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PREVALENCE OF DRUG RESISTANCE IN PATIENTS WITH PULMONARY TUBERCULOSIS PRESENTING FOR THE FIRST TIME WITH SYMPTOMS AT CHEST CLINICS IN INDIA*.

Part I.

FINDINGS IN URBAN CLINICS AMONG PATIENTS GIVING NO HISTORY OF PREVIOUS CHEMOTHERAPY.

(Indian Council of Medical Research)

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INTRODUCTION.

IT is generally accepted that information on the prevalence of drug resistance is essential for countries which contemplate mass chemotherapy programme for tuberculosis (International Union against Tuberculosis, 1961). In India in 1964, information on this subject was confined to certain limited areas only (Tuberculosis Chemotherapy Centre, Madras, 1959, 1960, 1964 ; Frimodt-Moller, 1962 ; Menon, 1963 ; Balbir Singh, 1964). Therefore, the Indian Council of Medical Research (I.C.M.R.) launched a series of investigations to determine the prevalence of drug resistance in tuberculous patients reporting for the first time with symptoms at chest clinics ; chest clinics were chosen since they are an obvious starting point for any mass chemotherapy programme. A special sub-committee of the Indian Council of Medical Research (see footnote) was constituted to organise the execution of these investigations, and a Central Laboratory set up on the premises of the Tuberculosis Chemotherapy Centre, Madras, to undertake all the necessary bacteriological investigations.

*This investigation was originally planned by Drs. P.K. Seo, J.B. Selkon, and K.K. Mathan, and executed through a special sub-committee of the Indian Council of Medical Research consisting of Drs. N.L. Bordia (Chairman), B.K. Sikand, P.K. Sen and M.D. Deshmukh. Dr. P.R.J. Gangadharam, Senior Research Officer, Tuberculosis Chemotherapy Centre, Madras, was seconded to look after the work of the Central Laboratory as well as to co-ordinate the work of all the participating centres.

The following doctors participated from the various Centres : Dr. J.L. Bhatia (Amritsar), Dr. R. Susaimary (Bangalore), Dr. M.D. Deshmukh (Bombay). Dr. N.N. Sen (Calcutta), Dr. B.K. Sikand (Delhi). Dr. N.K. Menon succeeded by Dr. D. Umapathy Rao (Hyderabad), Dr. M.A. Hamid (Madras), Dr. P.A. Deshmukh (Nagpur) and Dr. J.P. Karan (Patna).

The Central Laboratory was situated in the premises of the Tuberculosis Chemotherapy Centre, Madras, and was afforded all the necessary facilities by the Director of the Centre (Dr. H. Stott succeeded by Dr. N.K. Menon) and the Head of the laboratory (Dr. L. Eidus succeeded by Dr. S. P. Tripathy).

The analyses were undertaken and the report prepared by Dr. P.R.J. Gangadharam. assisted by Mr. K. Mohan (Statistical Assistant) and Miss V. Devaki (Research Assistant). This report should be referred to as : Indian Council of Medical Research First Drug Resistance Investigation. Reprints can be obtained from the Director, Tuberculosis Chemotherapy Centre, Chetput, Madras-31.

This paper reports the findings of the first of these investigations, and presents information regarding the prevalence of drug resistence in patients reporting for the first time with symptoms at 9 urban chest clinics and denying history of previous anti-tuberculosis chemotherapy. Subsequent investigations deal with the prevalence of drug resistance among *all* patients, with or without a history of previous chemotherapy, reporting for the first time with symptoms at the same urban clinics and in certain semi-urban clinics ; the findings will be reported later.

PLAN AND CONDUCT OF THE INVESTIGATION.

Participating centres. – Nine urban centres from different parts of the country, all having air connections with Madras participated in this investigation. These centres are situated at :

1. Amritsar (R.B. Sir Gujjarmal Kesardevi Tuberculosis Sanatorium),
2. Bangalore (Lady Willingdon Tuberculosis Demonstration and Training Centre),
3. Bombay (Tuberculosis Clinic, J.J. Group of Hospitals),
4. Calcutta (K.S. Ray Tuberculosis Hospital*),
5. Delhi (New Delhi Tuberculosis Centre),
6. Hyderabad (Institute of Diseases of Chest and Tuberculosis),
7. Madras (Government Tuberculosis Demonstration and Training Centre),
8. Nagpur (Tuberculosis Control and Training Centre), and
9. Patna (Tuberculosis Demonstration and Training Centre).

