

**Determination of *Mycobacterium tuberculosis* drug sensitivity in mazandaran province health center in 2002**

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**Background:** Tuberculosis (TB) is one of the most important infectious disease and the main cause of mortality in the developing countries (4). There have been educational program in order to prevent it, not only has not been eradicated in the recent years, but also has an increasing rate in the HIV patients.

Also due to episode of drug resistant species, it become serious treatening problems (6). The reason of drug resistant can be due to improper drug prescription, improper distribution of the drug when needed, wrong classification of the patients, the lack of supervision and the improper consumption of prescribed drug against TB by the patients (6). Drug resistant leads to failure in treatment which is followed by unaffordable expenses (6). Also, the patients with drug resistant species are the source of spreading the organism in the environment. Hence, study on the drug sensitivity of such organism provides guideline on the pattern of epidemiology study, as a result help reduce the episode of drug resistant species in the society.

**Methods:** Sputums from the individuals referring to health center suspected of having TB were collected in sterile containers, decontaminated by PETROPH method and contracted, then inoculated on to the Levenson jansons medium and incubated at 37 cente-grade for 4 weeks. A smears were prepared from the collected samples Ziehl Neelson staining was performed and looked for the presence of mycobacterium. Culture media were observed for the growth of *Mycobacterium tuberculosis* (TB), The culture positive plates were sent to Masih daneshvari TB center in Tehran, to be confirmed for the growth of TB and doing antibiogram.

**Results:** In this study, on 45 TB growth cultures, drug sensitivity was done for 4 essential drugs that is isoniazid (INH), refampin (Ref), ethambutol (Eth) and stereptomycin (ST) which are used against TB.

The pattern of sensitivity from 45 samples are as follows:

Eth 44 samples sensitive and 1 sample resitant (2.2%), INH 40 samples sensitive and 5 resistant (11%), Ref 43 samples sensitive and 2 resitant (4.4%), ST 31 samples sensitive and 14 resistant (31%). Was seperately 4 to 1, and combined resistant to two drugs that is INH and ST 2 samples and to three drugs that is to INH and Eth and ST 1 sample.

**Conclusions:** The results of this study indicate that, resistant to Eth, IHN, Ref and ST is 2.2%, 11%, 4.4%

and 31% respectively, which reveals an increasing pattern of TB drug resistant. Hence , increase in educational training of the physicians and paramedical personals for the proper short course treatment and implementation of directly observed treatment short course (Dots) strategy considered as prior issue is proposed.

Also determining of drug sensitivity which is not common in our country is recommended in order to prevent the recurrent of the disease and failure of treatment.

## MB 03

**Isolation of griseofulvin resistant strains of dermatophytes in Isfahan**

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**Background:** The emerge of drug resistance in dermatophytes would affect the incidence of infection in the society and causes difficulties both physician and patient. With report of cases of griseofulvin resistant dermatophytes, the use of new antifungal drugs is recommended which are more expensive or somehow rare in Iran. Therefore, the necessity of griseofulvin sensitivity pattern of dermatophytes in Isfahan is perceived which could lead to a more effective and less expensive treatment for recalcitrant dermatophytoses.

**Methods:** 50 isolates of the most prevalent dermatophytes in Isfahan were isolated from patients and then the standard homogenized suspension from them were prepared for future inoculation.

The minimum inhibitory concentration (MIC) of griseofulvin was determined by modified microdilution method for each isolate and then results were compared and analysed with standard values of MICs of dermatophytes and resistant strains identified.

**Results:** All 100% tested isolates had MIC mode of <0.25, 90% had < 8 and 50% ranged between <0.25-1  $\mu$ g/ml. From all isolates, 10% of them including three *Trichophyton verrucosum*, one *Microsporum canis* and one *T mentagrophytes* had MIC out of standardized range therefore, they considered as griseofulvin resistant isolates.

**Conclusions:** Although MIC values of drugs at in vivo and in vitro are somewhat different but in vitro values could be used as additional parameters in the decision making of treatment for dermatophytoses, in particular it's recalcitrant types or in areas which the resistant species may have high prevalence.

