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Non-tuberculosis Mycobacteria in Africa

1. Isolation and Identification *

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In tropical and subtropical countries, the presence of non-tuberculosis mycobacteria may invalidate case-finding programmes; experience has shown that many of the acid-fast bacilli discovered on examination of sputum specimens are non-tuberculosis mycobacteria—either photochromogens, scotochromogens, unpigmented or rapid growers (Groups I to IV, respectively, of Runyon's classification) or saprophytes.

Studies have recently been undertaken to determine the frequency of various types of non-tuberculosis strains in different parts of Africa. This paper describes the first of these studies, devoted to the isolation and identification of non-tuberculosis mycobacteria from seven countries.

Of 18 568 cultures examined at the Central Tuberculosis Laboratory, Nairobi, in 1961-64, 1.9% were non-tuberculosis strains. However, valid conclusions as to prevalence cannot be drawn from this figure, since some specimens came from tuberculosis patients and others from general population surveys. An earlier comparison, based on 7580 cultures from tuberculosis patients and 657 from a random survey, had shown a significant difference in the frequency of non-tuberculosis strains, the figures being 1.1% and 19.8%, respectively.

Of the identification tests studied, the formamidase test was found very useful for differentiating saprophytic mycobacteria from the other non-tuberculosis mycobacteria, particularly the rapid growers. This test is discussed in greater detail in the third study of the series.

The problem of non-tuberculosis mycobacteria (atypical and saprophytic) is important for clinicians and epidemiologists as well as for bacteriologists. The clinical significance, frequency of isolation and methods of identification of these organisms have been described in a number of papers over the past ten years. These observations have shown that the frequency may range from a few per mille (Gernez-Rieux & Tacquet, 1959; Lányi, 1958) to nearly one-third (Jenkins, 1959; Homa-Lemiško, 1961).

Briefly, the position as regards non-tuberculosis mycobacteria may be summarized as follows. Subjects suspected of having tuberculosis may excrete in the sputum not only tubercle bacilli, capable of inducing tuberculosis when injected into guinea-pigs, but also other types of mycobacteria that are non-pathogenic to guinea-pigs and show various chemical and cultural differences. Some of these types seem to be associated with other diseases in humans, and have been referred to variously as anonymous, unclassified and atypical mycobacteria (Runyon, 1959a). Other types, including some saprophytic strains of mycobacteria, are pathogenic neither to humans nor to laboratory animals. Considerable confusion exists as to the distinction between " atypical " and " saprophytic " mycobacteria; for a clear discussion of this problem, the reader is referred to Mitchison & Selkon (1959). In the present paper, and the following two papers of the series (Zykov et al., 1967; [4] Zykov & Roulet, 1967 [5]), the term " atypical " is used to designate mycobacteria in Groups I to IV of Runyon's classification (Runyon, 1959b) and the term " saprophytic " is used to designate mycobacteria that resemble Runyon's Group IV in being rapid growers, but differ from it in having formamidase activity—a property found by Nagayama et al. (1961) to be characteristic of saprophytic mycobacteria.

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[4] See the article on page 939 of this issue.

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The reported prevalence of non-tuberculosis mycobacteria varies considerably from country to country. It was found by the National Tuberculosis Association that 0.5%-2% of patients suspected of tuberculosis excreted non-tuberculosis mycobacteria in the USA; this compares with 1% in Brazil (Magarão & Lorian, 1962), 0.6%-2.5% in England (Mitchison & Selkon, 1959; Public Health Laboratory Service, 1962), 3.3% in Poland (Zdunczyk-Pawelek & Blitek, 1963), 5% (1961) and 16% (1963) in Western Australia (Kovacs, 196-) and 13.6% in Romania (Nasta & Cioclov-Bogdanescu, 1959). It was also observed that, in some parts of the world, the most common mycobacteria isolated from humans were photochromogens (Manten, 1959; Oosterbaan, 1959; Käppler, 1962; Public Health Laboratory Service, 1962), and that, in other parts, unpigmented mycobacteria (Battey strains) were found to be responsible for infection in man and animals (Kovacs, 1960, and unpublished data [1]).

The data reported by the various authors referred to above are largely without epidemiological significance, since the specimens were obtained from different categories of people and the percentages often calculated in completely different ways. The only representative estimates available are, so far as is known, those from the WHO surveys in Africa, where random samples of entire populations were investigated, sputum specimens being examined by culture methods. In Nigeria, where such a survey was carried out in 1955-56 (WHO, unpublished data, 1957) it was found that 4.23% of the adult population excreted atypical mycobacteria in their sputum. In Sierra Leone, the corresponding prevalence was 1.39% in 1958 (WHO, unpublished data, 1959). In Mozambique, 39 strains of atypical mycobacteria were isolated from a random sample of 1390 people (over 5 years of age), thus giving a prevalence of 2.8% in the general population (WHO, unpublished data, 1962).

