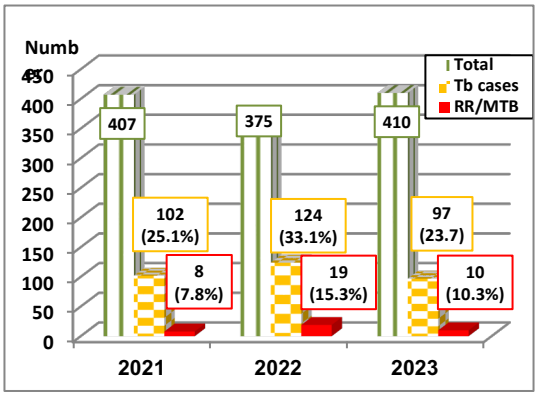

Figure 1. Distribution of all cases by year.

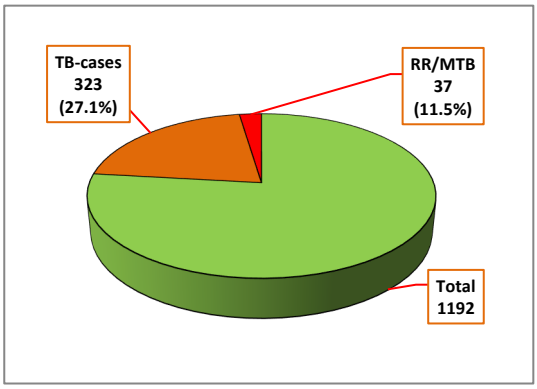

Figure 2. Distribution of all cases by test result.

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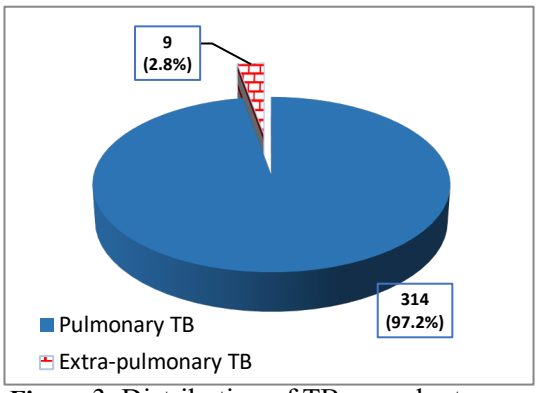

Figure 3. Distribution of TB cases by type.

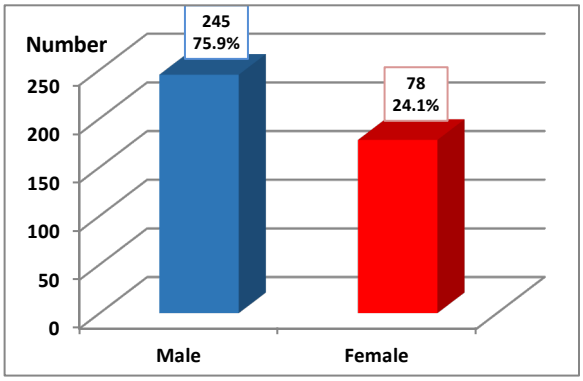

Figure 4. Distribution of TB cases by gender.

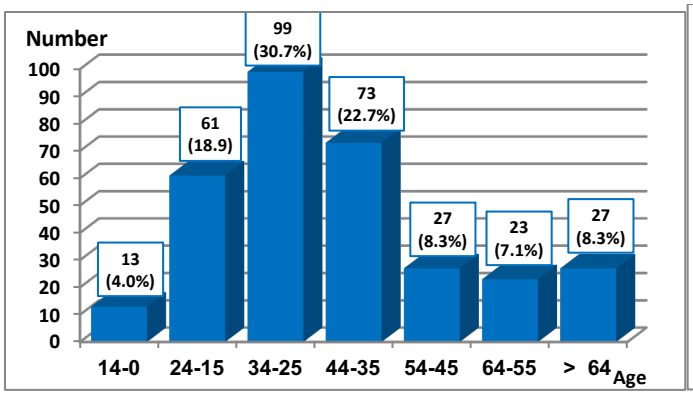

Figure 5. Distribution of TB cases by age.