Criteria for eligibility of patients. – A patient was eligible for inclusion in this investigation if he/she

(a) was aged 12 years or more,

(b) had resided in the area served by the clinic in the previous year, and was expected to continue there for another year,

(c) was attending the clinic for the first time and because of symptoms (patients detected by mass miniature radiography were not eligible),

(d) had radiographic evidence suggestive of tuberculosis (however, patients with evidence of only *minimal* disease, calcification, pleural thickening, hilar gland enlargement or fibrotic foci were not eligible), and

(e) had not received antituberculosis chemotherapy previously or had received it for less than 10 days.

* This institution was unable to provide adequate numbers of specimens speedily and consequently, specimens were also obtained from three other institutions in Calcutta-namely, the University of Calcutta Medical College, Tuberculosis Demonstration and Training Centre and B.R. Singh Railway Hospital.

Procedures followed by the participating centres.– At each of the 9 Centres, from each of 300 consecutive patients satisfying the above criteria, a specimen of sputum was collected in a sterile universal container (supplied by the Central Laboratory) under the direct supervision of the clinic staff. This specimen was then despatched by air as quickly as possible to the Central Laboratory in Madras ; if there was any delay, the specimen was stored in an ice-box or a refrigerator, wherever this was practicable.

Great stress was laid on the importance of obtaining accurate and detailed histories of previous antituberculosis chemotherapy. This was done by correspondence and by visits to each of the participating centres by Dr. Gangadharam of the Central Laboratory. The physicians-in-charge were asked to supplement their efforts, whenever possible, with those of other members of their staff. Furthermore, about 4 to 6 weeks later, a second interrogation was undertaken, with no knowledge of the sensitivity test results.

Investigations undertaken at the Central Laboratory.– The sputum specimens were processed by the Central, Laboratory usually on the day of receipt. On a few occasions (less than 2 per cent) when this was not possible, they were stored overnight in a refrigerator and processed the following day. The following investigations were undertaken :

(1) Direct smear examination using fluorescence microscopy, the positive results being graded as 3-plus (heavy), 2-plus (moderate) or 1-plus (scanty) (Holst, Mitchison and Radhakrishna, 1959).

(2) Culture examination for tubercle bacilli, employing the procedures described by Tuberculosis Chemotherapy Centre, Madras (1959) Growth typical of *Mycobacterim tuberculosis* was recorded as 3-plus (confluent), 2-plus (innumerable discrete colonies), 1-plus (100-20 colonies) or the actual number of colonies, if less than 20.

(3) Tests of sensitivity to isoniazid, streptomycin, PAS and thioacetazone* within 2 or 3 days of the culture becoming positive.

An inoculum suspension was prepared by adding approximately 2 mg. (moist weight) of bacilli, as judged by eye, to a ¼ oz. screw-capped bottle containing 0.5 ml. of sterile distilled water and 5 glass beads, and shaking the bottle mechanically for 1 minute. The resulting suspension (standard) was used for the isoniazid and streptomycin sensitivity tests, a 10-fold dilution of this suspension for the PAS sensitivity test as recommended by Yukon *et al.* (1960), and both the standard suspension and the 10-fold dilution for the thioacetazone sensitivity test. (In the case of the thioacetazone test, the drug was dissolved in triethylene glycol before incorporation in the medium, the final concentration of triethylene glycol being 0.5 per cent in all slopes ; also, an additional drug-free slope containing 0.5 per cent triethylene glycol was set up).

A standard (approximately 3 mm.) loopful of the appropriate suspension was inoculated on to Lowenstein-Jensen medium slopes containing the drug concentrations (expressed as the amounts added before inspissation) set out below, as well as on a drug-free slope as control.

* Thioacetazone is the recommended international non-proprietary name (see World Health Organization, 1962) for 4'-formylacetanilide thiosemicarbazone (thioacetazone, TB1-698).

