PHARMACOKINETICS OF SECOND LINE ANTI-TB DRUG *(LEVOFLOXACIN) IN MDR-TB* PATIENTS

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ABSTRACT

Tuberculosis, commonly known as TB, is the world's second deadliest disease caused by the bacterium Mycobacterium tuberculosis (MTB). The treatment for TB started in the year 1944 after the discovery of the antibiotic streptomycin. Over the century, the TB bacteria have evolved into the resistance forms, despite the use of effective drugs. In our study, We analysed the pharmacokinetic profile of Levofloxacin in MDR-TB patients. LFX is a synthetic broad spectrum antibacterial agent. It is the S-Isomer of the race mate. Plasma concentrations of levofloxacin were estimated using the validated methods by HPLC at NIRT. We had analysed the plasma concentrations of individual patients over different time intervals post single dosage. In case of LFX, about 10(40%) patients had their C_{max}> 12µg/ml, 3 patients showed sub therapeutic C_{max} value i.e. < 8 µg/ml and the remaining 12 patients (48%) had their C_{max} value within the range. This study is expected to have important clinical implications.

INTRODUCTION

TUBERCULOSIS

Tuberculosis, commonly known as TB, is the world's second deadliest disease. It is a chronic infection caused by the bacterium Mycobacterium tuberculosis (MTB). It is an aerobic, non-motile Bacillus that was first isolated by a German scientist Robert Koch in March 24, 1882. But there is evidence that this disease is known to mankind by several names throughout the history. Later in the year 1905, he was awarded the Nobel prize for his work in TB. X-rays developed by William Roentgen in 1985 and is an advanced diagnostic technique for many diseases including tuberculosis. TB bacterium is also called as 'Acid fast bacilli' . Because the cell wall of the

mycobacterium absorbs only a certain dye like Ziehl-Neelsen stainand is used for T staining procedure. In 1921, the French scientists Albert Calmette and Camille Guerin developed a vaccine for this disease called 'BCG vaccine' (Bacilli of Calmette and Guerin) which was named after the two scientists (Ananya Mandal, 2013).

Mycobacterium tuberculosis is an aerobic bacterium which primarily affects the lungs and the infection is called as Pulmonary TB. When this bacillus is gets into the blood stream, it passes through the other parts of the body and affects organs like kidney, spinal cord and brain and collectively known as Extra Pulmonary TB. The major symptoms of the TB bacteria infections include persistent cough for more than 3 weeks, persistent fever for more than 3 weeks, pain in chest and coughing up blood/sputum. In most of the cases, the person infected with TB bacterium will be asymptomatic. This condition is called as Latent TB Infection (LTBI). In this type, the bacilli remain dormant inside the person for many years. The chances of developing the disease latent during their life time if they are immunocompromised.

Different measures have been taken globally to find out the prevalence of this disease. WHO has been publishing a global report every year since 1997. In 2015, globally 10.4 million new TB cases were reported. About 60% new cases from India, Indonesia, Pakistan, China, Nigeria and South Africa. Because of the measures taken by WHO the TB incidence rate has been declined only by 1.5% from 2014-2015. However, the number of deaths remained the same as 2014.But it has been declined from 1.8million deaths in 2000 to 1.4 million deaths in 2015 (Global TB report 2016).

In the year 1944, first developed antibiotic to treat TB was streptomycin. Followed by that, more effective drugs have been discovered. Currently, there are more than 20 drugs have been developed and categorized based on its efficacy. The drugs that have the highest activity against the Mycobacterium tuberculosis are called First-line drugs. These drugs are given to the new TB patients, such as Rifampicin, Isoniazid, Pyrazinamide, Ethambutol, and Streptomycin.The second-line drugs are given to the patients with drug resistant TB bacilli. The major second line drugs include

Levofloxacin, Amikacin, Capreomycin, Kanamycin, and Moxifloxacin (www.tbfacts.org). The treatment period lasts for 6 months to 2 years or longer.

TB bacteria evolved and resisted to many effective drugs.There are mainly two different forms of drug resistant TB disease. They are multidrug resistant tuberculosis (MDR-TB) and extensively drug resistant tuberculosis (XDR-TB). Both the conditions are fatal and requires treatment for about 2years or longer. The researchers implicate inadequate testing and treatment as the primary reasons for the development of resistant forms. The inadequate treatment points towards the inappropriate dosages given to the patients. In this study, we analysed the pharmacokinetic profile of levofloxacin to find out the concentration of the drug that was absorbed in the patient's body. This drug is the most important drug used in the treatment regimen for MDR-TB patients.

HISTORY

Tuberculosis has been known to mankind since ancient times but in different names. An archaeological evidence shows that in the Old Testament books of the Bible dating to a time when the Israelites lived in Egypt, there are two probable evidence to tuberculosis. The ancient Hebrew word used in these passages is *schachepheth*, meaning 'wasting disease' (Virginia S. Daniel, 1999).

Many scientist believe that Africa is the 'home of TB bacilli'. Their assumptions are that when the people migrated from Africa to other places in the world they would have also carried the infectious bacilli along with them. But there is know archaeological evidence to prove it. However, there are evidence of prevalence of TB disease about 5,500 ago in Egypt. The analysis of unearthed Egyptian mummies from the Nile river valley showed the presence of tubercular decay in the spine of these mummies. This characteristics spine deformity is called Pott's disease. It is considered as the most common disease among Egyptian people in that era. (Thomas M. Daniel, 2006).

Around 460 BC, the Greek physician Hippocrates identified a most widespread disease of the times with the major symptoms of fever and coughing up blood (symptoms of modern TB). He understood

its clinical presentation well and found that the disease mostly affects people with the age group of 18 to 35 years and it was highly fatal. The disease was commonly called as ‘phthisis’ meaning ‘consumption’. Another Greek physician, Clarissimus Galen also wrote about the disease and recommended fresh air, milk and sea voyages for its treatment. (Tamanol, 1997).

