Original Article

Quantification of *Mycobacterium tuberculosis* and *Mycobacterium avium* complex in human immunodeficiency virus-infected individuals with and without tuberculosis

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Abstract

Context: For a dental practitioner, HIV-TB co-infection which is a lethal aliment is an occupational hazard by the virtue of abundant aerosol formation in day to day dental practice.

Aim: To assess the prevalence of TB co-infection among HIV patients. To assess the infectivity by culturing Mycobacterium Tuberculosis and Mycobacterium avium complex using Lowenstein Jensen medium and Middlebrook medium.

Setting and Design: One hundred and forty one recently diagnosed HIV seropositive patients were selected. They were divided into two group based on their clinical symptomatology. Sputum samples, CD4 counts and brief case history were collected from these patients.

Subjects and Methods: Sputum samples were homogenized using modified Petroff's method. The samples were cultured using Lowenstein Jensen and Middlebrook media. Cultures were interpreted after two weeks of incubation and the cultures were quantified based on their number of colonies produced on them.

Statistical Analysis: Descriptive statistical analyses followed by Chi square test were performed to assess the prevalence and variation of TB co-infection.

Results: Prevalence of TB in our sample is 25.53%. Higher CFU of MTB and MAC are obtained in patients symptomatic for TB. MB yields higher CFU than LJ.

Conclusion: Clinical symptoms alone cannot act as basis for suspecting TB in HIV patients. Mycobacterial cultures should be used as diagnostic aids and preferably both the mediums have to be used.

Keywords: HIV, Tuberculosis, MAC, LJ, Middlebrook, Quantification

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INTRODUCTION

Human immunodeficiency virus (HIV) thrives on death. During the early 1980s, numerous cases have been reported primarily from Africa with patients showing severe weakness, anorexia and vomiting in conjunction

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with a rare malignancy known as Kaposi's sarcoma, which showed an increase in incidence.[1] In 1983, Luc Montagnier and Robert Gallo led separate research teams successfully isolating a viral strain from such patients and named it HIV.[2,3]

HIV, as the name indicates, leads to immunosuppression by targeting CD4 cells in the circulation.[4] A decrease in the CD4 counts to a varying degree is seen in all the patients infected with HIV.[5] As a result of this immunosuppression, various opportunistic infections arise within the patients. Globally, candidiasis is the leading opportunistic infection; however, in India, candidiasis is paralleled by tuberculosis (TB) in prevalence.

TB, also known as "*The White Plague*," is a communicable, granulomatous, airborne bacterial infection caused by *Mycobacterium tuberculosis* (MTB). TB has been known to humankind since ancient times and is responsible for most deaths caused by any disease.[6] TB spreads through droplet formation, which is intensified by aerosols encountered regularly in a dental practice. As the organism is fastidious and highly contagious, additional care is necessary for patient management.

Unfortunately, the immunosuppression caused by HIV acts as an adjunct to TB, resulting in a coinfection and thereby an increase in incidence. TB classically presents as a persistent cough for more than 2 weeks. Weight loss, night sweats and fever along with cough are considered as the cardinal signs of TB. However, these clinical symptoms are subjected to an individual and geographical variation in case of an HIV-TB coinfection.

The WHO has proposed clinical guidelines to counter HIV-TB pandemic. These guidelines are based on taking a detailed history of the patient. Due to inconsistency in the CD4 counts, patient's clinical presentations and histories, clinical guidelines alone cannot be considered as a safe means of diagnosis.[7]

TB has been diagnosed since the early 20th century by sputum smear microscopy using Zeil–Neilson or acid-fast bacilli staining. Since the 1970s, culture of MTB using Lowenstein–Jensen medium (LJ) has been used as a gold standard for diagnosis.[8] A decade ago, with the advent of molecular diagnostic aids and complete identification of mycobacterial genome, nucleic acid amplification tests had been performed to diagnose TB with an accuracy of 90%.[9] In India, their sensitivity and specificity are overshadowed by their expensiveness and inaccessibility.

This situation demands the development of new parameters for diagnosing and predicting the possibility of TB development in HIV-positive patients, which are economical, easy, rapid to perform and reliable.

In the present cross-sectional study, correlation between CD4 counts of HIV-seropositive patients with culture positivity in terms of colony counts is performed to establish the predictive role of CD4 counts for TB in HIV-positive patients. Similarly, correlation between culture status and the presence of symptoms suggestive of TB is performed to assess the reliability of clinical symptoms in predicting TB in HIV-infected patients.