Drug.	*Drug concentration (µg./ml.)* Test strain.	H37Rv.
Isoniazid	0.025, 0.05, 0.1, 0.2, 1, 5, 50	0.025, 0.05, 0.1, 0.2, 1
Streptomycin	1, 2, 4, 8, 16, 32, 64	1, 2, 4, 8
Sodium PAS dihydrate	0.125, 0.25, 0.5, 1, 2, 4, 8, 16	0.125, 0.25, 0.5, 1, 2
Thioacetazone	0.25, 0.5, 1, 2, 4, 8, 16, 32	0.25, 0.5, 1, 2, 4, 8, 16, 32

With each batch of tests the standard sensitive strain of *Mycobacterium tuberculo*sis, H37Rv, was also set up as a control. The results of all the tests were read at the end of 4 weeks of incubation at 37°C. The results of isoniazid and thioacetazone sensitivity tests were expressed as MICs–that is, the minimum concentrations inhibiting growth (defined as 20 colonies or more), while those of streptomycin and PAS were expressed as resistance ratios–that is, the MIC of the test strain divided by the MIC of the strain H37Rv. If the MIC of isoniazid was 1 µg./ml. or the resistance ratio to streptomycin or PAS was 4, the test was repeated.

Definitions of drug resistance.– For isoniazid and streptomycin, the definitions employed are similar to those in use at the Tuberculosis Chemotherapy Centre, Madras, which have been found to be prognostically significant [Devadatta *et al.*, 1961; Tripathy *et* al. (in preparation), Tuberculosis Chemotherapy Centre, Madras (in preparation)].

Isoniazid.– MIC of 5 or more, *or* 1 followed by 1 or more in the retest.

Streptomycin.-Resistance ratio of 8 or more, *or* 4 followed by 4 or more in the retest.

For PAS and thioacetazone, satisfactory definitions of resistance are yet to be evolved for Indian tubercle bacilli. Thus, although cultures from untreated Madras patients are known to be frequently resistant *in vitro* to PAS (Selkon *et al., loc. cit.) and* to thioacetazone (Thomas *et al.*, 1961 ; Mitchison and Lloyd, 1964), the finding has had no effect on the outcome of treatment in the case of PAS (Tuberculosis Chemotherapy Centre, Madras, 1960, 1966) and only a slight effect in the case of thioacetazone (Tuberculosis Chemotherapy Centre, Madras, 1966). Consequently, for both these drugs, the findings ofsensitivity tests are presented in the form of distributions.

(4) A series of identification tests for *Mycobacterium tuberculosis :*

(a) Niacin production test, as described by Medveczky (1960). A positive result was indicated by the presence of pink colour within 10 minutes, and a negative result by the formation of a white precipitate.

(b) Qualitative catalase test, as described by Selkon and Mitchison (1959). A positive result was indicated by the liberation of oxygen bubbles within 60 seconds ; the degree of positivity was graded as 1-plus, 2-plus or 3-plus depending upon the number and speed of the evolution of the bubbles.

(c) Growth on Lowenstein-Jensen medium after incubation at 25°C. for 4 weeks.

(d) Pigmentation and morphology of growth after incubation at 37°C. for 4 weeks, in -the dark and in the light.

INTAKE OF SPECIMENS AND ASSESSMENT OF PREVIOUS ANTITUBERCULOSIS CHEMOTHERAPY.

The intake of specimens commenced in March 1964 and concluded in November 1965, the period of intake for the individual Centres varying from 12 to 18 months. In all, 2,707 sputum specimens were collected from the same number of patients and despatched to the Central Laboratory from the 9 Centres. Of these, 111 (4.1 per cent) have not been considered further in this report, 5 because they were from patients who were less than 12 years of age, 36 because the bottles had broken or the sputum leaked out during transit, and 70 because they were from patients whose history of previous chemotherapy was vague. The remaining 2,596 specimens are considered for the analyses.

