The Egyptian Journal of Hospital Medicine (January 2023) Vol. 90, Page 1488-1493

Molecular Detection of Tuberculosis Disease and Association between Drugs Taken with Some Liver Functions

Israa Jawad Jaber
College of Veterinary Medicine, Al-Muthanna University, Iraq
Corresponding Author: Israa Jawad Jaber, **Mobile:** 009647808957119, **E-mail:** Israa.jawad@mu.edu.iq

ABSTRACT

Background: Tuberculosis is one of most dangerous and infectious diseases that widespread in the world, as untreated severe cases lead to the death of infected patients. **Objective**: The current study aimed to isolate and diagnose *Mycobacterium tuberculosis* and to know the number of infections in the city of Samarra (Iraq), and whether there are associations between sex or age and disease. **Patients and methods**: A total of 50 specimens were collected from TB patients, out of which 25 were males and 25 were females. The diagnosis was made by the physician at the center based on chest x-rays, microscopy, biochemical tests and examinations. The confirmatory examination was carried out using a PCR test. **Results**: The age of the participants ranged from 20 to 45 years. The diagnosing bacteria from the number of the samples reached 123 samples collected from people suffering from chest symptoms who arrived at the Respiratory Disease Center in Samawah-Muthanna Governorate. The results of isolation and diagnosis by bacterial cultures, x-ray showed and PCR that 49 (39.9%) samples were for people with tuberculosis and 72 (60.1%) samples were not infected with the disease. The liver enzymes were found to be significantly low compared to the normal levels ($P<0.05$) in the studied patients. The number of the persons who completed the treatment and were cured of the sickness was discovered to be 24 (about 75%); we considered the importance of the moral difference (P value 0.463), because the significance level is larger than 0.05. **Conclusions**: High proportion of drug resistance to *M. tuberculosis* strain detected that could suggest the need to increase the efforts to the strengthen TB control program in the study area. There were significant variations observed in the drug resistance patterns between *M. tuberculosis* lineage.

Keywords: Tuberculosis, Liver function, Drugs course, Anti-tuberculosis, M.Tuberculosis, Case series, Al-Muthanna University.

INTRODUCTION

Tuberculosis is one of communicable infectious diseases caused by the *Mycobacterium tuberculosis* complex **(1).**

Bacteria *Mycobacterium tuberculosis* is the main cause of tuberculosis, that as one of oldest diseases which known as mankind. It's found in the mummies of the ancient Egyptians. many studies mention that this bacterium tends to the grow in the clumps despite its slow growth, so its generation time is from (11-30 hours), and its colonies grow after weeks on solid medium and this period is long compared to others bacteria. It is one of the types of bacteria whose generation time is less than an hour, and scientists has attributed that the length of the generation time for this bacteria occurs due to the components of its wall that prevent the passage of nutrients easily and it protects itself in this way from the substance secrete from the host organism against it, so its cell wall contains complex substances; waxy and fatty acids such as mycolic acid and layers of (Arabinogalactan) and (peptidoglycan), that are situated above the plasma membrane of the bacteria. Some scientists classified these bacteria as Gram-positive because they lack the phosphorylated fats, yet they are weak It is very pigmented or does not retain the crystal violet as a result of the high level of the waxy substances and myicolic acid **(2,3,4).**

Because of this, some scientists characterize it as neutral for chromium dye. Despite the fact that

Mycobacterium tuberculosis does not produce any toxins, other studies have shown that the bacteria remain for several years in the lungs because the immune system is the only organ that can contain them. This is evidence of the bacteria's significance because it possesses many key virulence factors; the growth of antibiotic resistance, the deteriorating state of health in big cities, and their virulence and severity **(5).**

During the last 100 years, tuberculosis has infected and killed more than 100 million people **(6).** Due to the fact that latent tuberculosis, the pathogen's natural reservoir, affects one third of population the world, it is a significant public health issue on a global scale. Additionally, 2 million people per year pass away from tuberculosis, which affects 9 million people with active cases. Countries account for more than 90% of tuberculosis cases. Africa, Southeast Asia, and Eastern Europe are the developing regions and the regions most concerned with this illness **(7).**

The BCG vaccine's insufficient protection against Mycobacterium TB, population shifts, and the existence of multidrug-resistant strains may be the cause. 70 years ago, there was no medicine to treat tuberculosis; today, there are 500,000 cases of the disease caused by multidrug-resistant strains and 27,000 widely drug-resistant. However, the first antibiotics, discovered in the 1950s and 1960s of the 20th century, are still the first

Received: 6/8/2022
Accepted: 6/10/2022

treatments for tuberculosis, particularly rifampicin and isoniazid [8].