The prevalences reported by various authors are not comparable, because the bacteriological methods used in different laboratories vary, and the sources of the cultures isolated are not representative. In some instances the frequency is based on the number of cultures isolated, while in others it is related to the total number of specimens examined; certain studies are based on groups of tuberculosis patients, while the material for other examinations originates from the general population. Therefore there is an acute need for information on the clinical, epidemiological and bacteriological characteristics of African non-tuberculosis mycobacteria, about which very little is known.

The main purpose of the present study was to investigate how frequently non-tuberculosis mycobacteria can be isolated from the routine specimens received in tuberculosis diagnostic laboratories in Africa. In addition, it was intended to compare the properties of strains of non-tuberculosis mycobacteria isolated from different parts of the continent.

MATERIAL AND METHODS

Material

A total of 18 568 of the sputum specimens examined in the Central Tuberculosis Laboratory, Nairobi, from 1961 to 1964 were found to be positive by culture, and were further investigated for the presence of non-tuberculosis mycobacteria. All these cultures were examined by the following tests: niacin, catalase, growth at room temperature and drug-sensitivity.

The cultures of mycobacteria were isolated from specimens sent to the Laboratory from various areas (Kenya, 15 057; Southern Rhodesia, 1812; Malawi, 1015; Zambia, 348; Zanzibar (now in Tanzania), 233; Basutoland (now Lesotho), 85; Seychelles Islands, 18).

The specimens received from Kenya as well as those from some of the other areas were to a large extent derived from tuberculosis patients under drug treatment, and many of these patients each had several specimens examined during the course of their treatment. No attempt was made to show whether a given specimen was the first, second, etc. from the patient in question; nor was it possible to determine which specimens pertained to the same person. It is therefore not known how many persons were examined, nor how many of them excreted tubercle bacilli. The term " frequency ", as used in this report, thus merely denotes how often certain types of mycobacteria were observed among the cultures isolated in the examining laboratory.

In order to study the frequency of isolation of non-tuberculosis mycobacteria from different population groups (apparently healthy persons and tuberculosis patients) it was necessary to include other groups of cultures, isolated in the laboratory, in the course of various WHO studies undertaken in Kenya (1959-62). The 8237 cultures isolated from 44 898 specimens examined during that period of time were divided into two groups: those isolated from tuberculosis

[1] N. Kovacs, paper presented to the Sixth International Congress on Diseases of the Chest of the American College of Chest Physicians, Vienna, August 1960.

patients (7580 cultures) and those isolated from the general population (657 cultures).

The typing of the non-tuberculosis mycobacteria was based on the examination of 216 strains collected during the period from July 1962 to August 1964 in the tuberculosis laboratories in Lagos (157 strains) and Nairobi (59 strains). These strains were selected for further investigation on the basis of the negative results which they gave in the tests for niacin and for growth at room temperature. Of the 59 strains isolated at the laboratory in Nairobi, 25 were from persons in Kenya, 7 from Southern Rhodesia, 4 from the Seychelles Islands, 1 from Basutoland and 2 each from Malawi and Zanzibar. It was also decided to include the group of 14 strains of non-tuberculosis mycobacteria isolated from domestic animals in Kenya.

Methods

Ziehl-Neelsen method. A smear was made, as usual, on two-thirds of the slide. After flame-fixing, the slide was covered with carbol fuchsin, heated until steam rose and then left for 5 minutes. After the slide had been rinsed in running water the smear was twice decolorized in 25% sulfuric acid for 2½ minutes and then rinsed again. The slide was kept in methylated spirit for 2 minutes and then counter-stained in 1% malachite green solution for 10 seconds.

Culture method. Two parts of 4% sodium hydroxide were added to 1 part of sputum in a universal container and shaken on an electrical shaker for 15 minutes. The container was kept at 37°C for 30 minutes and then centrifuged for 15 minutes at 3000 rev/min. The supernatant liquid was discarded and the sediment neutralized with 1 drop of 8% hydrochloric acid and incubated at 37°C for 8 weeks on Löwenstein-Jensen medium.

Catalase test. Reagents for the test were as follows:

(*a*) 30% (v/v) Hydrogen peroxide in distilled water;

(*b*) 10% (v/v) Tween 80 in distilled water.

