Molecular Genetics of Drug Resistance of Mycobacterium Tuberculosis

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gene were detected in 7 out of 41 specimens (17.1%), of which 5 had also mutation in the kat G gene (Table 5). Mutations in the rpsL gene were detected in 8 out of 41 specimens (19.5%), of which 4 had also mutations in the kat G gene and 3 had also mutations in the inh A gene (Table 4), these results are in agreement with that of Heym et al. (1994)(24), Cooksey et al. (1997)(25) and Scarpellini et al. (1997)(26).

Conclusions and Recommendation:

Early diagnosis and effective treatment of tuberculosis, will prevent the progression of the disease and its spread in the community.

PCR is a rapid, sensitive and specific method for the diagnosis of pulmonary tuberculosis.

PCR-SSCP is a very rapid, sensitive and specific technique for detecting drug resistant mycobacterium tuberculosis.

This study is a preliminary investigation and opens up new field for further studies to modify the chemical structure of the most important effective antituberculosis drugs in order to be not affected by gene mutation and thus reducing the incidence of initial drug resistance.

References

Fiberoptic bronchoscopy and bronchoalveolar lavage was done for the sputum negative 10 cases stained by both Z-N. and R-A. stains and the BAL became positive by both stains. These results are consistent with many workers who reported that sensitivity of direct sputum stained smear did not exceed 78% (2,15).

The smear examination of the sputum though simple, rapid and economic, its estimate detection limit is $10^4$ bacilli/ml, while that of the culture is $10^2$ bacilli/ml, so some specimens with positive culture results have negative smear results(16). Smear sensitivity may be also influenced by the efficiency of decontamination, concentration, thickness of the smear and the training of examining eyes(17).

As regarding the culture methods used, the biphasic Middle-Brook (M.B.) medium showed 82.9% sensitivity, which is higher than that of L.J. medium (65.9%) (Table 1). The liquid broth phase in Co2 atmosphere of the M.B. medium optimizes the initial growth of low number of bacteria in the inoculum and the solid media enable easy isolation, and provides additional information on the species of mycobacterial isolates and on contaminating organisms(18). It also shortened the time required for recovery of mycobacteria as the mean detection time in days was 16.4 in comparison to that of L.J. medium which was 37.4 days (Table 3). These results are in agreement with those of Tortoli et al. (1993)(19) and Michael (1994)(17) who reported that L.J. medium is not the optimal method for primary isolation of mycobacteria and that biphasic M.B. medium is a satisfactory, complete, safe, simple and rapid system for mycobacterium recovery.

As regarding PCR technique we used tuberculosis DNA extraction kit which provides a protocol that is specific for mycobacterium tuberculosis by amplification of 164 bp or 123 bp DNA sequences from 65Kda heat shock protein gene contained in the IS986 insertion sequence respectively. In this study PCR detected 41 positive cases out of 47 patients with a sensitivity of 100% (Table 1), [if we consider the 6 cases which were negative by PCR but positive by direct smear and culture, were proved to be due to infection by mycobacteria other than tuberculosis, (MOTT) table (2)]. This result agrees with those obtained by many authors(20 & 21).

As regarding the incidence of initial drug resistance as detected by PCR-SSCP, we used the PCR to amplify 306 bp, 238 bp, 209 bp and 157 bp DNA sequences respectively from the rpsL, kat G, inh A and rpoB genes that encode the antituberculosis drugs susceptibility/resistance phenotype. The sensitive strains were 20 out of 41 positive cases by PCR (48.8%) and the initial resistance was present in 21 cases out of 41 patients (51.2%) (Table 4). These results are in agreement with those obtained by conventional method of culture and sensitivity by Metwally in 1980 (57.5%) (22) and El-Nassany in 1990 (54.17%) (23) Resistance to one drug was detected in 11 cases out of 21 cases of initial resistance (26.8%) and resistance, to more than one drug was detected in 10 cases out of 21 patients with initial resistance (24.4%) (Table 4). These results are in agreement with that of Telenti et al., (1993)(7) and Heym et al., (1994)(24) who reported initial resistance of 25.5% for one drug.

