DETECTION OF M. tuberculosis AND DRUG SENSITIVITY TEST FROM SPUTUM CULTURE OF PULMONARY TUBERCULOSIS PATIENTS IN DR SOETOMO GENERAL HOSPITAL BETWEEN 2003-2004

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ABSTRACT

In most developing countries, sputum culture and drug-sensitivity tests are not done routinely because lack of shortage of laboratories facilities for culture and drug-sensitivity tests. It is rarely possible, therefore, to prescribe regimens according to laboratory results. Most developing countries suffer from a shortage of financial resources to procure antituberculosis drugs even for new smear-positive pulmonary cases. In addition, the cost of the drugs used in the treatment of drug-resistant tuberculosis is generally, much higher than that of the drugs used in the short-course chemotherapy, and it is rarely possible to cover the cost of secondary drugs unless external financial resources are available. In 2003 there was 133 patients, 81 (60.90%) male and 52 (39.10%) female. The result of the M. tuberculosis culture was 19 positive, 9 (47.37%) male and 10 (52.63%) female with 2 (10.53%) patients positive for MDR-TB. The culture and sensitivity test for the first-line antituberculosis drugs (SIRE) was as follows: sensitive for isoniazid was 9 (47.37%), for rifampin was 16 (84.21%), for streptomycin was 10 (52.63%), and for ethambutol there was 12 (63.16%) patients. Resistance for isoniazid was 10 (52.63%), for rifampin was 3 (15.79%), for streptomycin was 9 (47.37%), and for ethambutol there was 7 (36.84%) patients. 2 patients (10.53%) were MDR-TB and the sensitivity tests result were resistance for all drugs (isoniazid, rifampin, streptomycin and ethambutol). In 2004 there was 56 patients, 90 (57.69%) male and 66 (42.31%) female. The result of the M. tuberculosis culture was 26 positive, 15 (57.69%) male and 11 (42.31%) female and no MDR-TB. The culture and sensitivity test for SIRE was as follows: sensitive for isoniazid was 23 (88.46%), for rifampin was 24 (92.31%), for streptomycin was 16 (61.54%), and for ethambutol there was 12 (46.15%) patients. Resistance for isoniazid was 3 (11.54%), for rifampin was 2 (7.69%), for streptomycin was 10 (38.46%), and for ethambutol there was 14 (53.85%) patients. Regular examination must be done routinely in all patients with pulmonary TB to identify the MDR-TB and treated properly using the second-line drugs.

Keywords: pulmonary tuberculosis, culture M. tuberculosis, drug sensitivity test, MDR-TB

INTRODUCTION

M. tuberculosis infection is considered as a great public health problem despite the development of antituberculosis drugs, which have been available for more than 50 years. In 2003, there were 8.8 million new cases of TB, of which 3.9 million were smear positive; 674,000 of the patients were coinfectcd with HIV. There were a total of 15.4 million cases, of which 6.9 million were smear positive. An estimated 1.7 million people died from TB, including 229,000 people coinfectcd with HIV (WHO 2006).

As a consequent to the recent resurgence of tuberculosis, the rapid diagnosis of patients with active disease has become a focus of interest. Culture results remain the “gold standard” for the presence of mycobacteria in a specimen during diagnosis and follow up. Of equal importance to the control of tuberculosis is the assurance of effective treatment, yet over the past two decades, there has been little progress in the ability to predict a patient's response to antituberculosis therapy. Assessment or prediction of treatment response is especially important in patients at high risk for treatment failure, such as those with multidrug-resistant tuberculosis or HIV infection or both, but remains essentially limited to clinical, radiographic, and conventional bacteriologic evaluation despite the inherent inaccuracies of these methods. The most widely accepted measure of treatment response in patients with pulmonary tuberculosis is the disappearance of acid-fast bacilli (AFB) from sputum, as assessed by microscopic examination and culture. Unfortunately, smear sensitivity depends on the patient,
the type and degree of pulmonary parenchymal involvement, and the skill of the microscopist, and it ranges from 22 to 80%. Specificity is reduced by the presence of nontuberculous mycobacteria or dead organisms. Sputum culture is superior to direct examination in detecting AFB, but because *Mycobacterium tuberculosis* is relatively slow growing, serial sputum cultivation is an inefficient means to assess treatment response (Cernoch 1994).

