Microbes and Infectious Diseases 2021; 2(4): 690-696

Microbes and Infectious Diseases

Journal homepage: https://mid.journals.ekb.eg/

Original article

Contribution of Xpert MTB/RIF® in the diagnosis of pulmonary tuberculosis in Ziguinchor-Senegal

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ARTICLE INFO

Article history:
Received 7 September 2020
Received in revised form 9 October 2020
Accepted 12 October 2020

Keywords:
Tuberculosis
Resistance
Rifampicin
Xpert MTB/RIF®

ABSTRACT

Background: Multidrug-resistant tuberculosis (MDR-TB) is resistant to isoniazid and rifampicin. It is favored by poverty and infection with the human immunodeficiency virus. This retrospective, descriptive study aimed to determine the contribution of Xpert MTB/RIF® in the diagnosis of multidrug-resistant pulmonary tuberculosis in a semi-urban environment at the Peace Hospital of Ziguinchor in Senegal during the period from April 2015 to August 2019. **Methods:** The study is retrospective, descriptive and carried out in the laboratory of Ziguinchor Hospital de la Paix, in southern Senegal, from April 2015 to August 2019. All respiratory specimens were subjected to Ziehl-Neelsen (ZN) stained smear microscopy and Xpert MTB/RIF® testing. **Results:** 180 samples, i.e. 6.4% were microscopy-negative but positive in Xpert MTB/RIF® testing. Thirty_two (32) patients (1%) are infected with rifampicin-resistant *Mycobacterium tuberculosis (M. tuberculosis)*, against 29% of patients (798/2794) infected with susceptible strains. **Conclusion:** Tuberculosis is no longer a "disease of the past". Having a high cost, Xpert MTB/RIF® testing must be associated with microscopy, which remains accessible in semi-urban areas and easy to perform.

Introduction

Multi-drug resistant tuberculosis (MDR-TB) is a particularly dangerous form of tuberculosis caused by a strain of *Mycobacterium tuberculosis*. It is defined by resistance to both isoniazid and rifampicin, the two most effective major anti-TB drugs [1,2].

Tuberculosis with anti-tuberculosis-resistant bacilli has become a major public health problem [3,4]. The incidence of MDR-TB varies considerably from one population and region to another. According to World Health Organization (WHO) estimates, there were 1.2 million TB deaths among HIV-negative people in 2018 and an additional 251000 deaths among HIV-positive people. Geographically, most TB cases in 2018 were in the WHO regions of South-East Asia (44%), Africa (24%) and the Western Pacific (18%), with smaller percentages in the Eastern Mediterranean (8%), the Americas (3%) and Europe (3%). Eight countries accounted for two thirds of the global total: India (27%), China (9%), Indonesia (8%), the Philippines (6%), Pakistan (6%), Nigeria (4%), Bangladesh (4%) and South Africa (3%) [5]. A contagious disease, tuberculosis is endemic in Senegal, with a high prevalence in West Africa, it is favoured by poverty and human immunodeficiency virus (HIV) infection [6-10]. Diagnostic methods have evolved over time. Indeed, microscopy often gives

DOI:10.21608/MID.2020.43109.1063
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false negative results while the culture of mycobacteria takes too long. These limitations have favoured molecular diagnostic methods, such as the Xpert MTB/RIF® test, which nowadays plays an important role in the diagnosis of the disease [11-12]. Early diagnosis is essential in order to reduce the delay in starting treatment and the high mortality caused by delays in treatment.

The Xpert MTB/RIF® test uses a real-time PCR (Polymerase Chain Reaction) technique to detect both the presence of mycobacteria and the possible presence of a mutation in the *rpoB* gene responsible for rifampicin resistance [11]. Within less than two hours, it allows identification and signals a possible resistance to rifampicin (mutation of the *rpoB* gene). The mutation in this region is responsible for resistance to rifampicin, which is a major antibiotic in the treatment of this disease, and rifampicin resistance is often synonymous with multi-resistance. This test reduces the median time to start treatment from 56 days to 5 days [13].

