SECONDARY BACTERIAL FLORA IN PATIENTS WITH PULMONARY TUBERCULOSIS -A PRELIMINARY REPORT

RATHNASABAPTHY, S. V., PARAMASIVAN, C. N., SHANMUGASUNDARAM, N., AND JAGANNATHAN, K.

Institute of Tuberculosis and Chest Diseases; Tuberculosis Research Centre(ICMR), Madras 600 031 and Stanely Medical College, Madras 600 001.

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ABSTRACT

Sputum samples from 100 smear positive or skiagram positive pulmonary tuberculosis patients were cultured for superinfecting or co-injecting bacteria. These patients were equally divided into five groups. This included Croup-I who are not treated; Group-II who are treated up to three months; Group-III who are treated for more than three but less than six months; Group-IV treated more than six months and lastly Group-V who have completed the prescribed treatment schedule of varying durations. Neisseria catarrhalis *and* Strep. viridans *predominated in all patients irrespective of group, other organisms isolated, were* Micrococci, E.Coli, Serratia, Proteus *and* Pseudomonas. *There was no significant difference in the pattern of organisms isolated from different group of patients. The antibiogram showed the usual susceptibility pattern*

Introduction

Superinfection and co-infection in pulmonary tuberculosis has been described as early as 1919 by Berghaff[1] *who reported influence as a factor in the reactivation of quiescent and healed pulmonary tuberculosis. This was followed by Crassford's report*[2] *on the occurence of active pulmonary tuberculosis with pneumococci pneumonia. Since then* Staphylococci, Pneumococci, micrococci, Salm. typhymurium, Candida-albicans. Aspergillus *species etc., have been reported to be associated with active and healed pulmonary tuberculosis*[3,4,5.] *More recently Milder and Rough*[5] *have reported a case of pulmonary tuberculosis being complicated by secondary infection with* Leqionella pneumophila[6].

These superinfecting organisms in pulmonary tuberculosis need to be observed carefully because of two factors. Firstly, after the introduction of potent chemotherapeutic drugs in the treatment of tuberculosis, many M. tuberculosis *strains have acquired resistance. Because of the availability of these drugs in the system even the normal respiratory commensals could acquire resistance and are likely to cause recurrent acute respiratory tract infections in these tuberculosis patients. Secondly, the animal experimental studies of Pointdexter*[7] *show that* Strep. Viridans *and* Staph.aureus *; can enhance the tissue invasion and pathogenic process of pulmonary tuberculosis.*

Considering the importance of these superinfecting organisms, the authors report here a preliminary study on 100 patients with different treatment profiles, the spectrum of bacteria super-infecting the respiratory tract of patients with pulmonary tubercuiosis and the antibiotic susceptibility pattern of these bacteria.

Material and Methods

Subjects

Patients attending the Institute of Tuberculosis and Chest Diseases at Madras formed the study population. The diagnosis of pulmonary tuberculosis was based on clinical findings, skiagram examination and sputum smear testing for acid-fast bacilli. Depend-

ing on the treatment status. the patients were equally divided into five groups as follows: Group-1 patients who are not treated; Croup-II patients treated for 0 - 3 months; Group-III patients treated for 3 - 6 months; Group IV patients treated for 6 months and Group-V patients who have completed the prescribed period of treatment. For all the patients, the following examinations were done with sputum specimens obtained at a single point.

Sputum smears for acid fast bacilli

This was done by the Ziehl Neelsen's acid fast staining procedure. [8]

Bacterial culture of sputum for secondary organisms

This was done by standard cultural procedures. The media used were chocolate agar, blood agar and MacConkey agar. The identification of the organisms was done by employing appropriate biochemical and serioloqical reactions.

Antibiotic susceptibility test

Stoke's disc diffusion method [9] *was employed for this purpose. The antibiotics tested against were streptomycin, tetracycline, ampicillin, gentamycin, kanamycin and erythromycin.*

Results

The age and sex distribution of the study patients are given in Table-l.

TABLE-1

Age and Sex distribution of patients in five treatment groups (n = 100)

Treat-ment Croups	Male			Female		
	Number	Age range	Mean	Number	Age range	Mean
I	12	20-63	41.3	8	12-27	20.6
II	12	19-65	34.0	8	20-45	28.7
III	19	16-60	33.6	1	< 2 7	27.0
IV	10	30-60	44.6	10	9-60	25.6
V	17	27-73	49.6	3	24-05	36.6

The smear and skiaqram findings are presented in Table 2.

TABLE-2

Smear and skiagram positivity among patients (n = 100)

Categqry	Groups I	II	III	IV	V
Smear positive	5	16	12	13	9
X-ray positive	15	4	8	7	11
Total	29	20	20	23	20

Distribution of non pathoaenic organisms and potential pathogens which were most frequently isolated from the different groups of patients is given in Table-3.

