

Detection of *Mycobacterium tuberculosis* and rifampicin sensitive -resistant patients diagnosed by the GeneXpert MTB/RIF test in Al-Diwaniyah province

Alaa Abdelkadhim Jawad¹, Hassan Shareef Hussain² and Hayder Naji Ayyez¹

¹Unit of Zoonotic Research, College of Veterinary Medicine, University of Al-Qadisiyah, Diwaniyah, Iraq

²Chest and Respiratory Clinic Diseases, Diwaniyah, Iraq

Email: alaa.jawad@qu.edu.iq

Abstract

To date, tuberculosis (TB) is still a disease of big concern to humanity because it leads to losses in various life aspects such as people lives and economics. Because the long time and hard efforts needed to diagnose the disease, we have conducted this study to better detect the disease in patients in Al-Diwaniyah province, Iraq. Moreover, we have also launched this research to identify rifampicin-sensitive and -resistant patients. To fulfill our aims, we have recruited GeneXpert system for performing the Xpert MTB/RIF test that identifies specific DNA sequences of *Mycobacterium tuberculosis* (MTB) and rifampicin-resistant patients. For this, 179 patients who visited The Chest and Respiratory Clinic Diseases, Diwaniyah, Iraq, were referred to submit sputum specimens to the GeneXpert. After the test had been run, 157 (87.7%) samples were negative, and 22 (12.3%) were positive to the bacterium. Out of 22 patients, 17 (77%) patients were sensitive, and 5 (23%) patients were resistant to the rifampicin. Our results indicate that the disease is still affecting the life of people in the province but has low percentage of rifampicin

resistance in those patients. This keeps encouraging the continuous use of this drug against this bacterium. Moreover, the results attract the health facilities to use GeneXpert as reliable tool to diagnose MTB and whether it is resistant or sensitive to rifampicin.

Keywords: GeneXpert, *Mycobacterium tuberculosis*, XpertMTB/RIF, Rifampicin

Introduction

To date, TB is still a disease of big concern to humanity because it leads to losses in various life aspects such as people lives and economics (1). Even with the continuous and advanced work that tries eliminating this disease, it keeps ranking number one among diseases that threatening public health especially in the countries that have bad health system and low hygienic awareness of people in these countries (2). Iraq is considered as one of the countries in the world that are endemic with this disease. However, in countries like United Kingdom, the disease cases keep rising every year, and this phenomenon reasons belong to lack of care to some people for example homeless, human immunodeficiency virus (HIV) patients, people who use drugs, diabetic people, and people exposed to immunotherapy (3, 4, 5). These difficulties in treatment and increasing the incidence of the disease are resulted from issues such as resistance of MTB to many drugs (6). The bacterium also escapes host immune response which adds extra

obstacles of curing the disease (7, 8). Controlling of the disease gains too much difficulty due to the missing of the useful vaccine (9). Using the conventional methods such as culturing, conventional polymerase chain reaction (PCR), and acid-fast stain to diagnose TB involve long time, multiple-step processing, and false results (10, 11). To overcome the diagnosis hustle, we have conducted Xpert MTB/RIF test on GeneXpert to specifically diagnose the presence of MTB and whether it is resistant or sensitive to rifampicin.

