INTRODUCTION
Tuberculosis (TB) is one of the most important infectious diseases caused by *mycobacterium tuberculosis*. It is the biggest killer among communicable diseases in most productive years of human life in developing countries that are densely populated. Nearly half of the world's TB burden lies in developing countries of South East Asia. In year 2016, an estimated 1.3 million people died due to TB and an additional 0.37 million deaths in HIV positive individuals.\(^1\)

Resistance to anti-tuberculosis drugs has posed a serious threat in curtailting this disease. Multidrug resistant tuberculosis (MDR-TB) is defined as the resistance against two primary drugs Isoniazid (INH) and Rifampicin (RIF).\(^2\) Incidence of MDR-TB is continuously rising globally.\(^3\) Poor compliance, substandard anti-TB drugs, and delay in treatment of MDR cases can develop into extensively drug resistant tuberculosis (XDR-TB). XDR-TB is defined as MDR with additional resistance to any fluoroquinolones and one of the second line injectable drugs.\(^4\) Drug resistance TB is a continuous threat to community and in year 2016, 0.49 million new cases of MDR TB were diagnosed.\(^1\)

Rapid, reliable and standardised drug susceptibility testing (DST) methods are crucial for effective treatment and prevention of transmission. The gold standard indirect DST technique takes longer time for reporting results as first the isolate is cultured from the processed specimen and then DST is carried out from positive growth by MGIT 960 TB system.\(^5\)

MGIT 960 TB system is an automated, non-radiometric, continuously monitoring system for detection of MDR-TB.\(^6\) MGIT 960 is considered as gold standard culture system in many parts of the world as it has reduced the turnaround time by rapid growth of MTB as compared to its growth in solid media.\(^7\) This system has been evaluated against the other methods for detection of bacterial growth and DST; and has shown sensitivity of 100% for RIF, and INH and specificity ranging between 89 to 100%.\(^8\)

DST of MTB is based on growth of *mycobacterium* in a drug containing tube and comparing it with a drug-free growth control tube. Continuous analysis of fluorescence emitted by growth of MTB is detected by this system automatically. However, the main disadvantage is that culture and DST is a two-step process; first the isolate is cultured and then in second step, processed for DST. Isolation of MTB takes about

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**Abstract**

**Objective:** To evaluate direct drug susceptibility testing on MGIT 960 system for detection of multidrug resistant tuberculosis from smear positive pulmonary specimens.

**Study Design:** Cross-sectional analytical study.

**Place and Duration of Study:** Microbiology Department, Armed Forces Institute of Pathology, Rawalpindi, from July 2016 to September 2017.

**Methodology:** Smear positive specimens were pretreated according to guidelines and then tested on MGIT 960 TB system for direct drug susceptibility testing (DST) of isoniazid and rifampin. Samples were also processed by gold standard indirect method, which comprises culture and then DST from positive growth by MGIT 960 TB system.

**Results:** Out of 108 specimens, 95 (88%) DST results were reportable. Out of 95 reportable specimens, 17 isolates were resistant to both isoniazid (INH) and rifampin (RIF) by direct DST. The sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy for INH were 92%, 93%, 82%, 97% and 92.6%, respectively; and 95%, 96%, 86.3%, 98.6% and 95.7%, respectively for RIF. Average time to report DST by indirect method was 23.6 ±3.9 days, while it was 11.4 ±2.7 days for the direct method.

**Conclusion:** Direct susceptibility testing on MGIT 960 system showed very good agreement when compared with indirect method. Time saving is crucial factor in initiation of early effective therapy, especially in drug resistant cases. Further studies on large scale are required for more accurate evaluation of this method.

