MYCOBACTERIA OTHER THAN TUBERCLE BACILLI IN SPUTUM SPECIMENS FROM PATIENTS IN MANAUS (AMAZONIA, BRASIL).

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SUMMARY

Mycobacteria which are most commonly isolated in pulmonary secretions of patients with respiratory symptoms living in the State of Amazonas are given. The high percentage of isolates (25,4) and the species obtained indicate a strong relationship of contamination of man by the environment. Among the species isolated, seven of them are considered potentially pathogenic mycobacteria. Since some of these species may interfere with present and further vaccination programs against Tuberculosis and Leprosy, these investigations indicate a necessity of carrying out studies of population awareness against specific PPD's prepared from the same species.

INTRODUCTION

Mycobacteria other than tubercle bacilli (MOTT), are isolated from human clinical specimens at frequencies that differ widely according to the geographic region. The majority of these isolates lack clinical significance, however some of the MOTT may cause severe and even life-threatening diseases in humans (Wolinsky, 1979). The MOTT species most frequently causing disease in humans are **M. avium** and **M. intracellulare**, both in the general population (Wolinsky, 1979) and in patients with the acquired immunodeficiency syndrome - AIDS (Collins, 1986). Other MOTT species often associated with pulmonary disease are **M. kansasii** (Anderson **et al.**, 1975), **M. szulgai** (Davidson, 1976), **M. malmoense** (Collins **et al.**, 1986) and **M. xenopi** (Wolinsky, 1979). Some species are more often associated with extrapulmonary disease such as **M. scrofulaceum** (Lincoln & Gilbert, 1972) as a cause of submandibular adenopathy in childreen, and **M. marinum, M. ulcerans** and **M. haemophilum** causing skin lesions (MacCallum **et al.**, 1948; Linell & Norden, 1954; Jolly & Seabury, 1972; Feldman **et al.**, 1974; Davis **et al.**, 1982). **M. fortuitum** and **M. chelonae**

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have been found as the most frequent cause of postchirurgical complications (Hand & Sanford, 1970; Foster **et al.**, 1978; Wallace **et al.**, 1983). The majority of these mycobacterial species are encounted in the environment (Kubin, 1984; Tsukamura, 1984) and consequently knowledge about the species most frequently isolated in a region is of great interest to doctors, clinical laboratories and epidemiologists.

Previously we reported the isolation of 8 mycobacterial species from 29,05% of hand and forearm washings of 241 voluntarees in the city of Manaus Amazonia (Salem **et al.**, 1988a). In pursuing our investigations on the distribution of mycobacterial species in the Amazonian region, we herein report data in MOTT isolates from sputum specimens.

MATERIAL AND METHODS

Population studied

Sputum specimens from 516 individuals with clinical and/or radiological evidence of pulmonary tuberculosis were studied. Among these subjects 144 had a previous history of tuberculosis while 372 were first time cases.

Treatment of sputum specimens

The specimens were digested and decontaminated using the method of Petroff, and then inoculated onto 2 slants of Lowenstein-Jensen medium and 1 slant of Lowenstein-Jensen pyruvate, as described by David **et al.**, 1989. Briefly, the specimens were treated with an equal volume of 1.0N sodium hydroxide for 20 minutes at 37°C in a centrifuge tube. An equal volume of sterile destilled water is added. The tube is stirred, and then it is centrifuged at 2.000 x g for 20 minutes. The sediment is neutralized with 4.0% sulfuric acid using bromothymol blue as a pH indicator. The neutralized sediment was then inoculated on 2 Lowenstein-Jensen and 1 Lowenstein-Jensen pyruvate slants (0,2 ml per slant). The inoculated slants were incubated at 37°C for 2 months before cultures were discarded as negative. Positive cultures were checked for purity using the Kinyoun acid fast staining procedure (David **et al.**, 1989), and were identified as described below.

Bacteriology study

All strains were examined in respect to their growth-rate, pigment production and ability to grow on Lowenstein-Jensen medium at 30°, 38° and 42°C. Growth inhibition in Lowenstein-Jensen medium supplemented with 5% sodium chloride (NaCL), 500 µg/ml of p-nitrobenzoic acid (PNB) 30.0 µg/ml of D-cycloserine (DCS), 250.0 µg/ml of hydroxylamine (NH_2OH), 10.0 µg/ml of thiosemicarbazone (Tb_1), and 2.0 µg/ml of tiophencarboxilic acid hydrazide (TCH) were also examined as recommended by CDC (Kent & Kubica, 1985) and the Pasteur Institute (David **et al.**, 1989). The biochemical properties studied were the production of niacin, reduction of nitrate, hydrolisis of tween 80, room temperature and heated catalase test (Kent & Kubica, 1985) arysulfatase, urease, B-glucosidae, and penicilinase (Lévy-Frébault **et al.**, 1982). When it was considered necessary, the mycolic acid composition was studied as described before, Daffé **et al.** (1983) and David **et al.** (1989).

