 DOI: 10.18231/2455-6807.2017.0012

Efficacy of genexpert in diagnosing MTB / RIF resistance in HIV seropositive and seronegative patients: a study in comparison

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Abstract

Aims and Objective: The aim of the present study is to compare diagnostic efficacy of Gene-X pert in HIV seropositive and HIV seronegative patients in detecting MTB in sputum and Rifampicin resistance and to treat MDR TB as early as possible so that patient can be initiated on DOTS-PLUS treatment promptly thereby reducing morbidity, mortality, occurrence of XDR and transmission of MDR in the community. Considering its public health importance present study was undertaken.

Materials and Method: This is an observational, prospective study conducted over a period of 14 months (Jan 15 to April 16) in the Dept. of Pulmonary Medicine, Shree Vasantrao Naik Govt. Medical College, Yavatmal, Maharashtra, India. We subjected 659 patients, who fulfilled the clinical criteria for RNTCP - MDRTB suspect. viz 1)Treatment failure, 2)-Retreatment case sputum positive at the end of 4 months, 3) - Contact of known MDRTB case, 4) - Sputum positive at diagnosis, retreatment case, 5) - Any follow up sputum positive, 6) - Other category (sputum negative retreatment cases) and 7) - HIV-TB cases. With all safety precautions two samples were collected in the designated microscopy centre and subjected for routine ZN staining and Gene X-pert MTB/RIF assay. Study subjects were divided into Group-1(HIV sero-Positive) and Group-2 (HIV Sernegative). All new cases (sputum positive, sputum negative and extra-pulmonary cases) have been excluded from the domain of this study.

Results: Total 659 patients were enrolled for the study at hand and were divided into 2 groups. In Group-1 (HIV seropositive) there were 157 patients, and in Group -2 (HIV seronegative) there were 502 patients. Mean age of the study population was 38.98 years. In group-1 Male to Female ratio was found to be 2.73:1 and in group-2 it was 2.28:1 respectively. In group-1(n=157) MTB detected in 59 patients out of these 3 patients were Rifampicin resistant and in Group-2(n=502) MTB detected in 302 patients out of which 43 were found to be Rifampicin resistant.

Conclusion: Hence, it can be concluded that Gene Xpert is a useful tool in detecting MTB(P= 0.0002817) and Rifampicin resistance(P =0.004288) in HIV seropositive as compared to HIV seronegative patients.

Keywords: Gene Xpert, Rifampicin Resistant, HIV Seronegative, HIV seropositive

Introduction

Newer tests for diagnosis of Tuberculosis (TB) are needed because of the difficulties associated with the current available test modalities to diagnose as well as to detect drug resistance. Traditionally, TB has been diagnosed through the use of chest X- ray, smear microscopy and through the culture. One of the most significant disadvantage of sputum culture being the time that it takes and the accuracy.(1)

Multidrug resistance is an increasing concern globally and directly threatens disease-control efforts in many countries. Globally, 3.6% of new TB cases and 20.2% of previously treated cases are estimated to have MDR TB. In India prevalence of MDR TB has been estimated to be 1-3% and 12-14% in new and previously treated cases respectively.(5) Every year nearly 5,00,000 new cases of multidrug resistance tuberculosis are detected and reported, and mis-diagnosis causes thousands of deaths, nosocomial and community transmission, and amplification of drug resistance.(1,2,3)

To resolve these issues, substantial efforts are being made to strengthen laboratory capacity to diagnose smear negative and multi drug resistance tuberculosis, including increased use of solid and liquid culture, conventional drug-susceptibility testing and line probe assays. Unfortunately, these tests requires extensive laboratory infrastructure and cannot be done outside of reference facilities and also it is not feasible in rural areas.(4,5)

In recent past, a real time PCR assay (MTB/RIF) that simultaneously detects Rifampicin resistance was developed on the Gene Xpert platform (Cepheid, Sunnyvale, CA,USA), which integrates sample processing and greatly simplifies testing. This Xpert MTB/RIF assay, showed excellent performance in multi-center study undertaken in reference laboratories. MTB/RIF assay detects M. Tuberculosis and RIF resistance by PCR amplification of the 81 bp fragment of the M. Tuberculosis rpoB gene and subsequent probing of this region for mutations that are associated with RIF resistance. The assay can be completed within two hours.(3,4)

The main objective behind this study is to compare diagnostic efficacy of Gene Xpert in HIV seropositive and HIV seronegative patients and treat MDR TB as early as possible so that patient can be initiated on DOTS-PLUS treatment early and morbidity, mortality, occurrence of XDR and transmission in the community can be reduced.

