Mycetoma Caused by *Actinomadura (Streptomyces) madurae*

THE FIRST SOUTH AFRICAN CASE AND THE RESULTS OF CHEMOTHERAPY

HESTER F. VISMER, J. G. L. MORRISON

SUMMARY

The actinomycete *Actinomadura madurae* has been isolated for the first time in South Africa from a case of mycetoma pedis. *A. madurae* and *A. pelletieri* are two closely related though separate pathogens which were formerly regarded as species of *Nocardia* and *Streptomyces*, and are now placed in a newly created genus *Actinomadura*. They have been isolated from river water by Transvaal botanists and by us from clinical cases of mycetoma. Irrigated soil possibly serves as a habitat selecting for pathogenicity in man. Surgery can be postponed or possibly avoided by correct identification of the causative agent and appropriate chemotherapy in the case of *A. madurae* infection.

S. Afr. Med. J., **48**, 433 (1974).

In 1891 Vincent isolated an organism from a case of mycetoma pedis in Algeria which produced large, soft, yellow-white grains. This was the first of its kind to be isolated, and Vincent[1] described it as *Streptothrix madurae* in 1894. Since then the organism has been recovered elsewhere and given several other names which can nowadays be regarded as synonyms for Vincent's species.

The development of more refined techniques for determining the chemical components in cell walls has led to a revision of the genera *Actinomyces*, *Streptomyces* and *Nocardia* in the past 10 years. The new characteristics so obtained are more stable, but must be regarded as supplementary to the older criteria.[2]

As applied to the aerobic actinomycetes, which include Vincent's species, whole-cell hydrolysates have shown that the cell walls of the two species of *Streptothrix* or *Streptomyces* causing mycetoma, viz. *pelletieri* and *madurae*, are peculiar in containing mesodiaminopimelic acid and madurose, a carbohydrate of undetermined structure. This distinguishes them from the other members of the *Streptomyces-Nocardia* group. Lechevalier and Lechevalier[3-5] have therefore placed them in a new genus, *Actinomadura*. Hence we recognise *Actinomadura madurae* and *Actinomadura pelletieri* as the two pathogenic organisms belonging to the newly named genus as causes of mycetoma.

Department of Dermatology, University of Pretoria
HESTER F. VISMER
J. G. L. MORRISON

Date received: 29 October 1973.

GEOGRAPHICAL DISTRIBUTION

Many authors agree that *Actinomadura madurae* (Vincent) Lechevalier and Lechevalier seldom causes actinomycotic mycetoma.[6,7] In 1963 Mariat,[8] in a world review, calculated that *A. madurae* was found in only 7,27% of 854 mycetoma cases.

A. madurae is most commonly found in Africa, although it is not restricted to this continent. Cases are also known from South and Central America. Only a few examples have been reported from the Mediterranean countries. African countries where *A. madurae* has already been found include Algeria, Mauritania, Senegal, Mali, Upper Volta, Niger, Nigeria, Chad, Sudan, Uganda, Zaïre, Somalia, Afars and Isas. Only one case has been reported from Madagascar.

A. madurae has been readily recovered from soil samples,[5] but recently a strain resembling the pathogenic strains has been isolated from river water in South Africa, in a tributary of the Jukskei near Johannesburg.[9] This lies in the same watershed as the region from which our patient came. It is interesting to recall that *A. pelletieri* (Laveran) Lechevalier and Lechevalier was isolated in the same region from river water in 1969, but only 3 cases of clinical infection have been reported in South Africa from this organism. This subject has been discussed elsewhere.[10]

CASE HISTORY

A 28-year-old Black man from the Pietersburg district had been injured by a thorn just below his left medial malleolus about 8 years before admission. As the lesions were painless he had not sought medical aid before. His foot gradually started swelling, and on admission to hospital it was indurated, grossly swollen, with multiple sinus openings from which there was a seropurulent discharge that contained granules (Fig. 1). These granules were yellow-white, soft, and larger than any that we have ever seen in mycetoma cases. No amyloid could be found.