MATERIALS AND METHODS

The present study was conducted at Mahatma Gandhi General Hospital and Panineeya Mahavidyalaya Institute of Dental Sciences Hospital and Research Center in Hyderabad. The study was approved by the institutional ethical committee (PMVIDS/OP/0018/2014). The present study included a sample of 141 individuals who were recently diagnosed with HIV. Brief case history, CD4 counts and sputum samples were collected from patients. Patients who were receiving antiretroviral treatment (ART) or antitubercular treatment (ATT) were excluded. Any patient with a prior history of TB was also excluded from the study.

The 141 individuals were divided into study and control groups based on their clinical symptomatology. Individuals with symptoms suggestive of TB such as cough, dyspnea, chest pain and hemoptysis were included under the control group and those without the symptoms were included under the study group, respectively.

Sputum samples were collected following the WHO protocol in the presence of an observer. Two milliliters of the stimulated sputum sample was collected in a wide-mouthed, sterile, screw-capped bottle held slight away from the mouth. Care was taken that saliva would not mix with sputum by instructing the patient to take a deep breath and expectorate the sputum directly into the sample collection bottle without touching its walls.[10] Blood samples were preferably collected on the same day or the next.

The sputum samples were homogenized following the Revised National TB Control Program guidelines using modified Petroff's method.[11] The conventional LJ medium was used for culture along with Middlebrook 7H11 (MB) medium which has greater sensitivity for *Mycobacterium*

Figure 1: *Mycobacterium tuberculosis* colonies on Lowenstein–Jensen medium

Figure 2: *Mycobacterium tuberculosis* colonies on Middlebrook medium

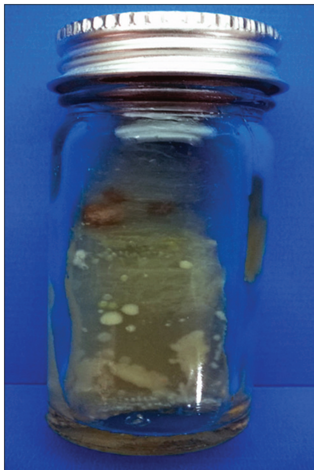

Figure 3: *Mycobacterium avium* complex showing smooth, waxy nonpigmented colonies on Middlebrook medium

Figure 4: *Mycobacterium avium* complex showing confluent growth and pigmented colonies on Middlebrook medium

avium complex (MAC) in comparison to LJ medium. The processed sputum sample was inoculated on one slant of LJ medium and MB medium each. The media were incubated at 37°C for 2 weeks.

At the end of 2 weeks of incubation, positive cultures were identified and speciation was performed based on colony morphology. MTB yielded rough, buff-colored colonies [Figures 1 and 2], whereas MAC yielded smooth, waxy, cream-colored colonies [Figure 3]. MAC also yield red-colored pigmented colonies [Figure 4]. Number of discrete colonies was counted, and as the dilution factor along with the volume of sample inoculated was known, number of colony forming units (CFU) was calculated using the formula:

$$\text{CFU} = \frac{\text{number of colonies}}{\text{volume tested}} \times \text{dilution factor}$$

Table 1: Age distribution among the control and study groups

	Control group (%)	Study group (%)	Total (%)
18-25 years	8 (10.13)	10 (16.13)	18 (12.77)
26-34 years	36 (45.57)	22 (35.48)	58 (41.13)
35-44 years	24 (30.38)	16 (25.81)	40 (28.37)
45-65 years	11 (13.92)	14 (22.58)	25 (17.73)
Total	79 (100.00)	62 (100.00)	141 (100.00)
Mean age	34.67±9.16	35.56±10.67	35.06±9.82

Table 2: Sex distribution within control and study groups

	Control group (%)	Study group (%)	Total (%)
Male	34 (43.04)	21 (33.87)	55 (39.01)
Female	45 (56.96)	41 (66.13)	86 (60.99)
Total	79 (100.00)	62 (100.00)	141 (100.00)

Chi-square test was performed to correlate the variation of CD4 counts with culture positivity using LJ and MB media. Variation of culture positivity with clinical symptomatology was also assessed. Statistical significance was set as $P < 0.05$.