It was not until the early 1990s, however, when outbreaks of multidrug-resistant tuberculosis were reported in patients with human immunodeficiency virus (HIV) infection in the United States and Europe, that the problem received international attention (Pablos-Mendez 1998). The problem of resistance results from treatment that is inadequate, often because of in irregular drug supply, inappropriate regimens, or poor compliance. Drug resistance is a potential threat to tuberculosis-control programs throughout the world. MDR-TB had been recognized as a serious issue for a long time. However, it had been given low priority in developing countries for two reasons: technical difficulties and funding. Today management of MDR TB is included as a recommended part of the new stop TB strategy and guidelines have also been revised to encourage countries to collect drug resistance surveillance data of patients in different treatment categories and to build capacity to diagnose and treat MDR TB in the full context of the directly observed treatment, short-course (DOTS) strategy (Mori 2007).

In this study, the sputum of patients with pulmonary TB were cultured and tested for sensitivity with first line antituberculosis drugs, streptomycin, isoniazid, rifampin, and ethambutol (SIRE).

**MATERIALS AND METHODS**

This cross sectional observational study was conducted at Male and Female Ward Lung Department Dr Soetomo General Hospital in Surabaya. The morning cough sputum was taken directly from patients with Pulmonary tuberculosis between 2003 – 2004 who fulfill the following criteria, 1) Clinical presentations and chest X-ray consistent with pulmonary tuberculosis, 2) Sputum shows positive smear for AFB on Ziehl-Neelsen staining (Anonymous 1969).

Digestion and concentration of sputum. The procedure of digestion and decontamination throughout the study was the N-acetyl-L-cysteine-sodium hydroxide combination according to the Standard procedure from Microbiology Clinics Laboratory Dr Soetomo General Hospital. Equal volumes of each specimen and the above solution were allowed to react for 15 min, followed by the addition of a phosphate buffer, pH 7.2. The mixture was then centrifuged and decanted. The sediment was resuspended in about 0.5 ml of buffer, and 1.0 ml of 0.2% ox albumin was added. The resuspended sediment was then used to seed the culture media and prepare slides. In some cases a few milliliters of sterile distilled water was added to thick, tenacious sputum in order to dislodge it from the container. The volumes of the sputum submitted varied greatly, ranging from a few milliliters to as high as 25 ml (Cernoch 1994).

Culture. The 7H10 supplement and dehydrated media were obtained from BBL (Middlebrook-Cohen 7H10 agar base no. 11422) and Difco (O.A.D.C. enrichment no. 0722-64), respectively. Lowenstein-Jensen (LJ) media were all prepared using standard formulas. The media were each dispensed into disposable, previously sterilized bottles and stored in a refrigerator until required. Refrigerated supplies of media were never more than 14 days old. The inoculated tubes of LJ media were incubated at 35 to 37 °C in a horizontal position in order to allow the inoculum to spread evenly over the surface of the medium and become fixed to the surface. Incubation was continued in incubator for a period of up to 12 weeks. During this period inspections were made at 3, 6, 8, or 12 weeks. Cultures showing evidence of growth at any time during this period were checked for morphology by making a smear and staining by the Ziehl-Neelse n procedure. All acid-fast organisms were biochemically tested for niacin and nitrate reduction. Confirmed organisms were then set up for sensitivity patterns of first-line antituberculosis drugs SIRE (Cernoch 1994).