Materials and Method

659 patients are subjected over a period of 14 months, from Jan 2015 – April 2016 in rural tertiary care hospital, which is a 700-beded teaching hospital with one DOTS PLUS unit and MDR-TB centre catering the 16 TB units of District. Study was conducted in Dept of Pulmonary Medicine, Shri Vasantrao Naik Govt. Medical College, Yavatmal, (Maharashtra) India. The prospective, observational study has been conducted after getting due approval from the institutional Ethical committee.

Clinical Screening for MDR-TB Suspect

The patients were enrolled in the study and grouped as per the RNTCP MDR suspect clinical criteria.[4]

Table 1: Clinical criteria for RNTCP MDR suspect

Sr. No	Clinical Criteria
1.	Treatment failure
2.	Retreatment case sputum positive at 4 months.
3.	Contact of known TB.
4.	Sputum positive at diagnosis, retreatment case
5.	Any follow up sputum positive
6.	Other category (sputum negative retreatment cases)
7.	HIV TB cases

All new cases are excluded (sputum positive, negative and extra pulmonary new cases) as per RNTCP norms. Patients have been divided into 2 Groups namely: Group-1) - HIV seropositive and Group-2) - HIV seronegative.

MDR-TB Confirmation: All the study patients with clinically suspected MDR-TB criteria have been referred to Xpert MTB/RIF assay lab which is under Dept. of Microbiology. With all precautions two sputum specimens collected from the patients in the designated microscopic centre. One sputum sample was subjected for ZN staining and other for Xpert MTB/RIF assay.

The Xpert MTB/RIF assay: Xpert MTB/RIF cartridge labelled with the corresponding specimen ID.1.0 ml expectorated sputum transferred to a conical, screw-capped tube using a sterile transfer pipette. 2.0 ml Xpert MTB/RIF Sample Reagent (2:1; v/v) added to the expectorated sputum using a sterile transfer pipette. The lid was replaced, and tube shaken vigorously for 10-20 times. The tube allowed to stand upright for 5 min at room temperature and again the tube was shaken vigorously for 10-20 times. The tube was allowed to stand upright for another 10 min at room temperature. Then specimens inspected as samples should be allowed to liquefied with no visible clumps of sputum. The Xpert MTB/RIF cartridge lid was opened. Using the sterile transfer pipette provided, aspirated the liquefied specimen into the transfer pipette until the meniscus is above the minimum mark and transferred the sample into the open port of the Xpert MTB/RIF cartridge. Closed the cartridge lid and test started as per Gene Xpert Dx System manufacturer instruction.[6]

The Xpert MTB/RIF assay results representative:

Each Xpert MTB/RIF cartridge includes reagents for the detection of MTB complex and RIF resistance as well as a sample processing control (SPC) to control for adequate processing of the target bacteria and to monitor the presence of inhibitor(s) in the PCR reaction.

The Probe Check Control (PCC) verifies reagent rehydration, PCR tube filling in the cartridge, probe integrity, and dye stability. The primers in the Xpert MTB/RIF assay amplify a portion of the *rpoB* gene containing the 81 base pair "core" region. Five differently coloured fluorogenic nucleic acid hybridization probes, called molecular beacons, interrogate the entire 81-bp core. Each molecular beacon was designed to be so specific that it does not bind to its target if the target sequence differs from the wild-type *rpoB* sequence by as little as a single nucleotide substitution. Since molecular beacons fluorescence only when they are bound to their targets, i.e. wild type *rpoB* sequence, the absence of any one of the five colors in the assay differentiate between the conserved wild type sequence and mutations in the core region that are associated with RIF resistance.

