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CHARACTERIZATION OF *Mycobacterium pulveris*, AN ENVIRONMENTAL MYCOBACTERIUM ISOLATED FROM A CLINICAL SAMPLE

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Summary

A rapidly growing nonchromogenic mycobacterium isolated from urine was identified as *Mycobacterium pulveris*, a nonpathogenic environmental species, using high performance liquid chromatography of cell wall mycolic acids. The procedure that allowed the characterization of the strain is reported and the significance of the isolation discussed.

Introduction

The identification of mycobacteria isolated from clinical samples is of paramount importance for the assessment of their clinical significance. The genus embraces a large number of environmental species, only some of which have at times been shown to play a pathogenic role (12). While for the more common species the identification strategy is well established and may be based on information reported by most manuals of clinical microbiology, for the uncommon ones relevant data are only available in their *species nova* description.

An unusual mycobacterium isolated from an urinary sample, could not be

speciated with conventional tests. We resorted to high performance liquid chromatography (HPLC) of cell wall mycolic acids and readily identified the isolate as *Mycobacterium pulveris*, a species previously found only in environmental samples.

Materials and methods

A few smooth, nonchromogenic colonies were grown on Lowenstein-Jensen medium from one voided urine specimen of a female outpatient who had asked for isolation of mycobacteria. Laboratory records showed that the same patient had requested the same isolation, at regular intervals, for the last ten years; the reason for such repeated requests was unknown. The centrifuged specimen was processed using the commercial decontaminating system Nekal BX (Biotest) and then inoculated on Lowenstein-Jensen slants and into the biphasic system Septi-Chek AFB (Becton Dickinson).

The identification of the isolate was first attempted using a wide panel of biochemical tests (Table 1) according to the conventional procedures recommended for the speciation of mycobacteria (5,7,9).

The HPLC analysis of cell wall mycolic acids, after extraction and derivatization to bromo-phenacyl esters, was performed as reported elsewhere (2,10); a high molecular weight internal standard (Ribi ImmunoChem), which is eluted at the end of the sequence, was added to the sample. The representative pattern of the strain was visually compared with each item of our library of *Mycobacterium* HPLC profiles; for a more accurate comparison single peaks were identified on the basis of their relative retention times (RRT).

The susceptibility of the strain was tested against the drugs recommended for rapidly growing mycobacteria (1) using the E-test (AB Biodisk) according to the manufacturer's recommendations. In short, the mycobacterium suspended in water to a turbidity of McFarland 0.5 was swabbed on plates of Middlebrook 7H11 medium (Difco) containing OADC enrichment (Difco). The strips with a preformed gradient of antimicrobials were laid on the surface of the medium once it had completely dried; the plates were then incubated at 36 °C in an atmosphere containing 5% CO_2. Plates were inspected daily until growth was visible, which took 7 days. The MIC was determined by the value read on the strip at the intersection with the elliptic inhibition halo.

Results

Conventional identification procedures (Table 1) did not allow to assign our

Table 1. Results of standard biochemical, cultural and inhibition tests on our isolate compared with the ones of the type strain of *Mycobacterium pulveris*.

	our strain	type strain	
		A	B
Growth at 28°C	+	+	
Growth at 37°C	+	+	
Growth at 45°C	-	-	
Growth rate	6 days	5 days	
Colony morphology[a]	S-W	S-W	
Niacin	-	-	
Nitrate reduction	+	+	
Catalase (68°C)	+		+
β-glucosidase	-		-
Tween 80 hydrolysis (10 days)	+	+	
Tellurite reduction	-		-
Arylsulfatase (3 days)	-	-	
Urease	+	+	
Semiquantitative catalase	-	-	
Acid phosphatase	+	+	
Photochromogenicity	-	-	
Scotochromogenicity	-	-	
MacConkey	-		-
Tolerance to:			
p-Nitrobenzoate (500 μg/ml)	-	+	
NaCl (5%)	+	+	
Thiophene-2-carboxylic hydrazide (5 μg/ml)	+	+	
Thiacetazone (10 μg/ml)	+		+
Hydroxylamine (500 μg/ml)	-	-	
Isoniazid (1 μg/ml)	-		-
Oleate (250 μg/ml)	-		-
p-Aminosalicylate (1 μg/ml)	+		+
Toluidine blue (300 μg/ml)	+		+

A: Tsukamura *et al*. (11).
B: Tests performed by us.
[a] S-W: smooth, wet.

