1. Introduction

Tuberculosis (TB) is the most important infectious disease in the world causing morbidity and mortality among adults[1]. In 2012, 8.6 million people developed TB for the first time, and 13% had TB with HIV infection[2]. Similarly, in 2012, 1.3 million people died from TB, and 320,000 deaths of TB patients were HIV positive[2]. Multi-drug-resistant tuberculosis (MDR-TB) is defined as tuberculosis resistant to at least two main first-line drugs isoniazid (INH) and rifampicin (RIF)[3]. The resistance to the two first-line anti-TB drugs has emerged as a serious threat to global health[4]. Molecular line probe assay (LPA) (Genotype MTBDRplus) has been recently approved for use in low income areas and can be used to screen smear-positive sputum specimens for rapid detection of rifampicin and isoniazid resistance in 1–2 days. Because of the high-risk transmission from person to person, emergence of MDR-TB and extensive drug resistant tuberculosis, the rapid detection of Mycobacterium tuberculosis and rifampicin (RIF) resistance in infected patients is essential for disease management. Culture is the gold standard for final determination, but it takes 2 to 8 weeks. Although smear microscopy for acid-fast bacilli (AFB) is rapid and inexpensive, it has poor sensitivity and a poor positive predictive value (PPV). Rapid identification is essential to initiate early treatment, improve patient’s outcomes, and more effective for public health interventions[5]. Therefore, molecular...
assays have been used to predict drug resistance in clinical specimens within one working day and are potentially the most rapid methods[6-10]. The GeneXpert MTB/RIF assay is a novel integrated diagnostic device that performs sample processing and semi-nested real-time PCR analysis in a single hands-free step for the diagnosis of tuberculosis and rapid detection of RIF resistance in clinical specimens[4,10]. The MTB/RIF assay detects *M. tuberculosis* and RIF resistance by PCR for 81 bp of the *M. tuberculosis rpoB* gene and subsequently probes this region for mutations that are associated with RIF resistance. The assay can generally be completed in less than 2 h[6,9]. The aim of the present study was to determine the sensitivity and specificity of the GeneXpert MTB/RIF assay for detection of resistance pattern of rifampacin, and line probe assay (Genotype MTBDR plus) as a rapid detection method for rifampacin and isoniazid resistance. The results obtained by the molecular assays were compared with the results of culture and drug susceptibility test.

2. Materials and methods

2.1. Study design and setting

A cross-sectional study was conducted at the National Reference TB Laboratory, National Public Health Laboratory, Khartoum, Sudan. A total of 126 specimens of suspected TB patients were collected from December 2011 to March 2015. All specimens showed acid fast bacilli (AFB) microscopically.

2.2. Sample procedure

The early morning deep coughed sputum specimens were collected in sterile containers from all participants after obtaining the written informed consent. Each sample was examined microscopically using Ziehl-Neelsen (ZN) staining[11], then the specimens that showed AFB microscopically were divided into two groups, one for GeneXpert MTB/RIF assay and line probe assay, and the other group for culture. Sputa were decontaminated according to Petroff’s method and aliquot of 0.1 mL was incubated at 37 °C on Lowenstein-Jensen medium (LJ) and then weekly tested for presence of the growth on LJ medium[12]. The strains which were identified as *M. tuberculosis* complex were tested for their susceptibility to isoniazid and rifampicin.

2.3. GeneXpert procedure

Briefly, the reagent was added at 2:1 ratio to clinical specimens. The closed specimen container was manually agitated twice during incubation period for 15 min at room temperature. The reagent sample mixture was transferred to the Xpert test cartridge. The cartridge was inserted into the GeneXpert device and the results generated automatically were read after 90 min[7].

2.4. Drug susceptibility test (DST)

DST was performed on the culture to identify *M. tuberculosis* complex (MTBC) strains.

2.5. Line probe assay (LPA)

Line probe assay was performed in three separate rooms, according to WHO recommendations[13]. Five hundred microlitres of processed specimen was used to perform the Genotype MTBDRplus (Hain Life science GmbH assay). Residual processed specimens were refrigerated at 2–8 °C overnight after DNA extraction to repeat the test if required.

2.6. Ethical approval

The study protocol was performed according to the Helsinki declaration and approved by the Faculty of Medical Laboratory Sciences, Ethic Committee of University of Khartoum. Informed written consent was obtained from each patient.