The final assessment of a patient's history of previous chemotherapy was usually based on the findings of the requestioning at 4 to 6 weeks ; however, for 9 per cent of the patients (in whom the requestioning had not been undertaken), this was based on the findings at the initial questioning. Further, only specific antituberculosis chemotherapy has been considered and treatment with unknown powders, tablets or injections, or with Ayurvedic, Unani or Homeopathic drugs has been disregarded. On this basis, 2,278 of the 2,596 patients were classified as having received no specific antituberculosis chemotherapy, 103 as having received less than 10 days of chemotherapy and 215 as having received 10 or more days of chemotherapy. In the next section, the findings are presented together for the former two categories (a total of 2,381 patients).

RESULTS.

Sex and age.– Of the 2,381 patients, 1,582 (66 per cent) were males, the proportions in individual centres ranging from 50 per cent in Patna to 76 per cent in Madras. The age distributions for the males and the females are presented in Table I, separately and for the 9 Centres combined.

Smear and culture results.– The results of direct smear examination and culture examination of sputum specimens are set out in Table II. In all, 74 per cent of the specimens yielded a positive smear, the proportions in the individual Centres varying from 67 per cent in Patna to 81 per cent in Amritsar. Examination by culture increased to 79 per cent the overall proportion of specimens with a positive result. Considering the individual Centres, the proportion with a positive culture varied from 68 per cent in Patna to 88 per cent in Hyderabad.

In all, 131 (6 per cent) specimens yielded a smear-positive culture-negative result, the proportions in individual Centres ranging from 3 per cent in Hyderabad to 10 per cent in Calcutta. Most of these specimens, namely 117 (89 per cent), were scantily positive on smear, while 10 (8 per cent) were moderately positive and the remaining 4 (3 per cent) heavily positive.

Contamination of the culture occurred in 111 (5 per cent) specimens, ranging from 2 per cent in Bangalore and Bombay to 9 per cent in Madras. It occurred in 5 per cent of 630 smear-negative specimens as compared to 4 per cent of 1,747 smear-positive specimens, a trivial difference.

TABLE I.

Distribution of the patients according to sex and age.

Centre.	MALES :					FEMALES :				
	Total.	Percentage of patients.				Total.	Percentage of patients.			
		Less than 25 years.	25-34 years.	3 5 - 4 4 years.	45 years or more.		Less than 25 years.	25—34 years.	35—44 years.	45 years or more
Amritsar	142	36	20	18	27	97	31	42	14	12
Bangalore	176	24	32	26	18	113	34	35	24	7
Bombay	199	31	37	15	17	71	53	28	11	6
Calcutta	202	23	31	21	24	71	39	39	17	4
Delhi	195	35	25	25	15	82	40	35	17	7
Hyderabad	166	27	25	22	26	118	34	43	17	6
Madras	222	23	30	24	23	67	39	37	12	12
Nagpur	179	23	38	21	18	81	36	48	16	5
Patna	101	33	27	14	27	99	47	34	10	8
All centres	1582	28	30	21	21	799	39	38	16	8

TABLE II.

Results of direct smear examination and culture examination of sputum specimens.*

Smear Culture.	PERCENTAGE OF SPECIMENS :									
	Amritsar.	Bangalore.	Bombay.	Calcutta.	Delhi.	Hyderabad.	Madras.	Nagpur.	Patna.	All centres.
Negative Negative	7	15	8	15	10	6	10	12	20	11
Negative Positive	11	14	13	15	21	15	13	12	10	14
Negative Contaminated	1	1	0	1	1	1	1	1	2	1
Positive Negative	4	6	7	10	4	3	4	4	8	6
Positive Positive	76	63	68	56	58	73	63	66	56	64
Positive Contaminated	2	1	2	3	6	2	8	5	3	4
Positive smear	81	70	76	70	68	78	75	75	67	74
Positive culture	87	79	83	71	79	88	76	78	66	79
Total specimens	239	289	269	273	277	282	268	260	200	2377

*Excluding 4 specimens for which, by oversight, direct smear examination was not undertaken.

Transit time.– Despite all efforts, long delays occurred on some occasions between the collection of the specimens and their arrival at the Central L&oratory. Defining 'transit time' as the interval (in days) between the date of collection of the sputum and the date of receipt of the specimen at the Central Laboratory, the mean transit time was 1.9 days for Bangalore, 2.1 for Hyderabad, 2.2 for Nagpur, 35 for Bombay, 4.0 for Patna, 4.2 for Amritsar, 5.9 for Calcutta and 8.5 for Delhi. (The Madras Centre was situated in the same compound as the Central Laboratory). These figures do not bear any relationship to the percentages of specimens with a positive culture or the percentages with a contaminated culture (Table II).