**RESULTS**

In 2003 there was 133 patients, 81 (60.90%) male and 52 (39.10%) female. The result of the *M. tuberculosis* culture was 19 positive, 9 (47.37%) male and 10 (52.63%) female. In 2004 there was 156 patients, 90 (57.69%) male and 66 (42.31%) female. The result of the *M. tuberculosis* culture was 26 positive, 15 (57.69%) male and 11 (42.31%) female (Table 1).
Table 1. The Sex Characteristic and Culture Result of the Patients from Pulmonary Ward Dr Soetomo General Hospital examined by the Microbiology Clinic Laboratory in 2003-2004

<table>
<thead>
<tr>
<th>Year</th>
<th>Sex</th>
<th>Number (Percentage)</th>
<th>Culture of M. tuberculosis</th>
<th>Year</th>
<th>Sex</th>
<th>Number (Percentage)</th>
<th>Culture of M. tuberculosis</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Positive (n)</td>
<td>Negative (% of total)</td>
<td></td>
<td></td>
<td>Positive (n)</td>
<td>Negative (% of total)</td>
</tr>
<tr>
<td>2003</td>
<td>Male</td>
<td>81 (60.90%)</td>
<td>9 (11.11%)</td>
<td>2004</td>
<td>Male</td>
<td>90 (57.69%)</td>
<td>15 (16.67%)</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>52 (39.10%)</td>
<td>10 (19.23%)</td>
<td></td>
<td>Female</td>
<td>66 (42.31%)</td>
<td>11 (16.67%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>133 (100%)</td>
<td>19 (14.29%)</td>
<td></td>
<td>Total</td>
<td>156 (100%)</td>
<td>26 (16.67%)</td>
</tr>
</tbody>
</table>

The culture and sensitivity test for SIRE in 2003 patients were as follows: sensitive for isoniazid was 9 (47.37%), for rifampin was 16 (84.21%), for streptomycin was 10 (52.63%), and for ethambutol there was 12 (63.16%) patients. Resistance for isoniazid was 10 (52.63%), for rifampin was 3 (15.79%), for streptomycin was 9 (47.37%), for ethambutol there was 7 (36.84%) patients and 2 patients (10.53%) were MDR-TB and the sensitivity tests result were resistance to all drugs (isoniazid, rifampin, streptomycin and ethambutol). The culture and sensitivity tests for SIRE in 2004 patients were as follows: sensitive for isoniazid was 23 (88.46%), for rifampin was 24 (92.31%), for streptomycin was 16 (61.54%), and for ethambutol there was 12 (46.15%) patients. Resistance for isoniazid was 3 (11.54%), for rifampin was 2 (7.69%), for streptomycin was 10 (38.46%), and for ethambutol there was 14 (53.85%) patients (Table 2).

Table 2. The result of the drugs sensitivity test for M. tuberculosis

<table>
<thead>
<tr>
<th>Drugs</th>
<th>2003 (19 patients)</th>
<th>2004 (26 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitive n</td>
<td>Resistance n</td>
</tr>
<tr>
<td>H</td>
<td>9 (47.37%)</td>
<td>10 (52.63%)</td>
</tr>
<tr>
<td>R</td>
<td>16 (84.21%)</td>
<td>3 (15.79%)</td>
</tr>
<tr>
<td>S</td>
<td>10 (52.63%)</td>
<td>9 (47.37%)</td>
</tr>
<tr>
<td>E</td>
<td>12 (63.16%)</td>
<td>7 (36.84%)</td>
</tr>
<tr>
<td>HR</td>
<td>2 (10.53%)</td>
<td>0</td>
</tr>
</tbody>
</table>

H= isoniazid; R= rifampin; S= streptomycin E= ethambutol; HR= isoniazid and rifampin