2.7. Statistical analysis

Statistical analysis was performed by using SPSS software for Windows (version 16.0).

3. Results

Approximately 67.5% of the patients were male, 64.3% of the affected patients were in the age group of 16–30 years. In the study, 57.1% of cases were previously treated, 19.8% were new cases and 23.1% were unknown (Table 1).

| Traits                  | n     | Percentage |
|-------------------------|-------|------------|
| Age group               |       |            |
| ≤ 15                    | 1     | 0.8%       |
| 16–30                   | 81    | 64.3%      |
| 31–45                   | 23    | 18.2%      |
| 46–60                   | 15    | 11.9%      |
| ≥ 60                    | 6     | 4.8%       |
| Gender                  |       |            |
| Male                    | 85    | 67.5%      |
| Female                  | 41    | 32.5%      |
| Patient case            |       |            |
| New                     | 25    | 19.8%      |
| Previously treated      | 72    | 57.1%      |
| Unknown                 | 29    | 23.1%      |

Among 126 RIF resistant isolates, missing WT (wild type) along with known mutations were detected in 38 isolates (30.2%). The RIF mutation was detected in codon S531L (28/126; 22.2%) followed by D516V mutation (6/126; 4.8%), H526Y mutation (5/126; 3.9%) and H526D mutation (0/126). Missing wild types with mutant probe among katG were found in 37 isolates (29.4%). Among 126 INH resistant isolates detected by MTBDRplus, katG mutations were found in 72 isolates (57.1%). Mutations in codon
S315T1 were detected in 35 INH resistant isolates (27.8%) or 35 of 72 (48.6%) katG mutants. Missing wild types with mutant probe among InhA were found in 1 isolate (0.79%), and mutations in InhA C15T were found in 1 INH resistant isolates (0.79%) (Table 2).

### Table 2

| Gene | Band | Gene region or mutation | MDR strain |
|------|------|-------------------------|------------|
| rpoB | WT1  | 506-509                 | 1          |
|      | WT2  | 510-513                 | 1          |
|      | WT3  | 513-517                 | 1          |
|      | WT4  | 516-519                 | 1          |
|      | WT5  | 518-522                 | 2          |
|      | WT6  | 521-525                 | 2          |
|      | WT7  | 526-529                 | 5          |
|      | WT8  | 530-533                 | 29         |
| MUT1 | D516V| 6                       |            |
| MUT2A| H526Y| 5                       |            |
| MUT2B| H526D| 6                       |            |
| MUT3 | S531L| 28                      |            |
| katG | WT   | 315                     | 37         |
| MUT1 | S315T1| 35                     |            |
| MUT2 | S315T2| 35                     |            |
| InhA | WT1  | 15/16                   | 1          |
|      | WT2  | 8                       | 1          |
| MUT1 | C15T | 1                       |            |
| MUT2 | A16G | 1                       |            |
| MUT3A| T8C  | 1                       |            |
| MUT3B| T8A  | 1                       |            |

In the present study, 126 smear-positive sputum specimens were tested. The resistant pattern using LPA revealed that 41 isolates (32.5%) were MDR (Figure 1) and 85 (67.5%) were sensitive, and the resistant pattern using GeneXpert showed 46 (36.5%) rifampicin resistant isolates (Figure 2) and 80 (63.5%) rifampicin sensitive isolates. The clinical isolates of *M. tuberculosis* were subjected to conventional DST, and the result showed that 42 (33.3%) were MDR and 84 (66.7%) were sensitive.

The results of LPA and GeneXpert were compared separately with that of DST (gold standard method), which showed that for LPA, the sensitivity was 92.9%, specificity was 97.6%, positive predictive value was 95.2% and negative predictive value was 96.4%. For GeneXpert, the sensitivity was 100%, specificity was 100%, positive predictive value was 100% and negative predictive value was 100%.

DST showed that 70 (55.6%) of specimens were sensitive to INH and RIF, 42 (33.3%) specimens were MDR, 4 (3.17%) specimens were sensitive to INH and resistant to RIF and 10 (7.9%) specimens were sensitive to RIF and resistant to INH. According to DST results, most of MDR cases were previously treated (37 (88.2%)), while 4 (9.5%) were new cases and 1 (2.3%) were unknown cases. GeneXpert and LPA are considered as rapid molecular tool with high accuracy for the detection of rifampicin resistant and MDR-TB.