Mutation in the kat G as whole was detected in 14 out of 41 cases (34.2%) (Table 5), of these 14 cases 4 had also alterations in the inh A gene. These results are in agreement with that of Heym et al., (1994)(24). Mutations in the inh A gene were detected in 7 out of 41 cases (17.1%), table (5), of which 3 had no concomitant kat G gene. Mutations in the rpoB
Mutations as a whole in kat G, inh A, rpo B and rpsL genes were detected in tuberculosis DNA from 14, 7, 7 and 8 specimens respectively (Table 5).

Table (5): Statistical distribution of the whole gene mutations conferring initial drug resistance as detected by PCR-SSCP

<table>
<thead>
<tr>
<th>Antituberculosis Drug</th>
<th>Location of resistant mutations</th>
<th>No.</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoniazid</td>
<td>kat G</td>
<td>14</td>
<td>34.2</td>
</tr>
<tr>
<td>Ethionamide</td>
<td>inh A</td>
<td>7</td>
<td>17.1</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>rpo B</td>
<td>7</td>
<td>17.1</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>rps L</td>
<td>8</td>
<td>19.5</td>
</tr>
</tbody>
</table>

**Discussion**

Tuberculosis-control programmes are compromised by the increased frequency of multidrug-resistant strains of mycobacterium tuberculosis. We used the polymerase chain reaction (PCR) and single-strand conformation polymorphism (SSCP) analysis techniques to establish the molecular basis of initial resistance in 47 patients of newly diagnosed pulmonary tuberculosis.

In this study, we used NALC-NaOH method for sample preparation. This method was reported to give the best recovery rate of mycobacteria when compared with different liquefaction and decontamination procedures(14).

Direct Z-N stained smear examination of the sputum on 3 successive days was positive in 33 cases out of 47 with sensitivity of 70.2%, which was confirmed by R-A stained smear examination of the sputum, and was negative in 14 cases (29.8%). Out of these 14 negative cases by Z.N. stain, 4 cases became positive by R.A. stain, so the total yield of R-A stained smear examination of the sputum on 3 successive days was 37 cases out of 47 with sensitivity of 78.7% (Table...
Table (3): Statistical distribution of the sensitivity and time of performance of different methods used for the diagnosis of tuberculosis.

<table>
<thead>
<tr>
<th></th>
<th>Z.N. Stain</th>
<th>R.A. Stain</th>
<th>L.J. Culture</th>
<th>M.B. Culture</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean time</td>
<td>&lt; 1 day</td>
<td>&lt; 1 day</td>
<td>57.4 day</td>
<td>16.4 day</td>
<td>&lt; 1</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>70.2%</td>
<td>78.7%</td>
<td>65.9%</td>
<td>82.9%</td>
<td>100%</td>
</tr>
</tbody>
</table>

Evaluation of initial drug resistance by PCR-SSCP:

The PCR was used to amplify 306 bp, 238 bp, 209 bp and 157 bp DNA sequences respectively from the rpsL, katG, inhA and rpoB genes that encode the antituberculosis drugs susceptibility/resistance phenotype. PCR-SSCP was used to determine the genotype of all 41 positive specimens (Figs. 1-4).

![PCR-SSCP analysis of katG gene mutations that mediate isoniazid resistance. Mutations are shown by altered strand mobility compared to the normal mobilities of the single strands (238 nucleotides). The amplification reactions were carried out by using the oligonucleotides primers katG (+) 5'-tgc cgc agc oac oac coc cc-3', and katG (-) 5'-tgc cgc gtc agc gcc cc gc-3' as described under Materials and Methods. R = isoniazid - resistant. S = isoniazid - sensitive.](image)