They were N.Catarrhalis, Strep. viridans, Micrococcus, E.Coli, Kiebsiella Serriatia, Proteus *and* Ps. aerugionsa. *Most of*

TABLE-3

Spectrum of bacterial flora isolated in different groups

Category	Groups I	II	III	IV	V
Organisms most frequently isolated	Neisseria Strep. viridans	Neisseria Strep. viridans	Neisseria Strep. viridans	Neisseria Strep. viridans	Neisseria Strep. viridans
Potential patho gens isolated in order of frequency*	Klebsiella E. Coli Proteus	Klebsiella Proteus Serratia	Klebsiella Pseudo-monas	Klebsiella Proteus Pseudo-monas	Klebsiella E. Coli Pseudo-monas

* from above downwards.

these were isolated in pure culture except Serratia *which was found always in combination with any one of the other organisms mentioned above. The results of the antibiotic susceptibility testing are given in Table-4.*

TABLE-4

Antibiogram pattern on a sample of 95 isolates

Strains	NO. tested	Streptomycin			Tetracycline			Ampicillin			Gentamycin			Kanamycin			Erythromycin		
		HS	MS	NS	HS	MS	NS	HS	MS	NS	HS	MS	NS	HS	MS	NS	HS	MS	HS
Strep.Viridans	54	47	6	1	34	8	12	44	5	5	54	0	0	52	2	0	54	0	0
Micrococci	7	7	0	0	1	1	5	3	2	2	7	0	0	7	0	0	7	0	0
E.Coli	3	3	0	0	0	1	2	1	1	1	3	0	0	3	0	0	3	0	0
Kelbsiella	13	13	0	0	11	0	2	12	0	1	13	0	0	13	0	0	13	0	0
Proteins	10	10	0	0	1	1	8	1	4	5	10	0	0	9	1	0	9	1	0
Pseudomonas	8	5	1	2	0	0	8	0	0	8	8	0	0	7	0	1	4	3	1

HS: Highly sensitive; MS: Moderately sensitive; NS: Non-sensitive.

Discussion

Though superinfection with bacterial, fungal and viral agents are well recognised in pulmonary tuberculosis, attending physicians of these patients have a general tendency to overlook these organisms. Animal experiments have revealed that Strep. Viridans *can act as a sensitizer which can interfere with' effective fibroblastic action in walling off* M. tuberculosis.. *This can enhance the spread of Mycobacteria and hence increase the tissue damage. The necrotic action of* Staph. aureus *and the systemic action of the toxic fractions from the secondary infecting organisms interfere with the effective reticulo endothelial response necessary to arrest the disease process in tuberculosis.*

Included in this study are five groups of patients, namely, the untreated, patients under treatment for varying periods of time and those who had completed treatment. Any difference in the pattern of superinfecting organisms and their susceptibility to drugs were looked for. It was found that Neisseria *and* Strep.viridans *predominated in all patients irrespective of the groups. They are in fact the predominant normal flora in the respiratory tract. But in the light of animal experimental model mentioned this finding gains importance. To get a clinical evidence for the experimental model, one needs to follow up the patients with predominant* Strep . Viridans *in their respiratory tract further in order to observe any differences in the severity of pathogenic process or prognosis of the disease process itself. In the absence of similar model for* Neisseria *this human study requires further follow up.*

Further, this study did not show any significant difference in the frequency and spectrum of bacteria isolated from different groups of patients. As this work is limited in scope and since the authors have not looked for other pathogens like Legionella *species, anaerobic bacteria, fungal and parasitic agents involved in respiratory illness. no firm conclusions can be drawn at present. The antibiogram results have revealed the usual pattern expected otherwise from patients with respiratory infections.*

This work underlines the importance of a more detailed investigation covering a wide range of micro organisms in pulmonary tuberculosis patients. This would provide more information about the effect *of these superinfecting organisms in augmenting the disease process in tuberculosis. Hopefully, a carefully planned future study with suitable controls will throw further light on the subject.*

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REFERENCES

1. Bergnoff, R.S., (1919) Influenza as a factor in the reactivation of quiescent and healed pulmonary tuberculosis. Am. Rev. Tuberc. **3,** 370-371.

2. Crossford, J.H., (1939). Pneumococcal pneumonia complicating pulmonary tuberculosis, treated with Mand B 693. Brit. Med. J.1, 606-609.

3. Kronenberger, F.L., Smith, H.S., (1966). The unusual occurence of an acid-fast saprophyte and Salmonella typhimurium in association with a pumonary lesion. Brit. J. Tubercl. **50,** 223-225.

4. Jain, S.K., Agarwal, R.L., Agarwal, M., (1982). Aspergillus infection in pulmonary tuberculosis. Indian. J. Med. Sci. **36,** 48-50.

5. Agarwal, S.K., Tewari, L., Singh, M., (1983). Non-tuberculous bacterial flora of sputum in pulmonary tuberculosis. Indian, J., Publ. Health, **27,** 55-59.

6. Milder, J.E., Rough R.R., (19221. Recurrent Legionnaires' Disease and active pulmonary tuberculosis. Am. Rev. Respir. Dis. **125,** 759-761.

7. Poindexter, H.A., (1943). Multiple bacteriological aetiology in clinical tuberculosis. Experimental observations. The Am. Rev. Tuberc. **47,** 635-646.

8. Bailey, W.R., Scott, E.G., (1974). Diagnostic Microbiology. The C.V. Mosby Company edition **3,** 197-199.

9. Bondi, A., Spauldin, E.H., Smith, E.D., and Dietz, C.L., (1947). A routine method for the rapid determination of susceptibility to peniciliin and other antibiotic. Am. J. Med. Sci. **214,** 221-225

Correspondence/request for reprints to: Dr. S.V. Rathnasabapathy, 42 Bazullah Road, T.Nagar, Madras-600 017.