**Key Words:** BACTEC MGIT 960. Culture. Direct drug susceptibility testing. Multidrug resistant tuberculosis.
In the year 2012, a multicenter study was carried out to get earlier results by direct DST method on MGIT 960 system on smear positive processed specimens omitting the first step of culture. Results of this study were convincing with 95 to 96% concordance with results obtained through conventional two-step indirect DST method on same system.10 The rationale of this study was to investigate whether direct susceptibility testing of INH and RIF on MGIT 960 system is feasible and expedites the detection of MDR TB when compared with the standard indirect method.

The objective of this study was to evaluate direct drug susceptibility testing on MGIT 960 system for detection of multidrug resistant tuberculosis from smear positive pulmonary specimens.

**METHODOLOGY**

This cross-sectional validation study was carried out at Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan. It is a tertiary care reference laboratory with BSL-3 facilities for MTB culture and sensitivity. Permission from Institutional Ethical Committee was taken for the study.

All the consecutive smear positive pulmonary specimens from fresh patients (patients who never took anti-TB treatment) were pretreated with standard sodium hydroxide-N-acetyl-L-cysteine method for digestion, decontamination, homogenisation and concentration.11,12 A smear was made from the deposit and stained with Ziehl-Neelsen stain. Smears were graded according to WHO guidelines depending on the number of acid-fast bacilli (AFB) observed under microscope.13 All the processed specimens were inoculated in BACTEC MGIT 960 system both for direct DST as well as for conventional indirect DST. *M. tuberculosis* H37Ra (ATCC 25177) was used as a sensitive strain, and institutional MDR strain was used as resistant strain for quality control (QC) testing in DST. These strains were inoculated each time when a new batch of DST was set up.

In direct DST, lyophilized antimicrobial mixture PANTA (Becton Dickinson Diagnostic Systems, Sparks, MD) containing polymyxin B, amphotericin B, nalidixic acid, trimethoprim, and azlocillin was reconstituted with 15 ml of SIRE supplement (not growth supplement) (Becton Dickinson Diagnostic Systems, Sparks, MD) and mixed thoroughly. A set of three MGIT tubes was prepared per specimen. One tube was labelled as GC, one for INH and the other for RIF. 800 ul of PANTA-SIRE supplement mixture was added into each of the three-labelled MGIT tubes. Then respective drugs were added in drug labelled tubes. In the INH-labelled tube, 100 ul of reconstituted lyophilized INH drug was added (0.1 µg/ml final concentration) and in RIF labelled tube, 100 µl of reconstituted lyophilized RIF was added (1.0 µg/ml).

Then 500 µl of the well-mixed smear positive processed specimen was inoculated in each of the two drug-containing tubes. For GC tube, 500 µl of 1:10 dilution of processed specimen was added. For the direct DST, an extended protocol, which is used routinely for indirect PZA DST setup, was followed since direct DST requires a 21-day protocol to complete the test.12 Tubes were then entered in the instrument. The first tube in the set carrier was always the growth control tube. When the GC reached the required growth unit (GU) value of 400 or more, the susceptibility set was removed after scanning, and an inventory report was printed. If GU value of the drug tube was less than 100, the test result was reported as susceptible (S); while if the GU value of the drug tube was 100 or more, the result was interpreted as resistant (R). In case the GU value of the control did not reach 400 within 21 days, the instrument indicated insufficient growth or contamination. The time it took for the instrument to complete the DST test was recorded.

In the indirect DST procedure, the processed smear positive specimens were inoculated in MGIT system for culture of bacteria, according to manufacturer’s recommendations. Once an inoculated specimen yielded positive growth indicated by signal on MGIT system, positive growth was subsequently processed for DST. Results of the indirect DST and time to complete the test were retrieved from the instrument and recorded. The time required for indirect DST was compared with that of direct DST for every specimen. The specimens which showed discrepant results between the direct and indirect methods were retested by repeating the indirect method.

Performance of direct DST by MGIT 960 TB system was analysed by using SPSS (Statistical Package for the Social Sciences), version 21 to calculate sensitivity, specificity, positive predictive value, negative predictive value and diagnostic accuracy. Chi-square test was used to find association between qualitative variables and p-value of <0.05 was considered as significant. In this study, sensitivity represents the ability of a test to detect true resistance, and specificity indicates the ability of a test to detect true sensitivity of an isolate.