## MB 04

**Detection of drug-resistant *M. tuberculosis* by oligonucleotide chip**

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**Background:** The resurgence of tuberculosis and the widespread emergence of multidrug-resistant *M. tuberculosis* has emphasized the importance of rapid and accurate diagnostic procedures. Recently, oligonucleotide chip has proven to be a useful tool in the rapid diagnosis of infectious disease. The purpose of this study was to detect rapidly and accurately specific mutations in the *rpoB*, *katG* and *rpsL* genes associated with rifampin, isoniazid and streptomycin resistance in *M. tuberculosis* using single oligonucleotide chip.

**Methods:** For detection of drug-resistance, 7 wild-type and 13 mutant-type probes for rifampin, 2 wild-type and 3 mutant-type probes for isoniazid, and 2 wild-type and 2 mutant-type probes for streptomycin were designed and spotted onto the glass slides. 55 cultured samples of *M. tuberculosis* were amplified by PCR, followed by hybridization and scanning. Direct sequencing was done to verify the results from the oligonucleotide chip and to analyze the types of mutations.

**Results:** 35 cases out of 40 rifampin-resistant strains (~88%) had mutations in the *rpoB* gene. One case had a new mutation (D516F, GAC  $\rightarrow$  TTC) and another known mutation together. 20 cases out of 42 isoniazid-resistant strains (~50%) had mutations in the *katG* gene and 7 cases out of 9 streptomycin-resistant strains (~78%) had mutations in the *rpsL* gene. In these results, oligonucleotide chip was confirmed to be able to detect the most frequent mutations from gene associated with rifampin, isoniazid and streptomycin resistance. The results proved that the drug-resistance detection probes were specific. When the results from oligonucleotide chip and DNA sequencing were compared, the types of mutations were exactly matched.

**Conclusions:** The diagnostic oligonucleotide chip with mutation specific probes for drug resistance is a very reliable and useful tool for the rapid and accurate diagnosis of drug resistance against rifampin, isoniazid and streptomycin in *M. tuberculosis*.

## MB 05

**Molecular analysis of isoniazid resistance in *Mycobacterium tuberculosis* isolates recovered from Korea**

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**Background:** Investigation of rifampin resistance is relatively straightforward. In contrast, resistance to isoniazid (INH) is associated with a variety of mutations affecting several genes such as catalase-peroxidase (*katG*), the enoyl acyl carrier protein reductase (*inhA*), alkyl-hydroperoxide reductase (*ahpC*), ketoacyl acyl carrier protein synthase (*kasA*), and NADH dehydrogenase (*ndh*). Because of considerable variance in the reported data, more information is needed about molecular mechanisms of INH resistance.

**Methods:** The *katG*, *inhA* and *ahpC* genes, in 71 isoniazid (INH)-resistant and 26 INH-susceptible *Mycobacterium tuberculosis* isolates, from Korea were examined by DNA sequencing and *MspI* restriction enzyme analysis. Of the 71 INH-resistant isolates, 25 were resistant to INH only, 33 were resistant to at least INH and rifampin (multidrug resistant) and 13 were resistant to INH and other drugs, excluding rifampin (multiple drug resistant).

**Results:** Mutations in the *katG* codon 315, *katG* codon 315/*inhA*, *inhA*, *ahpC* and *ahpC/katG* codon 315 were identified in 54.9, 2.8, 21.1, 5.6 and 1.4%, of the 71 INH-resistant isolates, respectively. Mutations in the *katG* and *inhA* were not found in all INH-susceptible isolates, but one INH-susceptible isolate had a mutation in the *ahpC* promoter region, a G→A substitution at position -46 (designated relative to the *ahpC* mRNA start site). There was no statistically significant difference ( $p > 0.05$ ) in the frequencies of these mutations for the INH monoresistant compared with the multidrug or multiple drug resistant isolates. Mutations in the *katG* codon 315 were associated with the high-level of INH resistance (MIC of  $>1 \mu\text{g/ml}$ ), whereas the mutation in the *inhA* promoter region was associated with the low-level of INH resistance (MIC of 0.2-1  $\mu\text{g/ml}$ ). The previously undescribed GGT→GAT (Gly→Asp) mutation in the *katG* codon 309 was found in two multidrug resistant isolates, but we cannot assess if this is predictive of INH resistance.