The SPC should be positive in a negative sample and can be negative or positive in a positive sample. The test result will be "Invalid" if the SPC is not detected in a negative test. Before the start of the PCR reaction, the GeneXpert Dx System measures the fluorescence signal from the probes to monitor bead rehydration, reaction tube filling, probe integrity and dye stability. The PCC passes if the fluorescence signal from the probes meets the assigned acceptance criteria. The results are interpreted by the Gene Xpert Dx System from measured fluorescence signals and embedded calculation algorithms and are displayed in the View Results window[6] as indicated below:

MTB Detected: MTB target DNA is detected; both controls, SPC and PCC, meet the assigned acceptance criteria. Lower Ct values represent a higher starting concentration of DNA template; higher Ct values represent a lower concentration of DNA template. In MTB DETECTED results "RIF Resistance DETECTED","RIF Resistance NOT DETECTED", or "RIF Resistance INDETERMINATE" will display on a separate line.

MTB Not Detected: MTB target DNA is not detected; both controls, SPC and PCC, meet the assigned acceptance criteria.

Invalid: Presence or absence of MTB cannot be determined: SPC does not meet acceptance criteria, i.e.

the sample was not properly processed, or PCR was inhibited

All the samples were subjected for Gene expert assay those who have found resistance for Rifampicin were subjected for culture and antiTB drug resistance (DRT). Before we get result of culture DRT MDR patients were put on category 4 regimens. That reduced the time for starting MDR treatment there by reducing the transmissions of MDR bacilli in the society.

Statistical Analysis: Statistical analysis was performed with the SPSS (version 16.0) software package. Numerical variables were summarized with mean ± standard deviation. The significance of differences among groups was assessed by the Student t test and analysis of categorical variables was examined by the chi square test. A value of P of <0.05 was considered significant for all statistical analysis.

Results

In both groups, males were pre-dominant, mean age is 38.98 and standard deviation 14.15.In group-2 clinical MDR suspect criteria, sputum positive at diagnosis (retreatment case) is dominating population followed by sputum negative retreatment cases, any follow up sputum positive treatment failure, contact of known MDR and retreatment cases sputum positive at 4 months. Gene Xpertss has detected MTB positivity (72.26%) and Rifampicin resistance (8.71%) in clinical criteria of sputum positive at diagnosis retreatment cases (criteria 4) followed by any follow up sputum positive.(criteria 5)

Table 1: Sex distribution in HIV seronegative and SERO positive patients

Sex	No of HIV Positive patients (n=157) (Group 1)	Percentage	No of HIV Negative patients (n=502) (Group 2)	Percentage
Male	115	73.25%	349	69.52%
Female	42	26.75%	153	30.48%
Total	157		502	

(P-value-0.3719)

Table 2: Age distribution in HIV Seronegative and SERO Positive patients

S. No	Age Group	No of HIV Negative PTS	No of HIV Positive PTS
1	1-10	6	1
2	11-20	44	10
3	21-30	119	28
4	31-40	142	68
5	41-50	83	34
6	51-60	63	15
7	61-70	37	1
8	71-80	8	0
	Total	502	157