RESULTS

Our sample includes 141 individuals recently diagnosed as HIV seropositive. Our sample distribution is depicted in Tables 1 and 2. Among the 141 subjects, 79 belonged to the control group and 62 belonged to the study group. Similarly, 55 were males and 86 were females. Within the control group, 34 were males and 45 were females, and within the study group, 21 were males and 41 were females.

Our sample was divided into four groups based on CD4 counts, namely, <200, 201–349, 350–500 and >501. A P = 0.508 was obtained which was not statistically significant. CD4 count distribution in our sample is shown in Table 3.

Comparative evaluation of culture positivity with respect to clinical symptomatology is presented in Table 4. Of the total 141 individuals, 36 showed culture positivity and 105 showed culture negativity. Within the control group, 21 individuals showed culture positivity and 58 showed culture negativity, whereas, among individuals of the study group, 15 showed culture positivity and 47 showed culture negativity. A P = 0.7473 was obtained which was not statistically significant.

Culture positivity for MTB using LJ medium and its variation with respect to CD4 counts and clinical symptomatology is described in Table 5. A P = 0.017 was obtained which was statistically significant. In the study group, 14 were positive of which five had CD4 counts <200 cells/mm^3, three had CD4 counts ranging between 350 and 500 cells/mm^3 and six had >501 cells/mm^3. In the control group, 15 were positive of which eight had CD4 counts <200 cells/mm^3.

Among the individuals positive for MTB using LJ medium, those with CD4 counts below 200 cells/mm^3 had a mean of 151.38 CFU. The mean CFU obtained from the control group and study group was 94 ± 166.19 and 81 ± 154.42, respectively. A P = 0.269 was obtained which was not statistically significant. Variation among the mean CFU of MTB with respect to CD4 counts and clinical symptomatology using LJ medium is depicted in Table 6.

Culture positivity for MAC using MB medium and its variation with respect to CD4 counts and clinical symptomatology is shown in Table 7. In the control group, four were positive of which one had CD4 counts ranging between 201 and 349 cells/mm^3 and three had CD4 count >501 cells/mm^3. In the study group, one was positive with CD4 counts ranging between 350 and 500 cells/mm^3. A P = 0.082 was obtained which was not statistically significant.

In the control group, the mean CFU of MAC was 541.50 ± 338.18. Similarly, the study group yielded a CFU of 744.00. Variation among the mean CFU of MAC with

Table 3: Distribution of CD4 counts in the sample

	Control group (%)	Study group (%)	Total (%)
<200	29 (36.70)	20 (32.26)	49 (34.77)
201-349	14 (17.73)	7 (11.29)	21 (14.89)
350–500	14 (17.73)	16 (25.80)	30 (21.26)
≥501	22 (27.84)	19 (30.65)	41 (29.08)
Total	79 (100.00)	62 (100.00)	141 (100.00)

P=0.508; not significant; no significant variation was found among CD4 counts with respect to clinical symptoms

Table 4: Culture status of the sample using both the media

	Control group (%)	Study group (%)	Total (%)
Positive	21 (26.58)	15 (24.19)	36 (25.53)
Negative	58 (73.42)	47 (75.81)	105 (74.47)
Total	79 (100.00)	62 (100.00)	141 (100.00)

P=0.7473; not significant; significant variation between culture positivity and clinical symptoms was not found

Table 5: Culture positivity for *Mycobacterium tuberculosis* using Lowenstein-Jensen medium with respect to their CD4 counts

	Control group (%)	Study group (%)	Total (%)
<200	8 (53.33)	5 (35.71)	13 (44.83)
201-349	5 (33.33)	0 (0.00)	5 (17.24)
350-500	1 (6.67)	3 (21.43)	4 (13.79)
≥501	1 (6.67)	6 (42.86)	7 (24.14)
Total	15 (100.00)	14 (100.00)	29 (100.00)

P=0.017; significant; culture positivity using Lowenstein Jensen medium showed significant variation with CD4 counts

Table 6: Mean colony forming units of *Mycobacterium tuberculosis* using Lowenstein-Jensen medium in control and study groups with respect to their CD4 counts