**Conclusions:** If mutations in the codon 315 of the *katG* and the promoter regions of the *inhA* and *ahpC* were considered, the sensitivity and specificity were 85.9 and 96.2%, respectively. If mutations in the codon 315 of the *katG* and the promoter region of the *inhA* were considered, the corresponding values were 80.3 and 100%, respectively. Therefore, mutations in both the *katG* codon 315 and the promoter region of the *inhA* are highly predictive of INH resistance in Korea.

## MB 06

**Comparison of bacT/ALERT system with egg-based media in sputum culture and drug susceptibility test of *Mycobacterium tuberculosis***

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**Background:** Liquid culture systems have been widely employed both for primary cultures of *Mycobacterium tuberculosis* from clinical specimens and for drug susceptibility test (DST) because of its greater sensitivity and faster turn-around time than the conventional egg-based culture methods. In this study, we compared the BacT/ALERT liquid culture system with Ogawa and Lowenstein-Jensen (L-J) media for sputum culture and DST of *M. tuberculosis* in a clinical mycobacteriology laboratory setting in Korea.

**Methods:** Sputum specimens were decontaminated with N-acetyl-L-cysteine (NALC)-4% NaOH and inoculated into the BacT/ALERT culture bottles and Ogawa media. *M. tuberculosis* grown in the liquid culture system were used for determining drug susceptibility to the first line drugs, isoniazid (INH), rifampicin (RIF), and ethambutol (EMB) by the BacT/ALERT system and L-J media containing drugs.

**Results:** Of 89 sputum samples examined, 66 (74.1%) were culture-positive by the BacT/ALERT culture system, while 58 (65.2%) were positive by Ogawa media. The mean time to culture-positive by the BacT/ALERT system was  $14.8 \pm 9.6$  days, which was significantly shorter than that by Ogawa media ( $26.3 \pm 9.1$  days). Of 38 *M. tuberculosis* cultures examined for drug sensitivity, the overall agreement rate was 84.2% in determining drug susceptibility between the two methods ranging from 78.9% for INH and EMB and 94.7% for RIF. The median time for DST was 7.7 days by the BacT/ALERT-based DST compared with 4 weeks by the L-J media-based test.

**Conclusions:** Although there were substantial discordant results in determining drug susceptibility to the first line drugs, the BacT/ALERT liquid culture method looks promising for use in primary cultivation of *M. tuberculosis* from sputum samples and presumptive DST in a clinical mycobacteriology of TB hospital.

## MB 07

**Comparison of drug resistance genotypes between beijing and non-beijing family strains of *Mycobacterium tuberculosis* in Korea**

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**Background:** Beijing family strains of *Mycobacterium tuberculosis* have been implicated for numerous multidrug resistant tuberculosis (MDR-TB) outbreaks worldwide. Since the majority of *M. tuberculosis* isolates in Korea belong to the Beijing family, we were interested in whether or not Beijing strains are prone to be MDR-TB and have different drug resistance genotypes, particularly resistance to isoniazid (INH) and rifampicin (RMP).

**Methods:** A total of 743 *M. tuberculosis* isolates including 241 drug sensitive and 502 drug resistant isolates (231 MDR-TB and 271 other drug resistant isolates) were included. Spoligotyping method was employed to identify the Beijing strains, and a reverse blot hybridization assay to compare drug resistance genotypes using 14 probes for *rpoB* gene associated with resistance to RMP and 7 probes for *katG*, *inhA*, and *ahpC* genes to INH.