Therefore, the current study aimed to isolate and diagnose Mycobacterium tuberculosis and know the number of infections in the city of Samarra and whether there are relationships between sex and disease, or age and disease, that is, there are moral differences in addition to a study [9]. The duration of treatment last from six to eight months, and the commonly used drug to treat tuberculosis are rifampicin and isoniazid. When an active case of the tuberculosis is detect in the presence of the germ in the sputum, the treatment that must be started depends to anti-tuberculosis drugs that given in a specific way and at a specific dose [10].

The current study aimed to isolate and diagnose *Mycobacterium tuberculosis* and to know the number of infections in the city of Samarra (Iraq), and whether there are associations between sex or age and disease.

PATIENTS AND METHODS

Between October 2020 and May 2021, 50 specimens were gathered from patients; 25 of whom were males and 25 of whom were females. The study was conducted in the Laboratories of Al-Hussein Teaching Hospital.

The physician at the facility made the diagnosis using chest x-rays, microscopes, biochemical tests, and physical examinations. The confirmatory examination was carried out using a PCR test.

Oral sputum was one of the specimens. All samples were collected, analyzed, and handled within a maximum of one hour at room temperature. Sputum was maintained on 4°C for 1-3 hours until processed or aliquoted and stored at -70°C for further evaluation. This was repeated for each day of collection. The entire bacterial genomes were extracted from the suspension created when sputum was submerged in distilled water as the eluent. Also, as soon as the samples were received, aliquots of material for PCR testing were created, and the bacterial genomes were extracted and kept at -70°C until use.

Additionally, all other clinical samples were kept at -20°C. The lyophilized primers in **Table 1** are dissolved by TE (Tris-EDTA) fluid buffer for making a stock fluid with a concentration at 100 pmoles/ml. After spinning and storing at 4°C overnight, the solutions for the primers were prepared by diluting the stock fluid by TE buffer to obtain the final working fluid (10 pmoles/Ml) to each primer. Multi-gene PCR products were examined using agrose gel electrophoresis.

Table (1): Primer and sequences of the studied samples.

Primer	Sequences	References
MPB	F 5'ATTGTGCAAGGTGAACTGAG'3 R 5'AGCATCGAGTCGATCGCGGA '3	5
MPB	F 5'ATTGTGCAAGGTGAACTGAG'3 R 5'AGCATCGAGTCGATCGCGGA'3	

AST, ALT, and alkaline phosphatase (ALP) as liver function tests were performed at the Central Chest and Respiratory Center in Al-Muthanna City, Iraq.

Ethical Consideration:

This study was ethically approved by the Ethical Committee of the Directorate of Health of Al-Hussein Teaching Hospitals. Written informed consent was obtained from all participants. This study was executed according to the code of ethics of the World Medical Association (Declaration of Helsinki) for studies on humans.

Statistical analysis

The collected data were introduced and statistically analyzed by utilizing the Statistical Package for Social Sciences (SPSS) version 20 for windows. Qualitative data were defined as numbers and percentages. Chi-Square test and Fisher's exact test were used for comparison between categorical variables as appropriate. Quantitative data were tested for normality by Kolmogorov-Smirnov test. Normal distribution of variables was described as means and standard deviation (SD), and independent sample t-test/Mann-Whitney test was used for comparison between groups. Spearman's correlation was utilized to examine the relationship between two variables. P value ≤ 0.05 was considered to be statistically significant.

RESULTS

The age of the participants ranged from 20 to 45 years.

The diagnosing bacteria from the number of the samples reached 123 samples collected from people suffering from chest symptoms who arrived at the Respiratory Disease Center in Samawah-Muthanna Governorate. The results of isolation and diagnosis by bacterial cultures, x-ray showed and PCR that 49 (39.9%) samples were for people with tuberculosis and 72 (60.1%) samples were not infected with the disease (Table 2).

Table (2): Primers found in the studied samples.

Primer	PCR product	TM	Positive Samples	Negative Samples
MPB64 inner primer	200bp	58C	(1-33)	(34-50)
MPB64 outer primer	240bp	55C	(1-26), (28-34) and (42-46)	(27-28), (35-40) and (47-49)

It is clear from **Table 3** that the infection rate of males is approximately equal to that of females, where the number of males infected reached 25 patients, i.e. 51%, but in females, the number of patients reached 24 patients, i.e. 49%, and there is no significant difference as the percentage of moral difference was 0.246.

