Drug Resistance in Mycobacterium Tuberculosis: A Four Years Experience

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K. A. Karamat,Shahid Rafi,Shahid Ahmad Abbasi (Department of Microbiology, Armed Forces institute of Pathology, Rawalpindi.)

Abstract

Objective: To assess the prevalence of drug resistance amongst the clinical isolates of M. tuberculosis.
Setting: Armed Forces Institute of Pathology, Rawalpindi.
Method: Four first line anti-tuberculosis drugs, isoniazid (INH), rifarnpicin (RIF), ethambutol (ETH) and streptomycin (STR) were tested on 300 isolates from clinical samples, by agar dilution method on Lowenstein Jensen medium. The sensitivities were interpreted by the resistance ratio method.
Results: One hundred and fifty eight (52.66%) isolates were found resistant to one drug at least. Among the resistant isolates, 79 (26.33%) were resistant to INH, 72 (24.0%) to RIF 84 (28.0%) to STR and 70 (23.33%) to ETH with or without resistance to other drugs. Multi-drug resistance (MDR) was found in 41 isolates (13.66%). **Conclusion:** To overcome this problem there is a need to establish centres at a number of places all over the country with professionals trained to handle the emerging problem of MDR. Each centre must be equipped with adequate facilities for susceptibility testing so that the resistance pattern can be ascertained and treatment regimens tailored accordingly (JPMA 49:262, 1999).

Introduction

It is estimated, that nearly one fifth of the world's population is infected with Mycobacterium tuberculosis, causing eight million new cases and three million deaths annually[1]. The increase in the Human Immune Deficiency Virus infection has led to a rise in the incidence of tuberculosis in North America and Europe, where, after thirty-five years of decline, the disease has re-emeged as an imortant health issue. The developing countries are by no means spared. A great majority of the cases and 95% of the deaths occur here[2]. Development of multi-drug resistant (MDR) strains has added fuel to the fire. Tuberculosis control relies upon case finding and treatment. Clinical diagnosis is confirmed by microscopy, culture and polymerase chain reaction (PCR)[3]. The latter, being a highly specialized technique, is practically non-available, Isolation of the organism on culture is the only means available for a definitive diagnosis and susceptibility testing, the only way to pick up a resistant strain. This facility too, is available for a comparatively small group of population. Paucity of diagnostic facilities, little understanding about the disease and meager finances cause delay in diagnosis, improper management and poor drug compliance. All these ultimately result in the spread of the disease, as well as the selection of drug resistant strains. These resistant strains were probably always there due to random genetic mutations. In USA, even in 1980, 7% resistant strains were reported2. In Pakistan, prevalence of primary resistance to RIF have been reported to be I 7%[4], This study has been undertaken to assess the prevalence of drug resistance amongst the clinical isolates of Mycobacterium tuberculosis tested at Armed Forces Institute of Pathology (AFIP), Rawalpindi and to have an idea of threat that lies ahead.

Material and Methods

All routine clinical specimens received for AFB culture in the department of Microbiology, AFIP, Rawalpindi, during January 1995 to December 1998 were included in the study. The specimens received were sputum, pUS, endometrium, urine, tracheo-bronchial secretions, pleural fluid, ascitic fluid and various others (Table 1). All sputa, pus and other samples likely to be contaminated by normal bacterial flora were homogenized decontaminated and concentrated by modified Petroffs technique before inoculation on the appropriate media. Sterile samples like CSF and bone marrow were dealt with as such. Direct microscopy was performed for acid fast bacilli on smears stained by Ziehl Neelsen method.

Every specimen was inoculated on three slopes of Lowenstein Jensen (Li) medium. Two containing glycerol and one with para nitrobenzoic acid (PNB). One of the glycerol containing slopes was incubated in the dark. The cultures were incubated aerobically at 37°C, kept for eight weeks, aerated twice a week and examined at regular intervals for growth.

Primary identification of mycobacterium tuberculosis was based on the acid-fast character on ZN staining, eugonic colony morphology on L.J. medium, absence of growth on L.J. medium containing PNB and non-pigment production. Growth on thiophen-2-carboxylic acid hydrazide medium, niacin production and a positive catalase test were used as confirmatory evidence[5].

All clinical isolates were tested against four first line drugs, streptomycin (STR), isoniazid (IN H), rifampicin (RIF) and ethamutol (ETH). Minimum inhibitory concentrations (MICs) were determined by the agar dilution method. Different concentrations of the drugs were prepared in L.J. medium and the organism suspension inoculated on the slopes. The concentrations of the drugs used were STR 1.25, 2.5, 5, 10, 20, 40 mgfL, INH 0.025, 0.05, 0.1, 0.2,0.4,0.8 mg/L, RIF 0.75, 1.5, 3.12, 6.25, 12.5, 25 mgIL, ETH 0.31, 0.62, 1.25,2.5,5, 10 mg/L. Resistance ratio method was employed to interpret the results using control strain H37 Rv. The inoculated media slopes containing antibiotic were incubated at 37°C in the same manner as adopted for primary isolation. The slopes were examined for growth after 2-3 weeks of incubation. The lowest concentration of the antibiotic showing no more than 20 colonies, was taken as the MIC or the end point. The ratio of 2 or less was taken as sensitive and 8 or more resistant. Ratio of 4 was considered doubtful and the test repeated[5].

Results

Mycobacterium tuberculosis was isolated from 300 of the 2672 samples tested. The isolates were from sputum, pus, endometrium, tracheobronchial secretions, lymph nodes, ascitic fluids and pleural fluids (Table 1).