Materials and Methods

Sampling and the Xpert MTB/RIF test

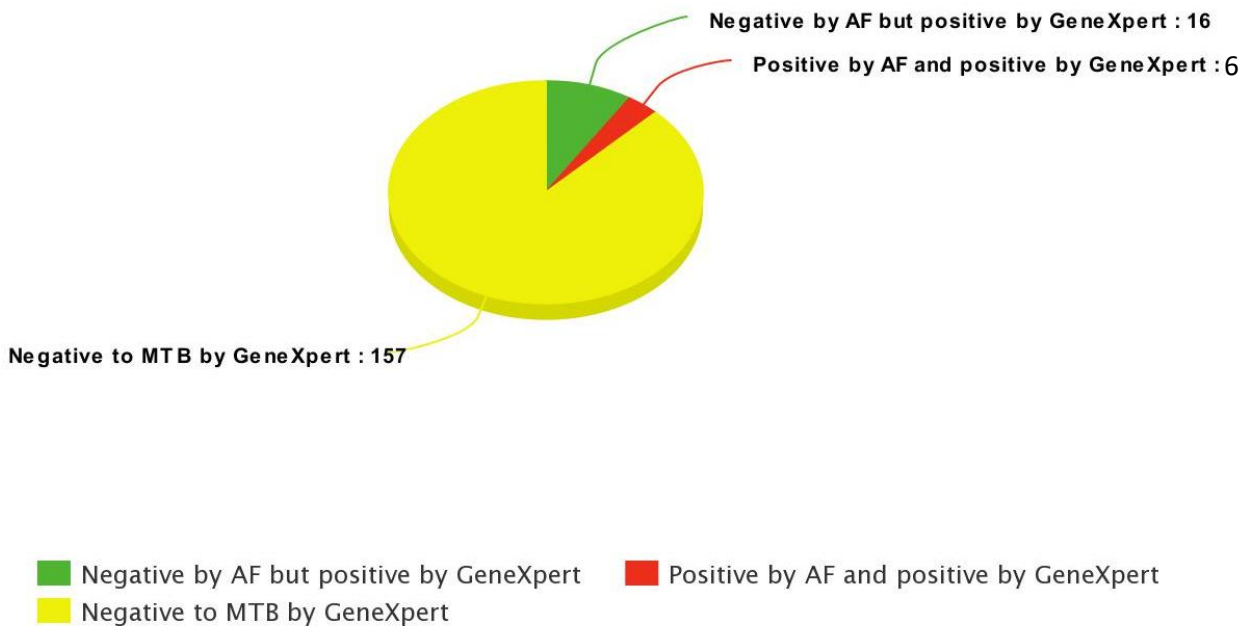
In The Chest and Respiratory Clinic Diseases, Diwaniyah, Iraq, Sputum from 179 patients were collected and assigned to acid-fast test (AF) and then GeneXpert that operates an automated real-time PCR to detect MTB and its resistant strains, Xpert MTB/RIF test. The test uses *rpoB* gene to identify these resistant strains. The test employs a fluorescent probe named Beacon. The test runs for 90 minutes. The whole test was carried out based on Cepheid GeneXpert system, USA, and the manufacturer protocol was followed. <https://www.meta-chart.com/pie> was used to generate the pie charts.

Result

To illustrate the results, 157 (87.7%) samples were negative, and 22 (12.3%) were positive to the bacterium. Only 6 out 22 patients were positive by AF. Out of 22 patients, 17 (77%) patients were sensitive, and 5 (23%) patients were resistant to the rifampicin, table 1 and figure 1 and 2

Table 1: The result of the study

Total Number of patients	+ve patients	-ve patients	Resistant strain to rifampicin	Sensitive strain to rifampicin
179	22 (12.3%)	157 (87.7%)	5 (23%)	17 (77%)