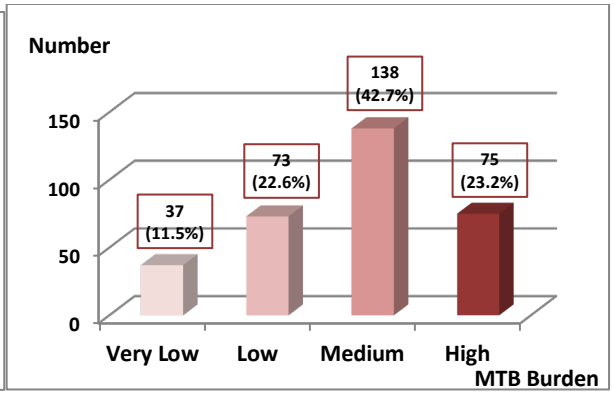

Figure 6. Distribution of TB cases by MTB burden.

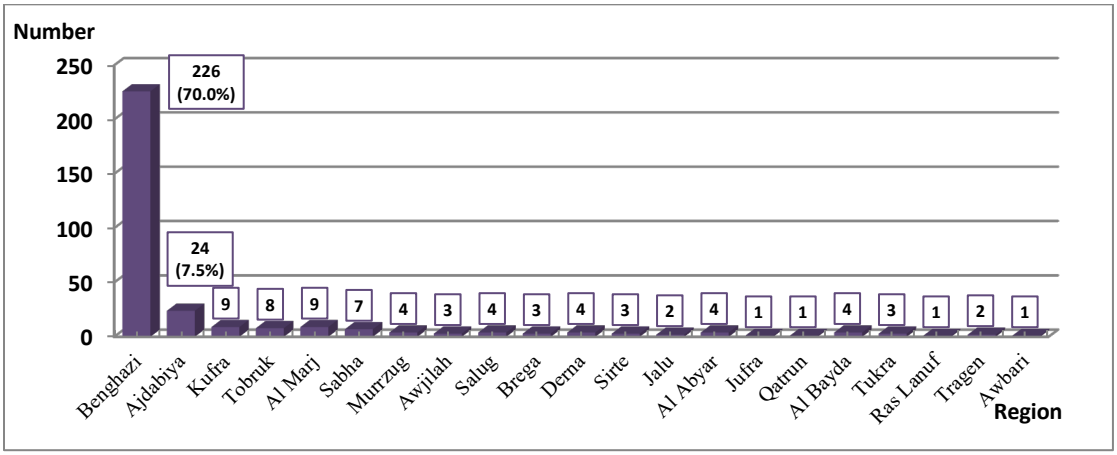

Figure 7. Distribution of TB cases by region.

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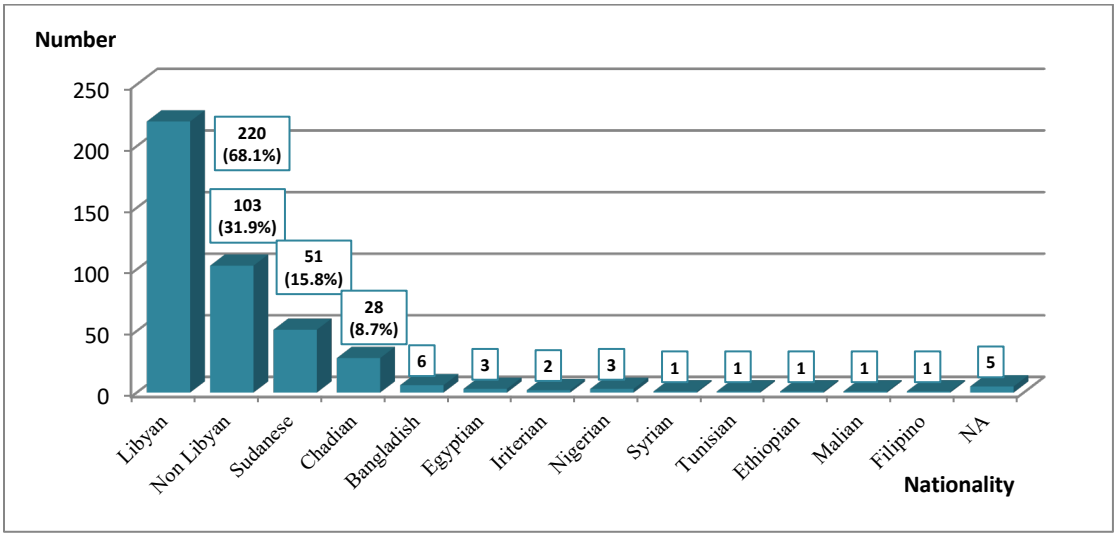

Figure 8. Distribution of TB cases by nationality.