Tween 80 dissolved in the minimum amount of warm water was made up to volume with distilled water and stored at 4°C. For the test, equal parts of hydrogen peroxide and Tween 80 were mixed. This solution was made up freshly for each batch of cultures to be examined, and used that day; any solution remaining was discarded. The peroxide-Tween solution was distributed in approximately 1-ml volumes in Kahn test-tubes. A new 3-mm loop (of 22 SWG nichrome wire) was prepared for each batch of tests (old loops tend to liberate oxygen from the peroxide). One-third to one-half a loopful of growth was scraped up from the culture with a sterile loop, and placed in the solution in the test-tube. The reaction was observed carefully for at least 60 seconds and the result was recorded as follows:

(−) : no bubbles liberated within 1 minute;

(+) : bubbles rising from the mass of bacilli one after the other, but slowly enough to be individually counted;

(++) : bubbles rising too fast to be counted, but not causing marked frothing;

(+++) : bubbles starting to rise from the mass of bacilli as soon as it is placed in the peroxide-Tween solution and producing marked frothing.

Niacin test (Konno's modified method). Reagents for the test were as follows:

(*a*) Benzidine, 3% in absolute ethanol, stored in a brown bottle at 4°C;

(*b*) Cyanogen bromide, 10% in water, also stored at 4°C.

The cultures were autoclaved for 15 minutes at a pressure of 15 lbf/in² (1.05 kgf/cm²). To the depression in a white porcelain tile were added 2 drops of the culture extract, 2 drops of benzidine and 2 drops of cyanogen bromide. The tile was gently shaken and the results were read as soon as colour developed (within one minute) and were recorded as follows:

Positive : purplish red colour.

Negative : blue to grey colour.

Sensitivity to mycobacteriophages. The following 7 mycobacteriophages were used for identification of non-tuberculosis mycobacteria: D-29, D-4, D-56, D-11, D-12, D-35 and D-28 (Zykov et al., 1967 [1]).

Formamidase test (Nagayama et al., 1961, modified by Dyhno et al., 1964). Cultures were grown on Löwenstein-Jensen medium. Two loopfuls of bacterial mass were suspended in 0.5 ml of phosphate buffer pH 7.2, to which was then added 0.5 ml of formamide solution (0.1 ml of formamide in 250 ml of distilled water); the test-tubes containing the mixture were then incubated at 37°C for 4 hours. Russel's method (1944) was used to determine the presence of ammonia, formation of a blue colour indicating a positive reaction, and the density of the colour indicating the quantity of ammonia liberated (Zykov & Roulet, 1967 [2]).

[1] See article on page 939 of this issue.

[2] See article on page 947 of this issue.

TABLE 1

FREQUENCY OF NON-TUBERCULOSIS MYCOBACTERIA ISOLATED AT THE NAIROBI LABORATORY (1961-64)

Origin of specimens	Year [a]												Total		
	1961 [b]			1962			1963			1964					
	No. of cultures	NTM [c]		No. of cultures	NTM [c]		No. of cultures	NTM [c]		No. of cultures	NTM [c]		No. of cultures	NTM [c]	
		No.	%		No.	%		No.	%		No.	%		No.	%
Kenya	1 977	60	3.0	4 709	61	1.3	4 140	89	2.2	4 231	94	2.2	15 057	304	2.0
Southern Rhodesia	296	1	0.3	494	5	1.0	533	9	1.7	489	5	1.0	1 812	20	1.1
Zambia	49	0	0	118	0	0	100	0	0	81	0	0	348	0	0
Malawi	135	0	0	337	3	0.9	353	7	2.0	190	0	0	1 015	10	1.0
Basutoland	0	—	—	5	1	(20.0) [d]	47	0	0	33	3	(9.1) [d]	85	4	4.7
Zanzibar	0	—	—	167	0	0	66	3	4.5	0	—	—	233	3	1.3
Seychelles Islands	0	—	—	0	—	—	18	5	(27.8) [d]	0	—	—	18	5	(27.8) [d]
Total (excluding Kenya)	480	1	0.2	1 121	9	0.8	1 117	24	2.1	793	8	1.0	3 511	42	1.2
Total	2 457	61	2.5	5 830	70	1.2	5 257	113	2.1	5 024	102	2.0	18 568	346	1.9

[a] The figures for each year refer only to the cultures that were examined between 1 January and 31 December.

[b] The niacin test was introduced as a routine method in November 1961; before that time, the identification of non-tuberculosis mycobacteria was based on growth at room temperature, catalase activity and pigmentation only.

[c] NTM = non-tuberculosis mycobacteria.

[d] The parentheses indicate percentages based on fewer than 50 observations.

Growth on broth and agar. The media used were as follows:

Broth: 10% P 3, 1% peptone, 0.5% NaCl.
Agar: As above, plus 2.5% agar.

A half-loopful of culture was added to each tube, which was incubated at 37°C for a week. Readings were made every day and tubes with growth were removed. Positive results were recorded as (+) or (++), according to the amount of growth. If no growth had been observed after a week, a negative result was recorded.

Growth at room temperature. While making sensitivity tests 2 tubes with Löwenstein-Jensen medium were seeded as controls. One of them was kept at room temperature. The reading was made after 4 weeks.