	Control group	Mean CFU	Study group	Mean CFU	Total	Mean CFU
<200	8	151.50	5	151.20	13	151.38
201-349	5	33.60	0	0	5	33.60
350-500	1	12.00	3	32.00	4	27.00
≥501	1	18.00	6	47.00	7	42.85
Mean CFU	94.00±166.19		81±154.42		87.724±157.87	

P=0.269; not significant. Variation of colony forming units using Lowenstein Jensen medium with respect to CD4 counts was not significant CFU: Colony forming units

Table 7: Culture positivity for *Mycobacterium avium* complex using Middlebrook 7H11 medium with respect to their CD4 counts

CD4 counts	Control group (%)	Study group (%)	Total (%)
<200	0 (0.00)	0 (0.00)	0 (0.00)
201-349	1 (25.00)	0 (0.00)	1 (20.00)
350-500	0 (0.00)	1 (100.00)	1 (20.00)
≥501	3 (75.00)	0 (0.00)	3 (60.00)
Total	4 (100.00)	1 (100.00)	5 (100.00)

P=0.082; not significant; culture positivity using Middlebrook medium showed no significant variation with CD4 counts

Table 8: Mean colony forming units for *Mycobacterium avium* complex using Middlebrook 7H11 medium with respect to their CD4 counts

CD4 counts	Control group	Mean CFU	Study group	Mean CFU	Total	Mean CFU
<200	0	0.00	0	0.00	0	0.00
201-349	1	36.00	0	0.00	1	36.00
350-500	0	0.00	1	744.00	1	744.00
≥501	3	710.00	0	0.00	3	710.00
Mean CFU	541.50±338.18		744.00		582.00±306.55	

P=0.746; not significant. Variation of colony forming units using Middlebrook medium with respect to CD4 counts was not significant
CFU: Colony forming units

respect to CD4 counts and clinical symptomatology using MB medium is presented in Table 8.

DISCUSSION

HIV is an incapacitating disease and causes numerous opportunistic infections, due to its immunosuppression, which makes it lethal to humanity. In 2014, the WHO reported that there were 35 million people all over the world living with HIV, and among them, 1.1 million people were diagnosed with TB. Along with MTB, various strains of nontuberculous mycobacteria (NTM) were isolated from HIV-TB-coinfected patients in recent years.[12,13] In India, TB is the second most common opportunistic infection in HIV-infected patients following candidiasis.[10]

In 2014, the WHO proposed a tetrad of clinical symptoms for diagnosing HIV-TB coinfection which includes cough, fever, night sweats and weight loss in a decreasing order of presentation.[13] According to Swaminathan *et al*., in the Indian population, there was variation in the clinical presentation of HIV-TB coinfection, with cough and weight loss being the most prevalent symptoms, whereas fever and night sweat being observed occasionally.[14]

CD4 counts have been used as a reliable indicator for immunosuppression in HIV patients, and hence, CD4 counts have been compared with the culture status in HIV-TB coinfection to assess the diagnostic reliability of this correlation. In 1997, Jones *et al*. stated that there is a reduction in the CD4 count with increasing culture positivity, but it is not statistically significant.[15] In 2015, Mishra and Rukadikar published similar results in the Indian population using LJ medium.[16]

In the present study, we have selected individuals who were not on ART or ATT because, in a cross-sectional study conducted by Wanchu *et al*., they reported that both ATT and ART influence the CD4 counts of patients.[17]

We have used MB medium in addition to LJ medium because, according to Thoen *et al*., it was not only more sensitive in detecting MTB than LJ but also useful in isolating colonies of MAC. Furthermore, it helps in differentiating rapid-growing mycobacteria from slow-growing mycobacteria.[18]

The prevalence of TB in our study based on clinical symptoms is 56.04%, which is higher than that reported by Aggarwal *et al*. and Swaminathan *et al*., who reported a prevalence of 31.25% and 43.58%, respectively. The difference in the prevalence can be attributed to the use of a smaller sample than our study.[14,19]

The prevalence of TB in the present study based on the use of a diagnostic test is 25.53%, which is higher than that reported by Sameer Singhal *et al*., who reported a prevalence of 11.2%. The decreased prevalence of their study could be attributed to the use of sputum smear microscopy for the detection of TB, which is a less sensitive method compared to cultures.[20,21] Mishra and Rukadikar reported a prevalence of 23.48%, which is very similar to our result. The similarity can be attributed to the use of LJ medium for culturing sputum sample.[16] Conversely, Swaminathan *et al*. reported a higher prevalence of 31.25%, and this could be attributed to the use of Mantoux test for diagnosis, which gives higher false-positive results when compared to cultures.[14]