**Results:** Of 743 *M. tuberculosis* isolates examined, 569 (77%) were Beijing strains. Proportion of Beijing strains was significantly higher among MDR-TB strains [82% (190/231)] than among drug sensitive strains [72% (173/241)] ( $p < 0.007$ ). Drug resistance genotype analysis showed that Beijing strains had significantly lower mutation rate at the codons 529-535 of the *rpoB* gene (42% vs. 66%) ( $p < 0.01$ ), higher at the 315 codon of the *katG* gene (65% vs. 49%) ( $p < 0.01$ ), and lower at the promoter region of the *inhA* gene (14% vs. 25%) ( $p < 0.05$ ) than non-Beijing strains.

**Conclusions:** More than 70% of *M. tuberculosis* isolates in Korea belong to the Beijing family strains, and the proportion of Beijing strains was greater among MDR-TB isolates than among drug sensitive strains. Significant difference in mutation rates at the *rpoB*, *katG*, and *inhA* genes between Beijing strains and non-Beijing strains may explain higher MDR-TB rate among Beijing strains.

## MB 08

**Directed evolution approach to a structural genomics project: Rv2002 from *Mycobacterium tuberculosis***

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Structural genomics/proteomics is one of the new challenges in the post-genome era. Its ultimate goal is to provide all the necessary information on three-dimensional structure of proteins. It is expected that structural genomics/proteomics will have a major impact on future drug discovery. Structural genomics/proteomics research in my laboratory is focused on human pathogens, including *Helicobacter pylori* and *Mycobacterium tuberculosis*. As an example, our recent work on Rv2002 from *M. tuberculosis* (Yang *et al.*, 2003) will be described. We engineered soluble mutants of this protein by green fluorescent protein-based directed evolution (Waldo *et al.*, 1999). We selected a triple mutant Rv2002-M3 with I6T/V47M/T69K mutations for further structural and functional characterizations. Three mutations lie outside the conserved regions. Our work indicates that the Rv2002-M3 protein has a high catalytic activity as a NADH-dependent 3 $\alpha$ , 20 $\beta$ -hydroxysteroid dehydrogenase and Rv2002 is likely to be involved in steroid metabolism in *M. tuberculosis*. One of the main difficulties of high-throughput structure analysis by both X-ray crystallography and NMR is overexpression of proteins in a soluble form. This work demonstrates that directed evolution is a powerful approach to overcoming this difficulty. Structures of other proteins from human pathogens will also be described.

**Trial for TB drug-susceptibility test using live and dead cell differentiation**

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**Background:** The main requirement for an anti-tuberculosis drug susceptibility test is the ability to make distinction between susceptible and resistant *M. tuberculosis* strains. Such a distinction by a traditional phenotypic strategy based on cultivation is quite time consuming method and other new molecular biological methods are too complicate for handling and quite expensive. To improve these defects we practically adapted LIVE/DEAD® BacLight™ Bacterial Viability Kit (Molecular Probes, Eugene USA) for anti-tuberculosis drug susceptibility test.

**Methods:** The minimum inhibitory concentrations (MICs) of the several anti-tuberculosis drugs were determined. And tested already have been known as anti-drug resistant strains using LIVE/DEAD method. Stained Bacteria cells were observed with fluorescence spectroscopy and measured with flow cytometry. These results are compared with conventional culture based anti-drug susceptibility tests.

**Results:** Live cells fluoresced green and dead cells fluoresced red. These results are almost similar in conventionally using 12 anti-tuberculosis drugs. Compared results with culture based methods (absolute and proportional method) showed high sensitivity and specificity.

**Conclusions:** BacLight assays for bacterial cell viability test, its very simple and rapid for diagnosis of anti-tuberculosis drug resistnace *M. tuberculosis* strains. It takes only 15 minutes but this method requires expensive equipments such as fluorescence microscopy and plate reader. But some more improvement of LIVE/DEAD® BacLight™ Bacterial Viability Kit based methods will provide more simple and accurate benefits for anti-tuberculosis drug susceptibility test.