DISCUSSION

In many developing countries drug resistant tuberculosis is increasing and is a significant threat to tuberculosis control because there are few drugs effective against M. tuberculosis. The mutant strains which are resistant to one anti-tuberculous drug and still sensitive to others is the basis of poly chemotherapy for treating TB which prevent selection of resistant mutants and leads to sterilization of the lesion. Worldwide accepted DOTS strategy seems to be an answer to control tuberculosis but it may also cause rapid emergence of drug resistance if patients with chronic disease are treated with a fixed regimen and not with a regimen based on their drug susceptibility profile. This is especially true for developing countries where drug resistance is high and information about previous history of treatment is not available. Today poly chemotherapy is matter of great concern due to emergence of resistant strains to multiple anti-tuberculous drugs. The results of the present study seems not far different from the global surveillance for antituberculosis-drug resistance in 1994-1997 and other studies. In this study the resistant for isoniazid range 11.56% to 52.63%, ethambutol 36.84% to 53.85%, streptomycin 38.46% to 47.37%, rifampin 7.69% to 15.79%, and MDR-TB 0% to 10.53%.

The global surveillance for antituberculosis-drug resistance, 1994-1997 resulted among patients with no prior treatment, a median of 9.9% of Mycobacterium tuberculosis strains were resistant to at least one drug (range 2 to 41%), resistant to isoniazid (7.3%) or streptomycin (6.5%) was more common than resistant to rifampin (1.8%) or ethambutol (1.0%). The prevalence
of primary multidrug resistance was 1.4% (range 0 to 14.4%) (Pablos-Mendez 1998).

The result of examination for drug resistance from patients with lung tuberculosis in Medan between 1993-1994 revealed resistance to ethambutol was 83.33%, to rifampin 63.33%, to isoniazid 56.67% and to streptomycin 46.65% (Tanjung 1996). The result of primary resistance to anti-tuberculosis drugs in BP4 Kodya Surabaya from the sputum of patient 26 patients were as follows: to isoniazid was 38%, to ethambutol 23%, to rifampin 15.3%, to streptomycin 3.8%, and MDR-TB (isoniazid and rifampin) revealed 11.53% (Siswantoko 1999).

The study conducted at BP4 Surakarta from 2332 cases by sputum materials from year 1995-1998 resulted of resistance to antituberculosis drugs as follows; primary resistance to isoniazid 4.87%, to rifampin 0.76%, to pyrazinamide 0.65%, to ethambutol 0.32%, to streptomycin 0.32% and kanamycin 0.11%. Secondary resistance to isoniazid 42.24%, to rifampin 13.35%, to pyrazinamide 3.11%, to ethambutol 3.41%, kanamycin 0.31% and streptomycin 0%. For the MDR from 1995 to 1998 tending to increase from 1.57%, 3.86%, 3.60% and 4.32% (Usman 1999).

The reported of drug resistance to antituberculosis chemotherapy at Dr M. Hoesin General Hospital in Palembang from the sputum of 178 cases revealed 48.9% for total resistance, 40.2% for primary resistance and 67.9% for secondary resistance. There were 30.3% for primary resistance to isoniazid, 15.6% for streptomycin and also to ethambutol, 4.9% for rifampin and 4.1% for pyrazinamide. The result for the primary resistance to 1 kind of drug was 20.5%, to 2 kinds of drug 11.5%, to 3 kinds of drug 5.7%, to 4 kinds of drug 2.5% and negative to 5 kinds of drug. Secondary resistance to isoniazid was 55.4%, to streptomycin 37.5%, to ethambutol 35.7%, to rifampin 30.4% and to pyrazinamide 17.9%. Secondary resistance to 1 kind of drug was 12.3%, to 2 kinds of drug 10.7%, to 3 kinds of drug 8.9%, to 4 kinds of drug 1.7% and to 5 kinds of drug 12.5%. The combine resistance to isoniazid and rifampin (MDR-TB) was 12.7% (Rasyid 2000).

