Drug susceptibility pattern of *Mycobacterium tuberculosis* isolates against conventional anti-tuberculosis drugs in Dhaka, Bangladesh

Abdul Mottalib, MSc, M.Phil, **Murad Hossain,** M.Phil, PhD, **Ibrahim Khalil,** MSc, PhD, **Sirazul Islam,** MSc, PhD, *Anwar Hossain,* MSc, PhD.

ABSTRACT

الأهداف: اختبار مدى حساسية عزلات المتفطرة السلية تجاه العقارات المضادة لمرض السل في العاصمة البنغلادشية دكا.

الطريقة: أُجريت هذه الدراسة في معهد بنغلاديش لأبحاث وتأهيل مرضى السكري بمستشفى اضطرابات الغدد الصماء وعمليات الأيض، بالإضافة إلى مركز مكافحة مرض السل، دكا، بنغلاديش وذلك خلال الفترة من أكتوبر 2008م إلى نوفمبر 2009م. لقد تم جمع 101 من عينات البلغم للمرضى الذين تم علاجهم سابقاً، بالإضافة إلى المرضى المصابين حديثاً، وقد تم تحليل هذه العينات بواسطة الفحص المجهري واختبار تصبغ زيهل نيلسين، وبعد ذلك تم الكشف عن حساسية العزلات تجاه 4 أنواع من مضادات مرض السل وعلى أساس ذلك تم جمع البيانات وتحليلها.

النتائج: أشارت نتائج الدراسة إلى ظهور المتفطرة السلية في 59 حالة (58.4%) من أصل 101 حالة، وقد تم اختيار 50 عزلة إيجابية لفحص المتفطرة السلية من أجل دراسة حساسيتها تجاه 4 أنواع من مضادات السل. لقد أظهرت 25 عزلة (50%) من أصل 50 عزلة حساسية تجاه كافة المضادات، فيما قاومت 25 (50%) من هذه العزلات واحد أو أكثر من المضادات، ولقد كان 37 مريضاً (74%) من أصل 50 مريضاً قد أصيبوا بالمرض حديثاً، فيما كان 13 (26%) قد تم علاجهم سابقاً. ومن أصل 37 حالة جديدة فقد أظهرت 14 عزلة (37.8%) مقاومة تجاه نوع واحد أو أكثر من المضادات، ومن أصل 13 حالة التي تم علاجها فقد قاومت 11 (84.6%) عزلة نوع واحد أو أكثر من هذه المضادات. ومن أصل 50 عزلة التي كانت نتائجها إيجابية فقد أظهرت 26% منها مقاومة تجاه الأيزونيازايد، و 12% تجاه الريفامباسين، و 22% تجاه الستريبتوميسين، و 20% تجاه الإيثامبيوتول، فيما أظهرت 8% من هذه العزلات مقاومة تجاه أنواع مختلفة من مضادات السل.

خاتمة: أشارت الدراسة إلى أنه ينبغي الحذر من خطر ظهور المتفطرة السلية في دكا، حيث أن انتشارها قد أصبح أكثر بخمس مرات من العقد الماضي. ولهذا يجب سن القوانين الصارمة التي تحد وتقي من مقاومة العزلات الجرثومية لمضادات السل.

Objectives: To investigate the drug susceptibility pattern of isolated *Mycobacterium tuberculosis (M. tuberculosis)* against conventional anti-tuberculosis drugs in Dhaka, Bangladesh.

Methods: Sputum samples from 101 suspected new and previously treated patients were collected and *M. tuberculosis* was identified by microscopic observation and Ziehl-Neelsen staining. Drug susceptibility was performed against 4 anti-tuberculosis drugs, and the obtained data was analyzed. This study was performed in the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders Hospital, Dhaka, Bangladesh between October 2008 and November 2009.

Results: Among 101 suspected, 59 (58.4%) cases were identified as *M. tuberculosis* and the drug susceptibility pattern of 50 positives isolates was studied against 4 anti-tuberculosis drugs. Out of these 50 isolates of *M. tuberculosis*, 25 (50%) were sensitive to all drugs, and 25 (50%) were resistant to one or more drugs. Among 50 positive patients, 37 (74%) were new cases, and 13 (26%) were previously treated cases. Among 37 new cases, 14 (37.8%) cases were resistant to one or more drugs, whereas 11 out of 13 (84.6%) treated cases were resistant to one or more drugs. Among the 50 positive isolates, 26% demonstrated resistance to isoniazid, 12% to rifampicin, 22% to streptomycin, 20% to ethambutol, and 8% to multi drug resistance.