Analyses were undertaken to study whether transit time influenced the frequency with which specimens with a *positive smear* result yielded a negative culture result. The findings are presented in Table III. For 4 of the 9 Centres, namely, Bombay, Calcutta, Hyderabad and Patna, there was some evidence that specimens with a transit time of 3 days or more were more often culture-negative than those with a transit time of 2 days or less, the proportions being 14 per cent and 7 per cent for Bombay ($P > 0.2$), 16 per cent and 6 per cent for Calcutta ($P = 0.4$), 12 per cent and 1 per cent for Hyderabad ($P < 0.01$), and 17 per cent and 5 per cent for Patna ($P=0.08$).

TABLE III.

Transit time related to culture negativity in specimens with a positive smear result.*

Centre.	3 DAYS OR MORE IN TRANSIT :		2 DAYS OR LESS IN TRANSIT :	
	Number of specimens with a positive smear.	Of these, per cent with a negative culture.	Number of specimens with a positive smear.	Of these, per cent with a negative culture.
Amritsar	171	5	21	5
Bangalore	49	8	154	8
Bombay	42	14	163	7
Calcutta	173	16	18	6
Delhi	151	6	36	8
Hyderabad	50	12	168	1
Nagpur	62	5	134	6
Patna	78	17	55	5

*For definition, see above.

Sensitivity test results.– Of the total of 2,381 specimens, 1,871 yielded a positive culture. Of these, 15 were found to contain atypical mycobacteria (and will be reported separately), and 13 had no sensitivity test results for any of the drugs on account of contamination in the test or drying-up of the culture. The remaining 1,843 cultures, all of which were identified as *Mycobacterium tuberculosis* are considered below. Of these, 99.4 per cent had a sensitivity result for isoniazid, 99.8 per cent for streptomycin, 99.5 per cent for PAS, and 98.6 per cent for thioacetazone.

Isoniazid and streptomycin.– Table IV presents the findings of isoniazid and streptomycin sensitivity tests in the 9 Centres. Considering all Centres together,

TABLE IV.

Findings of isoniazid and streptomycin sensitivity tests.

Centre.	Number of cultures with H or S sensitivity results.	PERCENTAGE OF CULTURES WITH RESISTANCE TO :					
		H only.	S only.	H+S.	H.	S.	One or both drugs.
Amritsar	**202**	*8*	*7*	*5*	*14*	*13*	*21*
Bangalore	**223**	*6*	*3*	*5*	*11*	*8*	*14*
Bombay	**221**	*10*	*9*	*10*	*20*	*19*	*29*
Calcutta	**191**	*7*	*8*	*11*	*18*	*20*	*27*
Delhi	**213**	*9*	*5*	*4*	*13*	*9*	*18*
Hyderabad	**245**	*9*	*3*	*6*	*15*	*10*	*18*
Madras	**217**	*6*	*5*	*7*	*13*	*12*	*18*
Nagpur	**196**	*5*	*4*	*6*	*11*	*10*	*15*
Patna	**130**	*14*	*9*	*4*	*18*	*14*	*27*
All centres	**1838**	*8.2*	*5.8*	*6.5*	*14.7*	*12.5*	*20.4*

H. = isoniazid S. = streptomycin.

the prevalence of drug resistence was 8.2 per cent for isoniazid *alone* and 5.8 per cent for streptomycin *alone,* the prevalence in individual centres ranging from 5 per cent in Nagpur to 14 per cent in Patna for isoniazid alone, and from 3 per cent in Bangalore and Hyderabad to 9 per cent in Bombay and Patna for streptomycin alone. Resistance to both isoniazid and streptomycin was observed in 6.5 per cent of the cultures, the corresponding proportions in the individual centres ranging from 4 per cent in Delhi and Patna to 11 per cent in Calcutta.

