

Drug Resistance of *Mycobacterium tuberculosis* isolated from Patients discovered in the Third National Tuberculosis Prevalence Survey in 1975 in Korea

Sang Jae Kim D Sc. and Sung. Chin Kim, M. D.

Korean Institute of Tuberculosis, Korean National Tuberculosis Association

—국문초록—

제3차 결핵실태조사에서 분리한 결핵균의 각종 항결핵제에 대한 감수성에 관한 연구

김 상 재 · 김 성 진

대한결핵협회 결핵연구원

1975년에 실시한 제3차 전국결핵실태조사에서 분리한 인형균주 270주에 대한 약제 감수성 검사(이차 약제 포함)를 실시하여 다음과 같은 성적을 얻었다.

1. 총 270주의 약제 감수성검사 성적을 보면 단계 또는 그이상 약제에 대한 내성률은 36.3%이었고, 단계 14.8%, 2제 13.0%, 3제 전내성이 8.5%로 나타났으며 INH 단독 약제 내성률은 12.6%였다.
2. 피검자 270명중 과거 복용사실이 없는 자는 179명(66.3%)인데 이 균의 단계 또는 그 이상 약제 내성률 즉 1차 내성률은 16.2%로 나타났다.
3. 남녀별 약제내성에서는 유의한 차이를 보이지 않았으나 도시와 농촌의 약제내성은 도시가 46.2%, 농촌이 30.1%로서 도시에서 높았다. 특히 다제내성이 도시에서 높았다.
4. 엑스선 진단별 약제내성은 단계 또는 그이상 약제내성률에 있어서 경증 24.3%, 중등증 33.6%, 중증 62.5%로 나타났다.
5. 과거 치료력이 없고 내성이생긴 환자(일차내성)의 연령별 내성률은 5-35세군이 21.9%, 39~65세군 16.3%, 그리고 65세 이상이 6.1%로써 최근감염 연령군인 저연령층에서 높게 나타났다.
6. 총 분리균 270주의 각 이차 약제에 대한 내성률은 에탐부롤 3.7%, 리팜피신 3.0%, 카나마이신 2.6%, 카프레오마이신 2.2%, 에치오나마이드 1.1%, 바이오마이신 1.1%로 비교적 낮은 내성을 나타내었다.

INTRODUCTION

The high prevalence of drug resistance has a double-edged meaning: it is associated with a high rate of treatment failure due

to improper or ineffective chemotherapy service, and it also refers to a wide use of corresponding drugs. The information on the prevalence of drug resistance is necessary not only for planning the mass chemotherapy programme but also to evaluate in part the performance of the over all chemotherapy service programme. Particularly

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the prevalence of primary drug resistance has an important epidemiological significance. Therefore an investigation of the prevalence of drug resistance was one of the primary objectives in the last two surveys in 1965 and 1970 and continuation of this study is natural in the 1975 survey.

From the 3rd survey we found 130 smear-positive cases and, by culture examination, 270 positive cases. We have investigated the drug sensitivity of these 270 isolates and the results obtained are presented with a discussion, comparing these results to past data.

MATERIALS and METHODS

The Strains: In the survey, laryngeal swabs and sputum specimens were collected from 3736 individuals whose chest X-rays showed some abnormality on field reading. Of these X-ray suspects, a number of the cases have been found to have normal chest X-rays on subsequent central reading. However, all the specimens from 3,736 suspected patients were subjected by microscopy and culture examination. The culture technique

employed in the survey was the oxalic acid method which had been introduced into the first survey according to the recommendations of WHO. However we examined the sputum specimens remaining after using the aforementioned procedure, by additional culture methods either by simple direct methods with 2,599 sputa or by concentration method with 729 sputa on 3% Ogawa, KIT or Löwenstein-Jensen medium. Animal inoculations using mice were done with 501 sputa. Smear and oxalic acid culture examination of 3,736 sputum specimens and 3,736 laryngeal swabs yielded 207 positive cases and additional culture examination and animal inoculation produced an additional 68 positive cases. The culture positive cases totaled 270. All of these isolates were confirmed as species of *M. tuberculosis* through identification tests. These 270 isolates were subjected to this study. All cultures were maintained on L-J medium.