Conclusion: The emergence of drug resistant *M. tuberculosis* isolates in Dhaka is alarming, which is currently 5-fold higher than last decade. Strict measures should be taken to control and prevent drug-resistant tuberculosis.

Tuberculosis (TB) is still a major health problem worldwide and the main cause of death by a single infectious agent, namely, *Mycobacterium tuberculosis. (M. tuberculosis).* It infects one-third of the world's population.[1] The disease, while generally curable, is becoming increasingly resistant to communally used antibiotics.[2] The emergence of *M. tuberculosis* is a problem in developed as well as in developing countries. The modern treatment of TB began in 1946 with the invention of streptomycin (SM). In 1952, the much more effective drug, isoniazid (INH) became available, and in 1970 rifampicin (RIF), became recognized as at least equal to INH. Drug resistant tuberculosis (DR-TB) has been reported since the early days of the introduction of antibiotics; however, the global magnitude of DR-TB has not been well studied until recently.[2] The detection of *M. tuberculosis* and its drug susceptibility pattern is increasingly being recognized as an important component of global TB control,[3] and it is critical to test the drug susceptibility of TB bacilli in order to guide therapy.[4] The first survey of drug resistance was released in 1998 and included data from 35 countries.[5] Multi-drug-resistant tuberculosis (MDR-TB) is an emerging problem of great importance to public health worldwide. Multi-drug-resistant (MDR) is defined as resistance to at least INH and RIF. Bangladesh is the most densely populated country in the world, and the increase in MDR-TB further complicates the situation. A rural-based study reported the prevalence of resistance as 10.9% to any anti-tuberculosis drugs; (INH, RIF, ethambutol [EMB] and SM) and MDR in 0.23% in new cases, and 5.6% in previously treated cases.[6] At present Bangladesh has the fourth highest number of TB cases among 22 countries considered with a high level TB infected in terms of death of at least one person every 10 minutes.[7] In Bangladesh, Dhaka is a crammed, overpopulated city, and highly susceptible to propagate tuberculosis. This study was thus pursued to investigate the current status of the susceptibility pattern of *M. tuberculosis* against commonly used anti-tuberculosis drugs in newly and previously treated tuberculosis patients in Dhaka, and to assist physicians in prescribing suitable drugs for the treatment of tuberculosis patients.

Methods. One hundred and one sputum samples were collected from 64 (63.4%) males and 37 (36.6%) females aged between 14-70 years. Samples

were collected randomly from patients attending the Tuberculosis Control and Training Centre, Chankharpool, Dhaka, and the Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM) Hospital, Dhaka for the treatment of tuberculosis between October 2008 and November 2009. Ethical approval was not needed as the collected samples were cultured for in vitro study only. However, we took the verbal consent of the patients. All collected specimens of sputum were examined.

Bacteriological procedure. The morning sputum samples (5-10 ml) were collected in McCartney bottles and processed the same day. They were then decontaminated, and the bacillary concentration was increased as described previously.[8] Two Lowenstein–Jensen (LJ) slants were inoculated and incubated at 37°C for 6-8 weeks. A smear was prepared from each of the processed samples on a grease-free slide and stained by carbol fuschin using the Ziehl–Neelsen technique. Slides were checked for acid fast bacillus (AFB) under a microscope. All isolates and standard (*M. tuberculosis* strain H37Rv) were freshly grown on LJ medium. All clinical isolates were defined as *M. tuberculosis* according to growth rates, pigmentation properties of colonies, nitrate reduction, and niacin accumulation tests.[9]

Drug susceptibility analysis. Several spade-fulls (2-5 mg) of growth were scraped from LJ slants, transferred to a sterile screw-cap tube containing 6-8 glass beads and 3 ml normal saline (0.85%) and mixed well on a vortex mixer. Turbidity was matched against McFarland standard no. 1. One hundred μl of prepared bacterial inoculum was inoculated on LJ medium with a drug in each quadrant. The *M. tuberculosis* strain H37Rv was used as a control in all sets of experiments and inoculated plates were incubated at 37°C for 3 weeks. Resistance was defined as growth on drug containing tubes greater than 1% of the growth of drug control medium for INH, RIF, EMB, and 10% for SM.[10,11] The critical drug concentrations were the following; INH 0.2 μg/ml, RIF 40 μg/ml, EMB 2.0 μg/ml, and SM 4.0 μg/ml.[12]

We used the term "new case" to refer to TB patients who have never received anti-TB drugs or whom had received them for no more than one month of treatment. The term "previously treated case" refers to patients who had received at least one month of anti-TB therapy in the past. The patients in the "previously treated cases" group in this study included relapse, treatment failure, patients returning after defaulting, and chronic cases.

