

## HUMAN PULMONARY INFECTIONS WITH BOVINE AND ENVIRONMENT (ATYPICAL) MYCOBACTERIA IN JOS, NIGERIA

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### SUMMARY

**Objectives:** To examine sputum specimens from patients with persistent bronchopulmonary disorders for mycobacterium species and to characterize the recovered isolates with a view to determining the extent of involvement of environmental mycobacteria in pulmonary infections.

**Design:** Analytical study using standard microscopy, culture and biochemical test for the identification of mycobacterium species.

**Setting:** Jos University Teaching Hospital (JUTH) and 2 referral hospitals: Plateau Specialist Hospital and Evangelical Churches of West Africa (ECWA) Evangel Hospital in Jos, Nigeria.

**Participants:** Three hundred and twenty nine (329) volunteer new patients seen at the chest clinic and general out patient departments with broncho-pulmonary disorders. Patients already on anti-tuberculosis were excluded from the study.

**Interventions:** Subjects were administered anti-tuberculosis drugs and or other treatment regimes after proper diagnosis

**Results:** Sixty-five (65) mycobacterial isolates were obtained and differentiated into human tubercle bacilli, bovine and or environmental (atypical) mycobacteria on the basis of nine identification tests. Of the 65 mycobacterial isolates subjected to the tests, 40 (61.54%) were identified as *Mycobacterium tuberculosis*, 10 (15.38%) as *M. bovis* and 15 (23.08%) as environmental mycobacteria. Among the environmental group, 9 (20.69%) were classified as *M. avium* 3 (3.45%) each as *M. kansasii* and *M. fortuitum*.

**Conclusions:** The study confirms the involvement of bovine and environmental mycobacteria in pulmonary infections. This may be related to the rising prevalence of HIV/AIDS globally. The need for adequate bacteriological analysis in current-day diagnosis of pulmonary tuberculosis is indicated.

**Keywords:** Environmental mycobacterium, pulmonary tuberculosis, sputum, Nigeria.

### INTRODUCTION

Environmental (Atypical or “anonymous”) mycobacteria have been documented to be involved in human pulmonary infections in both developed and developing countries<sup>1,2,3,4,5</sup>. Incidence rates of between 2-20% for atypical mycobacteriosis have been reported from various parts of the world<sup>5,6,7</sup>. In a World Health Organisation assisted survey of various parts of Nigeria in 1963, a 6% National Incidence rate of atypical mycobacteria was reported<sup>1</sup>. Also, in Lagos, Nigeria, Idigbe et al<sup>5</sup> reported an incidence rate of 11% for atypical mycobacteriosis. The only two reports of the involvement of bovine mycobacteria in human pulmonary infections in Nigeria are those of Alhaji and Schnurrenberger<sup>8</sup> who documented a 10% incidence rate in four of the then Northern States of the country and Idigbe et al<sup>5</sup> documented a 3.9% incidence rate in Lagos.

In the last few decades, the incidence of classical tuberculosis has been on the increase alongside tuberculosis-like disease caused by environmental mycobacteria. The apparent increase in the frequency of isolation of these mycobacteria from clinical specimens and the mounting evidence of their aetiology in severe pulmonary diseases in severe pulmonary diseases in man is currently generating concern as far as the accurate diagnosis and treatment of classical tuberculosis is concerned. Clinically and radiologically, the pulmonary diseases produced by environmental strains are indistinguishable from classical pulmonary tuberculosis, whereas the two diseases present fundamental differences in their epidemiology, management and prognosis<sup>5</sup>. It is therefore important that clinicians and bacteriologists appreciate the fact that not all Acid Fast Bacillus (AFB) found in sputum are *Mycobacterium tuberculosis*.

This paper reports the results of the differentiation of 65 mycobacteria isolates obtained from patients in Jos, Nigeria.

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## MATERIALS AND METHODS

### Study Population

Sputum samples were collected from three hundred and twenty-nine (329) new patients seen at the Chest Clinic and general out-patient department of Jos University Teaching Hospital (JUTH), Plateau Specialist Hospital and Evangelical Churches of West Africa (ECWA) Evangel Hospital all in Nigeria with broncho-pulmonary features including fever, loss of weight, persistent productive cough lasting for  $\geq 4$  weeks, haemoptysis and abnormal chest X-ray among others. The patients were screened for AFB in their sputum. The first sputum samples were collected on the first day of reporting to the hospital; thereafter first early morning sputum specimens were collected for 2 consecutive days.

### Bacteriological Analysis of Sputum Samples

The sputum samples were examined by microscopy and culture. The samples were first digested and concentrated by Petroff's method<sup>9</sup>. Smears of the final deposits from the various specimens were stained by the Ziehl-Neelsen (Z-N) method and examined under oil immersion using a binocular light microscope.