In the 18th century, the disease was called as ‘White Plague’. The prevalence rate in western Europe was 900 death/100,000. The transmission was mainly due to poor ventilation and over- crowded housing. At the beginning of the 18th century, the work of Theophile Laennec, who found the stethoscope, served as the stepping stone in understanding the pathogenesis of the disease. He found out the difference between the pulmonary TB based on the signs and symptoms (Duffin j.,1998). Only in 1839 the German naturalist J.L. Schonlein (Johann Lukas Schonlein) named the disease, “Tuberculosis”. The understanding of pathogenesis is further advanced by the demonstration of transmissibility by Jean-Antonie Villemin in 1865. He inoculated a rabbit with a small amount of purulent liquid from a tuberculosis cavity obtained from a dead patient Although the immune system of rabbit is resistant to the tuberculosis strain, the autopsy showed an extensive tuberculosis.

Robert Koch was the first one to elucidate the etiology of the disease. In 24 marches 1882, he demonstrated the tubercle bacilli along with his famous postulates (Koch-Henle postulates). Koch did not believe that the bovine (cattle) and human tuberculosis disease were similar. This delayed the recognition of transmission of TB disease to humans from the infected milk.

Fig 1: Robert Koch

In 1907, a paediatricians Clemens von pirquet extensively studied the use of the tuberculin and three years later demonstrated a diagnostic test in children with latent TB infection (LTBI) which is now called as the Tuberculin skin test (TST). Later in 1905, Robert Koch was awarded the Nobel prize in medicine and physiology for his work in tuberculosis. In 1906, the two French scientist Albert Calmette and Camile Gurein found the vaccine BCG (Bacilli of Calmette and Gurein) but it was only in 1921 (Calmette A., 1928). Now it is only vaccine available for TB.

MICROBIOLOGICAL CHARACTERISTICS

Mycobacterium tuberculosis (MTB) is an aerobic bacterium. It is a fairly large non-motile bacillus. It is distantly related to Actinomycetes. The size ranges from 2-4um length;0.2-0.5um width. It does not have an outer membrane and hence classified as gram positive. They have rather limited temperature range. Optimal growth temperature is 35-37 degree Celsius. They have slow generation time: 18-20hrs, it either remains dormant or grow intermittently. (Vinod Agarwal, 2010)

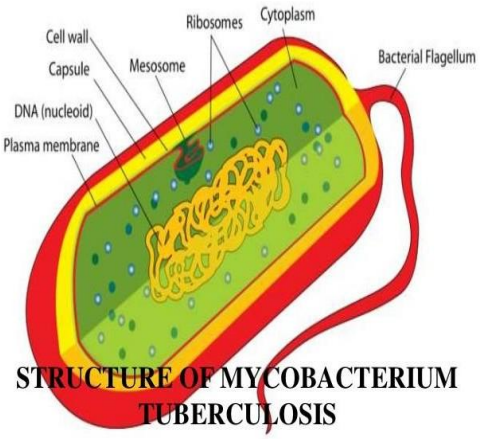
GENUS

The strain that causes tuberculosis belongs to the genus Mycobacterium. This genus Mycobacterium contains numerous species. They are divided into two

groups: slow growers and rapid growers. The most common species of the genus Mycobacterium or Mycobacterium tuberculosis that causes TB in human, M. bovis that causes TB in cows, M. avium, this bacillus causes TB like disease in AIDS patient.

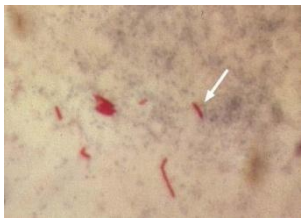
STRUCTURE

The cell wall of the MTB is waxy and thick, made up of various complex lipids and peptidoglycan. About 60% of cell wall is made up of lipids. There are three types of lipid found: Mycolic acid, Cord factor and wax d. This complex structure protects the bacteria from harse environment like from germicide and drying. Also because of the complex cell wall, the bacterium is unable to be stained by gram staining. Even the bile salts in human is unable to wash the cell wall.



STRUCTURE OF MYCOBACTERIUM TUBERCULOSIS

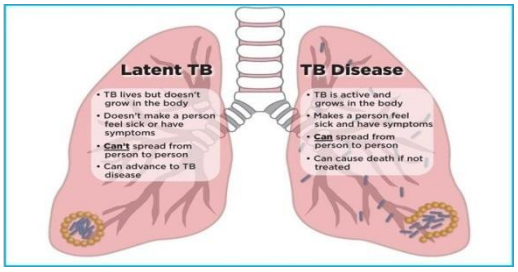
STAINING



SORTS OF TB

LATENT TB DISEASE

Latent TB occurs when an individual has the TB bacteria within their body, but the bacteria are present in very small numbers. they're kept in check by the body’s system and don't cause any symptoms.



MATERIALS AND METHODS

STUDY PATIENTS

The study population comprised of 25 MDR-TB patients had been treated at the government hospital of thoracic medicine, Tambaram, Chennai. Those patients who were all above 18 years of age and were newly started RNTCP category IV treatment considered eligible for this study.

INCLUSION CRITERIA

The patients should satisfy the following inclusion criteria:

Bacteriologically confirmed MDR-TB

Undergoing RNTCP CAT IV treatment regularly for minimum 7 days

Residing in Chennai and agree for the purpose of study

Willing for hospitalization for the purpose of study

Agree to give informed written consent

EXCLUSION CRITERIA

A patient will not have chosen for study if he/she comes under the exclusion criteria

Patients below 18 years of age were not included in the study

Patients who are seriously ill were excluded from the study

Patients with HIV-TB infecting were also excluded

Pregnancy women/breast feeding women were also excluded from the study

DOSAGE LEVEL

To facilitate procurement, distribution and administration of treatment to patients, the daily dosage was standardized for two or three body weight bands. The dosage of Levofloxacin

standardized for two body weight bands were 750mg and 1000mg for those with 31-40kg and 41- 75kg of body weight respectively. The patients received these dosages administered under direct observation on the days of PK sessions.

STUDY PROCEDURE INFORMED CONSENT

After seeing the eligible patients for the study, the study physician had informed the patients about the purpose of the study and the study procedures in their local languages. Simultaneously, the patients were provided with a copy of the patient information sheet and informed consent would have been obtained from all the patients.