### 4. Discussion

In the present study 19.8% of the TB patients were newly diagnosed for the first time, while 57.1% had history of TB reflecting the active transmission of TB. About 82.6% of TB patients were in the age group of 16 to 45 years. A previous study in Sudan revealed that 82% of TB patients aged under 50 years old[14]. In the developed countries, the most TB cases were found in Europe; it was more prevalent in elder people due to diabetes mellitus and among immunocompromised patients[15]. The present study revealed high prevalence of MDR among new cases and retreated cases which gives an indication of the presence of a serious problem attributed to either mismanagement of TB patients, wrong diagnosis, delay in diagnosis, wrong or interrupted treatment and mistreatment with both first and second line drugs.

The molecular LPA (Genotype MTBDRplus) was used to screen smear-positive sputum specimens for rapid detection of rifampicin and isoniazid resistance in 1–2 days. The GeneXpert MTB/RIF assay is a novel integrated diagnostic device for the diagnosis of TB and rapid detection of RIF resistance in smear-positive and smear-negative pulmonary and extra pulmonary specimens obtained from presumptive TB patients in 2 h. In the present study, RIF resistance was associated with mutation in the region of rpoB 530-533, mostly S531L mutation. Similar results were obtained in Sudan[14], South Africa[16] and Switzerland[17], which found that this mutation was more frequent in MDR-TB strains. The present findings provide the basis for rapid detection of rifampicin resistance. In addition, most INH resistant samples (98.58%) in this study were linked with katG gene, codon 315 (S315T1) as indicated in many high TB burden countries[18].

The study indicated that the molecular techniques were highly...
consistent with the conventional culture and DST method. Our results showed that 33.3%, 32.5% and 36.5% of samples were MDR when tested by DST, LPA and GeneXpert, respectively. The sensitivity, specificity, positive predictive value and negative predictive value of LPA were 97.6%, 100%, 97.6%, and 100%, respectively. These results were in agreement with the previous studies in Sudan [14] which revealed that the sensitivity and specificity of LPA and DST were 98.3% and 100%, respectively. Similar results were reported in studies in South Africa and Bangladesh [16,19], which compared the result of LPA with the DST method.

The sensitivity, specificity, positive predictive value and negative predictive value of GeneXpert were 100% and 100%, respectively. Similar results were reported in Greece [20]. However, previous studies indicated that the sensitivity of the MTB/RIF test for detecting Rif resistance was 94.4%–100% and the specificity was 98.3%–100% [21-23]. In addition, our study showed that there is no significant difference in sensitivity and specificity between GeneXpert and LPA and the golden method DST. Finally we conclude that the GeneXpert and LPA were accurate techniques for screening MDR-TB, and reduce the time for diagnoses.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgments

The authors acknowledge the staff of the National Tuberculosis Reference Lab and Tropical Medicine Research Institute for their support. This research was funded by the National Tuberculosis program, Sudan.

References

[1] Hiatt T, Nishikiori N. Epidemiology and control of tuberculosis in the Western Pacific Region: analysis of 2012 case notification data. Western Pac Survell Response J 2014; 5(1): 25-34.

[2] World Health Organization. Global tuberculosis report. Geneva: World Health Organization; 2013. [Online] Available from: http://apps.who.int/iris/bitstream/10665/91355/1/9789241564656_eng.pdf [Accessed on 8th December, 2016]

[3] Zumla A, Abubakar I, Raviglione M, Hoelscher M, Ditiu L, McHugh TD. Drug-resistant tuberculosis-current dilemmas, unsolved questions, challenges, and priority needs. J Infect Dis 2012; 205: 228-40.

[4] Centers for Disease Control and Prevention. Emergence of Mycobacterium tuberculosis with extensive resistance to second-line drugs worldwide, 2000-2004. MMWR Morb Mortal Wkly Rep 2006; 55: 301-5.

[5] Yousif SA, Abdel Rahim KA, Ahmed AO, Almaary KS, Mohamed AM. Diagnosis of pulmonary tuberculosis and detection of resistance to rifampin and isoniazid through direct molecular methods in stool samples. Ann Clin Lab Sci 2016; 46(6): 616-21.

[6] Blakemore R. Evaluation of the analytical performance of the Xpert MTB/RIF assay. J Clin Microbiol 2010; 48: 2495-501.

