ASSESSMENT OF THE RESULTS OF ERLICH ZIEHL-NEELSEN AND FLUOROCHROME STAINING PROCEDURES, BACTEC 460 AND LÖWENSTEIN-JENSEN CULTURING PROCEDURES IN THE DIAGNOSIS OF TUBERCULOSIS

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ABSTRACT
The Ziehl-Neelsen (ZN) and fluorochrome (FC) staining procedures and the Bactec and Löwenstein-Jensen (L-J) culturing procedures were used to test 340 clinical specimens for tuberculosis, and the diagnostic value of the fluorochrome procedure was investigated. Positive cultures were obtained from 34 specimens (10 %), of which 13 (3.8 %) tested positive in ZN, and 18 (5.3 %) in FC. Sensitivity of the ZN and FC staining results was found to be 38.2 % and 52.9 %, respectively. NAP (p-nitro-α-acetylamino-β-hidroxypropophenone) identified 32 (94.1 %) of the 34 strains as M. tuberculosis complex, and 2 (5.9 %) as Mycobacteria other than tuberculosis (MOTT) bacilli. Twenty-one (61.7 %) of the 34 culture-positive specimens grew only in Bactec 12 B medium, 2 (5.9 %) grew only in L-J medium, and 11 (32.3 %) grew in both Bactec and L-J media. The 32 M. tuberculosis complex strains’ sensitivities to streptomycin (STR), isoniazid (INH), rifampin (RIF), and ethambutol were assessed with the Bactec system. Four (12.5 %) of these strains were resistant to streptomycin, 9 (28.1 %) to isoniazid, 7 (21.8 %) to rifampin, and 6 (18.7 %) to ethambutol. Total drug resistance was 43.7 %. Six strains (18.7 %) were resistant to 1 drug, 5 (15.6 %) to 2 drugs, 2 (6.2 %) to 3 drugs, and 1 (3.1 %) to all 4 drugs, isoniazid plus rifampin resistance was seen in 18.7 %.

Introduction
Despite advances in diagnostic and therapeutic methods, tuberculosis, one of the oldest and most prevalent diseases in human history, today remains one of the most feared, particularly in developing countries. With the discovery of new drugs efficacious in its treatment, the second half of the 20th century saw a continuous drop in morbidity and mortality from tuberculosis in industrialized nations until 1985. The incidence increased dramatically with the spread of AIDS, by approximately 12 % between 1985 and 1995 (17, 18).
A new threat is the increase of multidrug-resistant (MDR) M. tuberculosis strains, which are resistant to the principal antituberculosis drugs, isoniazid and rifampin, and may be resistant to other antituberculosis drugs as well. Thus resistance has become a major issue, and the rapid diagnosis of cases and early initiation of treatment are needed to bring the disease under control (4,5).

In the present study, ZN staining and Löwenstein-Jensen (L-J) medium, which are in conventional use, and FC staining and Bactec culturing, which achieve rapid diagnosis, were compared, and the sensitivity of isolated M. tuberculosis complex...
strains to antituberculosis drugs was investigated.

**Materials and Methods**

Clinical specimens obtained from patients with a preliminary diagnosis of tuberculosis were sent to the Central Laboratory of the Medical Faculty of Dicle University for microbiological analysis. They were examined by microscopic and culturing methods, and their sensitivity to antituberculosis drugs was tested.

Clinical specimens such as mucus, bronchial lavage fluid, and abscess/pus sent to the laboratory to be analyzed for microbacteria were cultured after decontamination, and bodily fluids considered to have been collected under aseptic conditions, such as cerebrospinal fluid, synovial fluid, and pleural fluid, were cultured without undergoing decontamination, and preparations were made. For decontamination, specimens were transferred to 50-ml sterile centrifuge tubes, no more than 10ml per tube, at the same amount of 4 % NaOH+N-acetyl-L-cysteine-sodium citrate solution was added over it, and it was kept at room temperature for 15-20 minutes, during which time tubes were shaken every 4-5 minutes to homogenize the specimens. Afterwards, phosphate buffer solution (PBS: 0.067 M, pH6.8) was added for neutralization, with care taken that the tube contents did not overflow. Tubes were then stabilized and centrifuged at 3800g for 15 minutes. After the top liquid was carefully emptied into a container with disinfectant inside, 1-2ml PBS was added over the sediment specimens. After homogenization, preparations were made, and L-J and Bactec 12B culturing was performed. Before culturing, an antibiotic mixture containing polymyxin B (50 units/ml), azlocillin (10mcg/ml), nalidixic acid (20mcg/ml) trimethoprim (5.0mcg/ml), and amphotericin B (5.0mcg/ml) was added in order to reduce extra-micobacterial contamination (9,15).