Loopfuls of the various deposits were also evenly spread on to the surfaces of a pair of Lowenstein-Jensen (L-J) slopes, incubated at 37°C and observed daily on the first week and subsequently twice a week for 8 weeks. Slopes without visible growth after 8 weeks were discarded and recorded as negative, while slopes showing mycobacterial growth were recorded as positive. All such growths were checked for acid fast properties by Z-N microscopy. A case was taken to be positive when visible growth of pure colonies of AFB was obtained from at least one of the 3 sputum samples per case.

### Classification of Isolates

Subcultures of each of the primary isolates were carried out on fresh L-J slopes and the resultant growths were again checked for purity and acid fast properties by microscopy.

Differentiation of isolates into human, bovine and other mycobacteria was based mainly on the abilities of the isolates to produce niacin or reduce nitrates to nitrites during growth. Classification of environmental isolates using Runyon's<sup>10</sup> diagnostics scheme was done by testing all stains for rapidity of growth, pigment production and photochromogenesis. Strains growing in less than 7 days were classified as rapid growers while strains

growing after 7 days were classified as slow growers. Photochromogens were classified as Runyon group 1, scotochromogens as group 3 and rapid growers as group 4.

Identification of the Runyon group 3 strains was based on the catalase activity of their broth cultures after heating at 68°C for 20 minutes. The ability to hydrolyse Tween 80 and reduce nitrate to nitrite was examined in group 1 isolates. For group 3, isolates were screened mainly for their capability to reduce tellurite to tellurium within 3-4 days, while positivity for growth on MacConkey agar and the Arylsuphatase tests were used as differential tests in group 4 isolates.

A mycobacterium strain earlier identified as *M. tuberculosis* and a strain of *M. fortuitum* (NCTC 8573) were included in all the tests as positive and negative controls respectively.

### Statistical analysis

The data obtained were subjected to the Chi-squared test using a probability of  $P=0.05$  as the level of significance.

## RESULTS

A total of 65 isolates of mycobacteria were obtained. The isolates and control organisms were subjected to all the various culture and biochemical tests and the results (Table 1). In terms of Runyon's groupings, none of the isolates gave positive reactions in the scotochromogenesis test and since tests were repeated several times with consistent results, it was taken that none belonged to Runyon's group 2.

Three of the isolates were positive in the photochromogenesis test, developing various shades of yellow to orange pigments after exposure to light. Three strains were classified as Runyon group 1 mycobacteria. The 62 non-chromogenic isolates were differentiated into rapid and slow growers on the basis of their growth rate on L-J slopes. Three isolates consistently showed visible, well matured colonies within 4-5 days of incubation. These isolates were taken as rapid growers and classified as Runyon group 4 strains. With the other isolates, growths on LJ slopes were observed after 10-14 days incubation and were therefore classified as slow growers.

Essentially all mycobacteria were catalase positive. Results from repeated tests were consistent and showed that while nine of the isolates still had catalase activities after the heat treatment, 50 were

catalase negative. The nine catalase positive isolates were then classified as group 3 mycobacteria while the 50 negative isolates were assumed to be either *M. tuberculosis* or *M. bovis*. Ten of the 50 negative isolates were consistently negative in producing niacin and reducing nitrate to nitrite and were classified as *M. bovis*. The 40 isolates that produced niacin and reduced nitrate were classified as *M. tuberculosis*.

**Table 1** Characterization of mycobacteria isolates/strain

Tests	Isolates					Controls	
<b>A. Culture</b>							
Growth rate	S	S	S	S	R	S	R
Pigment production (light)	-	-	-	+	-	-	-
Pigment production (dark)	-	-	-	-	-	-	-
Growth on MacConkey agar	-	-	-	-	+	-	+
<b>B. Biochemical</b>							
Tween 80 hydrolysis (days)	+(10)	-	-	-	-	+(10)	-
Arylsulphatase test	-	-	-	+	-	-	-
Catalase test (60°C for 20 mins.)	-	-	+	+	+	-	+
Niacin production	+	-	-	-	-	+	-
Tellurite reduction	-	-	+	-	-	-	+
Nitrate reduction	+	-	-	-	-	+	-
Mycobacteria strain identified	a	b	c	d	e		
Number of strains	40	10	9	3	3		

Overall, of the 65 isolates 40 (61.54%) were identified as *M. tuberculosis*, 10 (15.38%) as *M. bovis*, 9 (13.85%) as *M. avium* and 3 (4.61%) each for *M. kansasii* and *M. fortuitum*.