Speed of growth and pigmentation. Each specimen was subcultured in 2 tubes of Löwenstein-Jensen medium. One tube was wrapped in black paper and both tubes were incubated at 37°C. The cultures were read daily. When growth was sufficient, both tubes were taken out of the incubator and the period of growth in days, as well as the colour of the colonies (cream, orange, etc.), was recorded. Both tubes were exposed to daylight for 6-8 hours. The following day any change in colour was recorded and the culture was identified as unpigmented, photochromogenic, or scotochromogenic.

Drug-sensitivity test. The method recommended by Canetti et al. (1963) was used.

RESULTS

Non-tuberculosis mycobacteria isolated in the laboratory at Nairobi in 1961-64

The analysis is based on 18 568 cultures from the routine flood of material through the laboratory from 1961 to 1964, for which the results of all examinations were available (niacin, catalase, growth at room temperature and drug-sensitivity). The majority of these cultures originated from Kenya (81.1%), while the remainder were sent to the laboratory from Southern Rhodesia, Malawi, Zambia, Basutoland, Zanzibar and the Seychelles Islands (Table 1).

Non-tuberculosis mycobacteria were isolated from 346 (1.9%) of the 18 568 specimens included in the analysis. The table shows a higher frequency of non-tuberculosis mycobacteria among specimens collected in Kenya (2.0%) than elsewhere in Africa (1.2%). This may be partly due to differences in the source of the materials—namely, survey of the general population as compared with that of groups of tuberculosis patients (see below).

Non-tuberculosis mycobacteria isolated from tuberculosis patients and general population

An analysis was made of 8237 cultures isolated from 44 898 specimens examined in 1959-62, in order to investigate the effect of different sources on the results. Table 2 shows that among specimens from a general survey population, non-tuberculosis mycobacteria were found in 19.8% of 657 cultures isolated,

TABLE 2

FREQUENCY OF ISOLATION OF NON-TUBERCULOSIS MYCOBACTERIA FROM TUBERCULOSIS PATIENTS AND FROM A GENERAL POPULATION SURVEY, AT THE NAIROBI LABORATORY, 1959-62

Year	Tuberculosis patients				Survey population			
	No. of specimens examined	No. of cultures isolated	Non-tuberculosis mycobacteria		No. of specimens examined	No. of cultures isolated	Non-tuberculosis mycobacteria	
			No.	%			No.	%
1959	4 322	922	5	0.5	3 587	234	29	12.4
1960	7 614	953	15	1.6	2 793	145	49	33.8
1961	10 229	2 552	31	1.2	3 991	238	29	12.2
1962	11 537	3 153	35	1.1	825	40	23	57.5
Total	33 702	7 580	86	1.1	11 196	657	130	19.8

while non-tuberculosis mycobacteria occurred only in 1.1% of 7580 cultures which came from tuberculosis patients.

The latter percentage is in agreement with the findings in Southern Rhodesia (1.1%), Malawi (1%) and Zanzibar (1.3%), whence specimens from tuberculosis patients only were sent to the laboratory in Nairobi for culture and sensitivity tests (Table 1).

Non-tuberculosis mycobacteria isolated from tuberculosis patients are very often found to be mixed with *Mycobacterium tuberculosis* var. *hominis*. Table 3 shows the frequency of "mixed cultures" among non-tuberculosis mycobacteria isolated in the Nairobi laboratory.

TABLE 3
FREQUENCY OF ISOLATION OF MIXED CULTURES AT THE NAIROBI LABORATORY, 1962-64

Year	Number of non-tuberculosis strains isolated	Mixed cultures [a]	
		No.	%
1962	70	10	14.3
1963	113	28	24.8
1964	102	13	12.7
Total	285	51	17.9

[a] The presence of non-tuberculosis mycobacteria is indicated by growth at room temperature, while a positive niacin test indicates the presence of *Myco. tuberculosis* var. *hominis*.

Identification and classification of the mycobacteria isolated in the Nairobi and Lagos tuberculosis laboratories

Using the tests described, it was possible to identify all 216 cultures of non-tuberculosis mycobacteria and to classify them (except for the saprophytic strains) according to Runyon's (1959b) classification.

The distribution of the non-tuberculosis mycobacteria isolated in the tuberculosis laboratories in Nairobi and Lagos is shown in Table 4. For all cultures examined it was as follows: photochromogens (Group I), 1.4%; scotochromogens (Group II), 8.8%; unpigmented (Group III), 41.2%; rapid growers (Group IV), 13.0%; and saprophytic strains, 35.6%.