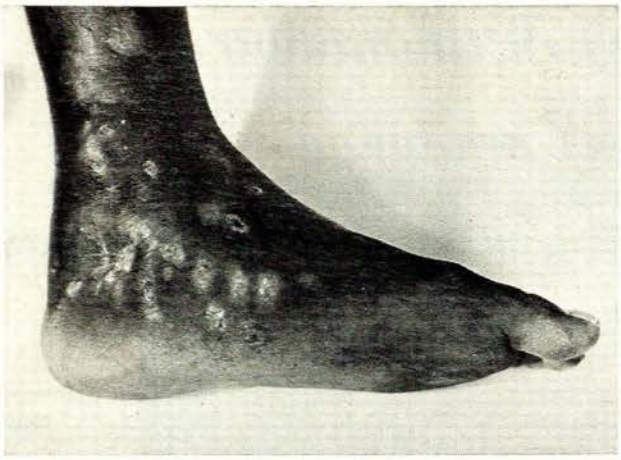

Fig. 1. Mycetoma caused by *A. madurae*.

Widespread bone involvement was seen on the X-ray films (Figs 2 and 3). The granuloma had infiltrated the tarsals, calcaneus, talus and lower ends of the tibia and fibula. The cortical shadows in these bones could not be identified. The spongy bone was sclerotic, with loss of the normal trabecular pattern. There were also multiple round areas of reduced density of about 5 mm diameter, representing sinuses in the bone.

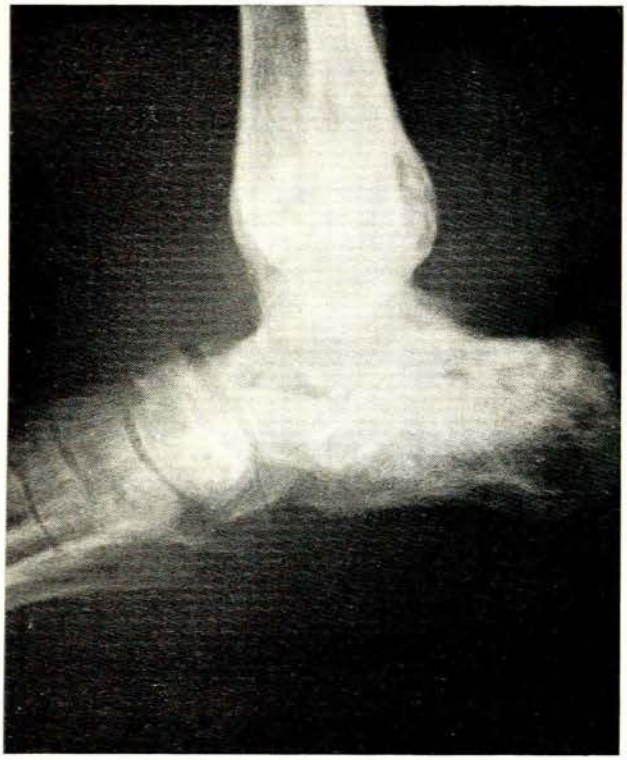

Fig. 2. X-ray film of the foot showing lytic areas representing sinuses in the bone and sclerosis of the spongy bone with loss of the normal trabecular pattern. Cortical shadows can not be identified in the affected bones.