Discussion

The main result of the current study is that, out of the 1192 total samples screened, 323 (27.1%) tested positive for MTB on the GeneXpert assay; of them, 37 (11.5%) were resistant to Rifampicin. The number of TB cases was recorded in three years: 2021 (102), 2022 (124), and 2023 (97); this difference was found non-significant (p=0.888). The prevalence of *M. tuberculosis* infection in this study was in agreement with a previous report in Egypt (27.6%) [22]. But in the other study, the prevalence rate of MTB positive among presumptive TB patients was (95.2%) [23]. In 2022, the estimated proportion of people with TB who had MDR/RR-TB was 3.3% among new cases and 17% among those previously treated [1]. The MTB burden in TB cases was medium (138), high (75), low (73), and very low (37); a non-significant difference was found (p=0.449). In our results, the majority of infected cases were pulmonary TB (97.2%), which contrasts with the study that reported the total number of TB cases in Libya during 2003 was 1559 cases. Of these, 51.0% were pulmonary TB, and the remaining 49.0% were extra-pulmonary TB cases [2] .

The notification data from the National Centre for Disease Control, Libya, in 2019 reported 2209 TB cases; the pulmonary cases were (56.63%) and extrapulmonary were (43.36%) [24]. The lowest rate of extrapulmonary TB cases in our study, may be due to converting these cases to the National Center of Disease Control (NCDS) to complete treatment . .

The TB infections are found to be dominant among the age group 25-44 (66.1%), but the lowest infection rate is in the age group 0-14 (4.0%); this difference was found to be non-significant (p = 0.578). In agreement with the study by Hassanin et al. (2023), which found the highest positive finding for MTB was observed in the age group 15-34 years (35.8%) and in 35-49 (19.7%) [22]. This could be ascribed to the wide range of motion of this age category subject to getting infected with TB. Obtaining expectorated sputum from children for detection of MTB tubercle is difficult, and its examination is of low yield; hence the low results for patients who are less than 14 years. Sputum induction has a higher yield than expectorated sputum in children [25]. The cases of TB patients in this study were (75.9%) male and (24.1%) female. This result agrees with some research papers [26], while there is a contrast with other studies [27]. This variance might be attributed to the higher male exposure to diverse elements such as smoking and alcoholism that constitute a hazard of getting infected by TB bacilli [22]. This contrasts with a study in Nigeria, which found the incidence rate of females was higher than males [28].

Benghazi is the region with the highest number of TB infections, followed by Ajdabiya and Kufra with a significant difference (p=0.048). This may be due to, primarily, the fact that it is the area of the hospital in which this study was conducted, and on the other hand, possibly because it is the second city in Libya in terms of population number or overcrowding. This agrees with studies that have found that a higher proportion of TB patients live in urban settings [29]. The highest of TB-infected cases were among Libyans (68.1%), while the non-Libyans were Sudanese (15.8%) and Chadian (8.7%). This difference

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was statistically significant (p=0.033). This result is similar to the study by Guyth et al. (2018) which observed the majority of cases (88%) were Libyans and (12%) were non-Libyan nationalities: Sudanese (2.1%) and Egyptian (1.9%) [27]. The most frequent nationalities among non-Libyan TB cases in our study were from neighboring African countries. In the WHO African Region, there is a high number of TB cases; the region accounted for 23% of new TB cases in 2022 [1]. In Libya, there should be mandatory screening procedures for all foreign-born people and immigrants through pre-entry screening for tuberculosis to identify TB active cases.

The microbiological detection of TB using WHO-recommended tests is critical because it allows people to be correctly diagnosed, is necessary to test for drug resistance, and ensures that the most effective treatment regimen (depending on the pattern of drug resistance) can be selected as early as possible [30].

Conclusion

This study reported the prevalence of tuberculosis and rifampicin resistance in suspected patients in Benghazi was 27.1% and 11.7%, respectively. This indicated an increase in the infection rate compared with previous studies. The researchers recommended further studies to determine the risk factors related to the TB infection among the population. Rapid and accurate methods of diagnosing TB infection are essential for early initiation of treatment, prevention, and improvement of patient outcomes.

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Conflict of interest:

The authors declare that there is no conflict of interest regarding the publication of this paper.

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