An informed consent form was required to be signed by all the potential subjects. These forms explained clearly the potential benefits and risks of participating in the study in simple English and also in Tamil. The respective physician would have witnessed the patient signing the informed consent. Also, in cases, if the patients were unable to read the form, an independent witness should have been present to ensure that the patient had received the complete information and fully aware of the further studies. The physician has also explained the patient that he/she is at liberty to abstain from participation in the study and to withdraw consent at any time.

ETHICS

The study was commended only after obtaining permission from the Institutional Ethics Committee of both sites. The procedures set out in the study protocol, pertaining to the conduct, evaluation and documentation, were designed to ensure that the principles of good clinical practice were adhered to. Patient privacy and confidentiality have been maintained throughout and after the study. Data from the study were stored in a secure manner. Patients identity were not revealed at any circumstances to anyone other than the Principal Investigator.

CONDUCT OF THE PK STUDY

The pharmacokinetic study was conducted at the National Institute for Research in Tuberculosis (NIRT), Chennai. The blood sample were collected from patients at the Government Hospital of Thoracic Medicine, Tambaram, Chennai after patients have had one week of treatment. Eligible patients would have been instructed to get admitted to the hospital ward at least a day prior to the start of the study.

On the day of study, the anti-TB drugs were administered to the patients under supervision. After that a sample of blood (2ml) was drawn by inserting an indwelling catheter and collected into a heparinised vacutainer. An initial blood sample was collected before the drug administration (i.e. 0 hour). After the drug administration, serial blood samples were collected at different time intervals 1hour, 2,4,6,8and 12hours. Blood was collected at 7-time points on the study day. A total of 14ml of blood was collected from those patients.

Whole blood samples were centrifuged immediately and plasma was separated and stored in-20^0C until drug estimation were undertaken.

MATERIALS REQUIRED CHEMICALS USED FOR LFX ASSAY

Pure Levofloxacin powder

Pure Moxifloxacin powder

Potassium dihydrogen phosphate

Acetonitrile

70% Perchloric acid

INSTRUMENTS USED FOR LFX ASSAY

High performance liquid chromatography column

Vortex

Centrifuge

Millipore

Micropipette

Ultrasonic cleaner

PREPARATION OF CHEMICALS MOXIFLOXACIN

Moxifloxacin was used as the internal standard for this process. It is prepared as a stock solution by dissolving 50 ug/ml in 0.1N HCL.

MOBILE PHASE

The buffer was prepared by dissolving 6.8g of Potassium dihydrogen phosphate powder in 1000ml of MilliQ water. The pH was then adjusted to 2.6 by adding 1N HCL drop wise. This method was also isocratic i.e. uses only one mobile phase. The Potassium dihydrogen phosphate buffer and Acetonitrile were taken in the ratio 80:20.

PREPARATION OF STANDARD SOLUTIONS

Initially, a stock standard (1mg/ml) was prepared by dissolving a pure powder of Levofloxacin in 0.1N HCL. The working standards of Levofloxacin in concentrations from 0.5, 1.0, 2.5, 10, 15ug/ml were prepared by appropriate dilution with pooled plasma.

PREPARATION OF SAMPLE SOLUTION

An aliquot of 100ul of blank or standard or sample was added to the eppendorf tube of 1.5ml capacity. Then 20ul of Moxifloxacin (Internal standard) and 50ul of 7% Perchloric acid were added. The solutions were mixed using a vortex for 3 minutes and centrifuged at 10,000 rpm for 10 minutes. After centrifugation, the supernatant was collected in another eppendorf tubes which 20ul was then injected into the HPLC.

CHROMATOGRAPHY SYSTEM

The HPLC used for the study of pharmacokinetics of Levofloxacin consisted of two pumps (LC-10AVP), Fluorescence detector (RF-10AXL) with built in system controller. The pharmacokinetic study of levofloxacin is isocratic method but the special feature of this HPLC is that the LC-10AVP can be used in both low and high-pressure gradient delivery modes. In this model, the samples were injected into the system via manual injector with the micro syringe. The sample was then passed through the column along with mobile phase. The column was provided with the temperature controller. The detector detects the components eluted from the column and outputs the signal data to a PC. The output signal was collected and data acquisition was done by the software class VP-LC work station.

The column used for this procedure is RP-18e short column. The fluorescence detector was set at an existing wavelength of 290nm and an emission wavelength of 460nm. Using the micro syringe an injection volume of 20ul was injected into the manual injector unit. The run time of a single sample was 7 minutes with a flow rate of 1.5ml/min. Unknown concentration were derived from linear regression analysis of peak height ratio vs. concentration curve. The linearly was verified using estimates of correlation coefficients

RESULT

25 Patients those who are living in and around Chennai were chosen for the study. Of the 25 patients, 4 patients were female and the rest were male. The medium age of the 25 patients was 41 (min.age-18; max.age-74). According to the standardized dosage, 8 patients received 750g of LFX; 17 patients received 1000g of LFX; other drugs given according to the MDR-TB treatment regimen. The table shows the demographic details of the study patients. The parameter does not vary between the drugs expect the dosage.

PARAMETERS	**LEVOFLOXACIN (n=25)**
Gender Male Female	21 4
Age (yr.*)	41(18-74)
Wt. (kg)*	50(33-75)
Hot(cm)*	160(152-180)
Patients with diabetic	10
Patients with BMI	

<18.5 >18.5	12 13
Dose (mg) [No of patients received dosage]	750[8] 1000[17]

The collected blood samples at different time intervals were centrifuged and the plasma was extracted and stored at 20℃. In HPLC, the standards of known concentration were run first and the retention time and heights of all were noted from the chromatograms obtained.