It appears that saprophytic mycobacteria were observed more frequently in the specimens in the Lagos laboratory (42.0%) than in the Nairobi labo-

TABLE 4
DISTRIBUTION OF NON-TUBERCULOSIS MYCOBACTERIA ISOLATED IN AFRICA, BY AREA OF ORIGIN

Group of mycobacteria	Nairobi laboratory								Lagos laboratory (Nigeria)		Total (both laboratories)		Excluding saprophytes and cultures of animal origin			
	Kenya	Southern Rhodesia	Basutoland	Malawi	Zanzibar	Seychelles Islands	Total Nairobi laboratory						Nairobi laboratory		Lagos laboratory	
							No.	%	No.	%	No.	%	No.	%	No.	%
Photochromogens [a]		1				1	2	3.4	1	0.6	3	1.4	2	5.7	1	1.1
Scotochromogens [a]	3	2	1		1		7	11.9	12	7.6	19	8.8	7	20.0	12	13.2
Unpigmented [a]	21	1		1		1	24	40.7	65	1.4	89	41.2	11	31.4	65	71.4
Rapid growers [a]	10	1		1	1	2	15	25.4	13	8.3	28	13.0	15	42.9	13	14.3
Saprophytic	9	2					11	18.6	66	42.0	77	35.6	—	—	—	—
Total	43	7	1	2	2	4	59	100.0	157	99.9	216	100.0	35	100.0	91	100.0

[a] According to Runyon's classification.

ratory (18.6%). The two laboratories also yielded different results in respect of the other groups: at the Lagos laboratory, 71.4% were classified as unpigmented mycobacteria (Group III), while at Nairobi only 31.4% belonged to that group. On the other hand, in the latter laboratory 42.9% were rapid growers (Group IV) as compared with 14.3% at Lagos.

The distribution by area of origin did not permit us to draw any conclusions about the predominant group, as the total number of cultures isolated was very limited.

Characteristics of non-tuberculosis mycobacteria isolated from humans

Table 5 shows the classification and different characteristics of non-tuberculosis mycobacteria isolated from people in Africa. One can see that there are three common characteristics in all five groups of non-tuberculosis mycobacteria (growth at room temperature, niacin negativity and high catalase activity). With regard to speed of growth, pigmentation and formamidase activity, the last-mentioned characteristic is very important as it permits saprophytes to be differentiated from mycobacteria of the four Runyon groups, particularly from the group of rapid growers. Study of the other characteristics showed that there was no significant difference between the five groups in sensitivity to isoniazid and *p*-amino salicylic acid (PAS), 96%-100% of strains being resistant to both drugs. The observed level of streptomycin-resistance was lower (65.2%-80%) in all five groups. It may be noted that while 99% of saprophytic mycobacteria can grow on nutrient agar and broth, only up to 32% of cultures from the other groups have this ability. The highest sensitivity to mycobacteriophages was also observed in the saprophytic group (35%).

Correlation between the results of the formamidase and catalase tests

Catalase activity is an important test for the identification of atypical and saprophytic mycobacteria. Table 5 shows the correlation between the formamidase and catalase activities of 202 non-tuberculosis mycobacteria isolated from humans: 61.9% (125 of 202 strains) of the catalase-positive cultures were found to be formamidase-negative. It can be seen that, with one exception (a scotochromogenic strain), all cultures belonging to Groups I-IV of non-tuberculosis mycobacteria were catalase-positive and formamidase-negative, whereas—again with one exception—all saprophytic cultures were catalase-positive and formamidase-positive.

Characteristics of non-tuberculosis mycobacteria isolated from domestic animals

Table 6 presents the results of various tests carried out on strains of mycobacteria isolated from domestic animals. Thirteen out of fourteen isolated cultures were identified as unpigmented mycobacteria (Group III); thus it was possible to compare their properties with those of human origin. The remaining culture was identified as saprophytic and was excluded from the analysis.

Comparison with unpigmented mycobacteria of human origin shows a similarity in such characteristics as catalase and formamidase activity, pigmentation and speed of growth. Differences were observed in the results of the niacin test (4 cultures isolated from domestic animals were positive) and ability to grow at room temperature (7 cultures isolated from domestic animals failed to grow on Löwenstein-Jensen medium). It appears that cultures isolated from humans are slightly more sensitive to mycobacteriophages than strains isolated from domestic animals, of which only one culture was lysed by mycobacteriophages.

Drug-sensitivity of non-tuberculosis mycobacteria isolated from humans and domestic animals

Tables 7 and 8 show the results of drug-sensitivity tests carried out with 190 cultures of non-tuberculosis mycobacteria of both human and animal origin. Data on the drug-sensitivity of the 26 other strains were not available. It was of interest to compare the cultures isolated in the Nairobi laboratory from humans and from domestic animals. This comparison of the two sources of material showed a marked absence of strains which are highly resistant to isoniazid and PAS among the cultures of animal origin. In contrast, a large proportion (36.4%) of cultures isolated from humans were highly resistant to isoniazid. The two groups of cultures showed a fairly similar pattern in respect of sensitivity to streptomycin.