Mean: 38.98786 Standard deviation SD: 14.15738

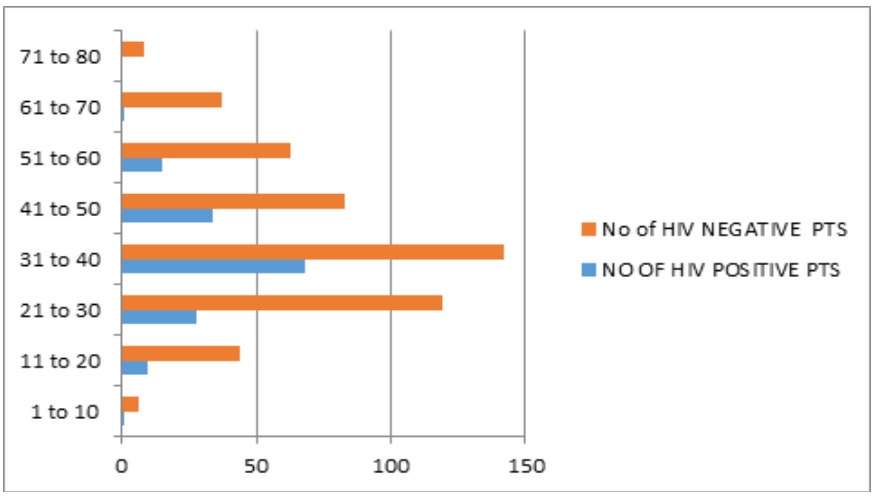

Fig. 1: Age distribution in HIV sero negative and sero positive patients

Table 3: Clinical criteria in HIV SERO Negative and SERO Positive patients

S. No	Clinical Criteria	No of HIV Positive Patients	No of HIV Negative Patients
1	Treatment failure	0	10
2	Retreatment case sputum positive at 4 months	0	2
3	Contact of known MDR	0	4
4	Sputum positive at diagnosis, retreatment case	0	310
5	Any follow up sputum positive	0	58
6	Other category (sputum negative retreatment cases)	0	118
7	HIV TB cases	157	0
	Total	157	502

Table 4: Numerical table showing MDR detected in clinically suspected patients

S. No	RNTCP MDR suspect criteria	No of Pts	MTB detected by gene xpert	MDR (Rif-resistance) detected by gene xpert
1	Treatment failure	10	4 (40%)	2(20%)
2	Retreatment case sputum positive at 4 months.	2	1 (50%)	0
3	Contact of known MDR.	4	2 (50%)	1(25%)
4	Sputum positive at diagnosis, retreatment case	310	224 (72.26%)	27 (8.71%)
5	Any follow up sputum positive	58	39 (67.24%)	9 (15.51%)
6	Other category (sputum negative retreatment cases	118	32 (27.11%)	4 (3.38%)
7	HIV TB cases	157	59 (37.57%)	3 (1.91%)
	Total	659	361 (54.77%)	46 (6.98%)

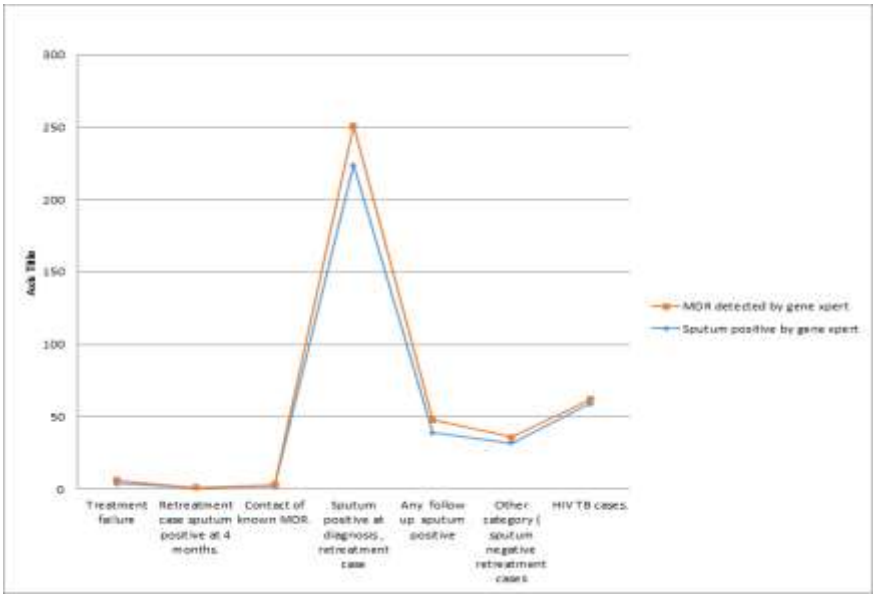

Fig. 2: Figure showing sputum positive and MDR Detected in clinically suspected patients

Table 5: Gene xpert results in HIV seronegative and seropostive patients

S. No	Patient Group	Total No of Patient	Result PF Gene Xpert	
			MTB Detected	RIF Ampicin resistant detected
1	HIV Positive	157	59 (37.57%)	3 (1.91%)
2	HIV Negative	502	302(60.15%)	43 (8.56%)
	Total	659	361(54.77%)	46 (6.98%)

(In case of MTB detection in HIV positive patient's p value -0.0002817)
(In case of Rifampicin resistance detection in HIV positive patient's p value - 0.004288)

Discussion

World Health Organization endorsed the use of Genexpert RIF/essay for the rapid diagnosis of TB and rifampicin resistance among the HIV infected with clinical suspicion of TB and HIV sero negative patient with clinical criteria RNTCP MDR suspect.[1] Considering the sensitivity and specificity of test it has the potential to complement the current reference standard of TB diagnostic in HIV sero positive and sero negative patients.