**RESULTS**

Out of 108 smear positive specimens, 47 (43.5%) were endobronchial washings, 43 (39.8%) sputum samples, 15 (13.8%) bronchoalveolar lavage, and 3 (2.7%) pleural fluid. From processed specimens, 95 (88%) DST results were reportable by both the methods. Thirteen results were not included in the final analysis. Out of invalid results, 5 specimens had contamination (X400 error), 4 did not reach the required threshold for GC (X200 error), 3 were identified as *mycobacteria* other than tuberculosis (MOTT) and 1 did not yield positive culture.
by direct method. The sensitivity, specificity, positive predictive value, negative predictive value, and diagnostic accuracy for INH and RIF are shown in Table I.

Out of 95 reportable results, 62 (65%) isolates were sensitive to both INH and RIF, while 17 (18%) isolates were MDR by direct DST. A total of 16 (16.8%) results showed mono resistance, 11 (11.5%) isolates showed resistance to INH, whereas 5 (5.2%) were resistant to RIF only by direct DST. By indirect DST, 66 isolates were sensitive to both drugs, 16 were MDR, 9 were resistant to INH only, and 4 were resistant to RIF. All the inconsistent results were retested by indirect method for confirmation.

Table I: Comparison of results of direct DST with indirect DST.

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Direct DST R</th>
<th>Indirect DST R</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
<th>DA</th>
</tr>
</thead>
<tbody>
<tr>
<td>INH</td>
<td>23</td>
<td>5</td>
<td>92</td>
<td>93</td>
<td>82</td>
<td>97</td>
<td>92.6</td>
</tr>
<tr>
<td>RIF</td>
<td>19</td>
<td>3</td>
<td>95</td>
<td>96</td>
<td>86.3</td>
<td>98.6</td>
<td>95.7</td>
</tr>
</tbody>
</table>

**PPV = Positive predictive value; NPV = Negative predictive value; DA = Diagnostic accuracy.**

The time to report results of positive cultures was analysed according to the degree of AFB smear positivity. Smear grades were 2+ 39 (41.1%) and 3+ 30 (31.6%) for the majority of specimens. The time interval taken to yield positive result by indirect DST was not significantly different between different smear grades (p=0.90). Overall, cultures were positive at an average of 12 days, with a range of 10 to 20 days, whereas average time to report indirect DST from isolated cultures was 9 days ranging from 6 days to 14 days. The total time required from the time the specimen was processed to the time when indirect DST results were available ranged from 15 to 34 days. Maximum results were available within 24 days. The time to complete direct DST from processed specimens was calculated according to the smear-positive grades, but it did not reveal any significant association (p = 0.94), similar to that noted in indirect DST. Results were ready within 7 to 19 days, out of which maximum results were ready within 12 days. (Table II).

### DISCUSSION

Early detection of MDR strains and timely management of patients are very crucial to control spread of these resistant strains in population. Basically, there were three main differences in the direct DST procedure compared to the conventional indirect DST procedure on MGIT 960 system.9 (i) Direct DST was a 4- to 21-day protocol, while indirect DST was a 4- to 13-day protocol; (ii) Growth control was diluted 1:100 in indirect DST, while in direct DST it was diluted 1:10; (iii) PANTA was added to the control as well as in the drug-containing MGIT tubes in direct DST; whereas in indirect DST, PANTA was not added. To the best of our knowledge, this is the third reported study on direct DST by MGIT 960 system for the detection of MDR TB from smear positive clinical specimens. The first study was carried out by Siddiqi et al. in 2012, which was a multicenter study and second study by Zhang et al. in 2015.10,14 However, in both the studies, authors only included smear positive sputum specimens; while in this study, the authors included all the smear positive pulmonary specimens from new patients. In another study, Demers et al. tested the susceptibility of pyrazinamide by direct method on MGIT 960 system.15