In Senegal, studies on the contribution of Xpert MTB/RIF® in the diagnosis of tuberculosis were conducted mainly in urban areas [14,15]. Based on this observation, we therefore undertook this work with the aim of determining the contribution of Xpert MTB/RIF® in the diagnosis of MDR pulmonary tuberculosis in a semi-urban environment at the Peace Hospital of Ziguinchor in Senegal.

Material and methods

The study took place at the Ziguinchor Peace Hospital located 450 km from Dakar in southern Senegal. It is a general hospital with several hospital departments, namely the Infectious Diseases Department, the Pneumology Department, the Paediatrics Department, the Surgery Department, the Gynaecology Department, the Medical Biology Laboratory.

With a hospitalisation capacity of 35 beds, the medical service welcomes tuberculosis patients. The hospital hospitalises an average of 94 patients/month for all departments combined. The biological analysis laboratory is equipped with an Xpert MTB/RIF® device, provided by the national TB control programme.

Since culture was not available, the Xpert MTB/RIF® test was used as a complementary diagnostic test in cases of suspected MDR-TB or HIV-associated TB.

This was a retrospective, descriptive study among a population of patients sent to the laboratory for microbiological confirmation. We analyzed clinical samples collected from April 2015 to August 2019.

According to the criteria of the WHO of 2013, the diagnosis of pulmonary TB was based on the positive result of at least one of the following tests: microscopy, Xpert MTB/RIF® test and culture [16].

The patients included in the study had clinical and radiological signs suspicious of TB received for the first time in consultation. Patients who relapsed were directly tested with Xpert MTB to detect possible secondary TB resistance.

This study involved 3456 patients with clinical symptoms and signs of pulmonary and/or extra-pulmonary TB. Among the samples analysed, sputum accounted for 80.84%.

With the advent of resistance, all samples were subjected to the Xpert MTB/RIF® test to detect possible resistance to rifampicin.

First we performed a smear examination with the ZN stain for AFB (acid-fast bacilli), then a first Xpert MTB/RIF® test on each sample and a second Xpert MTB/RIF® test on a second sample for microscopy positive and molecular biology negative tests was performed for confirmation.

The WHO-approved Xpert MTB/RF® test uses a real-time PCR technique that amplifies the 81 bp (base pair) region of the *rpoB* gene of *M. tuberculosis* [17]. Socio-demographic and biological data were collected and calculated using the statistical calculation software Excel version 2013.

Results

We analysed 2794 sputum samples, i.e. 80.84%. These samples came from pulmonary TB patients with signs of TB impregnation. These patients were given a chest X-ray to complete the diagnosis. In patients with extra-pulmonary tuberculosis, we analysed various pathological products, a total of 662 samples. There were 1736 men (62.1%) and 1058 women (37.9%), a sex ratio of 1.64. The most represented age group was between 1 and 20 years with a median equal to 5 and an average age of 14.62 +/- 22.51 years **(Figure 1).**

Microscopy remains an accessible technique with a relatively high sensitivity. One hundred and eighty (180) samples or 6.4% were microscopically negative but positive to the Xpert MTB/RIF® test. Twelve (12) samples or 0.43% were microscopy positive and molecular biology negative.

The HIV seroprevalence in our study was 23.15% (n = 647/2794). The majority of samples from

HIV positive subjects were microscopically negative with 98.6% (n=638/647) while 0.46% (n=3/647) of these samples were Xpert MTB/RIF® negative **(Table 1).**

Of the 2794 samples, 32 patients, i.e. a prevalence of 1%, were infected with rifampicin-resistant strains of *M. tuberculosis*, while 29% of the patients (798/2794) were infected with rifampicin-susceptible strains and the rest were negative **(Figure 2).**

Figure 1. Age distribution of patients.