Concentrations of Antituberculous Drugs used: Drug concentrations used for H37Rv and test strains are shown in Table 1.

Drug Sensitivity Test: The sensitivity of

Table 1. Drugs and their concentrations (r/mi) used.

Drugs	For H37Rv			For test strains		
Isoniazid (INH)	0.025	0.05	0.1	0.2	1	5
p-Aminosalicylic acid (PAS)	0.125	0.25	0.5	1	2	4
Streptomycin (SM)	2.5	5	10	10	20	40
Kanamycin (KM)	2.5	5	10	5	10	20
Capreomycin (CM)	5	10	20	10	20	40
Viomycin (VM)	10	20	40	20	40	80
Ethionamide (1321)	10	20	40	20	40	80
Ethambutol (EB)	0.315	0.625	1.25	0.625	1.25	2.5
Cycloserine (CS)	10	20	40	20	40	80
Rifampin (RFP)	2.5	5	10	5	10	20

the organisms to varying drug concentrations was determined by the indirect test on the L-J medium. The test was performed by using subcultures from the primary isolation medium. A loopful of bacterial mass (about 1 mg in wet weight) from a 2 to 3 weeks old growth on the L-J medium was placed in a 1/4 oz bottle containing 6 glass beads (2-3 mm in diameter) and 0.5 ml of sterile distilled water and the bottle was vigorously shaken on a voltex mixer for 1 min. An additional 4.5 ml of sterile distilled water was then added to this bacillary homogenate and a loopful of the suspension was inoculated onto the medium with a loop (3 mm external diameter) made from nichrome wire (24 SWG) after coarse bacillary clumps settled out. The inoculated media were incubated at 37°C for 4 weeks. The presence of 20 or more colonies on the drug

containing medium was taken as an indication that the strain is resistant. Interpretation of the test for the sensitivity to PAS and SM was made by the resistance-ratio, namely, the MIC of the test strain divided by the MIC of strain H37Rv. The sensitivity of INH and second line drugs was determined by the presence or absence of growth at the critical concentrations of each drug where strain H37Rv did not grow. The critical concentrations for INH, EB, 1321, RFP, CM, KM, VM, and CS were 0.2, 1.25, 40, 10, 20, 10, 40, and 20r/ml respectively.

RESULTS

The resistance to standard drugs of 270 isolates has been shown in Table 2 and Figure 1 comparing the data of the 1975 survey with those of the 1965 and 1970 sur-

Table 2. A Comparison of Drug Resistance Between the 1st, 2nd, and 3rd National Tuberculosis Prevalence Surveys.

		Survey Year		
		1965	1970	1975
Total Number of Cases Tested		17	132	270
Total Number of Resistant Cases		27(38.0)	50(37.9)	98(36.3)
Resistant to One Drug	INH	12(16.9)	17(12.9)	34(12.6)
	PAS	2(2.8)	2(1.5)	2(0.7)
	SM	7(9.9)	2(1.5)	4(1.5)
	SUB-TOTAL	21(29.6)	21(15.9)	40(14.8)
Resistant to Two Drugs	INH-PAS	2(2.8)	7(5.3)	18(6.7)
	INH-SM	3(4.2)	5(3.8)	17(6.3)
	SUB-TOTAL	5(7.0)	12(9.1)	35(13.0)
Resistant to Three Drugs	INH-PAS-SM	1(1.4)	17(12.9)	23(8.5)
Total Resistance to	INH	18(25.3)	46(34.9)	92(34.1)
	PAS	5(7.0)	26(19.7)	43(15.9)
	SM	11(15.5)	24(18.2)	44(16.3)

Table 3. Resistance to Standard Anti-Tuberculous Drugs of *M. Tuberculosis* Isolated from Patients Discovered by Radiographic Examination in the 3rd National Tuberculosis Prevalence Survey in 1975.