meta-chart.com

Positive Mycobacterium tuberculosis and resistant plus sensitive patients

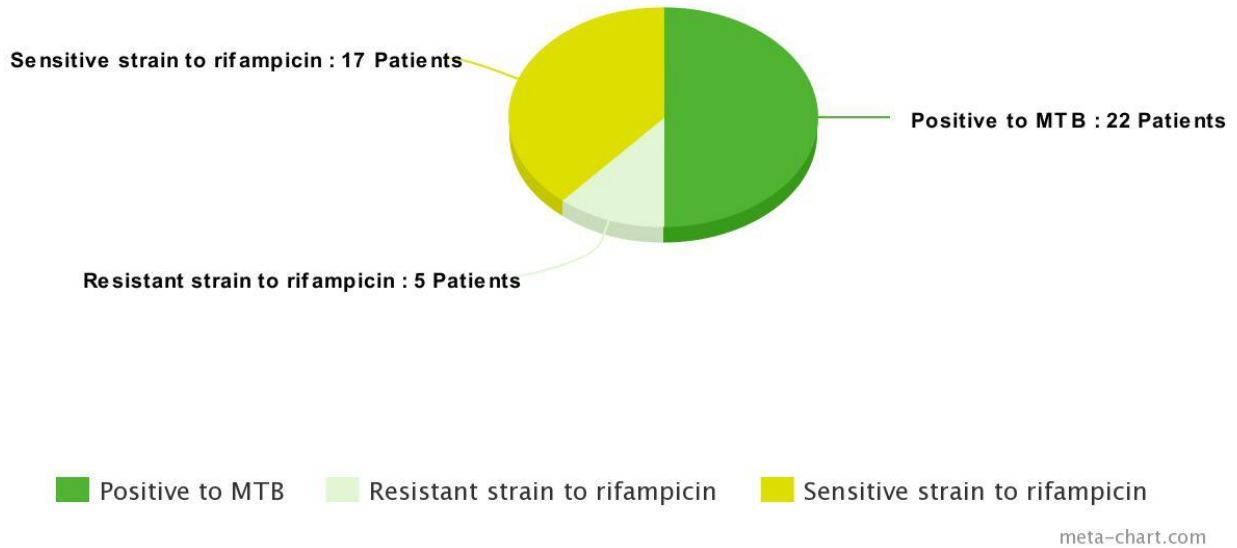


Figure 2: Positive MTB and resistant plus sensitive patients

Discussion

Tuberculosis is still a major disease that concerns humanity because it leads to losses in various life aspects such as people lives and economics (1). Using the conventional methods such as culturing, conventional polymerase chain reaction (PCR), acid-fast stain to diagnose TB involve long time, multiple-step processing, and false results (10, 11). This encouraged us to use a better with high accuracy technique that could identify both the bacterium and its resistance status in relatively short time. By using the GeneXpert, our results indicate that 157 (87.7%)

samples were negative, and 22 (12.3%) were positive to the bacterium. Only 6 out of 22 patients were positive by AF. Out of 22 patients, 17 (77%) patients were sensitive, and 5 (23%) patients were resistant to the rifampicin, table 1 and figure 1 and 2. Our results agree with (12) who found that using Xpert MTB/RIF test gave highly accurate results that overcome the MTB-negative smears. Moreover, our results come in agreement with (13) who declared that Xpert MTB/RIF is sensitive and specific test to diagnose MTB and the resistant strains in short period of time. Using Xpert MTB/RIF, (14) had found that the test was accurate and reliable in detecting MTB which matches our results. The current study results agree with (15) that recognized that Xpert MTB/RIF test was effective and sensitive in detecting MTB and RIF-resistant Mycobacteria in muscles. Our results come in agreement with (16) who found that the use of Xpert MTB/RIF gave high accurate result. Study results showed by (17) indicate the high accuracy of the Xpert MTB/RIF test which agrees with our study results. The Xpert MTB/RIF test performed using GeneXpert gives big hope of fast and accurate detection of MTB and rifampicin - resistant mycobacteria for better treatment.