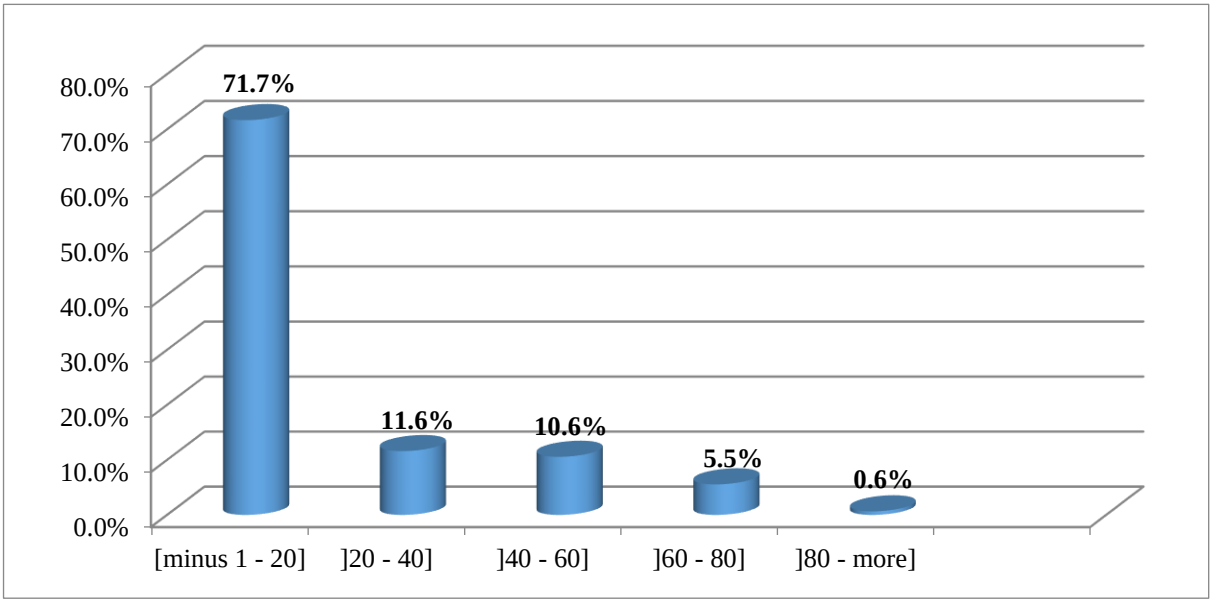
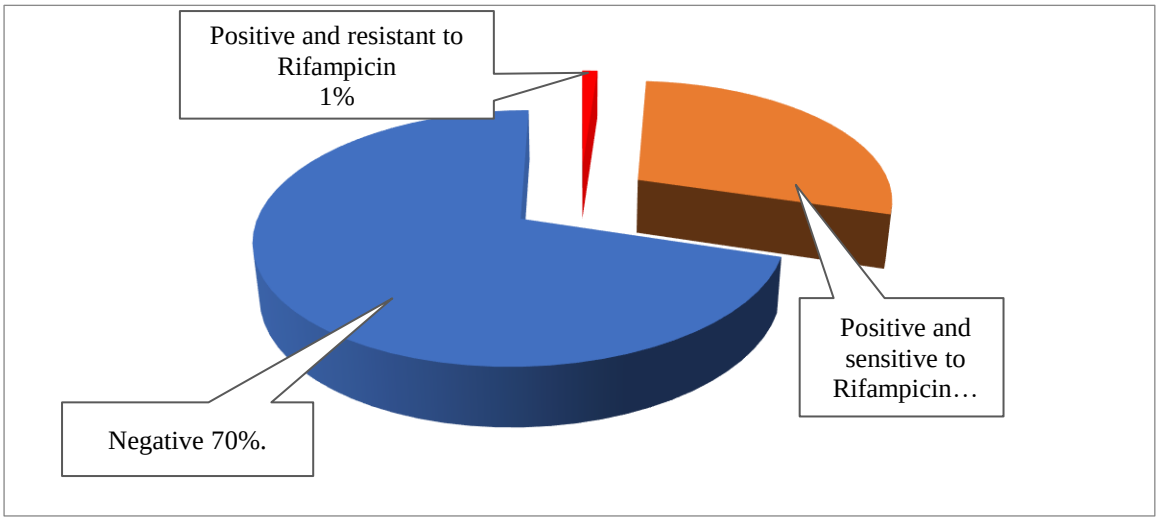


Table 1. Distribution of patients according to epidemiological and paraclinical characteristics and results of the Xpert MTB/RIF® test.