Table (3): Number and type of patients' samples.

Sex	Number of patients	%
Male	**25**	**51%**
Female	**24**	**49%**

Bacterial isolates were diagnosed based on the determination of the microscopic characteristics of the colonies and the microscopic characteristics of the prepared slides figures (1) represent the results of the microscopic examination of a positive sample.

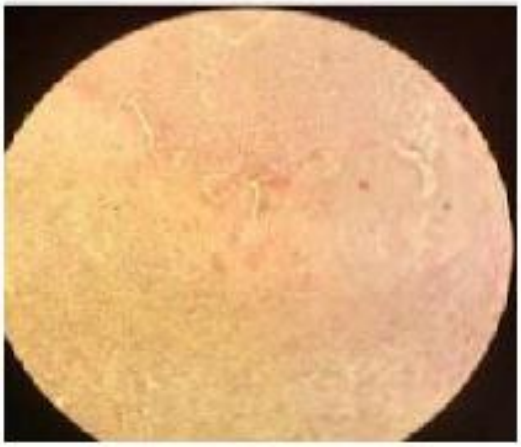

Figure (1): *M. Tuberculosis* examination under microscope.

The liver enzymes were found to be significantly low compared to the normal levels ($P<0.05$) in the studied patients. Before taking the medicine, ALP was 205.81 (SD 32.01) afterward, it was 150.14 (SD 20.21). Before taking the medicine, my AST was 43.45 (SD 12.35); after taking it, it was 27.21 (SD 8.13).

Before taking the medicine, ALT was 40.21 (SD 12.42); upon taking it, it really was 25.04 (SD 7.25).

The progression of 30 patients' cases was to determine the level of the bacteria resistances to anti-tuberculosis 1st and 2nd line drug.

The number of the persons who completed the treatment and were cured of the sickness was discovered to be 24 (about 75%); We considered the importance of the moral difference (P value 0.463), because the significance level is larger than 0.05. These isolates were considered sensitive to anti-tuberculosis drugs; there is no significant link between the two variables: illness and bacteria sensitivity to antibiotics (-0.154). This indicates that the connection is weak and inverse, and that its value should not be trusted.

There were 14 samples that failed treatment, accounting for 23.3% of the total. P value was 0.019, which means that there is a significant relationship between the two variables, sensitivity to antibiotics and disease, while the correlation value was -0.617 which means that the correlation is medium and inverse, and its value can be relied on as being average. There were total of 22 samples that were not subjected for treatment.

Retesting the specimens using a PCR assay verified the results. PCR products were visible on the gel in all of the samples, with no confusion.

Figure 2 shows Gel electrophoresis to optimization by different temperatures and Primers for PCR product (MPB64 inner primer) which show 200bp at 58c and (MPB64 outer primer) which show 240bp at 55c respectively; Agarose 1%, 10minate on 100 voltage and then lower to 70 volts, 60 minute).Visualized sub U.V light then stain by Ethidium bromide; Lane L: DNA ladder (1500-100) bp.

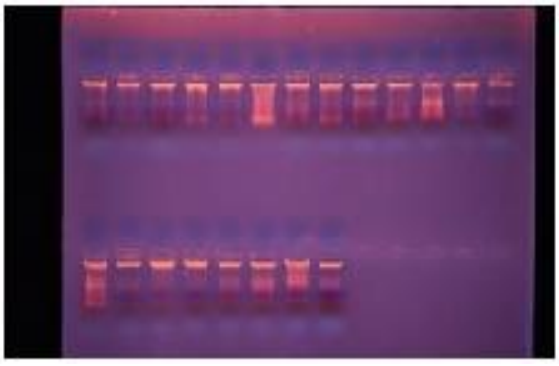

Figure (2): Ethidium bromide stain agarose gel electrophoresis appear that displays DNA from the bacteria which was extracted.

In **Figure 3** showed gel electrophoresis to PCR product ((MPB64 inner primer) that show 200bp Primer TM on (58C), (Agarose 1%, 10minate at 100 voltage and then lowered to 70 volts, 60min.) Visualized under U.V light

then stained by ethidium bromide. Lane L: DNA ladder (1500-100) bp, Lanes (1-21) represented positive; Lanes (N) represented Negative results.

Figure (3): Gel electrophoresis to optimization process with different temperatures and different Primers.