No statistical tests were used, only frequencies were calculated.

Results. A total of 101 sputum samples of patients were included in this study. Among them, 64 (63.4%) were males and 37 (36.6%) were females. Out of these 101 patients, 74 (73.3%) were smear positive, and 27 (26.6%) were smear negative for AFB by direct microscopy. Among these 74 smear positive cases, culture for mycobacteria were positive in 59 (79.7%) cases in LJ medium, contamination was present in 7 (9.5%), and no growth of mycobacteria in 8 (10.8%) cases. Then 50 isolates of *M. tuberculosis* were selected for drug susceptibility pattern against 4 anti-tuberculosis drugs. Out of these 50 isolates, 25 (50%) isolates were found sensitive to all drugs, and 25 (50%) isolates showed resistance to one or more drugs. **Table 1** summarizes the rates of drug resistance. The *M. tuberculosis* showed highest resistance to INH and resistance was found 26%, 22%, 20% and 12% of cases respectively for INH, SM, EMB, and RIF. From the resistance pattern of 25 isolates of *M. tuberculosis,* it appears that 13 (52%) were resistant to a single drug, 9 (36%) were resistant to 2 drugs, 3 (12%) resistant to 3 drugs, and only one case (4%) was found resistant to all 4 drugs. Out of 50 culture patients, 37 (74%) were new cases or untreated (no history of treatment) and 13 (26%) were treated. Among 37 new cases, 23 (62.2%) were sensitive to all drugs, and 14 (37.8%) were resistant to one or more drugs. Among 13 patients in the treated group, only 2 (15.4%) were sensitive to all drugs, and 11 (84.6%) were resistant to one or more drugs. Among 13 patients of treated groups, only 2 (15.4%) were sensitive to all drugs and 11 (84.6%) were resistant to one or more drugs. Out of these 11 treated cases, all cases were resistant to one drug, 8 (72.7%) cases were resistant to 2 drugs, 2 (18.2%) cases were resistant to 3 drugs, one (9.1%) case was resistant to 4 drugs and 4 (36.4%) cases were MDR.

Discussion. Tuberculosis has caused immense suffering for many years. People died helplessly, and civilizations were wiped out until man devised the idea to send tuberculosis patients to sanatoriums. An effective treatment for tuberculosis was introduced with the discovery of SM in 1946, para-aminosalicylic acid in 1949, and INH in 1952. Other anti-tuberculosis drugs discovered later improved the situation. However, unfortunately, the gradual development of resistance to these drugs was noted and problem of drug resistance made the situation more complicated. The mechanism of drug resistance to anti-tuberculosis drug was gradually understood, and the chromosomal basis of the mechanism was finally established. In general, drug resistance is mainly divided into 2 types: primary resistance and secondary (acquired) resistance. Primary resistance is that detected in isolates from patients who have never received anti-tuberculosis chemotherapy in the past or who have been treated for less than one month. Acquired drug resistance is that detected in isolates from patients with a record of previous treatment for tuberculosis for one month or more.[13]

The present study investigates the drug susceptibility pattern of *M. tuberculosis* in Bangladesh in this decade. According to the clinical demography of the studied patients, this experiment found that males (71%) were more susceptible than females (29%). In this study, the drug resistance of *M. tuberculosis* to at least one drug was found in 50% cases. The highest resistance (26%) was found to INH, which is the most popular drug in tuberculosis treatment. Resistance to SM was found in 22% cases, to EMB was found for 20% cases, and to RIF was found in 12% cases. Iqbal et al[14] from Pakistan reported drug resistance in 53% cases of tuberculosis, and resistance to INH was 26%, to RIF 28%, to SM 24%, to EMB 15%, and MDR was 16% of cases. Khan et al[15] from Saudi Arabia reported drug resistance in 29.7% cases of tuberculosis, and resistance to INH was 28.7%, to RIF 20.8%, to SM 22.8%, to EMB 6.9%, and MDR 20% of cases. The median prevalence of acquired resistance to at least one anti-tuberculosis drug (any resistance) was 18.4%, with the highest prevalence in Kazakhstan (82.1%). A significant increase in the prevalence of any resistance was observed in Botswana, Cuba, Switzerland, and the USA showed significant decreases. The prevalence of MDR significantly increased in Estonia, Lithuania, and Tomk Oblast (Russian Federation). Decreasing trends were significant in Slovakia, and the USA.[16] Effective tuberculosis control and management programs in developed countries have a favorable effect on the low incidence of acquired drug resistance, while our tuberculosis control and management programs are not so effective.