Variables		Staff	Xpert MTB/RIF® test	
			Positive	Negative
Sex	Male	1736***(62,13%)***	517 ***(18,50%)***	1219 ***(43,63%)***
	Female	1058***(37,87%)***	313 ***(11,21%)***	745 ***(26,66%)***
Samples	Expectorations	2794***(80,84%)***	786 ***(22,74%)***	2008 ***(58,10%)***
	Others	662 ***(19,16%)***	44 ***(1,28%)***	618 ***(17,88%)***
HIV serology	Positive	647 ***(23,15%)***	644 ***(23,04%)***	03 ***(0,11%)***
	Negative	2147***(76,84%)***	186 ***(6,66%)***	1961 ***(70,18%)***
Microscopy	Positive	650 ***(23,26%)***	638 ***(22,83%)***	12 ***(0,43%)***
	Negative	2144***(76,74%)***	180 ***(6,45%)***	1964 ***(70,29%)***

Figure 2. Results of the Xpert MTB/RIF® test.



Discussion

Socio-demographic profile

We received 3456 samples, of which 2794 were represented by sputum, i.e. 80.84%. The average age was 14.62 +/- 22.51 years. In Africa, and particularly in Senegal, the disease mainly affects the young population, which can be explained by promiscuity in households, but also by HIV infection among young people.

Microscopy and Xpert MTB/RIF® testing

Microbiological tests confirm the clinical diagnosis. Microscopic examination after ZN staining remains the most accessible and least expensive examination in semi-urban areas, with a more or less acceptable sensitivity [12]. Compared to the result of **Horo et al**. [18] who found a sensitivity of 85% with respect to the reference test, which is the culture. The variability of its diagnostic performance is related to the quality of the samples, the bacillary richness of the sputum, the dye used and the experience of the reader.

The use of a molecular test such as the Xpert MTB/RIF® test has improved the diagnosis of TB. In fact, 6.4% of the samples were negative on microscopy but positive on molecular biology, while 0.43% of the samples were positive on microscopy and negative on molecular biology. At the Dakar infectious disease clinic, **Diop et al.** found that the Xpert MTB/RIF® test was positive on negative smears in 55% of cases and in 89% of cases on positive smears [14]. In Turkey **Zeka et al.** found 68.9% (24/35) positivity in smear-negative lung samples [19]. Other studies have found a higher sensitivity of the Xpert MTB/RIF® test in smear negative specimens [20,21]. Our results confirm the high sensitivity of the Xpert MTB/RIF® test compared to microscopy, as demonstrated in most validation studies of the Xpert MTB/RIF® test [21,22].

Molecular tests have a sensitivity of over 95% for microscopically positive samples compared to only 60-70% for microscopically negative samples. The detection of nucleic acids increases the diagnostic specificity of tuberculosis; however, too low a sensitivity, especially for microscopically negative samples, makes it impossible to exclude TB in the case of a negative molecular test [23]. In our study 98.6% (n=638/647) of samples from HIV-positive subjects were negative on microscopy while 0.46% (n=3/647) of these samples were negative on the Xpert MTB/RIF® test. Compared to the same **Diop et al.** study where the performance of the Xpert MTB/RIF® test was significantly higher in HIV-negative patients (80%) than in HIV patients (54%). Our results show that the Xpert MTB/RIF® test performed better in HIV-negative patients. HIV infection complicates the diagnosis of tuberculosis by leading to pauci-symptomatic clinical forms and/or disseminated forms [24-30]. It reduces the prevalence of lung excavations and increases negative microscopy forms [31]. HIV infection, for example, leads to under-diagnosis of TB [32].

The test has been shown to be useful in the diagnosis of TB, especially in HIV-infected people [33-37].