Radiographic Finding	Total Cases Tested	Total Resistant Cases	Resistant to					
			INH	PAS	SM	INH PAS	INH SM	INH PAS SM
Inactive Tuberculosis and Other Types	30	8(26.7)	2(6.7)	0	0	2(6.7)	3(10.0)	1(3.3)
Minimal Tuberculosis	74	18(24.3)	10(13.5)	1(1.4)	2(2.7)	1(1.4)	2(2.7)	2(2.7)
Moderately Advanced Tuberculosis	110	37(33.6)	10(9.1)	1(0.9)	1(0.9)	8(7.3)	7(6.4)	10(9.1)
Far Advanced Tuberculosis	56	35(62.5)	12(21.4)	0	1(1.8)	7(12.5)	5(8.9)	10(17.9)
Total	270	98(36.3)	34(12.6)	2(0.7)	4(1.5)	18(6.7)	17(6.3)	23(8.5)

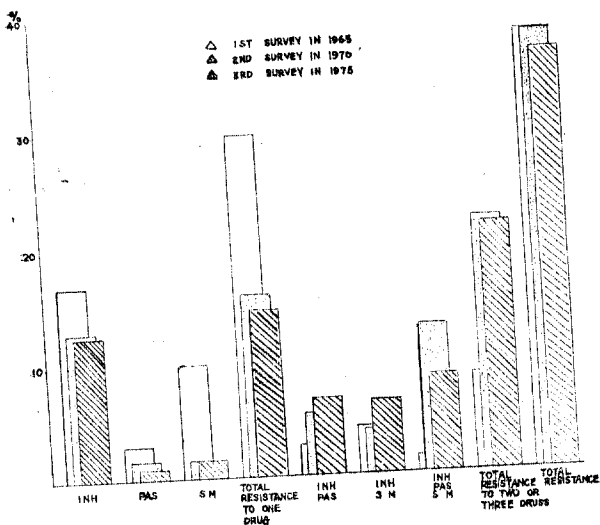


Fig. 1. A Comparison of Drug Resistance Between the 1st, 2nd and 3rd National Tuberculosis Prevalence Surveys.

veys. Of 270 isolates 98 or 36.3% were resistant to one or more of the standard drugs

Statistically there was no significant difference between the three surveys. Total single-drug resistance was 14.8% and this figure suggests a decrease in single-drug resistance when compared with those of the 1965 and 1970 surveys. Of the single-drug resistant isolates 12.6% were resistant to

INH. Resistance to PAS or SM alone appeared minimal. However, resistance to two drugs appeared to be on the increase. Resistance to three drugs had increased significantly in the 1970 survey but has remained stationary, Total resistance to INH, PAS, and SM was 34.1%, 15.9%, and 16.3% respectively. This data is similar with that of the 1970 survey but resistance to INH and PAS showed a significant difference when compared with the 1965 survey.

Drug resistance according to extent of disease classified by radiographic findings is shown in Table 3. From 30 inactive or other types of tuberculosis, 8 or 26.7% were resistant to one or more of the standard drugs and 6 or 20.0% out of 8 cases were found resistant to two or all three drugs.

Of 74 minimal, 110 moderately advanced, and 56 far advanced tuberculosis cases, 18 (24.3%), 37(33.6%), and 35 (62.5%) were found to be resistant to one or more of the standard drugs. The difference between the groups was statistically significant($P < 0.0005$). The progress of the disease and the prevalence of drug resistance seems to par-

Table 4 Drug Resistance of *M. Tuberculosis* Isolated from Patients with Previous Chemotherapy.