Figure 4 illustrates Gel electrophoresis for PCR product of (MPB64 inner primer) which show 200bp Primer TM at (58C), (Agarose 1%, 10min. at 100 voltage and then lowered to 70 volts, 60min.). Visualized sub U.V light then stained by ethidium bromide. Lane L: DNA ladder (1500-100) bp, Lanes (22-33) represented positive results; Lanes (34-50) represented Negative.

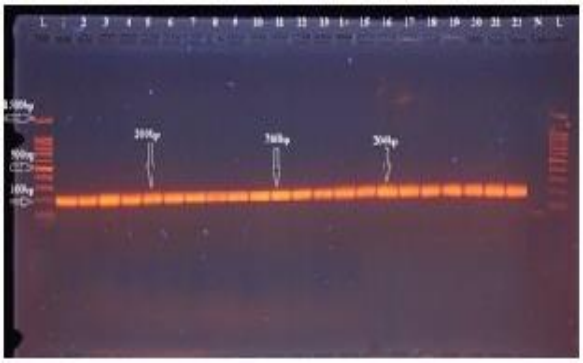

Figure (4): Gel electrophoresis to PCR product ((MPB64 inner primer) (1-19) lanes.

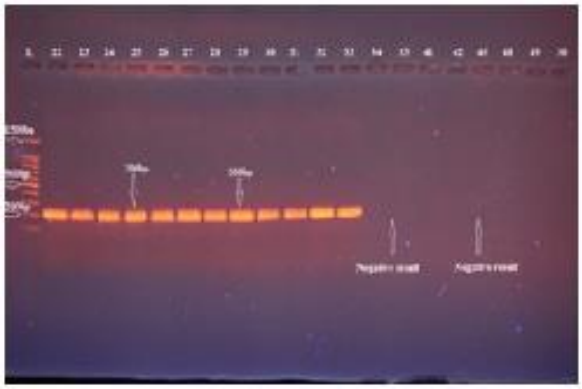

Figure (5): The Gel electrophoresis for PCR product of (MPB64 inner primer) Lanes (22-33).

Figures 6 and 7 illustrate Gel electrophoresis to PCR product ((MPB64 outer primer) which show 240bp Primer TM at (55C), (Agarose 1%, 10min. at 100 voltage and then lowered to 70 volts, 60min.).Visualized sub U.V light then stained by ethidium bromide. Lane L: DNA ladder (1500-100) bp, Lanes (1-20) and (21-46).

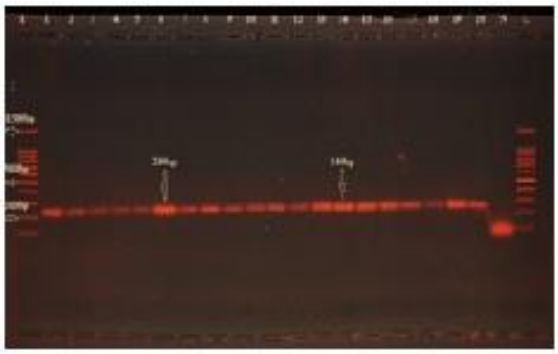

Figure (6): Gel electrophoresis to PCR product (MPB64 outer primer) Lane L: DNA ladder (1500-100) bp, Lanes (1-20) represented positive, lane N represent negative control.

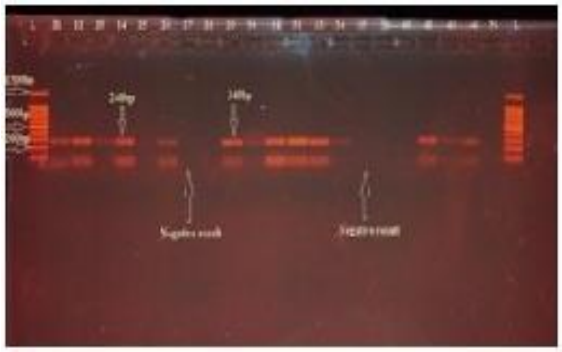

Figure (7): Gel electrophoresis to PCR product of ((MPB64 outer primer) (21-46 lenses).

DISCUSSION

The most common infectious disease in the world and the leading cause of mortality, tuberculosis, is still a major problem. In developing nations, tuberculosis, the world's most common infectious disease and the top cause of mortality overall, continues to be a serious public health problem [(11)]. There is minimal research on hematological factors in patients with pulmonary tuberculosis in the Iraqi community [(12,13)].