The total resistance to isoniazid was 14.7 per cent and the *total* resistance to streptomycin 12.5 per cent. Considering the individual centres, the prevalence of total isoniazid resistance ranged from 11 per cent in Bangalore and Nagpur to 20 per cent in Bombay, while the prevalance of total streptomycin resistance ranged from 8 per cent in Bangalore to 20 per cent in Calcutta. Finally, considering resistance to one or both drugs, Bombay had the highest prevalence (29 per cent) and Bangalore the lowest (14 per cent), the proportion for all the 9 Centres together being 20.4 per cent.

Resistance related to sex and age.– Analyses (not tabulated here) were undertaken to examine whether the sex or the age of the patient was associated with the finding of *(a)* isoniazid resistance (irrespective of streptomycin sensitivity results), *(b)* streptomycin resistance (irrespective of isoniazid sensitivity results) and *(c)* resistance to both isoniazid and streptomycin. Resistance to isoniazid was observed in 14 per cent of the males as compared with 16 per cent of the females, the corresponding proportions being 13 per cent and 11 per cent for resistance to streptomycin and 6 per cent and 6 per cent for resistance to both isoniazid and streptomycin. Next, in patients aged less than 25 years, 13 per cent had an isoniazid-resistant culture as compared with 17 per cent in those aged 25 to 34 years, 14 per cent in those aged 35-44 years and 14 per cent in those aged 45 years or more. The corresponding proportions were 12, 13, 11 and 12 per cent for streptomycin resistance and 6, 7, 7 and 5 per cent for resistance to both

isoniazid and streptomycin. Thus, neither sex nor age was associated with the finding of drug resistance.

Comparison of isoniazid-sensitive and isoniazid-resistant strains for catalase activity.– Qualitive tests for catalase activity were undertaken on 1,818 (99 per cent) of the 1,838 positive cultures with sensitivity tests ; of these, 1,549 were isoniazid-sensitive and the remaining 269 isoniazid-resistant. All except 19 (1 per cent) of the isoniazid-sensitive cultures were catalase-positive, a great majority (76 per cent) having 2-plus activity. In contrast, 25 per cent of the isoniazid-resistant cultures were catalase-negative, 25 per cent had 1-plus activity and the remaining 50 per cent had 2-plus activity.

PAS.– The findings of PAS sensitivity tests are set out, in the form of distributions (for reasons given on page 1620) in Table V. Considering all centres, 4.2 per cent of the cultures had a resistance ratio (RR) of 8 or more, 4.5 per cent an RR of 4 and the remaining 91.4 per cent an RR of 2 or less ; of 74 cultures with a high RR (namely, 8 or more), 45 (61 per cent) were resistant to isoniazid or streptomycin or both. In the individual centres, the proportion with an RR of 8 or more varied from 2 per cent in Hyderabad to 8 per cent in Calcutta.

TABLE V.

Findings of PAS sensitivity tests.

Centre.	Number cultures tested.	PERCENTAGE OF CULTURES WITH A RESISTANCE RATIO OF:		
		2 or less.	4.	8 or more.
Amritsar	202	*93*	*3*	*3*
Bangalore	222	*91*	*6*	*3*
Bombay	221	*90*	*5*	*6*
Calcutta	191	*87*	*5*	*8*
Delhi	213	*90*	*7*	*3*
Hyderabad	243	*94*	*5*	*2*
Madras	216	*93*	*2*	*5*
Nagpur	195	*92*	*4*	*4*
Patna	129	*95*	*3*	*3*
All centres	**1822**	***91.4***	***4.5***	***4.2***

Thioacetazone.– The findings of thioacetazone sensitivity tests, based on the standard suspension are set out in Table VI in the form of distributions (for reasons given on page 1620) and geometric means. Considering all centres, 15.5 per cent had an MIC of 4 µg./ml. or more, 22.1 per cent on MIC of 2 µg./ml. and 62.4 per cent an MIC of 1 µg./ml. or less. In the individual centres, the proportion with a high MIC (namely, 4 µg./ml. or more) varied considerably ; thus, it was 10 per cent or less in Amritsar, Delhi and Patna, 11 to 15 per cent in Nagpur, Hyderabad and Bangalore, 18 per cent in both Bombay and Calcutta and 30 per cent in Madras. These geographic differences in sensitivity are typified by the means (geometric) in the next column, which ranged from 0.89 µg./ml. in Amritsar to 2.38 µg./ml. in Madras.