Table 1. Prevalence of mycobacterium tuberculosis. 1995-98

Type of specimens	No. of specimen	Positive cases
a) Sputum	1231	209
b) Pus C/S	355	37
c) Endometrium	223	16
d) Urine C/S	185	4
e) Tracheobronchial secretion	124	14
f) Ascitic fluid	53	3
g) Pleural fluid	100	2
h) Miscellaneous	401	15
Total	2672	300

Out of a total 300 isolates tested, 158 (52.66%) were found resistant to one or more than one drug. Mono resistance to INH was found in 19 (6.33%) isolates, to RIF in 5 (1.66%) isolates, to ETH in 18 (6.0%) and to STR in 21 (7.0%) isolates. Two-drug resistance was seen in 56 (18.66%) isolates, twenty-two (7.33%) isolates were resistant to three drugs and 16 (5.33%) were resistant to all the four drugs. Resistance to INH and RIF both (MDR TB) with or without resistance to other drugs was found in 41 isolates (13.66%) (Tables 2 to 5).

Table 2. Resistance pattern.
(n=300)

Drug combinations	No. of isolates resistant	Percentage
Mono drug resistance	64	21.33
Two drug resistance	56	18.66
Three drug resistance	22	7.33
Four drug resistance	16	5.33
MDR TB	41	13.66

MDR: Multi drug resistance

Table 3. Resistance pattern.
Single drug resistance (n=300)

Antibiotics	No. of isolates resistant	Percentage
Isoniazid	20	6.66
Rifampicin	5	1.66
Ethambutol	18	6.0
Streptomycin	21	70

Table 4. Resistance pattern.
Two drug resistance (n=300)

Antibiotics	No. of isolates resistant	Percentage
INH and RIF	13	4.33
INH and STR	9	3.0
INH and ETH	6	2.0
RIF and ETH	8	2.66
RIF and STR	12	4.0
STR and ETH	8	2.66

Table 5. Resistance pattern.
Three drug resistance (n=300)

Antibiotics	No. of isolates resistant	Percentage
INH+RIF+ETH	4	1.33
INH+RIF+STR	8	2.66
INH+STR+ETH	4	1.33
RIF+ETH+STR	6	2.0

Discussion

Tuberculosis has been haunting mankind since ancient times but the tables turned fifty years ago, with the discovery of STR. Later INH, RIF, ETH and Pyrazinamide (PZI) were also added to the treatment regimens. These drugs, when administered in proper doses for a sufficient period, can cure over 90% of established cases[2]. Inappropriate prescription and poor drug compliance can lead to resistant tuberculosis, which if not properly and aggressively managed can lead to multi-drug resistance[2,6]. MDR TB is a serious hazard not only to the patient but to the community as well. The patients become reservoirs of MDR TB and spread the disease to their close contacts. The most vulnerable among them are the children. Outbreaks of MDR TB have occurred in hospitals as well as other institutes throughout the world[7]. In the USA over all resistance to INH is reported as 8.4%, RIF 3%, STR 6.2%, ETH 2.2%, Pyrazinamide 3% and MDR 2.2%. Rates of INH and SIR resistance were high in US born as compared with foreigners, whereas, rate of RIF resistance and MDR TB were similar. Among the US born, rate of RIF mono-resistance was higher in HIV positive patients[8]. This shows that in the US there is a decrease in the resistance rates of RIF, which was 15.8% reported during 1981-84[9]. In U.K., INH resistance was around 3% in 1981[10]. Reports from India show 37.3% resistance to RIF in 1986 as compared to 2.8% in 1980[11]. A study from South Africa showed 70% MDR TB[12]. This is due to the AIDS epidemic. Global surveillance carried out by W.H.O. for anti-tuberculosis drug resistance revealed, 22. 1% MDR in Latvia, the highest in the world today, followed by India 13.3%, U.S.A. and U.K. show a rate of 2% and 3.2% respectively. In U.K., the resistance against INH alone is 6.8%[13], which is twice than it was it 1981. In Pakistan a study in 1987 showed 32% isolates resistant to INH, 24% to STR and 9% to ETH[14]. Aziz et al in 1989 reported 17% resistance to INH[4]. Karamat et al in June 1995 reported 22.95% resistance to INH, 29.5 1% resistance of RIF, 24.59% against SIR and 22.95% against ETH[15]. There is a rise in the resistance rate of all the drugs tested in our study except RIF, which shows a decrease of 0.59% (Table 7). MDR strains (13.66%) are second only to Latvia (22.1%), the country reporting the highest MDR incidence in the world so far. India (13.3%) is close behind us[13]. These are very high rates as compared to the developed countries. This is most probably due to delay in the diagnosis, combined with inappropriate prescription and poor drug compliance, because of poverty and lack of understanding about the disease and its consequences. MDR TB is difficult to eradicate, maintaining a pool of such patients from which the disease keeps on trickling to the community. Tuberculosis suddenly emerged as an important health issue in U.S.A in 1995, which has been attributed to AIDS epidemic, causing a break down in the health care delivery system[16]. Tuberculosis and AIDS is a bad combination. TB spreads among AIDS patients very rapidly and chances of MDR TB are also increased[17]. In countries like Pakistan where TB exists in large population, if the HIV gets a foot hold the results will be devastating and with the frequent travel around the world this does not seem to be far away.

In conclusion, resistance against all the four primary anti-tuberculosis drugs, which is present all over the world is also high in our set up. The incidence of MDR TB is also high. It is strongly recommended that the TB control program in Pakistan should be strengthened and efforts made on early case finding and treatment to prevent MDR TB, which otherwise, is difficult to cure. There is a definite need to develop culture facilities at a number of centers, well distributed throughout the country, for accurate diagnosis and determination of drug sensitivity pattern. The threat posed by this disease must be visualized. It is more dangerous than AIDS. To acquire AIDS one has to exhibit a peculiar social behavior but tuberculosis is in the air around us.

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