Comparison of cultures isolated from human sources in the two laboratories (Tables 7 and 8) showed no difference in sensitivity to isoniazid and PAS, while cultures sensitive to streptomycin were found more often in the Nairobi laboratory (36.3%) than in the Lagos laboratory (18.9%).

DISCUSSION

During the period from July 1962 to August 1964 a total number of 216 strains of non-tuberculosis

TABLE 5

CLASSIFICATION OF NON-TUBERCULOSIS MYCOBACTERIA ISOLATED FROM HUMANS IN AFRICA

	Group according to Runyon's classification				Saprophytic mycobacteria
	Photochromogens	Scotochromogens	Unpigmented	Rapid growers	
Number of cultures isolated	3	19	76	28	76
Common characteristics:					
Catalase activity	+++ or ++	+++ or ++	+++ or ++	+++ or ++	+++ or ++ (one exception)
Niacin reaction	—	—	— (two exceptions)	—	—
Growth at room temperature	+	+ (one exception)	+	+	+
Differential characteristics:					
Speed of growth	≥ 6 days	≥ 6 days	≥ 6 days	≤ 5 days	≤ 5 days
Mean speed	17 days	13 days	12 days	4 days	2 days
Pigmentation	Pigmentation in light	Pigmentation in darkness	Pigmentation absent	Pigmentation absent or same in light and darkness	Pigmentation absent or same in light and darkness
Formamidase activity	—	— (one exception)	—	—	+, ++ or +++
Other characteristics:					
Drug-sensitivity:					
No. examined	3	18	66	23	66
Resistant to isoniazid	3 (100 %)	18 (100 %)	65 (98 %)	23 (100 %)	65 (98 %)
Resistant to PAS	3 (100 %)	18 (100 %)	66 (100 %)	22 (96 %)	64 (97 %)
Resistant to streptomycin	2 (67 %)	13 (72 %)	52 (79 %)	15 (65.2 %)	53 (80 %)
Microscopic and cultural: [a]					
0	2 (—)	17 (—)	54 (—)	17 (—)	47 (—)
1-3	— (2)	— (16)	— (43)	— (13)	1 (37)
4-99	— (—)	— (—)	3 (6)	5 (5)	6 (14)
100+	— (1)	— (1)	6 (14)	1 (5)	6 (10)
Total No. examined	2 (3)	17 (17)	63 (63)	23 (23)	60 (61)
Growth in nutrient media:					
Broth	0 (0 %)	3 (16 %)	19 (26 %)	9 (32 %)	75 (99 %)
Total No. examined	3	19	74	28	76
Agar	0 (0 %)	1 (5 %)	14 (19 %)	5 (18 %)	75 (99 %)
Total No. examined	3	19	74	28	76
Lysis by mycobacteriophages	0 (0 %)	3 (16 %)	12 (16 %)	6 (21 %)	27 (35 %)
Total No. examined	3	19	75	28	76

[a] The figures given in the table show the number of strains detected by direct smear microscopy, followed in parentheses by the number that yielded 0, 1-3, 4-99 or 100+ colonies on culture examination.

TABLE 6

NON-TUBERCULOSIS MYCOBACTERIA ISOLATED FROM DOMESTIC ANIMALS IN EAST AFRICA

Number of cultures isolated	13		
Common characteristics:			
Catalase activity	positive		
Pigmentation	absent		
Differential characteristics:			
Formamidase activity	negative		
Speed of growth	≥ 5 days		
Other characteristics:			
Niacin reaction:			
negative	9		
positive	4		
Growth at room temperature:			
absent	7		
present	6		
Drug-sensitivity:	isoniazid	PAS	streptomycin
sensitive	5	5	7
resistant	8	8	6
Growth in nutrient media:			
broth	0 (0%)		
agar	1 (8%)		
Lysis by mycobacteriophages	1 (8%)		

mycobacteria were examined in two tuberculosis laboratories: 157 strains in Lagos, Nigeria, and 59 strains in Nairobi, Kenya. According to the report from the Lagos laboratory (Beer & Davies, 1965) the frequency of isolation of non-tuberculosis mycobacteria (out of all cultures isolated) was 6%. But from some areas of Nigeria, the isolation of non-tuberculosis mycobacteria seems to be more frequent. For example, the examination of 871 specimens from Port Harcourt, Eastern Nigeria, showed that out of 133 cultures isolated 14 (10.5%) non-tuberculosis strains were found. Since, however, these strains probably included a number of saprophytic mycobacteria, the number of atypical mycobacteria is much less. Of 157 strains isolated in the Lagos laboratory and identified in this study, 66 (42.0%) cultures were classified as saprophytic mycobacteria. We therefore believe that the percentage of atypical mycobacteria

TABLE 7. DRUG-SENSITIVITY[a] OF NON-TUBERCULOSIS MYCOBACTERIA ISOLATED FROM HUMANS AND FROM DOMESTIC ANIMALS IN THE NAIROBI LABORATORY