CHROMATOGRAMS

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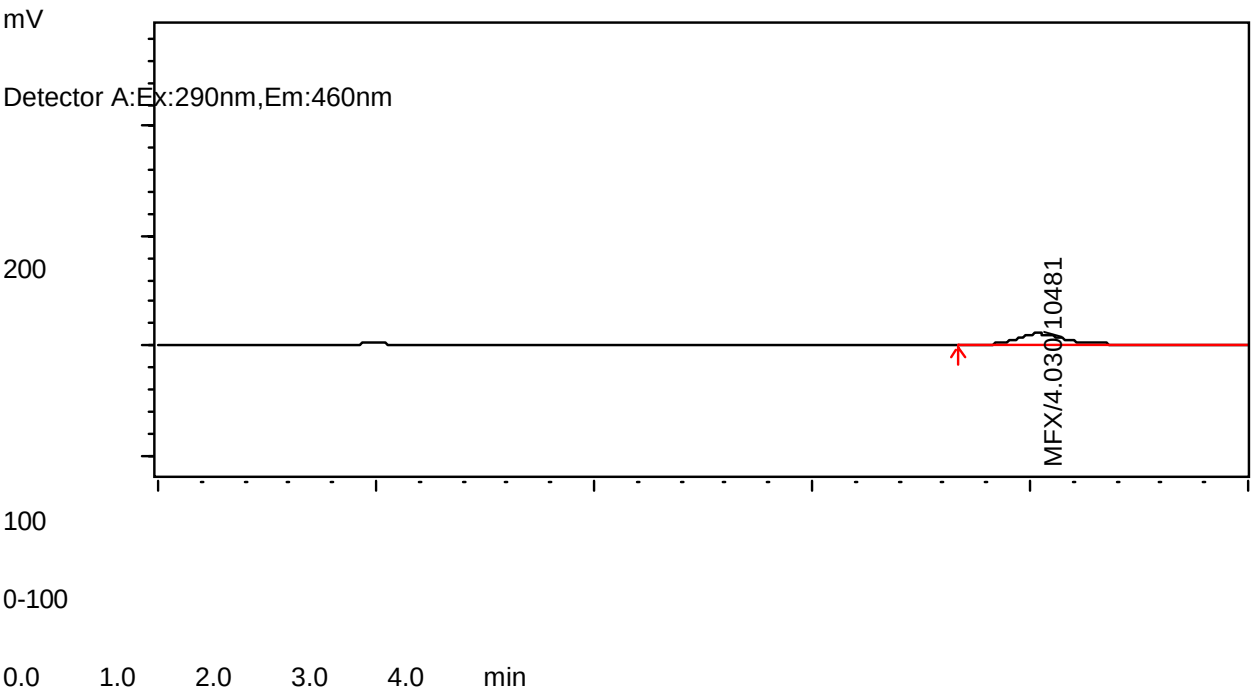
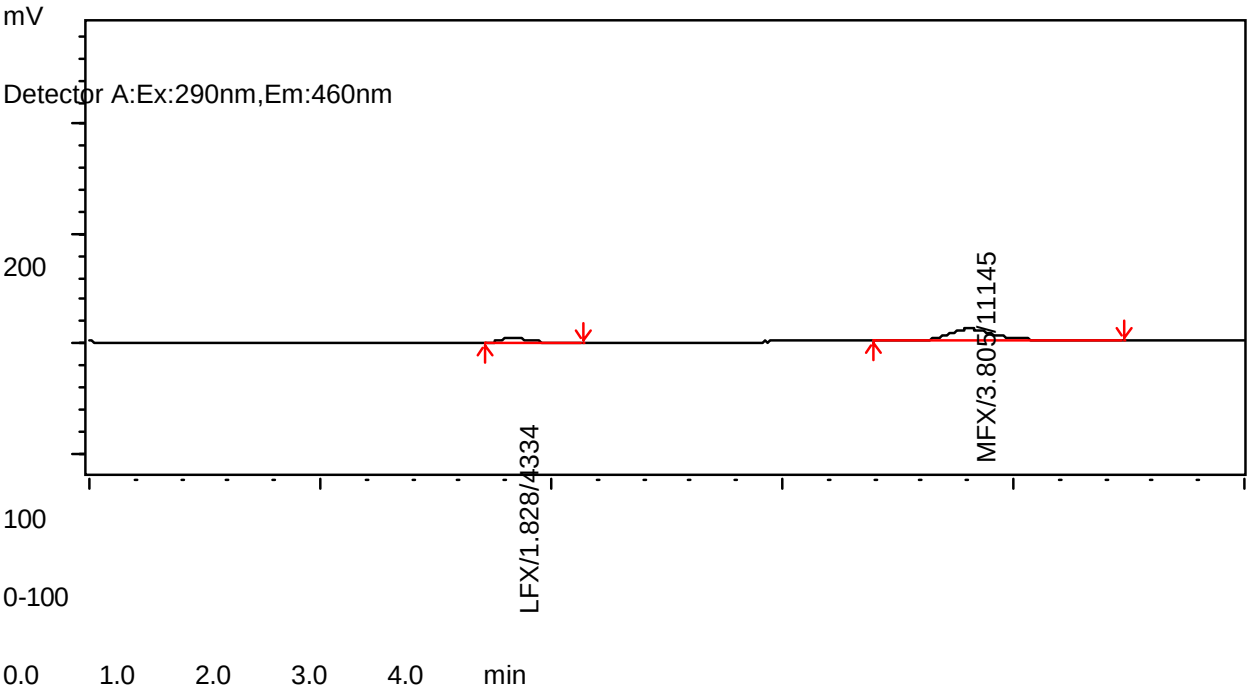
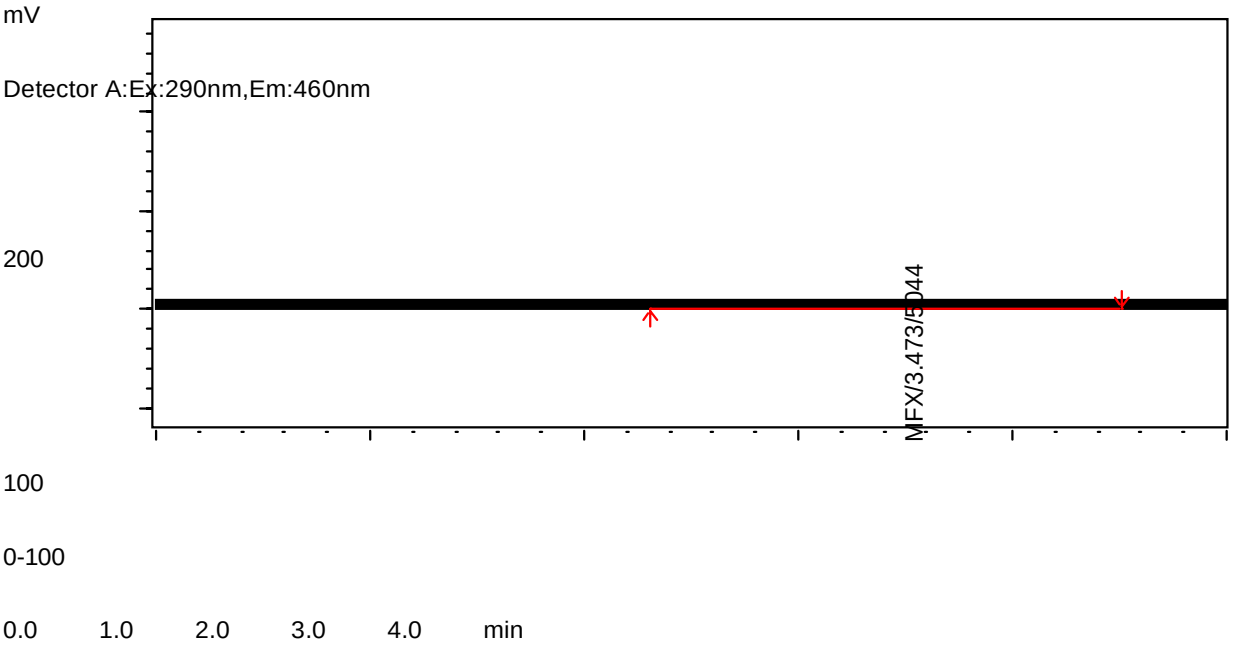