The infection rate of the males is approximately equal to that of females, where the number of males infected reached 25 injuries, i.e. 51%, but in females, the number of injuries reached 24 injuries, i.e. 49%, and there is no significant difference as the percentage of moral difference was 0.257 because of the significance level was larger than 0.05, and Pearson’s correlation

coefficient was found to find out the existence of an association between disease and sex, and the correlation coefficient was -0.109 that is, the correlation was very weak and unreliable because its value was very small. This is very logic, as tuberculosis is not limited to males or females [13], but to the both, and this is consistent with what he found that the infection rate for males is equal to the rate of infection for females, but it does not agree with what this study found In Baghdad [14], where the results showed that the incidence of the an infection in males was 30% higher than in females, which amounted to 20% [15,16]. Also, my study does not agree with a study conducted in Vietnam, which indicated that males are more susceptible to infection than females because of the difference in social relationships and negative behavior of community members [17].

The World Health Organization report [18] also indicated that the diagnosis of tuberculosis cases is more in males than in females [19], as well as the death rate resulting from it, the reason for this is due to the difference in exposure to the risk of infection [20]. Also, results of my current study agree to study conducted in India, which showed that the risk the developing the disease from infection to tuberculosis is more effective in men than women [21]. It also does not agree with previous studies [22,23] which showed that females are more sensitive to infection than males, while males In a study conducted by **Bhatt (2010)**, the male infection rate was 64%, while the female infection rate was 36%, and the reason for the lack of significant differences may be because the small size of the studied sample [24].

Aminotransferases (ALT, AST) levels increase considerably following the infection with Mycobacterium tuberculosis [25].

The rise in the liver enzyme levels did not correspond to increase in the bacterial burden. The use of drugs result in a significant reduction in the quantity of bacteria; particularly in groups receiving a combination of drugs [26,27] came to the same conclusion.

Because of incomplete patient data, the results obtained are definitely not representative of all tuberculosis patients [28]. Interactions between anti-TB treatments and other medications in people with comorbidities are another barrier [29].

CONCLUSION

High proportion of drug resistance to *M. tuberculosis* strain detected that could suggest the need to increase the efforts to the strengthen TB control program in the study area. There were significant variations observed in the drug resistance patterns between *M. tuberculosis* lineage. Thus, increased efforts in the timely evaluation of the drug application and resistance of the more robust diagnostic tools could help in an implementation of an effective TB control program.

Conflict of Interest: There was no conflict of interest.
Sources of Funding: None.
Authors' contribution: The authors contribute equally in this study.

REFERENCES

1. **SedrakA, Salem A, Amin T, Amin W (2022):** Cost effectiveness analysis of adopting Gene-Xpert® (GX) for the Diagnosis of Tuberculosis in Egypt. The Egyptian Journal of Hospital Medicine, 88:2452-8.
2. **Aldous W, Pounder J, Cloud J, Woods G (2005)**: Comparison of six methods of extracting Mycobacterium tuberculosis DNA from processed sputum for testing by quantitative real-time PCR. J Clin Microbiol., 43:2471-3.
3. **Mansour1 S, Abd Elsalam1 E, Rabie R, Ibrahim N (2021):**Study of Latent Tuberculosis among Type 1 Diabetic Children in Zagazig University Pediatric Hospital. The Egyptian Journal of Hospital Medicine, 84:2198-2202.
4. **Anon S (2011):** Implications for management of Mycobacterium tuberculosis infection in adult-to-adult live donor liver transplantation. Liver Int., 27(3):81-5.
5. **Chan R (2007)**: Genetic and phenotypic characterization of drug-resistant Mycobacterium tuberculosis isolates in Hong Kong. J Antimicrob Chemother., 59(10):866-73.
6. **Chryssanthou E, Angeby K (2012)**: The Geno Type(R) MTBDR plus assay for detection of drug resistance in Mycobacterium tuberculosis in Sweden. APMIS., 120(9):405-9.
7. **Fan X, Hu Z, Xu F, Yan Z, Guo S, Li Z (2003**): Rapid detection of rpoB gene mutations in rifampin-resistant Mycobacterium tuberculosis isolates in Shanghai by using the amplification refractory mutation system. J Clin Microbiol., 41(5):993-7.
8. **Heym B, Philipp W, Cole S (1996**): Mechanisms of drug resistance in Mycobacterium tuberculosis. Curr Top Microbiol Immunol., 215:49-69.
9. **Imperiale B, Cataldi A, Morcillo N (2011)**: Rapid detection of multidrug-resistant Mycobacterium tuberculosis by multiplex allele-specific polymerase chain reaction. Int J Tuberc Lung Dis., 15(8):496-501.
10. **Jin J (2012):**Evaluation of the GenoType(R) MTBDRplus assay and identification of a rare mutation for improving MDR-TB detection. Int J Tuberc Lung Dis., 16(3):521-6.
11. **Khosravi A, Goodarzi H, Alavi S (2012):** Detection of genomic mutations in katG, inhA and rpoB genes of Mycobacterium tuberculosis isolates using polymerase chain reaction and multiplex allele-specific polymerase chain reaction. Braz J Infect Dis., 16(2):57-62.
12. **Konno K, Oizumi K, Hayashi I, Oka S (1972):** Mode of action of rifampicin on Mycobacterium tuberculosis. Kekkaku., 47:255-60.
13. **Li J (2012):** The development of a GeXP-based multiplex reverse transcription-PCR assay for