Bactec 12B media cultured with clinical specimens were kept at 37° for 6 weeks, being checked twice a week for the first two weeks and once a week for the last four weeks with a Bactec TB 460 (Becton Dickinson Microbiology Systems, Cockeysville, Md.) automation apparatus. Cultures that failed to grow by the end of this period were considered negative. Cultures that grew underwent the NAP identification test, and strains identified as *M. tuberculosis complex* were subjected to antibiogram against streptomycin (2.0 µg/ml), isoniazid (0.1 µg/ml), rifampin (2.0 µg/ml), and ethambutol (2.5 µg/ml).

Preparations were stained by ZN and fluorochrome (Auramine-Rhodamine) techniques. The results of preparations and cultures were assessed by methodological investigation (1).

**Results and Discussion**

Clinical specimens and their distributions according to patient group are shown in **Table 1**.

Sixteen (4.7 %) of the 340 total specimens tested positive for acid-resistant bacteria in ZN staining, and 18 (5.3 %) tested positive in FC staining. Twenty-one (61.7 %) of the 34 culture-positive specimens grew only in Bactec 12B medium, 2 (5.9 %) grew only in L-J medium, and 11 (32.3 %) grew in both Bactec and L-J media (**Table 2**).

The 32 (94.1 %) of 34 micobacteria strains isolated from 340 clinical specimens were identified as *M. tuberculosis complex* and 2 (5.9 %) as Mycobacteria other than tuberculosis (MOTT) bacilli by the NAP technique.

ZN staining results were assessed with culturing, and ZN was found to have 38.2 % sensitivity, 99 % specificity, 81 % true positivity, and 93.5 % true negativity. FC staining results were assessed with culturing and FC was found to have 52.9 % sensitivity, 100 % specificity, 100 % true positivity, and 95 % true negativity.
TABLE 1

Clinical specimens and their distributions according to patient group

<table>
<thead>
<tr>
<th></th>
<th>Adult</th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Male</td>
<td>Female</td>
<td>Children</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>n</td>
<td>%</td>
<td>n</td>
</tr>
<tr>
<td>Sputum</td>
<td>166</td>
<td>113 (68)</td>
<td>50 (30)</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>62</td>
<td>2</td>
<td>5</td>
<td>8</td>
<td>55 (89)</td>
</tr>
<tr>
<td>Urine</td>
<td>43</td>
<td>14</td>
<td>26</td>
<td>60</td>
<td>3</td>
</tr>
<tr>
<td>Bronchoalveolar lavage fluid</td>
<td>13</td>
<td>8</td>
<td>62</td>
<td>4</td>
<td>31</td>
</tr>
<tr>
<td>Abscess/Pus</td>
<td>11</td>
<td>4</td>
<td>36</td>
<td>6</td>
<td>55</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>17</td>
<td>9</td>
<td>53</td>
<td>4</td>
<td>24</td>
</tr>
<tr>
<td>Biopsies</td>
<td>10</td>
<td>8</td>
<td>80</td>
<td>2</td>
<td>20</td>
</tr>
<tr>
<td>Peritoneal fluid</td>
<td>9</td>
<td>4</td>
<td>45</td>
<td>3</td>
<td>33</td>
</tr>
<tr>
<td>Gastric fluid</td>
<td>5</td>
<td>2</td>
<td>40</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Pericardial fluid</td>
<td>3</td>
<td>1</td>
<td>33</td>
<td>1</td>
<td>33</td>
</tr>
<tr>
<td>Synovial fluid</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>340</td>
<td>165 (48)</td>
<td>101 (30)</td>
<td>74</td>
<td>22</td>
</tr>
</tbody>
</table>

TABLE 2

Comparison of results from Bactec 12B and L-J media

<table>
<thead>
<tr>
<th></th>
<th>Only Bactec 12B Medium</th>
<th>Only L-J Medium</th>
<th>Bactec 12B+ L-J Medium</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sputum</td>
<td>13</td>
<td>2</td>
<td>7</td>
<td>22</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>3</td>
<td>-</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Biopsies</td>
<td>3</td>
<td>-</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Abscess/Pus</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>-</td>
<td>-</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Total</td>
<td>21 (61.7 %)</td>
<td>2 (5.9 %)</td>
<td>11 (32.3 %)</td>
<td>34</td>
</tr>
</tbody>
</table>

The Bactec system was used to assess the resistance of the 32 M. tuberculosis strains to streptomycin, INH, rifampin, and ethambutol. Four (12.5 %) were streptomycin resistant, 9 (28.1 %) were INH resistant, 7 (21.8 %) were rifampin resistant, and 6 (18.7 %) were ethambutol resistant. Total drug resistance was 43.7 %. Six strains (18.7 %) were resistant to 1 drug, 5 (15.6 %) to 2 drugs, 2 (6.2 %) to 3 drugs, and 1 (3.1 %) to all 4 drugs. Multidrug resistant (MDR) strains to INH plus rifampin were found in 18.7 %.