[7] Helb D. Rapid detection of Mycobacterium tuberculosis and rifampin resistance by use of on-demand, near-patient technology. J Clin Microbiol 2010; 48: 229-37.

[8] Huang WL, Chen HY, Kuo YM, Jou R. Performance assessment of the Genotype MTBDRplus test and DNA sequencing in detection of multidrug-resistant Mycobacterium tuberculosis. J Clin Microbiol 2009; 47: 2520-4.

[9] Aubry A, Sougakoff W, Bodzongo P, Delcroix G, Armand S, Millot G, et al. First evaluation of drug-resistant Mycobacterium tuberculosis clinical isolates from Congo revealed misdetection of fluoroquinolone resistance by line probe assay due to a double substitution T80A-A90G in gyrA. PLoS One 2014; 9(11): e113219.

[10] Mauya AK, Nag VL, Kant S, Kushwaha RS, Dhole TN. Genotypic analysis of multidrug-resistant tuberculosis isolates from extra pulmonary tuberculosis cases in tertiary care centers in Northern India. Int J Mycobacteriol 2016; 5(Suppl 1): 125-6.

[11] Centers for Disease Control and Prevention. Technical report: mastering the basics of TB controls. Development of a handbook on TB diagnostic methods. Atlanta: Centers for Disease Control and Prevention; 2011. [Online] Available from: https://www.cdc.gov/tb/publications/basics/TB_Basics_TB_control.pdf [Accessed on 8th December, 2016]

[12] Procop GW. Laboratory diagnosis and susceptibility testing for Mycobacterium tuberculosis. Microbiol Spectr 2016; doi: 10.1128/microbiolspec.TNM17-0022-2016.

[13] World Health Organisation. WHO policy statement: molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis. Geneva: World Health Organisation; 2008. [Online] Available from: http://www.who.int/tb/laboratory/line_probe_assays/en/ [Accessed on 8th December, 2016]

[14] Muataz ME, Elrayah IE, Awad Elkarm AI, Khalid FA, Egleaam A, Ibrahim NY, et al. Rapid detection of multi drug resistant tuberculosis using line probe assay (LPA) in Sudan. Euro Acad Res 2016; 3(10): 10755-68.

[15] Bajrami R, Mulliqi G, Kurti A, Lila G, Raka L. Comparison of GeneXpert MTB/RIF and conventional methods for the diagnosis of tuberculosis in Kosovo. J Infect Dev Cities 2016; 10(4): 418-22.

[16] Dheda K, Gumbo T, Gandhi NR, Murray M, Theron G, Udawadia Z, et al. Global control of tuberculosis: from extensively drug-resistant to untreatable tuberculosis. Lancet Respir Med 2014; 2(4): 321-38.

[17] Javed H, Jamil N, Jagielski T, Bakula Z, Tahir Z. Evaluation of genotype MTBDRplus assay for rapid detection of isoniazid and rifampin resistance in Mycobacterium tuberculosis clinical isolates from Pakistan. Int J Mycobacteriol 2016; 5(Suppl 1): 147-8.

[18] Leung KL, Yip CW, Yeung YL, Wong KL, Chan WY, Chan MY, et al. Usefulness of resistant gene markers for predicting treatment outcome on second-line anti-tuberculosis drugs. J Appl Microbiol 2010; 109(6): 2087-94.

[19] Aurin T, Munshi SK, Kamal SM, Rahman MM, Hossain MS, Marma T, et al. Molecular approaches for detection of the multi-drug resistant tuberculosis (MDR-TB) in Bangladesh. PLoS One 2014; 9(6): e99810.

[20] Ioannidis P, Papaventsis D, Karabela S, Nikolaou S, Panagi M, Rafatopoulou E, et al. Cepheid GeneXpert MTB/RIF Assay for Mycobacterium tuberculosis detection and rifampin resistance identification in patients with substantial clinical indications of tuberculosis and smear negative microscopy results. J Clin Microbiol 2011; 49(8): 3068-70.

[21] Boehme CC. Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 2010; 363: 1005-15.

[22] Boehme CC. 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. Lancet 2011; 377: 1495-505.

[23] Moura R. Rapid detection of Mycobacterium tuberculosis complex and rifampin resistance in smear-negative clinical samples by use of an integrated real-time PCR method. J Clin Microbiol 2011; 49: 1137-9.