Resistance to rifampicin

Despite the progress made in the fight against TB, the emergence of MDR-TB threatens to undermine this progress. It poses a serious threat to the success of the fight against TB. In our study, we noted 1% resistance to rifampicin. In **Diop et al.** study in Dakar, two cases of rifampicin resistance were detected. In Mali, **Toloba et al.** found one confirmed case of MDR-TB

out of 21 reported cases of tuberculosis, with a prevalence of 4.7% [38].

The first cases of MDR-TB were observed as early as the 1990s [39] and correspond to resistance to isoniazid and rifampicin, while XDR-TB corresponds to resistance to isoniazid, rifampicin, fluoroquinolones and at least one of the three injectable second-line anti-tuberculosis drugs: capreomycin, amikacin or kanamycin [40]. The detection of resistance is essential for any new case of TB in order to avoid primary resistance.

Limitations of the study

- Some of the results of Xpert MTB/RIF® were undetermined and therefore not usable.
- Frequent ruptures of Xpert MTB/RIF® cartridges.

Conclusion

Tuberculosis is no longer considered a "disease of the past". Its diagnosis in semi-urban areas is improved by reducing the time taken to deliver results and by increasing the sensitivity of microbiological diagnostic methods. With a high cost, the Xpert MTB/RIF® test must be associated with microscopy which remains accessible in semi-urban areas and easy to perform. The prescription of the Xpert MTB/RIF® test could be rationalized by a strong clinical suspicion in patients whose microscopic examination is non-contributory.

Conflict of interest: No conflicts of interest for all authors.

Financial disclosure: No funds have been received for this research.

References

1-**Van der Werf MJ, Langendam MW, Huitric E, Manissero D.** Multidrug resistance after inappropriate tuberculosis treatment: a meta-analysis. Eur Respiratory Soc 2012; 39(6):1511-1519.

2- **Zignol M, van Gemert W, Falzon D, Sismanidis C, Glaziou P, Floyd K, et al.** Surveillance of anti-tuberculosis drug resistance in the world: an updated analysis, 2007-2010. Bulletin of the World Health Organization 2012; 90(2): 111-119.

3-**Nathanson E, Lambregts-van Weezenbeek C, Rich ML, Gupta R, Bayona J, Blöndal K, et al.** Multidrug-resistant tuberculosis management in resource-limited settings. Emerging Infectious Diseases 2006; 12(9): 1389-97.

4-**Shah NS, Wright A, Bai G-H, Barrera L, Boulahbal F, Martín-Casabona N, et al.** Worldwide emergence of extensively drug-resistant tuberculosis. Emerging infectious diseases 2007; 13(3): 380.

5-**Organisation mondiale de la santé.** Global tuberculosis report 2019. 2019. Disponible sur: https://www.who.int/teams/global-tuberculosis-programme/tb-reports/global-report-2019.

6-**DeRiemer K, Kawamura LM, Hopewell PC, Daley CL.** Quantitative impact of human immunodeficiency virus infection on tuberculosis dynamics. American journal of respiratory and critical care medicine 2007; 176(9): 936–944.

7-**Martinson NA, Hoffmann CJ, Chaisson RE.** Epidemiology of tuberculosis and HIV: recent advances in understanding and responses. Proceedings of the American Thoracic Society 2011; 8(3): 288–293.

8-**World Health Organization**. Global tuberculosis report 2012. Geneva: World Health Organization; 2012. Available at: https://www.who.int/tb/publications/global_report/gtbr12_main.pdf.

9-**Mosso RA, Dore EDA, Kouakou HA, Assi SB, Bakayoko M, Bakayoko Y, et al.** Equipe de redaction du rapport final. :591.

10-PSN_TB-CI_ 2016-2020_Plan Principal.pdf [Internet]. Disponible sur: https://www.ansci.org/alliance/pdf/PSN_TB-CI_%202016 2020_Plan%20Principal.pdf. [cité Oct 7, 2020].

11-**Hervé C, Bergot E, Veziris N, Blanc F-X.** La tuberculose en 2015 : du diagnostic à la détection des formes résistantes. Revue des Maladies Respiratoires 2015; 32(8): 784-90.