Group of non-tuberculosis mycobacteria	Humans												Domestic animals											
	Isoniazid				PAS				Streptomycin				Isoniazid				PAS				Streptomycin			
	H	M	L	S	H	M	L	S	H	M	L	S	H	M	L	S	H	M	L	S	H	M	L	S
Photochromogens[b]	1		1			2					1	1												
Scotochromogens[b]	2	5				6	1			5		2												
Unpigmented[b]	5	6			1	9	1			5	3	3		7	1	5		7	1	5		4	2	7
Rapid growers[b]	3	11			1	11	2			6	4	4												
Saprophytic	5	4	1		2	6	1	1		4		6		1				1					1	
Total	16	26	2	0	4	34	5	1	0	20	8	16	0	8	1	5	0	8	1	5	0	4	3	7
	44				44				44				14				14				14			

[a] H = high drug-resistance. M = moderate drug-resistance. L = low drug-resistance. S = drug-sensitive.
[b] According to Runyon's classification.

TABLE 8

DRUG-SENSITIVITY [a] OF NON-TUBERCULOSIS MYCOBACTERIA ISOLATED FROM HUMANS IN THE LAGOS LABORATORY

Group of non-tuberculosis mycobacteria	Isoniazid		PAS		Streptomycin		Viomycin	
	R	S	R	S	R	S	R	S
Photochromogens [b]	1		1		1		1	
Scotochromogens [b]	11		11		8	3	7	3
Unpigmented [b]	54	1	55		44	11	34	21
Rapid growers [b]	9		8	1	5	4	4	5
Saprophytic	55	1	55	1	49	7	42	12
Total	132		132		132		129	

[a] R = Resistant; S = sensitive. Information about the degree of resistance was not available.
[b] According to Runyon's classification.

among the non-tuberculosis cultures isolated in the Port Harcourt area is approximately 6%, the remaining cultures (4%) being saprophytic mycobacteria.

Bearing in mind that 18.6% of the non-tuberculosis mycobacteria isolated in East Africa are saprophytic, the frequency of isolation of atypical mycobacteria may be taken to be approximately 1.6%. This makes the frequency of isolation of atypical mycobacteria approximately four times as high in Nigeria as in East Africa.

The question arose whether the frequency of isolation of non-tuberculosis mycobacteria was similar in the specimens from tuberculosis patients and in those from apparently healthy people. The answer to this question was obtained from the data accumulated in the Nairobi tuberculosis laboratory in 1959-62. It was found that the frequency of non-tuberculosis mycobacteria among the cultures isolated from the survey population in Kenya was 19.8%. The proportion of non-tuberculosis strains among the mycobacteria isolated was striking (33.8% in 1960 and 57.5% in 1962). The frequency of non-tuberculosis mycobacteria in specimens obtained from tuberculosis patients (1.1%) was 18 times lower than in the general population. This fact should be borne in mind when case-finding programmes with microscopic examination of sputum specimens are launched in tropical and subtropical countries, since 20% of the acid-fast bacilli discovered in this way may be non-tuberculosis mycobacteria.

Our findings are in agreement with the results of the above-mentioned WHO survey in 1957. During this survey 210 strains of non-tuberculosis mycobacteria were isolated; 75% of these strains came from a single village (Kadandani) where 22% of all the sputum samples collected gave cultures of non-tuberculosis mycobacteria. Our observations in Nigeria in 1963 also revealed a very high prevalence of non-tuberculosis mycobacteria among healthy people. Unfortunately, the above-mentioned difference in frequency of non-tuberculosis mycobacteria isolated from the survey population and from tuberculosis patients has not been taken into consideration in the reports of the other investigators, which may be one of the reasons why there is so great a discrepancy between the different results. Comparison is also made difficult by the fact that the frequency of non-tuberculosis mycobacteria was not always calculated from the number of cultures isolated or the number of persons examined. In most cases these figures included unidentified saprophytic mycobacteria as well. We found the proportion of saprophytic mycobacteria among non-tuberculosis strains isolated in different parts of Africa to vary, being 18.6% in East Africa and 42.0% in Nigeria. Excluding saprophytic mycobacteria and cultures isolated from domestic animals, we can compare the distribution according to Runyon's classification of atypical mycobacteria isolated in East Africa and Nigeria. The dominating group (71.4%) for Nigeria was unpigmented mycobacteria (Group III) while rapid growers (Group IV) were found more frequently (42.9%) among atypical cultures in East Africa. The latter figure needs further confirmation

as the total number of atypical mycobacteria isolated from people in East Africa was rather small (35 strains).