![PCR-SSCP analysis of rpsL gene resistant mutations. Mutations in the rpsL, gene conferring resistance to streptomycin are shown by altered strand mobility compared to the normal mobilities of the single strands (3.6 nucleotides). The amplification reactions were carried out by using the oligonucleotide primers rpsL (+) 5'-ccc acc acc aat cag cag gat gli-3', and rpsL (-) 5'-gca gca acc acc ggc oac gag -3' as described under Materials and Methods. R = streptomycin-resistant. S = streptomycin-sensitive.](image)

![PCR-SSCP analysis of inhA gene mutations that mediate isoniazid and ethionamide resistance. Mutations are shown by altered strand mobility compared to the normal mobilities of the single strands (209 nucleotides). The amplification reactions were carried out by using the oligonucleotide primers inhA (+) 5'-tgc tgc gac tgc acg tgc aa-3', and inhA (-) 5'-cga cgc ata cga ata cgc cgc-3' as described under Materials and Methods. R = isoniazid/ethionamide-resistant. S = isoniazid/ethionamide-sensitive.](image)
Table (1): Statistical analysis of the distribution of the results obtained by different methods for the diagnosis of pulmonary tuberculosis.

<table>
<thead>
<tr>
<th>Sputum smear</th>
<th>Stain</th>
<th>Culture</th>
<th>PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td></td>
<td></td>
<td>1+</td>
</tr>
<tr>
<td>17</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6#</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Negative*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>47</td>
<td>33</td>
<td>37</td>
</tr>
</tbody>
</table>

Sensitivity %   | 70.2  | 78.7  | 65.9 | 82.9 | 100  |

Z.N. = Ziehl-Neelsen stain  
R.A. = Rhodamine- Auramine stain

# = Mycobacteria other than TB  
* = Positive after staining BAL smears by Z.N. or R.A.

Table (2): Statistical distribution of the bacteriological and biochemical differentiation of the different mycobacteria.

<table>
<thead>
<tr>
<th>Test</th>
<th>M. Tuberculosis</th>
<th>MOTT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M.A.I.</td>
<td>M. Chelonei</td>
</tr>
<tr>
<td>Z.N. Stain</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>R.A. Stain</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>L.J. Culture</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Mean growth time</td>
<td>37.4 days</td>
<td>20.5 days</td>
</tr>
<tr>
<td>Colony morphology</td>
<td>Opaque &amp; Rough</td>
<td>Glossy</td>
</tr>
<tr>
<td>Pigment production</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Niacin test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Catalase at 68 C</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Urease</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Tuberculin test</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>PCR</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Total</td>
<td>41</td>
<td>4</td>
</tr>
</tbody>
</table>

MOTT = Mycobacteria other than tuberculosis.
M.A.I. = Mycobacterium Avium Intracelluar.
laroid instant image films.

**PCR-SSCP**

PCR was used to amplify a 306 bp DNA sequence from the rpsL gene, which encodes the ribosomal protein S12, in order to detect mutations that confer resistance to streptomycin\(^{(13)}\), a 238bp DNA sequence from the katG gene which encodes a heme-containing catalase peroxidase enzyme mediating susceptibility to isoniazid \(^{(14)}\), a 209 bp DNA sequence from the inhA gene in order to analyze codon-94 gene mutations which confer resistance to isoniazid and ethionamide\(^{(6)}\) and a 157 bp DNA sequence from the rpoB gene which encodes the B subunit of the RNA polymerase\(^{(7)}\).