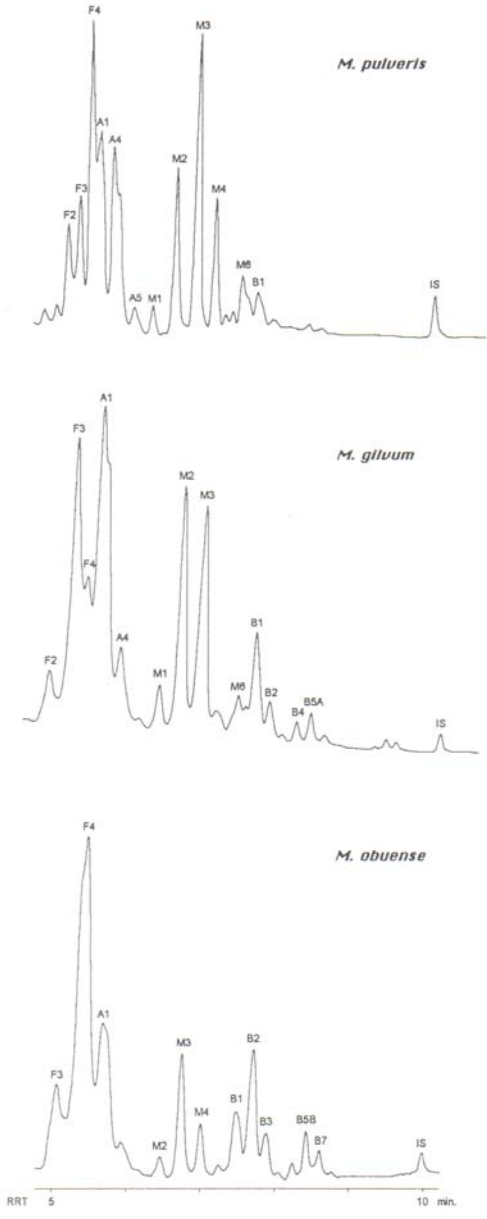

Figure 1. Representative patterns of bromo-phenacyl esters of mycolic acids determined by HPLC from *M. pulveris* (ATCC 35154), *M. gilvum* (NCTC 10742) and *M. obuense* (NCTC 10778). Peak identification according to Glickman *et al.* (3); IS = Internal Standard.

organism to any one of the species included in the major microbiology handbooks.

The mycolic acid profile of our isolate turned out to be very unusual; grossly, it was characterized by three early clusters of peaks. Only three profiles in our *Mycobacterium* library, which includes the HPLC pattern of 54 different species, present such a pattern (Figure 1): *M. pulveris*, *Mycobacterium gilvum* and *Mycobacterium obuense*. Owing to the rarity of such species, we had their representative profiles drawn from their reference strains only (ATCC 35154, NCTC 10742 and NCTC 10778). Our isolate showed 12 major peaks, which on the basis of RRTs corresponded to all 12 major peaks of *M. pulveris;* a partial correspondence was found with 7 of the 11 peaks of *M. obuense* and with 10 of the 13 of *M. gilvum*. Our strain also showed the best agreement in the relative peak heights with *M. pulveris*. Furthermore, *M. gilvum* and *M. obuense* have colonies with a yellow pigmentation (12) while the colonies of our isolate were, besides being wet and mucous, not pigmented, a very unusual appearance within the genus *Mycobacterium* but typical of *M. pulveris*.

All results of conventional tests were either in agreement with the ones reported by Tsukamura et al. (11) in the *species nova* description or, when previously not investigated, gave identical results in tests we performed in parallel both on our isolate and the reference strain.

Table 2. MICs of our isolate compared with the ones of the type strain of *Mycobacterium pulveris*.

Drugs	MICs (μg/ml)	
	our strain	type strain
Amikacin	0.38	0.50
Cefoxitin	>256	128
Ciprofloxacin	0.012	0.004
Doxycycline	0.032	0.023
Imipenem	>32	>32
Tobramycin	6	4
Trimethoprim-sulfamethoxazole	>32	>32

The activities of all antimicrobials tested did not reveal significant differences between the two strains, with very low MICs for amikacin, ciprofloxacin and doxycycline (Table 2).