In the present study, we observed that of 36 (25.53%) individuals who were positive for TB, only 33 (23.41%) were reported positive using LJ medium. Our results are similar to those of Naveen and Peerapur, who reported that of 120 individuals who were positive for TB in their study, only 65 were positive with LJ medium.[21] This difference in positivity could be due to the interpretation of the cultures at the end of the 2nd week because the peak positivity of LJ medium is in the 4th week. The cultures on LJ medium are interpreted by the end of the 2nd week as there is an increased chance of contamination on further incubation. By this observation, we understand that the use of LJ medium alone could lead to misdiagnosing few patients as TB negative.

In the present study, we observed that of 36 (25.53%) individuals who were positive for TB, only 25 (17.73%) were reported positive using MB medium. From this observation, we could infer that MB is less sensitive for TB when compared to LJ medium. These results are in agreement to those of Naveen *et al*., who stated that MB medium shows only 69% sensitive for TB in comparison to LJ medium.[22] The decreased sensitivity could be due to the

absence of carbon dioxide production from the medium, which is need for mycobacterial growth.

Among the 36 individuals who were culture positive in our study, 14 (38.89%) had CD4 counts <200 cells/mm^3 while 10 (27.77%) had CD4 counts above 500 cells/mm^3. This variation yielded a $P = 0.837$ which is not significant, indicating that decrease in CD4 count does not result in an increase in culture positivity among the individuals. Our results are in conflict with Jones *et al*., who reported TB positivity with CD4 counts below 200 cells/mm^3 and above 500 cells/mm^3 while showing a significant variation.[15] This difference could be due to the use of LJ medium alone in their study.

Few cultures from severe immunocompromised individuals showed fungal growth within 2 days of inoculation on LJ medium. After 2 weeks of inoculation, rough, buff-colored colonies developed amid the fungal growth. They were reported as MTB positive. This interpretation is in accordance with Mwaura *et al*., who reported that *Candida* (19.18%) is the most common fungal organism isolated from sputum samples of TB patients, and appearance of fungal growth on LJ medium should not be interpreted as contamination if MTB colonies are seen within 2 weeks of inoculation.[23]

In the previous studies, the tuberculous colonies were graded according to the number of discrete colonies that are obtained on the slopes; however, in the present study, we have expressed the colonies in the form of CFU, as a known volume of sputum with a standardized dilution was used to inoculate the media. Our results show that using LJ medium subjects of the control group (94 ± 166.19) yielded higher colony counts for MTB in comparison to the study group (81 ± 154.42). A $P = 0.269$ was achieved indicating a nonsignificant variation of mean CFU among the control and study groups. Our search through the literature shows few studies that expressed MTB and MAC in the form of CFU; however, we cannot compare our results with them as the former studies quantified the cultures as CFU only in secondary cultures.

CONCLUSION

If an individual is diagnosed with HIV-TB coinfection, it is safe to assume that the severity of the disease increases with the appearance of clinical symptoms and the degree of immunosuppression. As TB shares clinical symptoms with numerous opportunistic infections, clinical symptoms alone should not be used as the basis of suspicion. NTM such as MAC show similar clinical presentation to MTB and they do not respond to ATT; hence, if a subject is nonresponsive for ATT, it indicates not only multidrug-resistant MTB but also MAC.

Asymptomatic patients and those who are not suspected of TB along with those who are not qualified for ATT are also showing culture positivity, indicating a potential threat. Accurate diagnosis helps the patient to receive proper treatment before the disease advances to a refractory stage, and it also helps the dental practitioners prevent cross-contamination.

Clinical symptoms can be used as basis for suspecting MAC alone in HIV patients, and MB medium has to be used if MAC is suspected. The mean CFU reflects the severity of disease better than grading the cultures based on number of colonies.