Primers used in PCR-SSCP mutational screening were as follow: rpsL (+) 5' - ccc acc aft cag cag csg gt-3' and rpsL (-) 5' - gtc gag cga acc cgg aat ga -3' (Ta = 60 C)\(^{(13)}\); kat G (+) 5' - tgc ccc agc aac acc cac cc-3' and kat G (-) 5' - atg tcc cgc gtc agg gcg tc-3' (Ta = 60 C)\(^{(14)}\); inh A (+) 5' - tgc tcg aac tcg acg tgc aa-3' and inh A (-) 5' - cga agc ata cga ata cgc cga-3' (Ta = 52 C)\(^{(6)}\) and rpo B (+) 5' - tgc aac tgc acg ccg acc a-3' and rpo B (-) 5' - tcg accg cga tea agg agt -3' (Ta = 55 C)\(^{(7)}\). PCR was performed as usual except for the inclusion of 5 \(\mu\)g of \(\text{dCTP (3000 CY/mmol)}\) in the reaction. After PCR, the products were diluted into a solution of 10 mM acetic acid and 0.1% SDS. A diluted PCR product was mixed with an equal volume of sequencing/denaturing buffer (95% formamide, 20 mM EDTA, 0.05% bromophenol blue, 0.05% xylene cyanol), heated for 10 min. at 94 C to denature the PCR products, quickly chilled on ice to prevent, renaturation and loaded immediately into a 6% non denaturing polyacrylamide slab gel containing 10% glycerol in 0.6 x TBA buffer. Electrophoresis was conducted at 50°C. the gels were dried and exposed overnight for autoradiography.

**Results**

This study was carried out on 47 patients with newly diagnosed sputum or BAL positive pulmonary tuberculosis by direct smear microscopy stained with Ziehl-Neelsen and confirmed by Rhodamine-Auramine stain. All patients were not receiving any antituberculous drugs before the study. 30 patients were males (63.8%) and 17 patients were females (36.2%) with the mean age of 43.6 ± 10.2 years.

The presenting symptoms were prolonged cough (89%), low grade fever and weight loss (57%), night sweats (42%) and haemoptysis (14%) with overlap of symptoms. Radiological findings were cavitary lesion (43%) and pneumonic patches (57%). Tuberculin test using 2 tuberculin units of PPD-RT23 tween 80 was positive in 41 cases (87.2%) with a diameter of induration of 10 mm or more and was negative in 6 patients (12.8%).

Sputum direct smear on 3 successive days stained by Ziehl-Neelsen stain was positive in 33 cases out of 47 (70.2%) which was confirmed by R.A. stain, and was negative in 14 cases (29.8%). Out of these 14 negative cases by Z.N. stain, 4 cases became positive by R.A. stain i.e. the total yield of positivity by R.A. stain was 37 out of 47 cases (78.7%). The remained 10 negative cases of sputum smear stained by both Z.N. and R.A. staining, for whom fiberoptic bronchoscopy and BAL were done and after concentration, BAL smear stained by Z.N. stain became positive which was also confirmed by R.A. stained smears (Table 1).
tion 2 (1% sodium dodecyl sulphate (SDS), 25 mM Tris-Hcl pH 8.0, 10 mM EDTA, and 100 ug/ml proteinase K) and the mixture was gently incubated at 55 C for 24 hours. Proteins were salted-out by adding a saturated solution of sodium chloride (approximately 6M, solution 3) and placing the mixture on ice for 15 minutes. The proteins were pelleted by centrifugation for 15 minutes at the maximum setting of a microcentrifuge. The supernatant was transferred to another microcentrifuge tube and 2 volumes of absolute ethanol were added and the DNA was precipitated at - 20° C overnight. The preparation was centrifuged for 20 minutes in a microcentrifuge at the maximum setting to pellet the DNA. The DNA pellet was washed in 75% ethanol and reprecipitated by centrifugation. After draining excess liquid, the precipitated DNA was placed in 30% of nuclease free sterile water and allowed to dissolve for several days at 4 C. The concentration of DNA was determined by comparing the intensity of its ethidium bromide fluorescence to those of known amounts of standard DNA.

**Polymerase Chain Reaction (PCR)**

The PCR was performed in an air thermocycler from Idaho Technologies (Idaho, USA), using the thermostable DNA polymerase from Thermos aquaticus, Taq DNA polymerase (Perkin Elmer Cetus, Norwalk, Conn., USA). The final composition of the PCR mix (50 µl) was 10 mM Tris-HCl (pH 8.3) at 25 C, 50 mM NaCl, 1.5 mM MgCl2, 0.25 ug/ml BSA, 0.2 mM of each deoxynucleoside triphosphate (dATP, dCTP, dGTP, and dTTP), 0.4 mM each primer, and 2 units of Taq polymerase. For all pipetting, positive displacement pipette tips (Microman) were used. The reactions containing the positive control were prepared in a separate room in the laboratory to minimize contamination and product carryover.