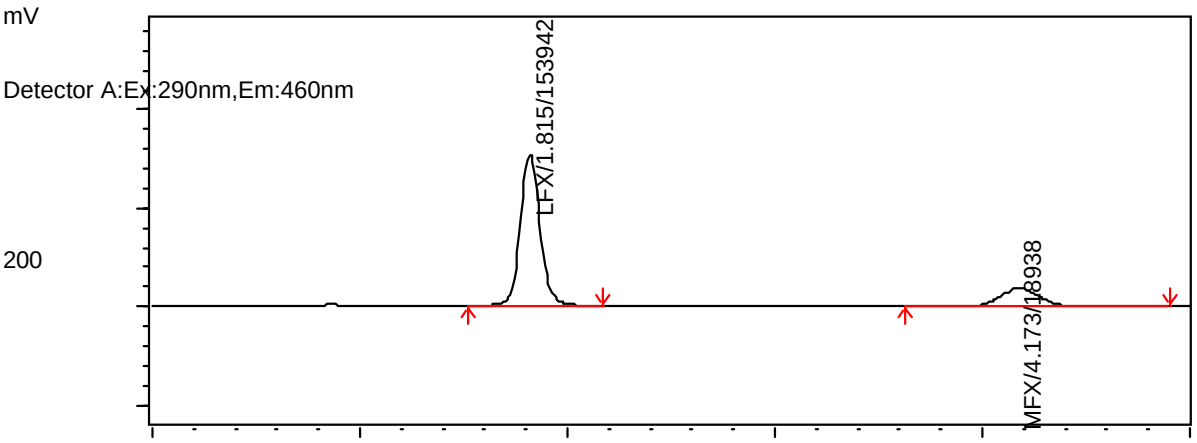
STD 15.0µG/ML

STD 0.5µG/M



0 HOUR

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MFX/4.173/18938
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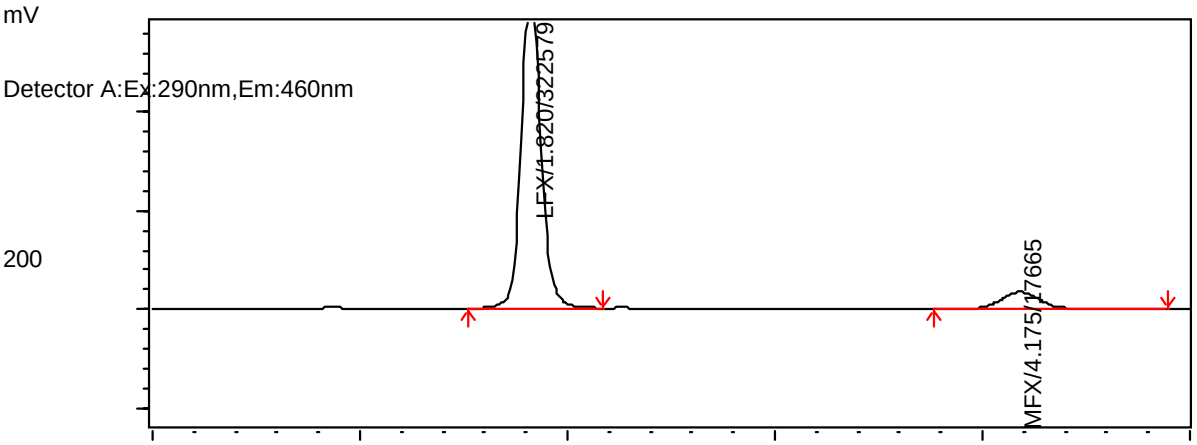
2 HOURS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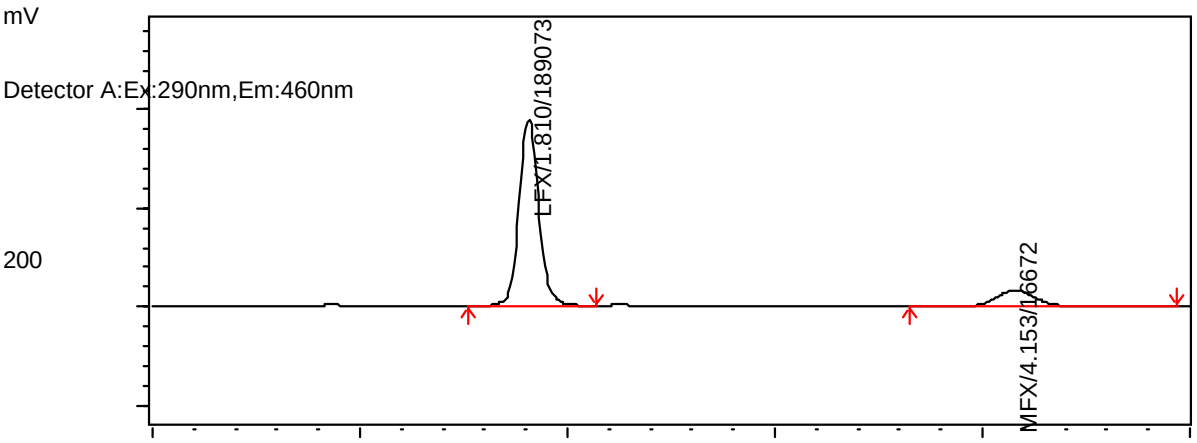