Our study establishes that asymptomatic patients with CD4 counts above 350 cells/mm^3 who do not qualify for chemotherapy are culture positive for MTB, indicating a need in increasing the range for CD4 counts eligible for chemotherapy. It emphasizes on the need for diagnosing TB through LJ and MB cultures, which has taken a back seat in comparison to sputum smear microscopy which is less sensitive and National Assessment and Accreditation Council tests which are less economic and accessible to the general population.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Altman L. New Homosexual Disorder Worries Health Officials. New York Times; 1982.
2. Smith AJ, Walker DM. The origins of the human immunodeficiency viruses: An update. J Oral Pathol Med 1992;21:145-9.
3. Sharp PM, Hahn BH. Origins of HIV and the AIDS pandemic. Cold Spring Harb Perspect Med 2011;1:a006841.
4. Costin JM. Cytopathic mechanisms of HIV-1. Virol J 2007;4:100.
5. Parisi E, Glick M. Immune suppression and considerations for dental care. Dent Clin North Am 2003;47:709-31, vii.
6. Rothschild BM, Martin LD, Lev G, Bercovier H, Bar-Gal GK, Greenblatt C, *et al. Mycobacterium tuberculosis* complex DNA from an extinct bison dated 17,000 years before the present. Clin Infect Dis 2001;33:305-11.

7. Padmapriyadarsini C, Narendran G, Swaminathan S. Diagnosis and treatment of tuberculosis in HIV co-infected patients. Indian J Med Res 2011;134:850-65.
8. Singhal R, Myneedu VP. Microscopy as a diagnostic tool in pulmonary tuberculosis. Int J Mycobacteriol 2015;4:1-6.
9. India. Revised National Tuberculosis Control Program. Training module for Medical Practitioners. New Delhi; 2010.
10. World Health Organization. Guidelines on Standard Operating Procedures for Laboratory Diagnosis of HIV-Opportunistic Infections. New Delhi: World Health Organization; 2001.
11. India. Revised National Tuberculosis Controlled Program. Diagnosis of Smear Positivity Pulmonary TB. DOTS; 2009.
12. India. Revised National Tuberculosis Control Program. Annual Status Report. New Delhi; 2014.
13. WHO. HIV Prevention, Diagnosis, Treatment and Care for Key Populations. Switzerland: World Health Organization; 2014.
14. Swaminathan S, Sangeetha M, Arunkumar N, Menon PA, Thoma B, Shibi K, *et al*. Pulmonary tuberculosis in HIV positive individuals: Preliminary report on clinical features and response to treatment. Indian J Tuberculosis 2002;49:189-94.
15. Jones BE, Oo MM, Taikwel EK, Qian D, Kumar A, Maslow ER, *et al*. CD4 cell counts in human immunodeficiency virus-negative patients with tuberculosis. Clin Infect Dis 1997;24:988-91.
16. Mishra S, Rukadikar A. Prevalence of pulmonary tuberculosis in HIV positive individuals, its sensitivity and association with CD4 counts. Int J Curr Microbiol Appl Sci 2015;4:292-304.
17. Wanchu A, Kuttiatt VS, Sharma A, Singh S, Varma S. CD4 cell count recovery in HIV/TB co-infected patients versus TB uninfected HIV patients. Indian J Pathol Microbiol 2010;53:745-9.
18. Thoen CO, Himes EM, Jarnagin JL, Harrington R Jr. Comparison of four culture media for isolation of *Mycobacterium avium* complex from porcine tissues. J Clin Microbiol 1979;9:194-6.
19. Aggarwal A, Arora U, Bajaj R, Kumari K. Clinico-microbiological study in HIV seropositive patients. J Indian Acad Clin Med 2005;6:142-5.
20. World Health Organization. Improving the Diagnosis and Treatment of Smear-Negative Pulmonary and Extrapulmonary Tuberculosis among Adults and Adolescents. Switzerland: World Health Organization; 2007.
21. Singhal S, Mahajan SN, Diwan SK, Gaidhane A, Quazi ZS. Correlation of sputum smear status with CD4 counts in cases of pulmonary tuberculosis and HIV co-infected patients – A hospital based study in rural area of central India. Indian J Tuberc 2011;58:108-12.
22. Naveen G, Peerapur BV. Comparison of the Lowenstein-Jensen medium, the Middlebrook 7H10 medium and MB/BacT for the isolation of *Mycobacterium tuberculosis* (MTB) from clinical specimens. J Clin Diagn Res 2012;6:1704-9.
23. Mwaura EN, Matiru V, Bii C. Mycological findings of sputum samples from pulmonary tuberculosis patients attending TB clinic in Nairobi, Kenya. Viral Mycol 2013;2:10D0119.