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Mycobacteria other than *M. tuberculosis* isolated from clinical material in Kinshasa (Congo)

BY

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Summary — From a series of 1,953 cultures of which 174 were positive for mycobacteria in Kinshasa, 18 strains of non human mycobacteria were isolated.

There was 1 intermediate strain, 7 strains of scotochromogens, 3 strains of *M. terrae*, 5 strains of Battey group organisms and 2 strains of *M. fortuitum*.

With the exception of the bovine strain none of these strains were recognized as etiologic agents of disease.

In this report we give the results on the identification of atypical mycobacterial strains isolated at Lovanium University during the past two years.

Material and methods

Decontamination of clinical specimens was done with the trisodium phosphate technique. All strains that were suspected of being different from classical *M. tuberculosis* were sent to the Institute for Tropical Medicine in Antwerpen. The identification techniques used have been described elsewhere. (Pattyn et *al.*, 1964, 1965, 1966, 1967).

As a rule, when an unknown strain of acid fast bacilli is received in the laboratory, a suspension is prepared from it and the following media inoculated :

- Loewenstein : 2 tubes at 37 °C, one of which is incubated in the dark. 1 tube at 42° and one at 44.5 °C.
- Loewenstein containing 1 gamma INH/ml.
- Loewenstein containing 25 gamma TCH/ml (thiophene-2-carboxylic acid hydrazide).
- I dubos oleic acid agar in a petridish to obtain isolated colonies. If necessary pure colonies may be subcultured. Colony morphology is observed and the nomenclature of Fregnan and Smith (1962) is used.
- A tween hydrolysis medium (Wayne *et al.*, 1964) is inoculated with a loopful of mycobacteria from the original tube. If insufficient growth is available the tween hydrolysis test is performed later on from a subculture.
- The growth on the loewenstein tubes at 37 °C and eventually from the tubes incubated at the higher temperatures is used to perform the niacin production, nitrate reduction and arylsulfatase tests.

— In the case the strain is not *M. tuberculosis* or *M. bovis*, amidase tests (Bönicke 1962) are done.
— Catalase activity at room temperature and after heating is tested (Kubica e.a. 1966).
— Other tests are performed if indicated, such as growth on Mc Conkey agar or agglutination tests.

Results

Eighteen strains were examined (table 1). Their origin and identification was as follows :

— « Intermediate » strain or *M. bovis* (strain 836)

This strain was dysgonic on Loewenstein medium and gave rough colonies (RR) on Dubos oleic acid agar. Niacin production was positive, but nitrate reduction negative. It was sensitive to INH 0.1 gamma/ml and sensitive to TCH. It was guinea pig virulent.

TABLE 1

Grouping of atypical strains isolated

	Sputum	Gastric washings	Number
« Intermediate »	1	—	1
M. spec. scotochromogen			
— amidases O	1	1	2
— amidases « 3 »		1	1
— amidases « 3, 5, 6 »		3	4 (*)
M. terrae		3	3
M. battey group	1	4	5
M. fortuitum		2	2
			18

(*) 1 strain on a tube inoculated with pleural exsudate.

— *Scotochromogens* (strains 744, 795, 796, 839, 863, 867)

Scotochromogens have been subdivised by Bönicke (1962) on the basis of amidase tests into three groups :

— *M. aquae* var. *non-ureolyticum* : no amidase activity

— *M. aquae* var. *ureolyticum* : desamination of ureum and an unnamed group with amidase activity for three amides : ureum, nicotinamide, pyrazinamide (3, 5, 6 group).

Of the seven scotochromogenic strains isolated, 2 were *M. aquae* var. *non-ureolyticum,* 1 was *M. aquae* var. *ureolyticum* and 4 were of the 3, 5, 6 group.

All colonies were of the SmSy type on Dubos oleic agar. One strain of the 3, 5, 6 variety produced colonies which can still be called SmSy but with a slightly different appearance. The internal structure is rather granular and the edges are very much intented, giving an arborescent appearance.

These strains are eugonic on Loewenstein medium : niacin production, nitrate reduction and arylsulfatase tests are negative.