Radiographic Finding	Number of Cases Examined	Total Resistant Cases	INH	PAS	SM	INH PAS	INH SM	INH PAS SM
Inactive Tuberculosis & Others	3	2(66.7)	0	0	0	0	2(66.7)	0
Minimal Tuberculosis	10	8(80.0)	6(60.0)	0	0	1(10.0)	0	1(10.0)
Moderately Advanced Tuberculosis	39	26(66.7)	4(10.3)	1(2.6)	1(2.6)	7(18.0)	5(12.8)	5(20.5)
Far Advanced Tuberculosis	39	33(84.6)	12(30.8)	0	0	7(18.0)	5(12.8)	9(23.1)
Total	91	69(75.8)	22(24.2)	1(1.1)	1(1.1)	15(16.5)	12(13.2)	18(19.8)

Numbers in Parenthesis represent Percent.

Table 5. Drug Resistance of *M. Tuberculosis* Isolated from Patients without Previous Chemotherapy.

Radiographic Finding	No. of Cases Examined	Total Resistant Cases	INH	PAS	SM	INH PAS	INH SM	INH PAS SM
Inactive Tuberculosis & Others	28	6(21.4)	2(7.1)	0	0	2(7.1)	1(3.6)	1(3.6)
Minimal Tuberculosis	63	10(15.9)	4(6.4)	1(1.6)	2(3.2)	0	2(3.2)	1(1.6)
M. A Tuberculosis	71	11(15.5)	6(8.5)	0	0	1(1.4)	2(2.8)	2(2.8)
F. A Tuberculosis	17	2(11.8)	0	0	1(5.9)	0	0	1(5.9)
Total	179	29(16.2)	12(6.7)	1(0.6)	3(1.7)	3(1.7)	5(2.8)	5(2.8)

Numbers in Parenthesis represent Percent.

allel each other. Thus, as we expected, the more advanced the disease the higher the prevalence of drug resistance was found. In minimal tuberculosis single drug resistance was 17.6% but multiple-drug resistance was 6.8%. However, a higher prevalence of multiple-drug resistance was observed in moderately or far advanced tuberculosis (22.8% or 39.3% respectively) than those of single-drug resistance (10.9% or 23.2% respectively).

Drug resistance of 91 isolates from patients having a history of previous chemotherapy was 75.8% as shown in Table 4. Of these 49.5% were resistant to two or three drugs and 26.4% to one drug. There was no

significant difference between the groups at varying stages of disease development.

Primary drug resistance of isolates from 179 patients who did not have antituberculous drugs previously was 16.2% as shown in Table 5. Single-drug resistance was 8.9% and resistance to two or three drugs was 7.3%. Differences in primary drug resistance according to the extent of disease were no longer significant.

When the patients were grouped by age of 30 year intervals, 5-35, 36-65, and more than 65, Primary drug resistance in these groups showed significant differences as shown in Table 6. Within the 5-35, 36-65, and more than 65 age groups, 14(21.9%),

Table 6. Primary Drug Resistance by Age Groups

Age groups	Total number of cases tested	Total resistant cases	Resistant to					
			INH	PAS	SM	INH+PAS	INH+SM	INH+PAS+SM
5-35	64	14(21.9)	5(7.8)	0	1(1.6)	1(1.6)	4(6.3)	3(4.7)
36-65	80	13(16.3)	7(8.8)	1(1.3)	1(1.3)	1(1.3)	1(1.3)	2(2.5)
>65	33	2(6.1)	0	0	1(3.0)	1(3.0)	0	0
Total	179	29(16.2)	12(6.7)	1(0.6)	3(1.7)	3(1.7)	5(2.8)	5(2.8)

Numbers in parenthesis represent percent.

Table 7. A Comparison of Drug Resistance Between Urban and Rural Areas.