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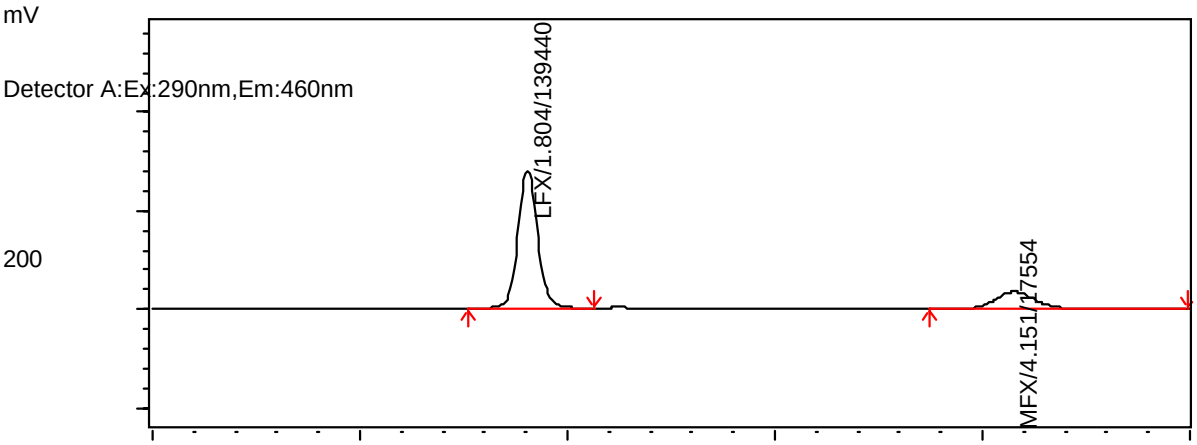

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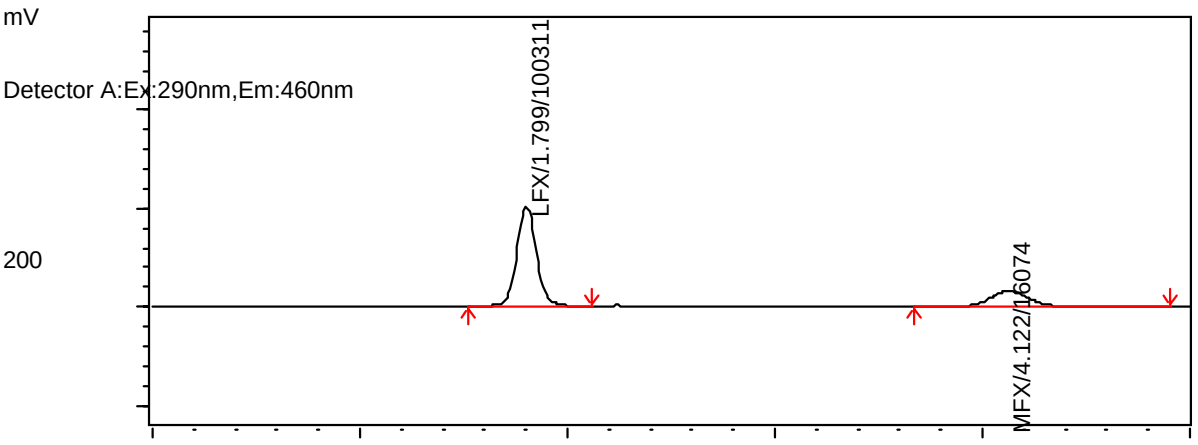
mV
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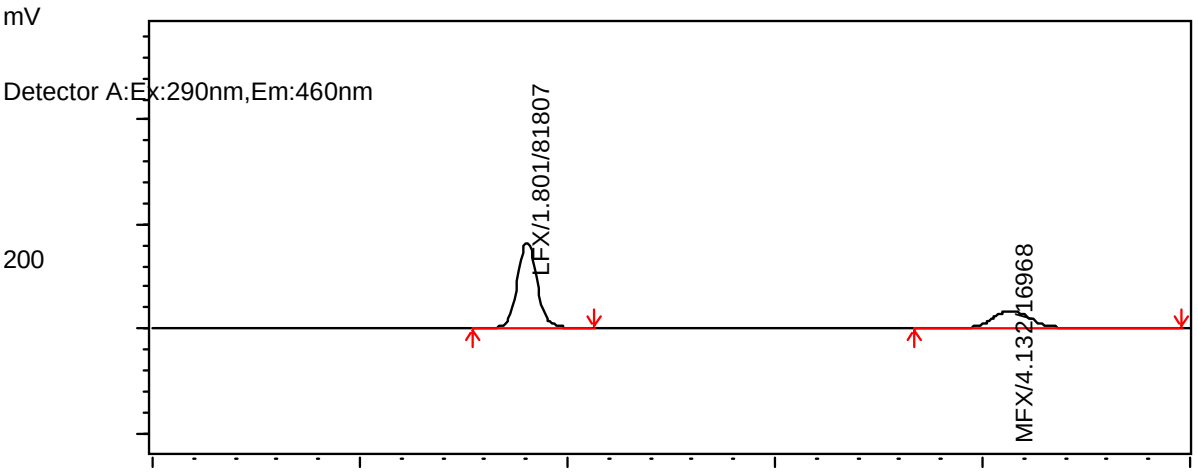
12 HOURS


mV
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LFX/1.801/81807
200
MFX/4.132/16968
100
0-100
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1.0
2.0
3.0
4.0
min

DETERMINATION OF PLASMA DRUG CONCENTRATION

Plasma concentration of LFX were determined using HPLC validated methods. The concentrations were tabulated below.