None are positive in the tween hydrolysis test. Only 2 of the 7 strains (both with amidases 3, 5, 6) grow at 42 °C. No agglutination was obtained with scrofulaceum, Gaüse and Lunning serotype autisera (Schaeffer, 1965).

— *Battey group* (798, 837, 864, 865, 866)

Five strains were non pigmented slow growers. They produced SmS colonies on Dubos oleic agar, were negative in niacin production, nitrate reduction, and rapid tween hydrolysis tests. Two strains were positive in the arylsulfatase test. Amidases were present for nicotinamide and pyrazinamide (amides 5 and 6 of Bönicke 1962). Two strains multiplied at 42 °C, none at 44.5 °C, two strains were inoculated into chickens (1 mg wet bacill I. V.) with negative results.

— *M. terrae* (*strains* 745, 746, 841) (Wayne, 1966)

These strains also are non pigmented slow growers, Runyon group III. Colony morphology is of a type not described by Fregnan and Smith. We call them SmG for : Smooth, granular. This morphology was identical with that of the colonies produced by 3 other strains of *M. terrae* received from G. P. Kubica.

Catalase is thermoresistant.
Niacin production is negative.
Nitrate reduction is positive.
Rapid tween hydrolysis is positive.
Arylsulfatase is positive in 2 strains.
Only 1 strain grows at 42 °C not at 44.5 °C.
Amidase tests give a 5,6 response (characteristic for Runyon group III organisms), for 2 strains, 1 strain also shows urease activity.
All strains are resistant to INH at 0.1, 0.2 and 1 gamma/ml.

— *M. fortuitum* (799-800)

In two instances *M. fortuitum* was identified.

The more pertinent characters leading to this diagnostic are :

Rapid grower.
Colony morphology SmF.
No development at 42 °C and 45 °C.
Catalase positive.
Resistance to 5 gamma/ml INH.
Nitrate reduction and arylsulfatase positive.
Growth on Mac Conkey agar within 3 days positive.
Amidase tests : 1, 3, 5, 6.

Discussion

In a previous study from these laboratories (Hermans-Boveroulle *et al.*, 1965) we mentioned the relative frequency in Kinshasa of *M. bovis* strains producing niacin or *M. tuberculosis* nitratase negative.

The patient from whom strain 836 was obtained lived in the proximity of a cattle farm.

Van den Abbeele (1962) has mentioned the existence in the Congo of tuberculosis due to the bovine bacillus.

The remaining 17 strains are considered as contaminants since they were isolated only once as a few colonies on the medium. As can be seen from table 1 the great majority originated from gastric juices. Gastric washings are probably the main source of non significant so called atypical mycobacteria.

On the whole during the same period 157 strains of *M. tuberculosis* were isolated from 1953 specimens cultured. This gives a frequency of 0.8 percent of contaminant atypical mycobacteria.

Table 2 summarizes the findings of several analogous studies in Africa.

The highest frequency of atypical organisms was in Senegal; whereas Stottmeyer *et al.* in South Africa had the lowest frequency : 0.37 percent, in this work however only sputum specimens were considered.

The frequency of the different species isolated also differs considerably from one region to another.

In our study scotochromogenic and group III organisms were most frequently isolated.

Stottmeyer *et al.* (1966) found in their material mostly group III organisms (2 strains of which were even considered as pathogenic). Again must be stressed however that these authors only worked with sputum specimens.

Ganse and Lefèvre (1966) reported the highest frequency of rapid growers and scotochromogens whereas Bonnardot and Salle *et al.* (1965) most frequently found scotochromogens.

Lester (1939) and Boveroulle and Pattyn (1964) advanced the hypothesis that many so called atypical strains were contaminants arising in the laboratory.

The finding of groups of identical contaminants also in this study gives again support to this hypothesis.