Areas	Total Cases Tested	Total Resistant Cases	Resistant to					
			INH	PAS	SM	INH PAS	SM INH	INH PAS SM
Urban	104	48(46.2)	16(15.4)	0	1(1.0)	8(7.7)	8(7.7)	15(14.4)
Rural	166	50(30.1)	18(10.8)	2(1.2)	3(1.8)	10(6.0)	9(5.4)	8(4.8)
Total	270	98(36.3)	34(12.6)	2(0.7)	4(1.5)	18(6.7)	17(6.3)	23(8.5)

Table 8. A Comparison of Drug Resistance by Sex.

Sex	Total Cases Tested	Total Resistant Cases	Resistant to					
			INH	PAS	SM	INH PAS	INH SM	INH PAS SM
Male	177	65(36.7)	18(10.2)	2(1.1)	3(1.7)	14(7.9)	12(6.8)	16(9.0)
Female	93	33(35.5)	16(17.2)	0	1(1.1)	4(4.3)	5(5.4)	7(7.5)
Total	270	98(36.3)	34(12.6)	2(0.7)	4(1.5)	18(6.7)	17(6.3)	23(8.5)

13(16.3%), and 2(6.1%) showed resistance to one or more of the standard drugs respectively. Therefore, as the group age increased primary drug resistance decreased.

A comparison of drug resistance between urban and rural areas has been made as shown in Table 7. Drug resistance of urban patients was 46.2% (48) out of 104 cases and that of rural patients, 30.1% (50) out of 166 cases. The difference between two groups was significant (p 0.01). There was no sta-

tistically significant difference in single drug resistance between the two groups, but there was a significant difference (p 0.025) in two or three drug resistance (29.8% in urban and 16.3% in rural).

Drug resistance comparing males and females is seen in Table 8. However, no significant difference has been recognized. 270 patients who are excreting tubercle bacilli have been grouped into 4 age groups of 15 year intervals and drug resistance in these

Table 9. A Comparison of Drug Resistance by Age Groups. (with and without previous chemotherapy)

Age Groups	Total Cases	Total Resistant	Resistant to					
			INH	PAS	SM	INH PAS	INH SM	INH PAS SM
5-19	28	8(28.6)	3(10.7)	0	0	2(7.1)	0	3(10.7)
20-34	58	27(46.6)	11(19.0)	0	1(1.7)	3(5.2)	5(8.6)	7(12.1)
35-49	71	29(40.9)	9(12.7)	0	1(1.4)	7(9.9)	5(7.0)	7(9.9)
50	113	34(30.1)	11(9.7)	2(1.8)	2(1.8)	6(5.3)	7(6.2)	6(5.3)
Total	270	98(36.3)	34(12.6)	2(0.7)	4(1.5)	18(6.7)	17(6.3)	23(8.5)

groups have been compared as shown in Table 9. Of the 28 the 5-19 age group or 28.6% were resistant to one or more of the standard drugs, 46.6% of the 20-34, 40.9% of the 35-49, and 30.1% of the over 50 age group, showed resistance. Differences between age groups were significant.

Drug resistances to second-line drugs have been investigated along with the standard drugs. Of 270 isolates 3.7% were resistant to ethambutol, 3.0% to rifampin, 2.6% to kanamycin, 2.2% to capreomycin, 1.1% to ethionamide, and 1.1% to viomycin.

DISCUSSION

The prevalence of single and multiple-drug resistance to the standard drugs in patients discovered in the three surveys in 1965, 1970, and 1975 showed observable trends. There was no substantial increase or decrease in total resistance between the three surveys. However, the prevalence of resistance to single or multiple drugs showed differing trends during the three survey years. For example the resistance to a single standard drug has shown a decrease in the 1970 survey when compared with that of the 1965 survey, but remained stationary

between the 1970 and 1975 surveys. However, the resistance to two or three drugs had increased in the 1970 survey when compared with that of the 1965 survey, but again remained stationary between the 1970 and 1975 surveys. This phenomena suggests that before 1965 single-drug regimen might have been used widely or a wide use of combined therapy was of an initial stage. Since 1962 at which the national tuberculosis programme was initiated, combined therapy has been used widely. In comparing total resistance to each of the standard drugs the resistance to INH and PAS had clearly increased in the 1970 survey, but has since remained unchanged. However there was no significant difference in SM resistance between the three surveys. This phenomena suggests a wide distribution of INH and PAS since 1962 when the NTP was first initiated.