Table 2 shows the age and sex distribution of the identified cases with environmental and bovine mycobacteria.

**Table 2** Age and sex distribution of cases with environmental and bovine mycobacteria

Age (yrs)	Environmental		Bovine	
	Males	Females	Males	Females
<15	-	-	-	-
14-24	2	-	-	-
25-34	5	3	1	1
35-44	2	1	4	-
45-54	1	1	2	-
55-64	-	-	2	-
<65	-	-	-	-
<b>Total</b>	<b>10</b>	<b>5</b>	<b>9</b>	<b>1</b>

**DISCUSSION**

The involvement of environmental mycobacteria strains in human pulmonary infection in the developed and developing countries has been frequently reported. In these papers, incidence rates of 2-10% for atypical mycobacteria were noted<sup>4,5,11,12</sup>. Data in the present study confirms the involvement of environmental mycobacteria in human pulmonary infection in Jos. An incidence rate of 23.08% for human atypical mycobacteriosis reported for various parts of the world<sup>6,7</sup> but lower than the prevalence rate of 26.6% for atypical tuberculoid bacilli earlier reported by Allanana et al<sup>12</sup> in Jos. Strains of *M. kansasii*, *M. avium* and *M. fortuitum* were isolated from various patients within the period. Idigbe et al<sup>13</sup> in a report from Lagos not only confirmed the involvement of atypical mycobacteria in human infections but indicated a progressive increase in the prevalence of atypical mycobacteria from 14.6% in 1982 to 30.1% in 1992. Again, strains of *M. kansasii*, *M. avium*, *M. fortuitum* and *M. xenopi* were isolated from various patients. The resurgence of tuberculosis worldwide has been attributed to the rising prevalence of HIV/AIDS. At the end of 2001, an estimated 40 million people globally were living with HIV according to the Joint United National Programme on HIV/AIDS<sup>14</sup>. In Nigeria, a prevalence rate 5% was documented within the period<sup>15</sup>.

However, despite this involvement of atypical mycobacteria, results from this study have incriminated *M. tuberculosis* as the most prevalent causative agent of the cumulative number of positive TB cases detected in Jos. This situation is not different from earlier reports by Allanana et al<sup>12</sup> in Jos, Idigbe et al<sup>13</sup> in Lagos and some other parts of the world<sup>16</sup>.

Isolation of species of atypical mycobacteria from patients in this study suggests the pathogenic role of such atypical mycobacteria in these patients. The most important feature of atypical mycobacteria is the ability of some of them to produce tuberculosis-like disease frequently resistant to usual tuberculosis therapy<sup>17,18</sup>. In most of Nigeria and other developing countries, diagnosis of tuberculosis is usually based on such symptoms as fever, loss of weight, persistent productive cough, haemoptysis and an abnormal chest X-ray. Where laboratory facilities are available the presence of acid fast bacilli in clinical specimens is also used in diagnosis. However, observations from this study and several other studies seem to further highlight the increasing awareness that the presence of acid fast bacilli in sputum, X-ray abnormality or mere

isolation of mycobacterium colonies on L-J slopes is not enough to establish a case of classical pulmonary tuberculosis. Identification of the mycobacterium isolate is inevitably necessary.

A significant observation in this study was that all the 15 cases of atypical mycobacteriosis before bacteriological identification of the isolates were queried as pulmonary tuberculosis (PTB) and sent to the laboratory for AFB microscopy. A positive AFB microscopy result and other relevant clinical data would result in the patients being placed on anti-tuberculosis regimens. This result in most of the cases would have been little or no response to therapy after prolonged treatment.

Evidence from the study also indicated the involvement of bovine mycobacteria in human pulmonary infections in Jos. An prevalence of 15.4% was documented.

Other reports of the involvement of bovine mycobacteria in human pulmonary infections in Nigeria were those of Alhaji and Schurrenberger<sup>8</sup> who documented a 10% incidence rate in four of the Northern States of the country and Idigbe *et al*<sup>5</sup> who reported a 3.9% incidence rate of bovine mycobacteriosis in Lagos. Results of the present study in the Northern part of Nigeria revealed a prevalence rate of 15.4% for bovine mycobacteriosis. Thus, it would appear that the prevalence of bovine mycobacteriosis is higher in the Northern than the Southern part of the country.

A more detailed epidemiological survey of various parts of the country would be needed to confirm this observation. However, it is likely that the hypothesis could be correct because culturally a significant proportion of the Northern population are nomadic pastoralist and thus have more frequent contact with domestic animals than the Southern population. Several workers have associated bovine mycobacteriosis with cattle<sup>17,19</sup>.

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