TABLE 2

Comparison of results of several studies on atypical mycobacteria in Africa

Authors	No specimen/ no positives	Decontamination procedure	Number of atypical mycob. by Runyon's groups.	
Stottmeyer e. a.	4.000 sputa / 2.000	P-D(*)	14 { I: 1; II: ; III: 5; IV: 7; noc: 1 }	0.37 %
Cause-Lefèvre	2.246 sp + g. w. / 535	CPBr	51 { I: 4; II: 20; IV: 26; B: 1 }	2 %
Bonnardot e. a.	1.330 sp + g. w. / 387	CPBr	18 { I: ; II: 12; III: ; IV: 6 }	1 %
	1.727 sp + g. w. / 172	CPBr	40 { II: 39; IV: 1 }	2 %
Salle, Sodhi	? sp / 115	NaOH	4 { I: 1; II: 2; III: 1 }	
This study	1.953 sp + other / 157	Na_3PO_4	17 { I: 1; II: 7; III: 7; IV: 2 }	0.8 %

(*) P-D = pancreatin desogen.
CPBr = cetyl pyridinium bromide.

In connection with the finding of two strains of *M. fortuitum* we must mention the frequent isolation of this species in Kinshasa from abcesses (Vandepitte *et al.*, 1962).

During the period of isolation of the above mentioned strains however no strains of *M. fortuitum* were handled in the laboratory.

Although the isolation of atypical acid fast organisms remains low in Africa it may be useful for diagnostic laboratories in the tropics to apply also the niacin or nitrate reduction tests to all isolations of mycobacteria to confirm the diagnosis of *M. tuberculosis*.

Résumé — **Mycobactéries autres que *M. tuberculosis* isolées à partir de matériel clinique à Kinshasa (Congo).**

Dans une série de 1.953 cultures faites à Kinshasa et dont 174 positives pour mycobactéries, 18 souches autres que *M. tuberculosis* furent trouvées.

Parmi les 18 souches il y eut 1 souche « intermédiaire », 7 souches de mycobactéries scotochromogènes, 3 souches de *M. terrae*, 5 souches de mycobactéries du groupe Battey et 2 souches de *M. fortuitum*.

A l'exception de la souche bovine aucune autre souche n'a pu être considérée comme agent étiologique d'une affection humaine.

Samenvatting — **Mycobacteriën andere dan *M. tuberculosis* in het klinisch materiaal te Kinshasa (Kongo).**

Te Kinshasa werden in een reeks van 1.953 kulturen, waaronder 174 positief voor mycobacteriën, 18 stammen gevonden die geen *M. tuberculosis* waren.

Deze stammen werden geïdentificeerd als 1 « intermediaire » stam, 7 stammen scotochromogene mycobacteriën, 3 stammen *M. terrae*, 5 stammen van de Battey groep en 2 stammen *M. fortuitum*.

Met uitzondering van de *M. bovis* stam werden alle andere als saprophytaire kontaminanten aanzien.

Zusammenfassung — **Andere Mycobacteria als *M. tuberculosis* aus klinischem Material in Kinshasa (Kongo) isoliert.**

In einer Reihe von 1.953 in Kinshasa vorgenommenen Kulturen, von denen 174 für Mykobakterien positiv waren, wurden 18 Stämme, die nicht *M. tuberculosis* waren, gefunden.

Unter den 18 Stämme war 1 Stamm « intermediäre », 7 Stämme skotochromogener Mykobakterien, 3 Stämme *M. terrae*, 7 Stämme Mykobakterien der Gruppe Battey und 2 Stämme *M. fortuitum*.

Mit Ausnahme des Rinderstammes konnte kein einziger Stamm als menschenpathogen anerkannt werden.

Resumen — **Otras micobacterias con el *M. tuberculosis* aisladas del material clinico en Kinshasa (Congo).**

En una serie de 1953 cultivos llevada a cabo en Kinshasa resultaron positivos para micobacterias 174, habiéndose hallado 18 cepas distintas al *M. tuberculosis*.

Entre las 18 cepas hubo 1 de *M. bovis*, 7 de micobacterias scotocromógenas, 3 de *M. terrae*, 5 del grupo Battey y 2 de *M. fortuitum*.

Con la excepción de la cepa bovina ninguna otra ha podido incriminarse como responsable de una afección humana.

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