In the present study the mean age of study patients is 38.98 years (SD: 14.15). In similar study done by Hyder A et al, observed the same age group.[7] In the current study Male: Female ratio in Group1 and Group-2 is 2.73: 1 and 2.28:1 respectively. Similar observations have been made by Grant Theron et al. was 2.1:1.[8] Above observations has highlighted again HIV TB exposure in Male and in economically productive age group.

In Group-1, 157 Sero positive patients have been included and in Group-2, 502 sero -negative patients fulfilling the clinical criteria of MDR suspect of which critera No-4 has highest number 73.26% (Tabel 4); Fig. 1, followed by any follow sputum positive, criteria-5 (67.24%). Similar observation has been found in study conducted by Dagnra A.V. et al.[9]

The highest no. of MTB detected by Gene Xpert has been found in patients of MDR suspect criteria-4 i.e. 72.26%, followed by 67.24% in criteria-5, 50% in criteria-3, 50% incriteria-2, 40% in criteria-1, 37.57% in criteria-7 & 27.11% in criteria-6.

The highest no. of rifampcin resistance detected by Gene Xpert has been found in patients of MDR suspect criteria-3 i.e. 25%, followed by 20% in criteria-1, 15.51% criteria-5, 8.71% criteria-4, 3.38% criteria-6, 1.91% in criteria-7.

Amongst 659 patients, 157 were HIV positive and 502 were HIV negative. In group-1 genxpert has detected smear positivity in 59 (37.57%) and rifampcin resistance in 3 (1.91%) patients. Where as in Group-2 patients out of 502 MTB detected in 302 (60.15%) and Rifampcin resistance in 43 (8.56%)(Table 5). In case of MTB detection in HIV positive patients P value is 0.0002817 and in case of rifampcin resistance detection in HIV positive patient's p value is 0.004288.

Total 659 sample we observed, 8(1.30%) treatment failure, of which 2 (0.32%) had MDR –TB positive, retreatment case 283 (46.16%) of which 25 (4.07%) MDR-positive. The study of Jeon et al, 2011(41), shows that inadequate treatment has contributed to the high prevalence of MDR and XDR-TB. In Korea the prevalence of MDR-TB has been estimated to be up to 10 times higher after unsuccessful treatment.[10] The present study shows similar results with these studies. Delayed diagnosis, delayed recognition of drug resistance, inappropriate chemotherapy regimens, inadequate or irregular drug supply, and poor compliance by both patients and clinicians have each been reported as a reason for inadequate treatment. A few studies in review specified the reasons for inadequate treatment for e.g.; defaulting treatment receiving previous, treatment in prison, and being given fewer than four drugs.[10]

In our study among total sample 3(0.49%) patients had history of family contact of which 1(0.16%) MDR-TB positive. TB prevalence among contacts is very high. The odds ratio of having a family member with TB and developing TB is estimated at 13.4 further highlighting the importance of close TB contact and TB risk.[11]

The number of studies shows the association between HIV and TB. Some studies show that HIV co-infection is the most potent immunosuppressive risk factor for developing active TB disease.[12] HIV co-infection greatly increases the chances of reactivation of latent infection of TB[13] and increases the rapid TB progression following primary infection or re-infection with TB.[14] Individual studies conducted in both high[15] and low burden TB countries[16] have attributed increasing TB incidences to HIV infection. Our study confirms the significant association between HIV and TB as is found in previous studies. But the co-relation between HIV infection and drug resistance TB remains controversial. According to Cohn et al, 1997,[17] though the association of MDR TB with AIDS has been well documented during outbreaks,[18] the role of HIV infection as a risk factor for the development of drug resistant TB in other settings was not clear.[19] In Kenya, Malawi, Tanzania, Cote d'Ivoire, and France, drug resistance was not associated with HIV infection.[20] One study in France[21] and one in Italy[22] did not find any association with this variable. On the contrary, in a survey of eight metropolitan areas of the United States, HIV infection was associated with resistance to drug resistant TB, both within and outside New York City area.[23]