The PCR was carried out for 40 cycles under the following cycling conditions: denaturation at 94 C for 1 sec., annealing at 60° C for 1 sec., and extension at 72° C for 15 sec. PCR cycling was preceded by a 25 sec. Initial denaturation at 94° C and was followed by a 15 sec. of terminal (long) extension at 62° C. PCR primers used for detecting the mycobacterium tuberculosis complex amplify a 164 bp DNA sequence from the evolutionary conserved 65 kDa heat shock protein gene(11). The plus-sense primer sequence was as follows: 5'-cta ggt cgg gac ggt gag gcc agg-3' (nucleotide position 91-114). The minus-sense primer sequence was as follows: 5i-cat tgc gaa gtg att tact ceg gag tgg-3' (nucleotide position 254-231). PCR primers specific for the repetitive DNA sequence 1S986 were also used to amplify a 123 bp DNA sequence from the mycobacterium tuberculosis complex(12). The plus-sense primer sequence was as follows: 5' - ctc gtc cag cgc cgc llc gg-3' (nucleotide position 770-789). The minus-sense primer sequence was as follows: 5'- ccg aga ctc gta ggc gtc gg-3' (nucleotide position 892-873). The 164 bp or 123 bp PCR products, expected from positive samples, were then analyzed by agarose gel electrophoresis.

Prior to electrophoresis, DNA samples were mixed with the loading buffer (0.05% bromophenol blue, 0.05% xylene cyanol, and 30% glycerol). Electrophoresis was performed onto 2% agarose gels prepared in 1 x TAE buffer (40 mM trizma base, 10 mM Glacial acetic acid, 0.02 M EDTA) in a DNA electrophoresis subcell from Molecular Bio-Products (MP, San Diego, CA, USA). Molecular Biology grade agarose was obtained from Sigma (St. Louis, MO, USA). DNA was stained with ethidium bromide at a concentration of 0.5 ug/ml, visualized on a UV transilluminator, and photographed with an MP-4 camera system from Fotodyne (Walnut Ridge Drive, Hartland, WI, USA) using po-
Drug resistance in mycobacterium tuberculosis occurs by random single step spontaneous mutations at the gene loci of the chromosomes. Such mutations can alter one or more genes involved in effective drug action affecting the primary drug target or the transport system or may cause increased synthesis of the target enzyme, thus rendering the drug less effective to inhibit mycobacterial growth(3). The rapid availability of information concerning drug susceptibility patterns is important not only because it makes it possible to select adequate multidrug regimens but also because it contributes to minimizing the spread of drug-resistant strains. Since isolation, identification and susceptibility testing by conventional procedures can take 4 to 8 weeks, it would be a great advantage to be able to obtain accurate results in a shorter time (4). In the last few years there has been a dramatic increase in the applications of molecular biology techniques to various aspects of mycobacteriology(4).

It is now known that high-level resistance to isoniazid is associated with mutations to the catalase-peroxidase gene (kat G)(5), and that cross resistance to isoniazid and ethionamide results from mutations in the (inh A) gene(6). Likewise, it has been shown that resistance to rifampicin (rpoB) gene(7), streptomycin (rpsL) gene(8) and fluoroquinolones (gyr A) gene(9) stems from missense mutations in the genes encoding bacterial RNA polymerase, certain ribosomal subunits, and DNA gyrase respectively. This improvement in the knowledge of the resistance mechanisms employed by mycobacterium tuberculosis has resulted in development of tests for predicting resistance rapidly.

**Aim of the work**

This study aims at the identification of mycobacterium tuberculosis in sputum samples by PCR-based testing and the detection of mutations conferring initial resistance to the current antibacterial drugs using polymerase chain reaction-single strand conformation polymorphism (PCR - SSCP) techniques. The PCR results are compared to those obtained by conventional smear and microbiological methods.