References

- 1- Dallenga T., Linnemann L., Paudyal B., Repnik U., Griffiths G. and Schaible U.E. (2017). Targeting neutrophils for host-directed therapy to treat tuberculosis. *Int. J. Med. Microbiol.* pii: S1438-4221(17)30329-6.
- 2- da Silva D.A., Rêgo AM., Ferreira N.V., de Andrade M.A.S., Campelo A.R., Caldas P.C.S., Pereira M.A.S., Redner P., de Pina L.C., Resende F.C., Pissinatti T.A., Lopes C.A.A., Kugelmeier T., Perea J.A.S., de Souza I.V., da Silva F.A., Campos C.F., Fandinho Montes F.C.O. and Antunes L.C.M. (2017). Detection of mycobacterial infection in non-human primates using the Xpert MTB/RIF molecular assay. *TB(Edinb)*.107:59-62.
- 3- Zumla A., Raviglione M., Hafner R. and von Reyn C.F.(2013). Tuberculosis. *N Eng.l. J. Med.* 368(8):745-55.
- 4- Rabie H. and Goussard P.(2016).TB and pneumonia in HIV-infected children: an overview. *Pneumonia (Nathan)*. 8:19.
- 5- Barry, C. E., Boshoff, H., Dartois, V., Dick, T., Ehrt, S., Flynn, J., et al.(2009). The spectrum of latent tuberculosis: rethinking the biology and intervention strategies. *Nat. Rev. Microbiol.* 7: 845–855.
- 6- Al-Humadi H.W., Al-Saigh R.J. and Al-Humadi A.W. (2017). Addressing the Challenges of Tuberculosis: A Brief Historical Account. *Front Pharmacol.* 8:689.
- 7- Subbian S., Tsenova L., O'Brien P., Yang G., Koo M. S , Peixoto B and et al. (2011). Phosphodiesterase-4 inhibition alters gene expression and improves isoniazid mediated clearance of MTB in rabbit lungs. *PLoS. Pathog.*7:e1002262. doi: 10.1371/journal.ppat.1002262
- 8- Tobin D. M., Roca F. J., Oh S. F., McFarland R., Vickery T. W., Ray J. P. and et al. (2012). Host genotype-specific therapies can optimize the inflammatory response to mycobacterial infections. *Cell.* 148: 434–446.
- 9- Yeboah-Manu D., Asare P., Asante-Poku A., Otchere I.D., Osei-Wusu S., Danso E., Forson A., Koram K.A. and Gagneux S. (2016). Spatio-Temporal Distribution of MTB Complex Strains in Ghana. *PLoS One.* 11(8):e0161892.

- 10- Ko Y., Song J., Lee S.Y., Moon J.W, Mo E.K., Park J.Y., Kim J.H., Park S., Hwang Y.I., Jang S.H., Jhun B.W., Sim Y.S., Shin T.R., Kim D.G., Hong J.Y., Lee C.Y., Lee M.G., Kim C.H., Hyun I.G., Park Y.B. (2017). Does repeated pleural culture increase the diagnostic yield of MTB from tuberculous pleural effusion in HIV-negative individuals? PLoS One. 12(7):e0181798.
- 11- European Centre for Disease Prevention and Control/WHO Regional Office for Europe. TB surveillance and monitoring in Europe 2015. Stockholm, Sweden. <http://ecdc.europa.eu/en/publications/Publications/tuberculosis-surveillance-monitoring-Europe-2015.pdf>. Accessed October 21st 2016.
- 12- Lombardi G., Di Gregori V., Girometti N., Tadolini M., Bisognin F. and Dal Monte P. (2017). Diagnosis of smear-negative TB is greatly improved by Xpert MTB/RIF. PLoS One.12(4):e0176186.
- 13- Huang H., Zhang Y., Li S., Wang J., Chen J., Pan Z. and Gan H. (2017). Rifampicin Resistance and Multidrug-Resistant TB Detection Using Xpert MTB/RIF in Wuhan, China: A Retrospective Study. Microb. Drug Resist. 2017 Oct 20.doi: 10.1089/mdr.2017.0114.
- 14- Chen C., Yang C.G., Gao X., Lu Z.Z., Tang F.X., Cheng J., Gao Q. and Cárdenas V. (2017). Community-based active case finding for TB in rural western China: a cross-sectional study. Int. J. Tuberc. Lung Dis. 21(11):1134-1139
- 15- Wen H., Li P., Ma H. and Lv G. (2017). Diagnostic accuracy of Xpert MTB/RIF assay for musculoskeletal tuberculosis: a meta-analysis. Infect. Drug Resist. 10:299-305.
- 16-Khapharde S., Raizada N., Nair S.A., Denkinger C., Sachdeva K.S., Paramasivan C.N., Salhotra V.S., Vassall A. and Hoog A.V. (2017). Scaling-up the Xpert MTB/RIF assay for the detection of TB and rifampicin resistance in India: An economic analysis. PLoS One.12(9):e0184270.
- 17- Chikaonda T., Nguluwe N., Barnett B., Gokhale R.H., Krysiak R., Thengolose I., Rosenberg N.E., Stanley C., Mpunga J., Hoffman I.F., Hosseinipour M., Scott L. and Stevens W. (2017). Performance of Xpert® MTB/RIF among TB outpatients in Lilongwe, Malawi. Afr. J. Lab Med. 6(2):464.