RESULTS

From the pulmonary secretions of the 516 patients studied, 125 strains of M. **tuberculosis** and 139 MOTT strains were isolated. Three of the **M. tuberculosis** isolates were mixed cultures 1 with **M. avium-intracellulare** and 2 with **M. terrae**. Eight of the Mott isolates were mixed cultures: 4 with **M. avium-intracellulare** and **M. terrae**; 2 with M. terrae and **M. gordonae**; 1 with **M. fortuitum** and **M. terrae**, and 1 mixed **M.avium-intracellulare** and **M. gordonae**.

In this series of patients, the frequency of isolation of MOTT was thus 26.16% (139/516) and their distribution is depicted in Table 1. The species most frequently isolated were **M. avium-intracellulare** and **M. fortuitum** (7.36% and 6.20% of all specimens cultured).

Among the 10 species identified, 7 were of potentially pathogenic (opportunistic) mycobacteria; **M. avium-intracellulare** (38 strains-7.36%); **M. fortuitum** (32 strains-6.20%); **M. asiaticum** (8 strains-1.55%); **M. scrofulaceum** (7 strains-1.36%); **M. chelonae** (2 strains-0.39%); **M. szulgai** (1 strain-0.19%); and **M. malmoense** (1 strain-0,19%). Nine of the isolates could not be identified to the species level, and their study is under way.

DISCUSSION

This study showed that MOTT were often isolated from sputum specimens since these organisms were found in 131 out of 516 specimens examined (25.39%). That MOTT may be widely distributed in the region was reported before in a study of hand and forearm washings where the frequency of isolates was 29.05% (Salem, 1988). In both studies **M. avium-intracellulare** was the species most frequently encountered.

The overall frequency of MOTT isolates in Manaus was significantly higher than reported in France with 3,74% of all cultures (David, 1986) and elsewhere in Brasil-Rio de Janeiro, with 6.5% of all cultures (Andrade, 1976). In the southern part of Brasil MOTT has been seldom reported (Gontijo **et al.**, 1971; Correa, 1971; Fonseca & Gontijo, 1974; Barreto, 1980).

The frequent isolation of these MOTT in Manaus may be related to environmental conditions as the city is located in the Amazonian forest next to the Amazonian river, at 03 degrees of latitude and 60 degrees of longitude. The clinical and immunological significance of exposure to these mycobacteria was not established during this study, however skin infection caused by **M. avium-intracellulare** and **M. fortuitum** were reported before (Salem **et al.**, 1988b). Consequently, this and our previous studies (Salem **et al.**, 1988a and 1988b)), indicate that further investigations on the distribution of MOTT in the environment, and of their clinical and immunological significance in men are necessary as they may induce immunity reactions interfering with current and further vaccination programs against Tuberculosis and Leprosy (WHO-Geneva, 1980).

Considering the relatively higher number of sputum specimens examined, it is worth mentioning that **M. bovis** and **M. africanum** were not isolated.

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RESUMO

São apresentadas as espécies micobacterianas mais comumente isoladas de secreções pulmonares de pacientes com sintomatologia respiratória, residentes no Estado do Amazonas. O alto percentual de isolamentos (25,4%) e as espécies obtidas indicam uma forte relação de contaminação do homem pelo meio-ambiente. Entre as espécies isoladas, 7 são consideradas micobactérias potencialmente patogênicas. Isto indica a necessidade de realizar-se estudos de sensibilização na população, através de PPDs específicos para as espécies relatadas já que, algumas destas espécies podem interferir com os programas atuais e futuros de vacinação contra a Tuberculose e Hanseníase.

Table 1. Mycobacterial species, and their distribution among 131 isolates from 516 sputum specimens.

Mycobacterium	asiat.	av.intra.	chel.	flav.	fort	gord	mal.	scrof.	szul.	ter.	not id.	total
asiaticum	8											8
avium-intracellulare		33										33
chelonae			2									2
flavescens				1								1
fortuitum					31							31
gordonae		1				10						11
malmoense							1					1
scrofulaceum								7				7
szulgai									1			1
terrae		4			1	2				20		27
not identified											9	9
Total	8	38	2	1	32	12	1	7	1	20	9	131

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