Malabsorption of anti-TB drugs has been documented for HIV-positive patients, which could increase the risk for acquired rifampicin resistance.[24] In settings where HIV infection is linked to socio-economically vulnerable populations, treating the element poorly and superficially as also lack of access to proper treatment may contribute to the development of drug resistance.[25] In our study at hand HIV co-infection was found independently associated with MDR TB.

It has in present study that significant association between HIV infection and MTB detection ($p<0.005$) and significant association between rifampicin resistance in seropositive patients and seronegative pts.

($p<0.005$) (Table 5). At least 3 studies from Africa and one study from US reported data indicating that HIV infection may be associated with anti TB drug resistance.[26] One of these studies from Mozambique demonstrated an association between HIV infection and resistance to Isonozised and streptomycin and one from Ethopiya demonstrated an association between Anti TB drug resistant and HIV infection among patient newer previously treated for TB. Although the evidence for HIV infection as a specific risk factor for multi drug resistant among the patient with TB is variable. HIV infection has been associated with accrued Rifampicin resistance among the patients with TB in controlled clinical trials and other study.[26] In the present study, several factors were found to be associated with acquired Rifampicin resistance, poor adherence to TB treatment, advanced immune suppression, treatment of other opportunistic infection, co treatment with anti-retroviral medications and the use of intermittent Tb treatment regimes. HIV infection has also been associated with gastro intestinal mall absorption of Anti- TB medication.[26] There is definite geographic overlap between HIV infection and MDR TB.

Conclusion

There is definite geographic overlap between the HIV infection and The MDR-TB, it is humbly inferred. Hence, it can be concluded that Gene Xpert is a useful tool in detecting Rifampicin resistannce in HIV sero-positive as compared to HIV sero-negative (P =0.004288) and MTB detection (P= 0.0002817).

Acknowledgment

State Tuberculosis Society of Maharashtra for supplying the Gene Xpert Machine to the medical college.

Conflict of Interest: None.