The findings with the 10-fold dilution also showed a wide variation in sensitivity, as may be seen from the geometric means in the last column of Table VI ; again, Amritsar had the lowest mean (0.57 µg./ml.) and Madras the highest (1.35 µg./ml.).

TABLE VI.

Findings of thioacetazone sensitivity tests.

Centre.	STANDARD SUSPENSION :					10-FOLD DILUTION :
	Number of cultures tested.	Percentage of cultures with an MIC (µg./ml.) of :			Geometric mean of M1Cs.	Geometric mean of MICs.
		1 or less.	2.	4 or more.		
Amritsar	**199**	*73*	*17*	*10*	**0·89**	**0.57**
Delhi	**211**	*77*	*13*	*9*	**0·91**	**0.62**
Patna	**129**	*77*	*13*	*10*	**1·01**	**0.65**
Nagpur	**196**	*70*	*17*	*13*	**1·03**	**0.64**
Hyderabad	**243**	*65*	*20*	*15*	**1·09**	**0.69**
Bombay	**221**	*63*	*19*	*18*	**1·31**	**0.80**
Bangalore	**219**	*58*	*31*	*15*	**1·39**	**0.87**
Calcutta	**187**	*57*	*26*	*18*	**1·57**	**0.94**
Madras	**213**	*31*	*39*	*30*	**2·38**	**1.35**
All centres	1818	62.4	22.1	15.5	1.24	0.77

Patients who had received specific antituberculosis chemotherapy for 10 or more days.– Of the 215 patients who had received specific antituberculosis chemotherapy for 10 or more days, 166 had a positive culture with a sensitivity test result for at least one of the drugs. Resistance to isoniazid was observed in 25 per cent of these cultures and to streptomycin 14 per cent, including 11 per cent in which resistance to both the drugs was observed.

DISCUSSION.

India is now in the early stages of her national tuberculosis control programme, which aims at offering efficacious chemotherapy to all cases of pulmonary tuberculosis detected by direct microscopy. Since chest clinics are an obvious starting point for such a programme, a precise knowledge of the prevalence of drug resistance in patients reporting *for the first time* at such clinics would be of great value. The present report gives information on this subject for 9 urban clinics, among patients giving no history of antituberculosis chemotherapy or a history of at most 9 days.

At the outset, it must be emphasised that this investigation should not be regarded as a national survey of drug resistance, like, for instance, the surveys reported from England (Fox *et al.*, 1957 ; Miller *et al.*, 1966). Thus, no statistical sampling procedures were employed, the choice of the 9 urban Centres being governed solely by administrative reasons, such as good air connections to Madras, where the Central

Laboratory was situated. Furthermore, it is likely that, even in the limited areas catered to by these clinics, not all tuberculous patients were covered ; for instance, in Tumkur district, Banerjee and Anderson (1963) observed that only 11 of 21 patients with a positive smear reported at chest clinics on their own for treatment.

The prevalence of drug resistance in this study was quite high–namely, 14.7 per cent to isoniazid and 12.5 per cent to streptomycin, including 6.5 per cent with resistance to both the drugs. In other words, as many as 20.4 per cent of the patients had organisms resistant to one or both of these very potent drugs. These figures are fairly similar to those reported from Hong Kong (Hong Kong/British Medical Research Council Drug Resistance Survey, 1964), Ghana (Bell and Brown, 1960) and East Africa (East African/British Medical Research Council Pretreatment Drug Resistance Report, 1963), but are appreciably higher than those reported from England (Miller *et al., loc. cit.),* U.S.A. (United States Public Health Service, 1964, Hobby *et al.,* 1964) and Canada (Armstrong, 1966).