Table; Plasma concentration of LFX

Patient SI.NO	Plasma Concentration LFX						
	0 hour	1 hour	2 hours	4 hours	6 hours	8hours	12hours
1	1.27	1.32	10.02	9.81	6.5	5.51	3.06
2	1.01	1.18	1.35	9.5	9.82	7.47	5.11
3	2.9	3,83	6.58	11.81	10.34	7.18	6.64
4	2.33	4.77	4.82	12.77	12.5	12.09	9.66
5	1.89	12.38	13.68	11.77	7.95	6.67	4.72
6	1.71	2.75	7.47	7.96	6.65	4.89	3.65
7	1.62	6.38	20.24	13.5	9.87	7.96	6.19
8	5.07	18.18	16.64	10.76	10.55	8.95	7.82
9	0.93	3.92	11.55	7.15	6.94	5.64	3.56

10	2.05	13.55	15.15	10.35	9.27	7.42	5.03
11	1.01	2.21	7.28	7.17	6.7	5.84	5.64
12	17.03	14.23	11.32	9.29	9.16	8.78	5.63
13	9.93	16.62	14.98	9.68	9.16	7.56	6.4
14	16.74	22.87	20	17.97	15.35	14.86	11.57

15	0.74	N. A	12.99	9.68	6.81	5.46	3.24
16	3.2	12.68	14.68	13.67	10.65	8.15	5.7
17	3.16	16.67	12.74	10.7	8.92	7.63	6.59
18	2.58	2.59	3.97	10.7	12.97	12.11	9.69
19	1.68	9.5	10.17	11.47	9.55	7.97	6.68
20	1.86	12.19	8.96	8.85	6.57	5.82	5.17
21	2.0	9.68	10.87	10.7	8.6	5.5	5.13
22	2.04	2.34	11.29	9.77	7.89	6.28	4.92
23	1.61	4.95	7.06	7.53	6.91	5.7	5.28
24	4.18	4.06	3.38	2.61	2.48	2.45	1.73

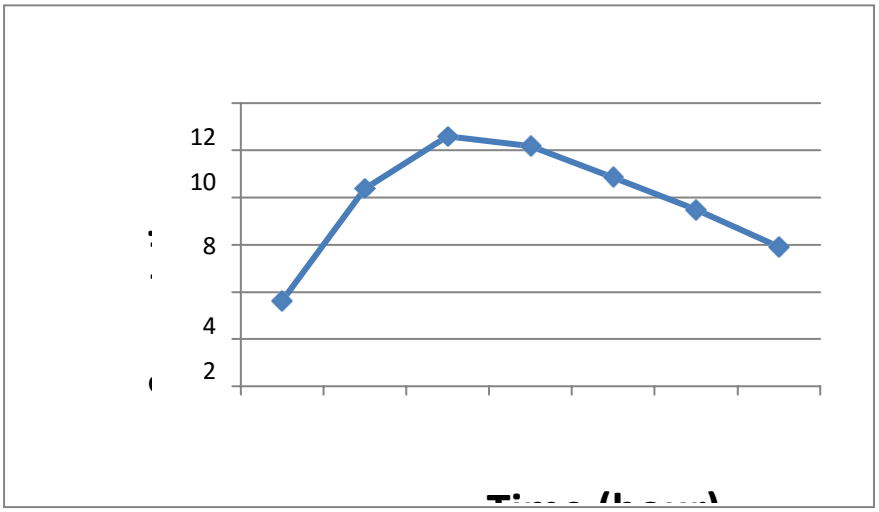
25	1.73	1.94	7.56	9.55	9.34	9.18	8.87

*NA- sample not collected

To calculate the mean LFX drug concentration of the 25 patients over 12hours along with the standard deviation

Table: Mean and Standard deviation of LFX drug concentration

Time (hours)	Mean	Standard deviation
0 hour	3.61	4.31
1 hour	8.37	6.21
2 hours	10.59	4.69
4 hours	10.19	2.75
6 hours	8.86	2.51
8 hours	7.48	2.50
12 hours	5.91	2.22



Pharmacokinetic profile of LFX

The above calculated mean LFX drug concentrations were plotted in the graph with the time (hour) on X-axis and the mean drug conc. (µg/ml) on the Y-axis. From the graph, the peak plasma concentration i.e. C_{max} was found to 10.59µg/ml obtained at the 2nd hour after drug concentration.

The calculated mean C_{max} and T_{max} of both the drugs were tabulated in the above table

Table: Pharmacokinetic parameter

Parameters (mean of 25 patients)	LFX
C_{max}(µg/ml)	10.59
T_{max}(hours)	2

DISCUSSION

The therapeutic range of levofloxacin is (8-12) µg/ml. The control of tuberculosis has become a global challenge due to the emergence of drug resistance tuberculosis. One of the main reason for that is the inadequate administration of effective treatment of drug susceptible. TB which led to the development of resistance (Paramacivan, C.N. and Venkatraman, P., 2003). For effective treatment of MDR-TB, the pharmacokinetic profile of second line drugs in Indian patients. This prospective study for the first time has provided the pharmacokinetic data of second-line anti-TB drugs .

A previous study in Tanzanian subjects initiating MDR-TB treatment reported that the concentrations of Levofloxacin was frequently below the expected range and requires further optimization of treatment regimen (J.L, Johnson et al, 2006). In contrast to that study, our study reported that maximum patients (48%) had the required range of LFX drug but about 40% patients had concentrations above the range. 12% patients showed sub-therapeutic C_{max} value. Patients with higher BMI having lower C_{max} was only common. But in our study, patients with higher BMI showed lower C_{max} than that of patients with lower BMI, which was non-significant because relating to the body weight, the patients should have higher concentration. Since this study was conducted in only a small group of patients, to be conclusive further study has to be done on a large population. This study is expected to have important clinical implication.