According to history of previous treatment for tuberculosis, confirmed cases were divided into 2 groups:

Table 1 - The rate of drug resistance to *Mycobacterium tuberculosis* among isolates collected from suspected cases in Dhaka, Bangladesh.

Drug	No. of resistant isolates	Total number of isolates	Drug resistance (%)
Isoniazid	13	50	(26)
Rifampicin	6	50	(12)
Ethambutol	10	50	(20)
Streptomycin	11	50	(22)
Multi drug resistant	4	50	(8)

new cases and treated cases. Out of 50 tuberculosis positive cases, 74% were new cases, and 26% were previously treated cases. A similar study was carried out in Duzce, Turkey by Ozturk et al,[17] and according to their report, new cases comprised 83.8%, and treated cases 16.2%.

In this study, drug susceptibility pattern of new cases was carefully analyzed. Out of 37 new cases, 62.2% were sensitive to all drugs, and 37.8% were resistant to one or more drugs. The drug resistance potency of 37 new cases against INH, RIF, EMB, SM and MDR were 16.22%, 8.10%, 8.10%, 13.51% and 2.7%. Similarly, Ozturk et al[17] from Turkey reported resistance to INH as 8%, to RIF as 4.8%, to SM as 11.3%, and MDR as 4.8% of cases. Among 14 resistant isolates in new cases, 12 (85.7%) cases were resistant to one drug, only 2 (14.29%) cases were resistant to 2 drugs and no strain was resistant to 3 or 4 drugs. Drug resistant patterns of treated patients were also analyzed. Among 13 patients of the treated group, only 2 (15.4%) were sensitive to all drugs and 11 (84.6%) were resistant to one or more drugs. The *M. tuberculosis* resistance was terrific among these 11 treated cases where 72.7% cases were resistant to at least two drugs and 36.4% were MDR.

Our results indicate that drug resistance rate was higher in treated cases (84.6%) than in new cases (37.8%). Treatment of tuberculosis without the benefit of susceptibility information may increase the risk of treatment failure, and may cause *M. tuberculosis* resistance to additional drugs. In Bangladesh, especially in Dhaka city, most TB hospitals, TB control centers, and physicians treat their patients only after microscopic examination. Such examinations alone cannot ensure proper treatment. This study supports the suggestion for accurate diagnosis through culture and sensitivity testing, which will help in determining drug susceptibility patterns of *M. tuberculosis* and prescribing proper drug (s) for the treatment of infected patients.

Drug resistance in *M. tuberculosis* is known to be caused by mutations in relatively restricted regions of the genome.[18] Mutations associated with drug resistance occur in rpoB for RIF, katG and the promoter region of the mabA (fabG1)-inhA operon for INH, embB for ethambutol, pncA for pyrazinamide, rpsL and rrs for streptomycin, and gyrA for fluoroquinolones such as ofloxacin and levofloxacin.[18] However, due to lack of funding facilities and limited laboratory facilities, studies on the molecular basis of drug resistance of these isolated strains were not pursued. Also, due to lack of sufficient funding, a limited patient number was included in this study. Further studies are needed to reveal the detailed figures on the drug susceptibility pattern of *M. tuberculosis* in Bangladesh.

In conclusion, the emergence of *M. tuberculosis* isolates resistant to anti-tuberculosis agents in Dhaka is alarming and strict measures to control and prevent drug-resistant tuberculosis are urgently needed. The incidence of *M. tuberculosis* resistance has increased 5-fold in Dhaka compared with the results of an earlier study.[6] The drug resistance rate was found higher in treated cases than in new ones, and the highest resistance of *M. tuberculosis* was found to INH. This study provides information on the present state of drug susceptibility patterns of *M. tuberculosis*, which will help physicians to choose proper drugs for the treatment of the *M. tuberculosis* infected patients, and thus will decrease the risk of treatment failure. This study also shows the necessity of developing new drugs to combat against MDR strains of *M. tuberculosis*.

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Related topics

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