Considering next the findings in the 9 individual centres, wide variation in the prevalence of drug resistance was observed, cities like Bombay, Calcutta and Patna having a high prevalence and Bangalore and Nagpur a low prevalence. The figure for Delhi, namely, 13 per cent to isoniazid is similar to 14 per cent reported by the New Delhi Tuberculosis Centre (1962) and to 16 per cent reported by Balbir Singh *(loc. cit.)* for the same area ; for Hyderabad, the proportion for isoniazid resistance, namely, 15 per cent was the same as reported by Menon *(loc. cit.).* The proportions for Madras, namely, 13 per cent for isoniazid and 12 per cent for streptomycin, are considerably higher than the 3.7 per cent and 4.3 per cent, respectively, reported by the Tuberculosis Chemotherapy Centre, Madras (1964) ; since the bacteriological techniques employed were the same in both laboratories, the lower figures from the Tuberculosis Chemotherapy Centre are presumably due to the very intensive interrogation of the patients at the Tuberculosis Chemotherapy Centre and consequent exclusion of almost all the patients with acquired drug-resistance.

In the absence of clinically meaningful definitions of resistance to PAS and thioacetazone, it is difficult to interpret the findings of tests of sensitivity to these drugs. However, an interesting feature concerning thioacetazone sensitivity has emerged from this study, and may have implications on the value of thioacetazone for the treatment of tuberculosis in India. This is the existance of large difference in thioacetazone sensitivity between the 9 Centres, with cultures from the Madras patients being considerably less susceptible than cultures from the other centres.

The reliance to be placed on the findings of an investigation of this type depends on the technical standards maintained in the laboratory and the accuracy of histories of previous chemotherapy. The use of a central laboratory for all the Centres, instead of individual laboratories at each of the 9 centres, ensured uniformity of procedures and standards. Furthermore, special investigations undertaken concurrently in the Central Laboratory and the well-established laboratory at the Tuberculosis Chemotherapy Centre, Madras, showed that the standards of smear and culture examination and sensitivity tests were as highly satisfactory in the former as in the latter. Finally, adequate

identification tests were undertaken to confirm that the positive cultures isolated were typical tubercle bacilli of the human type.

As regards histories of previous chemotherapy, there are reasons to believe that these may not always have been complete and accurate. Firstly, as the work connected with this investigation constituted an additional responsibility for the centres, sufficient staff and time were not always available in some of the centres for interrogating the patients carefully. Secondly, the regulations in some centres (with hospital facilities) put a premium on patients not having had previous chemotherapy, and might consequently have led some patients to conceal the truth. Thirdly, lack of proper utilization of existing home visiting facilities and non-availability of 9 per cent of the patients for the requestioning at 4 to 6 weeks are also likely to have affected the accuracy of the histories of previous chemotherapy. (In this context, it is interesting to note that, of 2,329 patients who at the initial questioning, denied having received any chemotherapy previously 202 (8.9 per cent) confessed, at the requestioning at 4 to 6 weeks, to having received chemotherapy for at least 10 days).

In view of these limitations, it is specially emphasised that the figures for resistance in the present investigation *must not* be regarded as indicative of the prevalence of *primary* drug resistance in the urban clinics, but as a mixture of true primary drug resistance and an unknown amount of acquired drug resistance. Nevertheless, the findings are of considerable importance to the clinicians-in-charge of the 9 centres as they indicate the magnitude of drug resistance they can expect in patients reporting to them for the first time with symptoms, but claiming that they had no previous antituberculosis chemotherapy. Finally, this study has shown that, despite numerous organizational problems involved in a vast developing country like India, it is possible to conduct a co-operative investigation involving several centres. This finding is encouraging for the conduct of future co-operative investigations in India.

SUMMARY.

1. A co-operative study was undertaken to estimate the prevalence of drug resistance in tuberculous patients who reported for the first time with symptoms at 9 urban chest clinics in different parts of India, having received no chemotherapy previously or received it for, at the most, 9 days.

2. At each centre, one specimen of sputum was collected from each of about 300 consecutive patients and despatched, by air, to a Central Laboratory at Madras.

3. The prevalence of resistance, based on over 1800 positive cultures were 14.7 per cent to isoniazid and 12.5 per cent to streptomycin, *including* 6.5 per cent to both the drugs.

4. Large differences were observed between the 9 Centres in the susceptibility to thioacetazone.

Besides the officers-in-charge, several other staff members of the participating centres contributed to the success of this investigation. Competent technical assistance

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