CONCLUSION

In conclusion, the variation of drug concentration between different groups of patients were studied. The sub-therapeutic drug levels in patients for LFX drugs are likely to pose a problem in sub-set of patients. Measuring drug levels would help clinicians achieve better patient management. This would help them when altering drug doses, and can be correlated with treatment outcome and adverse reactions.

REFERENCES

Agarwal, R., Malhotra P., Awasthi A., Kaka N., Gupta D. (2005) 'Tuberculous Dilated Cardiomyopathy: an under –recognized entity?' – BMC Infect. Dis., Vol.5, pp29.

Ananya Mandal (2013) 'History of Tuberculosis' News Medical.

Axelrod, S., Ostinato, H., Enders, J., Schlegel, B., Brinkmann, V., Kaufmann, SH., Haas, A. and Schaible, UE. (2018) 'Delay of phagosome maturation by a mycobacterial lipid is reversed by nitric oxide' Cell Microbiology, Vol.10, pp.1530-1545.

American Thoracic Society, CDC, Infectious Disease Society of America (2003) 'Treatment of tuberculosis' MMWR Recommendations and Reports, Vol.52 (RR-11), pp.1-77.

Bardou, F., Raynaud, C., Ramos, C., Lanelle, G. And Lanelle, M. A. (1998) 'Mechanism of Isoniazid uptake in Mycobacterium tuberculosis' Microbiology, Vol.144, pp.2539-2544.

Barry, CE., III, Boshoff, HI., Dartois, V., Dick, T., Ehrt, S., Flynn, J., Schnappinger, D and Wilkinson, RJ., Young, D. (2009) 'The spectrum of latent tuberculosis: rethinking the biology and intervention strategies' Nat Rev Microbiol, Vol.7. pp.845-855.

Bernstein, J., Lott, W.A., Steinberg, B.A. and Yale, H.L. (1952) 'Chemotheraphy of experimental tuberculosis. V. Isonicotinic acid hydrazide (nydrazid) and related compounds' American review of Tuberculosis, Vol.65, No.4, pp.357-364.

Calmette A., (1928) ‘On preventive vaccination of the new-born against tuberculosis by B.C.G.’ Br J Tuberc, Vol.22, pp.161-165.

CDC report (2016) ‘Highlights from the 2016 Treatment of Drug-Susceptible Tuberculosis Guidelines’

Cole, S. and Riccardi, G. (2011) ‘New tuberculosis drugs on the horizon’ CurrOpin Microbial, Vol. 14, pp.570-576.

Centres for Disease Control and Prevention (CDC) (2010). ‘Tuberculosis Elimination: Multi drug Resistance Tuberculosis (MDR TB)’

Centres for Disease Control and Prevention (CDC) (2016) ‘Tuberculosis (TB)’

Daniel TM (1997) ‘Captain of death: the story of tuberculosis’ Rochester NY: University of Rochester Press.

David, H. L. (1970). ‘Probability Distribution of Drug-Resistant Mutants in unselected Populations of Mycobacterium tuberculosis’ Applied Microbiology, Vol.20, No.5, pp.810-814.

Davis, R. And Bryson HM (1994) ‘Levofloxacin: a review of its antibacterial activity, pharmacokinetics and therapeutic efficacy’ Drugs, Vol.47, pp.677-700.

Duffin J. (1998) ‘To see with a better eye: A life of R.T.H. Laennec’ Princeton, NJ: Princeton University Press.

Elzinga, G., Raviglione, M.C., Maher, D., Raviglione and Maher (2004) ‘Scale up: meeting targets in global tuberculosis control’ Lancet Vol.363, No.9411, pp.814—819.

Falzon, D., Jaramillo, E., Schunemann, H., Arentz, M., Bauer, M. and Bayona, J. (2011) ‘WHO guidelines for the programmatic management of drug resistant tuberculosis’ EurRespir Journal, Vol.38, pp.516-528.

Fish, D. N. and Chow, A. T. (1997) ‘The Clinical Pharmacokinetics of Levofloxacin’ Springer, Vol.32, pp.101.

Gaby E. Pfyffer (2015) ‘Mycobacterium: General Characteristics, Laboratory Detection, and Staining Procedures’ Clinical Microbiology.

Gale, E. F, Cundliffe, E., Reynolds, P. E., Richmond, M. H., and Waring, M. J. (1981) ‘The molecular basis of antibiotic action’ John Wiley and Sons, Inc., New York.

Gerber F., Krummen M., Potgeter H., Roth A., Siffrin C., and Spoendlin, C. (2004) ‘Practical aspects of fast reversed-phase high-performance liquid chromatography using 3µm particle packed columns and monolithic columns in pharmaceutical development and production working under current good manufacturing practice’ Journal of Chromatography, Vol.2, pp.127—133.

Global tuberculosis report (2015) WHO/HTM/TB/2015.22. Geneva: World Health Organization.

Hemanth Kumar, A.K., Sudha, V., Geetha Ramachandran (2016) 'Simple and rapid liquid chromatography method for simultaneous determination of levoflozxacin in plasma' SAARC Journal of TB Lung disease & HIV/AIDS., Vol.13., No.2, pp.28-33.

Hemanth Kumar AK., Sudha, V., Geetha Ramachandran (2016) 'Pharmacokinetics of Isoniazid, Rifampicin in adults with pulmonary tuberculosis' Int J Tuberc Lung, Vol.20, No.9, pp.1236-1241.

26.J. L. Johnson, D.J., Hadad, W.H. Boom, C.L. Daley, C.A. Peloquin, K.D. Eisenah, D.D. Jankas, & S.M. Debanne (2006) 'Early and Extended early bactericidal activity of levofloxacin, gatifloxacin and moxifloxacin in pulmonary TB' Int J Tuberc Lung Dis, Vol.10, No.6, pp.605-612.

Kanabus, Annabel (2016) 'Information about tuberculosis' GHE.,

Karger, B.L, Berry, L.V. (1971) 'Rapid Liquid Chromatographic separation of steroids on columns heavily loaded with stationary phase' ClinChem, Vol.17, No.8, pp.757-764.

Kaufman, SHE. (2001) 'How can immunology contribute to the control of tuberculosis?' Nat Rev Immunol, Vol.1, pp.20-30.