Discussion

The isolation of a nontuberculous mycobacterium from urinary specimens is always difficult to interpret, the main question being its role as an agent of infection and disease. Species identification may assist towards an answer as it is known that some species are more likely than others to be of clinical significance.

M. pulveris is a rapidly growing acid fast bacillus previously isolated only from house dust (11) and not considered to be a pathogen for humans (8). Several circumstances of the present case seem to confirm the lack of clinical significance of *M. pulveris*: (i) the organism was isolated only once and few colonies grew in culture; (ii) the reported prevalence of *M. pulveris* in house dust may well account for a contamination in the course of collection.

The results of susceptibility testing concurred to strengthen the identification of the isolate, as they were in agreement with those of the reference strain (Table 2). The epsilometer test has been validated for a wide spectrum of organisms and is giving promising results with rapidly growing mycobacteria (4).

The identification of rare organisms is at times an esoteric exercise: conventional tests alone are in fact not sufficient, and the more sophisticated techniques, like the recognition of species-specific genomic sequences (6), or the analysis of cell wall mycolic acids, rely on comparisons with known signature characteristics (nucleotide sequences or lipidic profiles) which are not readily available so that the isolate has to be directly compared with the reference strains; this granted, the lipid analysis approach appears to be the more economical.

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References

1. Brown BA, Swenson JM, Wallace RJ, Jr. Agar disk elution test for rapidly growing mycobacteria. In: HD Isenberg (ed), Clinical microbiology procedure handbook, American Society for Microbiology, Washington 1992; p. 5.10.1-5.10.11.

2. Butler WR, Thibert L, Kilburn JO. Identification of *Mycobacterium avium* complex strains and some similar species by high-performance liquid chromatography. J Clin Microbiol 1992; 30:2698-704.

3. Glickman SE, Kilburn JO, Butler WR, Ramos LS. Rapid identification of mycolic acid patterns of mycobacteria by high-performance liquid chromatography using pattern recognition software and a *Mycobacterium* library. J Clin Microbiol 1994; 32:740-5.

4. Hoffner SE, Klintz L, Olsson-Liljequist B, Bolmstrom A. Evaluation of E-test for rapid susceptibility testing of *Mycobacterium chelonae* and *Mycobacterium fortuitum*. J Clin Microbiol 1994; 32:1846-9.

5. Kent PT, Kubica GP. Public health mycobacteriology. A guide for the level III laboratory. U.S. Department of Health and Human Services, Atlanta, 1985, p. 207.

6. Kirschner P, Springer B, Vogel U, Meier A, Wrede A, Kiekenbeck M, Bange FC, Böttger EC. Genotypic identification of mycobacteria by nucleic acid sequence determination: report of a two-year experience in a clinical laboratory. J Clin Microbiol 1993; 31:2882-9.

7. Roberts GD, Koneman EW, Kim YK. *Mycobacterium*. In: A Balows et al. (eds.), Manual of clinical microbiology, 5th ed., American Society for Microbiology, Washington, D.C. 1991; p. 304-39.

8. Shinnick TM, Good RC. Mycobacterial taxonomy. Eur J Clin Microbiol Infect Dis 1994; 13:884-901.

9. Silcox VA. Identification of mycobacteria. In: HD Isenberg (ed), Clinical microbiology procedures handbook, American Society for Microbiology, Washington 1992; p. 3.11.1-3.11.11.

10. Tortoli E, Bartoloni A, Burrini C, Mantella A, Simonetti MT. Utility of high-performance liquid chromatography for identification of mycobacterial species rarely encountered in clinical laboratories. Eur J Clin Microbiol Infect Dis 1995; 14:240-3.

11. Tsukamura M, Mizuno S, Toyama H. *Mycobacterium pulveris* sp. nov., a nonphotochromogenic mycobacterium with an intermediate growth rate. Int J Syst Bacteriol 1983; 33:811-5.

12. Wayne LG, Kubica GP. Family *Mycobacteriaceae* CHESTER 1897, 63[AL]. In: PHA Sneath et al. (eds.), Bergey's manual of systematic bacteriology, The Williams & Wilkins Co., Baltimore 1986; p. 1435-57.

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