Most of the available methods for DST of MTB are very slow and cumbersome other than commercial molecular methods.16 Although commercially available molecular assays yield earlier results with good sensitivity and specificity, yet these molecular tests are costly and expertise is required.17 Couple of other DST methods such as nitrate reductase assay (NRA), thin layer agar (TLA), and microscopic observation drug susceptibility (MODS) on smear positive clinical specimens have also been studied in last two decades.18-20 Although these methods are inexpensive but are laborious, and take longer time to yield results with increased risk of aerosol generation and contamination.

In this study, out of 108 specimens, 5 (4.6%) were contaminated (X400 error), still in the acceptable range for a laboratory. These results are consistent with the study by Zhang et al. but in disagreement with the study by Siddiqi et al. in which majority of the specimens, which were not reportable, had X200 error. In this study, out of total 95 specimens, individually INH had 88 (92.6%) while RIF had 91 (95.8%) concordance. The results of this study are similar to study by Siddiqi et al. in which there was 95.1% concordance for INH and 96.1% for RIF.

In this study, majority of the isolates had 2+ and 3+ smear grades; a finding similar to studies by Siddiqi et al. and Zhang et al. However, time to report DST had no

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**Table II: Average time for detection by both methods and average time saved (days).**

<table>
<thead>
<tr>
<th>Smear index</th>
<th>No. of specimens</th>
<th>Average TT-DST (Mean ± SD)</th>
<th>Average time saved</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Indirect Method</td>
<td>Direct Method</td>
<td></td>
</tr>
<tr>
<td>1+</td>
<td>14</td>
<td>23.8 ±3.4</td>
<td>11.57 ±2.9</td>
</tr>
<tr>
<td>2+</td>
<td>39</td>
<td>23.4 ±3.8</td>
<td>11.33 ±2.9</td>
</tr>
<tr>
<td>3+</td>
<td>30</td>
<td>23.1 ±3.7</td>
<td>11.12 ±2.2</td>
</tr>
<tr>
<td>4+</td>
<td>12</td>
<td>24.3 ±5.2</td>
<td>11.9 ±3.2</td>
</tr>
</tbody>
</table>

**TT-DST: Total time to drug susceptibility testing results, SD: Standard deviation.**
significant association with grades of smear in both direct and indirect methods. Siddiqi et al. also observed this finding in their study, but Zhang et al. reported a significant relation of smear grades with time to report DST. Average time to report DST for indirect method was 23.6 days while it was 11.4 days for the direct method. These results were similar to the studies done by Siddiqi et al. and Zhang et al.

The main focus of this study was time-saving by direct method when compared with indirect method. Overall time-saving by direct method in our study was 12 days. This study showed slightly more time-saving as compared to studies by Siddiqi et al. and Zhang et al. in which time savings were 8 days and 10.5 days, respectively. The most likely reason could be that it was a single centre study and also the number of specimens was not too large. This time saving is very important as it can help the clinicians in initiation of early therapy and controlling the spread of MDR isolates.

Direct DST has two main disadvantages: Firstly, there may be no or poor growth of mycobacteria, in which case the indirect DST becomes mandatory thus further delay in diagnosis and therapy. Secondly, the presence of NTM can give invalid results, especially in areas where NTM has high prevalence. This study has two main limitations: Firstly it was carried out at one setup and number of specimens was not too large as compared to previous study by Siddiqi et al., which was a multicenter study with 360 clinical specimens. Secondly, the representation of MDR isolates was small (18%) of the total isolates.

CONCLUSION

Direct DST is a reliable method with very good agreement when compared with indirect method. The time saving is significant, which can help in initiation of early effective therapy, especially in MDR cases. Further studies on large scale and on other drugs are needed to know its exact accuracy and reliability with INH and RIF and other drugs.

REFERENCES