12-**Ninet B, Roux-Lombard P, Schrenzel J, Janssens J-P.** Nouveaux tests pour le diagnostic de la tuberculose. Revue des Maladies Respiratoires 2011; 28(6): 823-33.

13-**Boehme CC, Nicol MP, Nabeta P, Michael JS, Gotuzzo E, Tahirli R, et al.** Feasibility, diagnostic accuracy, and effectiveness of decentralised use of the Xpert MTB/RIF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. The lancet 2011; 377(9776): 1495–1505.

14-**Diop SA, Massaly A, Ka D, Manga NM, Fortes-Déguénonvo L, Ndour CT, et al.** Utilisation du test GeneXpert pour le diagnostic de la tuberculose au service des maladies infectieuses du CHNU de Fann. Pan Afr Med J [Internet]. 2016;23. Disponible sur: http://www.panafrican-med-journal.com/content/article/23/244/full/. [cité oct 7, 2020]

15-**Touré NO, Wayzani M, Thiam K, Cissé MF, Mbaye FB.** Apport de l'Xpert MTB/RIF dans le diagnostic étiologique des pleurésies tuberculeuses. Revue des Maladies Respiratoires 2017; 34(7): 758-64.

16-**Organisation mondiale de la Santé .** Définitions et cadre de notification pour la tuberculose– Révision 2013. Organisation mondiale de la Santé; 2014. Disponible sur: https://www.who.int/tb/publications/definitions/fr/.

17-**World Health Organization.** WHO endorses new rapid tuberculosis test. A major milestone for tuberculosis diagnosis and care. Geneva, Switzerland: WHO, 2010. Available at: https://www.who.int/tb/features_archive/new_rapid_test/en/.

18-**Horo K, N'Guessan R, Koffi M-O, Kouamé-N'Takpé N, Koné A, Samaké K, et al.** Test Xpert ® MTB/RIF et dépistage des nouveaux cas de tuberculose pulmonaire en routine dans une zone de haute endémicité tuberculeuse. Revue des Maladies Respiratoires 2017; 34(7): 749-57.

19-**Zeka AN, Tasbakan S, Cavusoglu C.** Evaluation of the GeneXpert MTB/RIF assay for rapid diagnosis of tuberculosis and detection of rifampin resistance in pulmonary and extrapulmonary specimens. Journal of clinical microbiology 2011; 49(12): 4138–4141.

20-**Tortoli E, Russo C, Piersimoni C, Mazzola E, Dal Monte P, Pascarella M, et al.** Clinical validation of Xpert MTB/RIF for the diagnosis of extrapulmonary tuberculosis. European Respiratory Journal 2012; 40(2): 442–447.

21-**Boehme CC, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, et al.** Rapid molecular detection of tuberculosis and rifampin resistance. New England Journal of Medicine 2010; 363(11): 1005–1015.

22-**Bates M, O'Grady J, Maeurer M, Tembo J, Chilukutu L, Chabala C, et al.** Assessment of the Xpert MTB/RIF assay for diagnosis of tuberculosis with gastric lavage aspirates in children in sub-Saharan Africa: a prospective descriptive study. The Lancet infectious diseases 2013; 13(1):3 6–42.

23-**Dinnes J, Deeks J, Kunst H, Gibson A, Cummins E, Waugh N, et al.** A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. In: NIHR health technology assessment programme: executive summaries. NIHR Journals Library. Southampton (UK). 2007.

24-**Corbett EL, Charalambous S, Moloi VM, Fielding K, Grant AD, Dye C, et al.** Human immunodeficiency virus and the prevalence of undiagnosed tuberculosis in African gold miners. American journal of respiratory and critical care medicine 2004; 170(6): 673–679.

25-**Badri M, Wilson D, Wood R.** Effect of highly active antiretroviral therapy on incidence of tuberculosis in South Africa: a cohort study. The Lancet 2002; 359(9323): 2059–2064.

26-**Archibald LK, Dulk MO den, Pallangyo KJ, Reller LB.** Fatal Mycobacterium tuberculosis bloodstream infections in febrile hospitalized adults in Dar es Salaam, Tanzania. Clinical Infectious Diseases 1998; 26(2): 290–296.