**Subjects and Methods**

This study was carried out on 47 adult patients with active newly diagnosed pulmonary tuberculosis from those patients admitted to the chest department of Benha University Hospital during the period from March 1996 to March 1997. Full history taking, clinical and radiological examination of the chest as well as tuberculin testing were carried out for each patient. Expectorated sputum on three successive days or bronchoalveolar lavage (BAL) specimens were collected from each patient.

**Smear Examination, Culturing And Biochemistry**

After decontamination, liquefaction and concentration, sputum or BAL smears were stained by Ziehl-Neelsen stain and directly examined. Smear examination was confirmed by Rhodamine-Auramine staining using ultra-violet microscopy. Culturing mycobacteria was performed on both the biphasic Middle-Brook (M.B.) and Lowenstein-Jensen (L.J.) media. Biochemical identification of mycobacteria was also performed at the clinical pathology department, Benha Faculty of Medicine.

**DNA preparation**

Sputum samples from all 47 patients were digested and decontaminated with an equal volume of 4% (Wt/Vol) solution of NaOH and 2.9% solution of sodium citrate containing 0.1 gm of N-acetyl-L-cysteine (NALC). Bacterial pellets were collected by centrifugation at 4000 g for 15 minutes, resuspended in 110 μl of solu-
Molecular Genetics of Drug Resistance of Mycobacterium Tuberculosis

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Abstract

Sputum or bronchoalveolar lavage (BAL) specimens from 47 patients (30 males and 17 females) with active newly diagnosed pulmonary tuberculosis, who were not receiving any antituberculous drugs before the study, were analysed by Ziehl-Neelsen (Z.N.), Rhodamine-Auramine (R.A.) Staining, Middlebrook (M.B.), Lowenstein- Jensen (L.J.) Cultures and PCR. Mutations conferring multidrug resistance were detected by single-strand conformation polymorphism (SSCP) analysis. Out of the 47 sputum specimens, 41 cases were found to be PCR positive for mycobacterium tuberculosis by amplification of 164 base pair (bp) or 123 bp DNA sequences from the 65 Kda (Kilo Dalton) heat shock protein gene contained in the IS986 insertion sequence, respectively. The remaining 6 cases were found negative by PCR, which proved by different identification methods to be mycobacterium other than tuberculosis (MOTT). All the 47 cases were positive by direct smear and/or culture of the sputum in 37 cases and of BAL in 10 cases. Of the PCR-positive patients, 8, 10, 14 and 16 patients were negative by M.B. culturing, R.A. staining, Z.N. staining and L.J. culturing respectively. Out of the 41 PCR positive patients, 20 patients (48.8%) had organisms sensitive to all drugs tested, while 21 patients (50.2%) had organisms resistant to one or more of the antituberculous drugs. Mutations in kat G gene conferring resistance to isoniazid were found in 14 out of 41 specimens (34.2%) of which, 4 had also alterations in the inh A gene which confer resistance to both isoniazid and ethionamide. Mutations in the inh A gene were detected in 7 out of 41 specimens (17.1%), of which 3 had no concomitant kat G gene. Mutations in the rpo B gene, conferring resistance to rifampicin were detected in 7 out of 41 specimens (17.1%), of which 5 had also mutations in the kat G gene. Therefore, mutations conferring rifampicin resistance appeared to be associated with those conferring isoniazid resistance. Mutations in the rpsL gene, conferring resistance to streptomycin were detected in 8 out of 41 specimens (19.5%). This work is a preliminary investigation and opens up new field for further studies to modify the chemical structure of the most important effective antituberculous drugs in order to be not affected by gene mutation and thus reducing the incidence of initial drug resistance.

Introduction

Pulmonary tuberculosis is still one of the major health problems in developing countries including Egypt. Tuberculosis also remains an important cause of morbidity, mortality and health care expenditure(1). Detecting pathogenic mycobacteria in clinical samples by conventional methods is either low in sensitivity and/or specificity or time consuming. The highly contagious nature of tuberculosis necessitates rapid identification of mycobacterium tuberculosis to be available(2).