Result of drug resistance reported from Microbiological Laboratory at Persahabatan General Hospital in Jakarta 1994-1997 from sputum of 600-800 cases, showed primary and secondary resistance to isoniazid were 4.75-5.92% and 33.33-38.46%, to rifampin 1.33-1.6% and 29.19-33.96%. Primary resistance to streptomycin 2.1-2.93% and ethambutol 0-0.53% (Aditama 2000).

The study in Padang from the new patient and after finishing the medication of lung tuberculosis, resulted that the new patient group showed 41.6% resistance to INH, 37.5% to rifampin, 58.3% to ethambutol. From the patients which completed the drugs, the result was 72.0% for INH, 64.0% for rifampin and 84.0% for ethambutol. The MDR-TB from the new patient group was 33.3% and 48.0% for the group completed the medication (Erly 2001).

Bhatti et al. reported that resistance to isoniazid was 34%, streptomycin 40.6%, ethambutol 3.8% and rifampicin 4.5% (Bhatti 1988). In Mayo Hospital at Lahore in 1999, drug sensitivity tests showed isoniazid resistance in 25%, streptomycin 19%, rifampicin 15%, ethambutol 12% and pyrazinamide 32%. Another study from Bangladesh, in 2000 reported an overall resistance of 30% to one or more drugs. It showed resistance of 15.8% to INH, 11% to RIF, 6.9% to STM, 3% to EMB, and 4% to PZA in clinically suspected untreated patients. These figures seem to be quite low as compared the present study (Miah 2000). Drug resistance reported from India is also high to these drugs with the exception of RMP, which is 25%, while MDR was reported to be 8.1%, which is low as compared to the overall resistance in the present study (Hemvani 2001).

Recently, drug sensitivity study at the University of Santo Tomas Hospital from 2003-2007, reported from 359 specimens with positive TB culture, the result of sensitivity test were as follows: overall sensitivity rate for he four drugs (HRES) was 48.74%, one-drug resistance was 23.12%, three-drug resistance was 15.04%, four-drug resistance was 3.06%, and five-drug resistance (HRESZ) was 0.28%. Among the one-drug resistance, rifampicin was the highest at 11.42% followed by isoniazid 6.41%. Two-drug combination resistance to H-R was found at 6.13%, while three-drug resistance rate was highest with H-R-E combination at 3.06%. Susceptibility had an increasing trend from 20% to 60% in 2004 and a decreasing trend of resistance to one drug. Trends for two-drug resistance was increasing 2004 to 2005 and 2006 to 2007 while, for three-drug resistance an increasing trend initially from 2003 to 2005 and a plateau from 2006 onwards (Visperas 2008).

Report from Pakistan at the Department of Medicine Liaquat University Hospital Hyderabad/Jamshoro & Institute of Chest diseases Kotri from 2005 to 2007 were as follows.

Among all culture positive cases, 20 (40%) were sensitive to all five drugs, 9 (18%) resistant to one drug, 11 (22%) resistance to two drugs, 7 (14%) resistant to three drugs, 3 (6%) resistant to four drugs and none of them was resistant to all five drugs. Total case resistant to isoniazid were 23 (46%) with 9 (18%) primary and 14 (28%) having secondary resistance. Resistance to
ethambutol was observed in 15 (30%), with primary resistance in 2 (4%) and secondary in 13 (26%) cases. Resistant to streptomycin were 9 (18%) with primary in 2 (4%) and secondary resistance in 7 (14%) cases. Resistance to pyrazinamide was seen in 4 (8%), having primary in 1 (2%) and secondary in 3 (6%) cases (Muzaffar 2008).

CONCLUSION

All studies and reports mentioned above indicate that drug resistance has increased significantly in the last two decades and needs to be controlled. Therefore larger studies from many centers on a national level are needed to establish overall prevalence of drug resistance in this country. Regular examination from all patients with pulmonary TB must be done routinely to identify the MDR-TB and then treated properly using the second-line drugs.

REFERENCES