If the chemotherapy programme is implemented effectively, the primary drug resistance will decrease due to successful negative conversion of positive cases. Thus no drug resistant bacilli can be evolved and transmitted to the community. The absence of differences in the prevalence of drug resistance between the 1970 and 1975 surveys

reflect in part no difference in the over all treatment services during that time. The effectiveness of a chemotherapy service programme can also be estimated by observing the prevalence of primary drug resistance because usually the prevalence of primary drug resistance in developing countries is higher than in technically developed countries suggesting ineffective treatment services in the former than in the latter. In the 1975 survey primary drug resistance has been found to be 16.2% which is still high enough to suggest a high prevalence of drug resistant cases in the pool of positive cases. This figure is also comparable with those of other developing countries but not with those of technically developed countries. This figure can not be compared directly with 23.9% of the 1970 survey because many of the cases found during the survey as having no history of previous chemotherapy were reconfirmed and many of these cases had previous chemotherapy, however, such reconfirmation data was not gathered in the 1970 survey. Drug resistant organisms usually evolve as a result of failure of initial drug therapy, thus, reduction of such cases thru improvement of treatment service will cause a decrease in the prevalence of primary drug resistance.

The higher prevalence of drug resistance in urban patients than in rural patients suggests that urban patients have a greater accessibility to these drugs than do rural patients. The factors affecting the lower rate of drug resistance in the less than 19 and the over 50 years old age groups than those in the other groups are rather unclear. However 14.3% and 21.2% of patients had a history of chemotherapy in the less than

19 and the over 50 age groups while 36.2% and 26.8% of patients had a history of chemotherapy in the 20-34 and the 35-49 age groups respectively. These suggest that the proportion of patients having a history of previous chemotherapy seem to be closely related to the prevalence of drug resistance.

The prevalence of resistance to second-line drugs is not a matter of grave concern yet, therefore, we can introduce these drugs into the mass chemotherapy programme to strengthen the drug regimen.

SUMMARY

The sensitivity to standard and second-line antituberculous drugs has been investigated on 270 isolates of *M. tuberculosis* which were obtained from patients discovered in the 3rd national tuberculosis prevalence survey in 1975 in Korea. The results obtained are as follows.

1. Of 270 isolates 36.3% were resistant to one or more of the standard drugs. The resistance to one drug was 14.8% and of these the resistance to INH alone was 12.6%. The resistance to two or all three drugs was 13.0% or 8.5% respectively.
2. Of 179 isolates from patients without previous chemotherapy 16.2% were resistant to one or more of the standard drugs.
3. There was no significant difference in drug resistance between males and females, however, significant differences have been observed between urban and rural patients as the resistance of the former was 46.2% while that of the latter was 30.1%. The difference is mainly due to a larger prevalence of resistance to two or three drugs in urban areas than that in rural areas.

4. The drug resistance according to extent of disease was 24.3% in minimal tuberculosis, 33.6% in moderately advanced tuberculosis, and 62.5% in far advanced tuberculosis.

5. Drug resistance of age groups less than 19 and over 50 years (28.6% and 30.1% respectively) were significantly lower than those for age groups 20-34 and 35-49 (46.6% and 40.9% respectively).

6. Of 270 isolates 3.7% were resistant to ethambutol, 3.0% to rifampin, 2.6% to kanamycin, 2.2% to capreomycin, 1.1% to ethionamide, and 1.1% to viomycin.

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