References

1. Blakemore, R .Evaluation of the analytical performance of the EXPERT MTB/RIF assay. J Clin. Microbiol.2010;48:2495-2501.
2. Boehme C. C. Rapid molecular detection of tuberculosis and rifampicin resistance. N. Engl. J. Med. 2010;363:1005—1015.
3. Boehme C.C. et al, Feasibility, diagnostic accuracy, and effectiveness of decentralized use of the XPERT/MTB test for diagnosis of tuberculosis and multidrug resistance. A multicenter implementation study. Lancet 2011;377;1495—1505.
4. WHO. 2010. Global tuberculosis control: WHO report 2010. World Health Organization, Geneva, Switzerland
5. Munje et al Multidrug-resistant TB among previously treated TB cases: A retrospective study in Nagpur, India Indian Journal of Tuberculosis Volume 62, Issue 4, October 2015, Pages 207–210.
6. Gene Xpert Dx System Operator Manual. Cepheid Inc., Sunnyvale, CA, USA (2011).
7. Hyder et al. HIV/AIDS in Pakistan: the context and magnitude of an emerging threat. J Epidemiol Community Health 1998;52:579-585 doi:10.1136/jech.52.9.57.
8. Theron G, Peter J, van Zyl-Smit R, Mishra H, Streicher E, et al. (2011). Evaluation of the Xpert(R) MTB/RIF Assay for the Diagnosis of Pulmonary Tuberculosis in a High HIV Prevalence Setting. Am J Respir Crit Care Med. Epub Ahead of print. doi: 10.1164/rccm.201101-0056OC.
9. Dagnara AY, Vidal Nicole, Naima Fabin Roch, Prince david Mireille, Peeters martin, et al; July 2009; volume 9 issue 4, page 646-652.Genetic characterization of HIV-1 strains in Togo reveals a high genetic complexity and genotypic drug-resistance mutations in ARV naive patients.
10. Pablo Méndez A, Raviglione M C, Laszlo A. et al Global surveillance for anti-tuberculosis drug resistance, 1994–1997. N Engl J Med 1998;338:1641–1649.
11. Global TB control report 2012. Available at [http://apps.who.int/iris/bitstream/10665/75938/1/9789241564502_eng.pdf]. Accessed 12 December 17, 2014.
12. E. L. Corbett, C. J. Watt, N. Walker et al., "The growing burden of tuberculosis: global trends and interactions with the HIV epidemic," Archives of Internal Medicine, vol. 163, no. 9, pp. 1009–1021, 2003.
13. H. C. Bucher, L. E. Griffith, G. H. Guyatt et al., "Isoniazid prophylaxis for tuberculosis in HIV infection: a meta-analysis of randomized controlled trials," AIDS, vol. 13, no. 4, pp. 501–507, 1999.
14. P. A. Selwyn, D. Hartel, V. A. Lewis et al., "A prospective study of the risk of tuberculosis among intravenous drug users with human immunodeficiency virus infection," The New England Journal of Medicine, vol. 320, no. 9, pp. 545–550, 1989.
15. S. D. Lawn, L. G. Bekker, K. Middelkoop, L. Myer, and R. Wood, "Impact of HIV infection on the epidemiology of tuberculosis in a peri-urban community in South Africa: the need for age-specific interventions," Clinical Infectious Diseases, vol. 42, no. 7, pp. 1040–1047, 2006.
16. K. DeRiemer, L. M. Kawamura, P. C. Hopewell, and C. L. Daley, "Quantitative impact of human immunodeficiency virus infection on tuberculosis dynamics," American Journal of Respiratory and Critical Care Medicine, vol. 176, no. 9, pp. 936–944, 2007.
17. Cohn DL, Bustreo F, Raviglione MC. Drug-Resistant Tuberculosis: Review of the Worldwide Situation and the WHO/IUATLD Global Surveillance Project. Clinical Infectious Diseases 1997;24 (Suppl 1) S121-30.
18. Fischl MA, Uttamchandani RB, Daikos GL, et al. An Outbreak of Tuberculosis Caused by Multiple-drug-resistant Tubercle Bacilli among Patients with HIV Infection. Annals of Internal Medicine 1992;117 177-83.
19. Nunn P, Felten M. Surveillance of Resistance to Antituberculosis Drugs in Developing Countries. Tubercle and Lung Disease 1994;75 163-167.
20. Githui W, Nunn P, Juma E, et al. Cohort Study of HIV-positive and HIV-negative Tuberculosis, Nairobi, Kenya: Comparison of Bacteriological Results. Tubercle and Lung Disease 1992;73 203-209.
21. Robert J, Trystram D, Truffot-Pernot C, et al. Surveillance of Mycobacterium tuberculosis drug resistance in France, 1995–1997. Int J Tuberc Lung Dis2000;4:665–72.
22. Girardi E, Antonucci G, Tronci M, et al. Drug resistance patterns among tuberculosis patients in Rome, 1990–1992. Scand J Infect Dis1996;28:487–91.

23. Gordin FM, Nelson ET, Matts JP, Cohn DL, Ernst J, Benator D, Besch CL, Crane LR, Sampson JH, Bragg PS, El-Sadr W. The Impact of Human Immunodeficiency Virus Infection on Drug Resistant Tuberculosis. American Journal of Respiratory and Critical Care Medicine 1996;154(5) 1478-1483.
24. Patel KB, Belmonte R, Crowe HM. Drug malabsorption and resistant tuberculosis in HIV-infected patients. N Engl J Med. 1995;332:336–7.
25. Suchindran S, Brouwer ES, Van RA. Is HIV infection a risk factor for multi-drug resistant tuberculosis? A systematic review. PLoS ONE. 2009;4:e5561.
26. Wells CD, Cegielski JP, Nelson LJ, Laserson KF, Holtz TH, Finlay A, Castro KG, Weyer K. HIV infection and multi drug resistance Tuberculosis: The Perfect Strom. J Infect Dis. 2007 August 15; 196 suppl 1: S 86-107.