A new test (developed by Nagayama et al., 1961, and modified by Dyhno et al., 1964) based on formamidase activity, for the differentiation of saprophytic mycobacteria from those belonging to Runyon's groups I-IV was found to be very useful and simple enough for routine work. In particular, the results of this test make it possible to distinguish Runyon's rapid growers from the saprophytes. However, we found it impossible to distinguish atypical mycobacteria from tubercle bacilli on the basis of their formamidase positivity as described by Muftic (1964). The catalase activity of saprophytic mycobacteria is often used to characterize them. Of 77 saprophytic mycobacteria cultures in our study, 94.8% had the highest catalase activity (+++). However, very similar results were observed in all groups of atypical mycobacteria. Analysis showed no correlation between catalase and formamidase activity. The latter is of great value for identification, as it is found in saprophytic mycobacteria only.

Mallmann et al. (1964) stated that mycobacterial diseases of domestic animals should be taken into consideration when the epidemiological problem of typical mycobacteria in humans is examined. On the basis of these considerations, 14 strains of mycobacteria isolated from domestic animals in Kenya were included in our study. It was of interest to discover whether the properties of strains belonging to the same groups in Runyon's classification are similar when isolated from humans and from animals. The results of such a comparison revealed differences in that the cultures isolated from animals did not yield growth at room temperature (7 strains) or on simple media (nutrient broth and agar), and exhibited a high resistance to mycobacteriophages and a low resistance to isoniazid, PAS and streptomycin. The last observation is of great importance as it has hitherto been considered that one of the most important criteria for the identification of atypical mycobacteria is a high degree of resistance to antituberculosis drugs (Meissner, 1958). Our observations on both groups of cultures (of animal and human origin) showed that this holds true for non-tuberculosis mycobacteria isolated from humans only, and not for those of animal origin. The absence of strains isolated from animals with a high degree of resistance to the above-mentioned drugs may be due to two facts: (1) that highly resistant strains excreted by tuberculosis patients are unable to infect domestic animals owing to lack of virulence, and (2) that antituberculosis drugs are not used for the treatment of tuberculosis among animals.

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RÉSUMÉ

Dans les pays tropicaux et subtropicaux, la présence de mycobactéries atypiques ou saprophytes dans les produits d'expectoration peut fausser les résultats des examens destinés au dépistage de la tuberculose. L'expérience montre que nombre de bacilles acido-résistants découverts à cette occasion sont en fait des mycobactéries autres que *Mycobacterium tuberculosis:* photochromogènes, scotochromogènes, mycobactéries non pigmentées, mycobactéries à croissance rapide (correspondant respectivement aux groupes I à IV de la classification de Runyon) ou souches saprophytes. Plusieurs enquêtes ont été récemment menées en différentes régions d'Afrique en vue de rechercher la fréquence de ces divers types de mycobactéries. Le présent article — le premier d'une série de trois — décrit les techniques mises en œuvre au Laboratoire central de la tuberculose de Nairobi, Kenya, pour l'isolement et l'identification de ces souches dans des échantillons de crachats en provenance de 7 pays: Kenya, Rhodésie du Sud, Malawi, Zambie, Tanzanie, Lesotho, îles Seychelles.

Sur 18 568 cultures obtenues de 1961 à 1964, on a identifié des mycobactéries autres que *Myco.tuberculosis* dans 1,9% des cas. On ne doit attribuer à ce chiffre qu'une valeur relative, les échantillons ayant été recueillis à la fois chez des sujets souffrant de tuberculose et chez des personnes examinées au cours d'opérations de dépistage systématique. Des investigations antérieures ont d'ailleurs montré une différence significative en matière de prévalence des souches atypiques et saprophytes selon que les examens portent sur des échantillons fournis par des

tuberculeux (prévalence: 1,1% sur 7580 cultures) ou obtenus lors des opérations de dépistage au sein d'une collectivité (prévalence: 19,8% sur 657 cultures).

Parmi les procédés d'identification utilisés, l'épreuve d'activité de la formamidase s'est révélée très prometteuse pour différencier les mycobactéries saprophytes des mycobactéries atypiques, et en particulier des souches à croissance rapide. Les modalités de cette épreuve sont exposées en détail dans le troisième article de la série.

Sur 14 cultures isolées à partir d'animaux domestiques d'Afrique orientale, 13 appartenaient au groupe des mycobactéries non pigmentées (groupe III de la classification de Runyon) et 1 au groupe des mycobactéries saprophytes. Ces 13 souches se différenciaient de leurs homologues d'origine humaine par les résultats de l'épreuve à la niacine, par une moindre aptitude à la croissance à la température ambiante et par une sensibilité moins accentuée aux mycobactériophages.

Très peu de souches d'origine animale ont fait preuve d'une résistance à l'isoniazide et au PAS; en revanche, une notable proportion (36,4%) des souches d'origine humaine étaient résistantes à l'isoniazide. Dans les deux groupes, la sensibilité à la streptomycine était de caractère très semblable.

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