simultaneous detection of sixteen human respiratory virus types/subtypes. BMC Infect Dis., 12(5):189.

14. **Little S (2001):** Amplification-refractory mutation system (ARMS) analysis of point mutations. Curr Protoc Hum Genet., 9(9):8-9.
15. **Liu Y (2012):** Development of a multiplex tetra-primer amplification refractory mutation system PCR to detect three single nucleotide polymorphisms associated with breast cancer. Sci SinVitae., 7(1):42. doi: 10.1360/052011-628.
16. **Ma X (2006)**: rpoB gene mutations and molecular characterization of rifampinresistant Mycobacterium tuberculosis isolates from Shandong Province. China J Clin Microbiol., 44(9):3409-12.
17. **Marttila H (1998):** Rapid detection of rifampin-resistantMycobacteriumtuberculosis by sequencing and line probe assay. Scand J Infect Dis., 30(6):129-32.
18. **Marttila H (1999):** Line probe assay in the rapid detection of rifampin-resistant Mycobacterium tuberculosis directly from clinical specimens. Scand J Infect Dis., 31(5):269-73.
19. **Miller L, Crawford J, Shinnick T (1994):** The rpoB gene of Mycobacterium tuberculosis. Antimicrob Agents Chemother., 38(8):805-11.
20. **Newton C (1989):** Analysis of any pointmutation in DNA. The amplification refractory mutation system (ARMS). Nucleic Acids Res., 17(4):2503-16.
21. **O'Riordan P (2008**): Rapid molecular detection of rifampicin resistance facilitates early diagnosis and treatment of multi-drug resistant tuberculosis: case control study. PLoS One, 3(2):317.
22. **Rastogi N, David H (1993):** Mode of action of antituberculous drugs and mechanisms of drug resistance in Mycobacterium tuberculosis. Res Microbiol., 144(10):133-43.
23. **Siddiqi S (2012):** Direct drug susceptibility testing of Mycobacterium tuberculosis for rapid detection of multidrug resistance using the Bactec MGIT 960 system: a multicenter study. J Clin Microbiol., 50(7):435-40.
24. **Somoskovi A, Parsons L, Salfinger M (2001):** The molecular basis of resistance to isoniazid, rifampin, and pyrazinamide in Mycobacterium tuberculosis. Respir Res., 2(5):164-8.
25. **Tho D (2008**): Comparison ofMAS-PCR and GenoTypeMTBDR assay for the detection of rifampicin-resistant Mycobacterium tuberculosis. Int J Tuberc Lung Dis., 12(9):1306-12.
26. **Traore H (2007):** Low-cost rapid detection of rifampicin resistant tuberculosis using bacteriophage in Kampala Uganda. Ann Clin Microbiol Antimicrob., 282(8):16288-94.
27. **Wang Q (2007):** A newly identified 191A/C mutation in the Rv2629 gene that was significantly associated with rifampin resistance in Mycobacterium tuberculosis. J Proteome Res., 6(3):4564-71.
28. **Yip C, Chan M, CheungW*et al*. (2012):**Randomblinded rechecking of sputum acid-fast bacilli smear using fluorescence microscopy: 8 years' experience. Int J Tuberc Lung Dis., 16(2):398-401.
29. **Zhang C (2013):** A novel multiplex tetra-primer ARMS-PCR for the simultaneous genotyping of six single nucleotide polymorphisms associated with female cancers. PLoS One, 8(2):621.