27-**Banda HT, Harries AD, Welby S, Boeree MJ, Wirima JJ, Subramanyam VR, et al.** Prevalence of tuberculosis in TB suspects with short duration of cough. Transactions of the Royal Society of Tropical Medicine and Hygiene 1998; 92(2): 161–163.

28-**Mwachari C, Batchelor BIF, Paul J, Waiyaki PG, Gilks CF.** Chronic diarrhoea among HIV-infected adult patients in Nairobi, Kenya. Journal of Infection 1998; 37(1): 48–53.

29-**Mtei L, Matee M, Herfort O, Bakari M, Horsburgh CR, Waddell R, et al.** High rates of clinical and subclinical tuberculosis among HIV-infected ambulatory subjects in Tanzania. Clinical infectious diseases 2005; 40(10): 1500–1507.

30-**Swaminathan S, Paramasivan CN, Kumar SR, Mohan V, Venkatesan P.** Unrecognised tuberculosis in HIV-infected patients: sputum culture is a useful tool. The International Journal of Tuberculosis and Lung Disease 2004; 8(7): 896–898.

31-**Pozniak AL, MacLeod GA, Ndlovu D, Ross E, Mahari M, Weinberg J.** Clinical and chest radiographic features of tuberculosis associated with human immunodeficiency virus in Zimbabwe. American journal of respiratory and critical care medicine 1995; 152(5): 1558–1561.

32-**Wood R, Middelkoop K, Myer L, Grant AD, Whitelaw A, Lawn SD, et al.** Undiagnosed tuberculosis in a community with high HIV prevalence: implications for tuberculosis control. American journal of respiratory and critical care medicine 2007; 175(1): 87–93.

33-**Chheng P, Tamhane A, Natpratan C, Tan V, Lay V, Sar B, et al.** Pulmonary tuberculosis among patients visiting a voluntary confidential counseling and testing center, Cambodia. The International Journal of Tuberculosis and Lung Disease 2008; 12(3): S54–S62.

34-**Day JH, Charalambous S, Fielding KL, Hayes RJ, Churchyard GJ, Grant AD.** Screening for tuberculosis prior to isoniazid preventive therapy among HIV-infected gold miners in South Africa. The international journal of tuberculosis and lung disease 2006; 10(5): 523–529.

35-**Kimerling ME, Schuchter J, Chanthol E, Kunthy T, Stuer F, Glaziou P, et al.** Prevalence of pulmonary tuberculosis among HIV-infected persons in a home care program in Phnom Penh, Cambodia. The International Journal of Tuberculosis and Lung Disease 2002; 6(11): 988–994.

36-**Mohammed A, Ehrlich R, Wood R, Cilliers F, Maartens G.** Screening for tuberculosis in adults with advanced HIV infection prior to preventive therapy. The International Journal of Tuberculosis and Lung Disease 2004; 8(6): 792–795.

37-**Shah S, Demissie M, Lambert L, Ahmed J, Leulseged S, Kebede T, et al.** Intensified tuberculosis case finding among HIV-Infected persons from a voluntary counseling and testing center in Addis Ababa, Ethiopia. JAIDS Journal of Acquired Immune Deficiency Syndromes 2009; 50(5): 537–545.

38-**Toloba Y, Ouattara K, Soumaré D, Kanouté T, Berthé G, Baya B, et al.** Tuberculose multi-résistante (TB-MR) en milieu carcéral noir africain : expérience du Mali. Revue de Pneumologie Clinique 2018; 74(1): 22-7.

39-**Frieden TR, Sherman LF, Maw KL, Fujiwara PI, Crawford JT, Nivin B, et al.** A multi-institutional outbreak of highly drug-resistant tuberculosis: epidemiology and clinical outcomes. Jama 1996; 276(15): 1229–1235.

40-**Cox HS, Sibilia C, Feuerriegel S, Kalon S, Polonsky J, Khamraev AK, et al.** Emergence of extensive drug resistance during treatment for multidrug-resistant tuberculosis. New England Journal of Medicine 2008; 359(22): 2398–2400.

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