Microscopic examination is used in the diagnosis of tuberculosis, especially pulmonary tuberculosis, because of its ease, low cost, rapid results, and determination of bacterial density in the examination field; nonetheless, microscopic results should be supported by culturing. If the staining procedure gives results compatible with culturing, this provides information on the value of the preparation in diagnosis.

The early diagnosis and treatment of tuberculosis patients are the most effective means of protecting the community from this disease. Therefore, it is urgent that laboratory methods that will confirm the diagnoses of patients thought to have contacted tuberculosis, that have high sensitivity and specificity, that give rapid results, and that are easy and inexpensive enter routine use.

While the search for a rapid, sensitive
continues, direct microscopy and culturing are in wide use, and much attention is given to the usefulness of preparation in diagnosis (19). Rickman and Moyer (14) used L-J culturing and fluorochrome staining in a study on 14509 clinical specimens, and reported a sensitivity of 82.4 %, a specificity of 99 %, a positive predictive value of 98.9 %, and a negative predictive value of 97.7 %.

In a study on 10468 clinical specimens, Murray et al. (13) found that FC staining had 39 % sensitivity, 99.9 % specificity, 95 % positive predictive value, and 97.5 % negative predictive value. Lipsky et al. (11) reported 33 % sensitivity and 99.8 % specificity for FC staining.

The sensitivities of ZN and FC found in the present study were higher than those reported by Murray and Lipsky and lower than that reported by Rickman. ZN staining was able to correctly diagnose 38.2 % of true patients with positive cultures, while FC staining accurately diagnosed 52.9 %. In the literature, there is a wide gap between sensitivities, and only a narrow gap between specificities. The gap between sensitivities may be explained by factors that may affect frotty sensitivity, such as type, decontamination, concentration, and decolorization procedures, frotty densities, the number of bacilli in the specimen, and the experience of the person performing the assessment.

The delay of diagnosis and drug-sensitivity results is a major factor in tuberculosis developing resistance. Prolonged inappropriate treatment of a resistant case is an extremely serious matter in terms of infection of people in the vicinity of the patient, hence the importance of more rapid isolation, identification, and sensitive test results.

Lazslo et al. (10) tested drug sensitivity in 245 M. tuberculosis complex strains by conventional and radiometric methods. By the conventional method, they found 134 (55 %) of the strains were resistant to at least 1 drug, 88 (36 %) to INH, 76 (31 %) to STR, 22 (9 %) to RIF, and 21 (8.5 %) to EMB. By the radiographic method, they found 73 (30 %) had resistance to SM, 84 (34 %) to INH, 22 (9 %) to RIF, and 4 (1.6 %) to EMB. Hua et al. (7) found resistance to STR, INH, RIF, and EMB to be 11.9 %, 84.4 %, 17.6 %, and 23.3 % respectively; they found MDR-TB to be 17 %. Moore et al. (12) carried out a study in all 50 states of the USA, and found INH resistance to be 8.4 %, RIF resistance to be 3 %, and concomitant resistance to both to be 2.2 %, MDR-TB prevalence reported in studies in various countries ranges from 1.6 % to 14.4 % (4).

In studies carried out in Turkey: Bengisu et al. (2) found INH, RIF, and STR resistances of 10.5 %, 6.9 %, and 7.0 %, respectively, and MDR-TB of 5.8 %. Taso et al. (16) investigated drug resistance in 393 clinical specimens, and found total resistance rates of 22.6 %, 20.6 %, 8.6 %, and 10.1 % against INH, RIF, STR, and EMB, respectively, and an MDR-TB rate of 18.5 %. Karabay et al. (8) found 105 (49.1 %) out of 214 M. tuberculosis strains to be resistant to at least one drug. They determined total resistance of 27.1 % to INH, 21.5 % to RIF, 29 % to STR, and 10.3 % to EMB. They found an MDR-TB rate of 11.6 %. These studies from Turkey display multiple-resistance rates ranging from 5.8 % to 18.5 %. In the present study, resistance to STR, INH, RIF, and EMB was found to be 12.5 %, 28.1 %, 21.8 %, and 18.7 %, respectively, while MDR-TB was 18.7 %.

As compared to antituberculosis drug resistances in various parts of the world and in Turkey, the Diyarbakir region is found to have the highest (18.7 %). These rates demonstrate that resistance is a serious
problem in tuberculosis prevalent regions and that treatments should be guided by the
determination of regional resistance results. Early diagnosis and sensitivity to antituber-
culosis drugs are of great importance in bringing tuberculosis under control and
treating patients efficaciously. To supply a contribution for early diagnosis of tuber-
culosis, we wish to emphasize that FC staining procedure gave more results com-
patible with culturing in our study.

REFERENCES

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