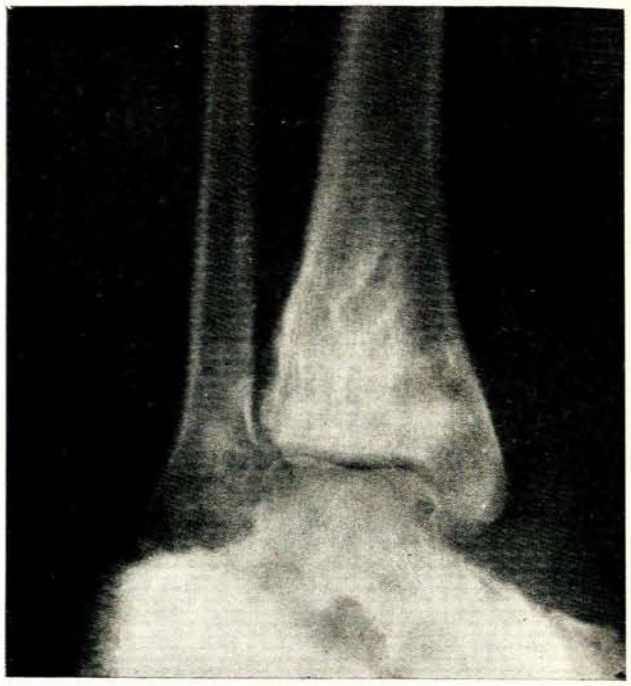

Fig. 3. X-ray film of the ankle showing the same bony changes as in Fig. 2.

Histopathology

The lesions consist mainly of vascular granulation tissue with lymphocytes and histiocytes as the predominant cells. The granules are surrounded by collections of polymorphonuclear leucocytes and are widespread in the granuloma. The epidermis extends down the sinus openings for a short distance, but there is no pseudo-epitheliomatous hyperplasia above the granuloma. Some of the granules have an eosinophilic central area on haematoxylin and eosin staining. The filaments are found around this area and are deeply stained with haematoxylin (Fig. 4). The outer filament layer is dense, and is surrounded by a homogeneous eosinophilic area. In some parts this has a radially toothed appearance, as described by Lynch *et al.*[7] (Fig. 5).

Treatment

The patient was treated medically, despite the widespread bone involvement, to avoid amputation. It has been suggested that *A. madurae* is one of the organisms most sensitive to antibiotics among those that cause actinomycotic mycetoma.[7]

Initial therapy consisted of Bactrim, potassium iodide and intramuscular penicillin and streptomycin. The swelling subsided, the sinuses dried and the patient could walk more easily. He was discharged after 10 weeks, on ampicillin, Bactrim and potassium iodide for home treatment, but was readmitted 3 weeks later with his foot swollen and the sinuses once again draining.

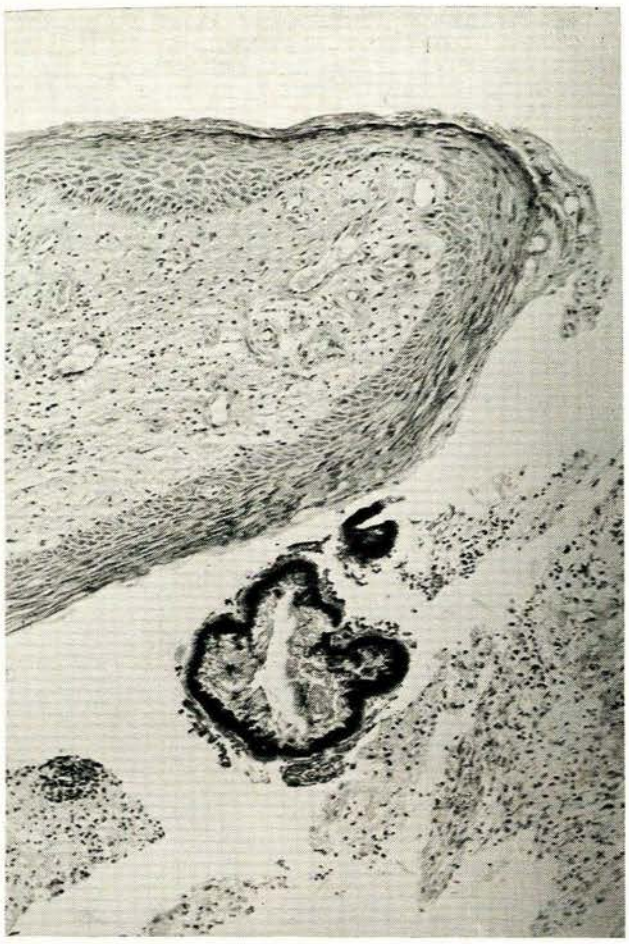

Fig. 4. A granule in a sinus with the epidermis extending down the sinus wall.

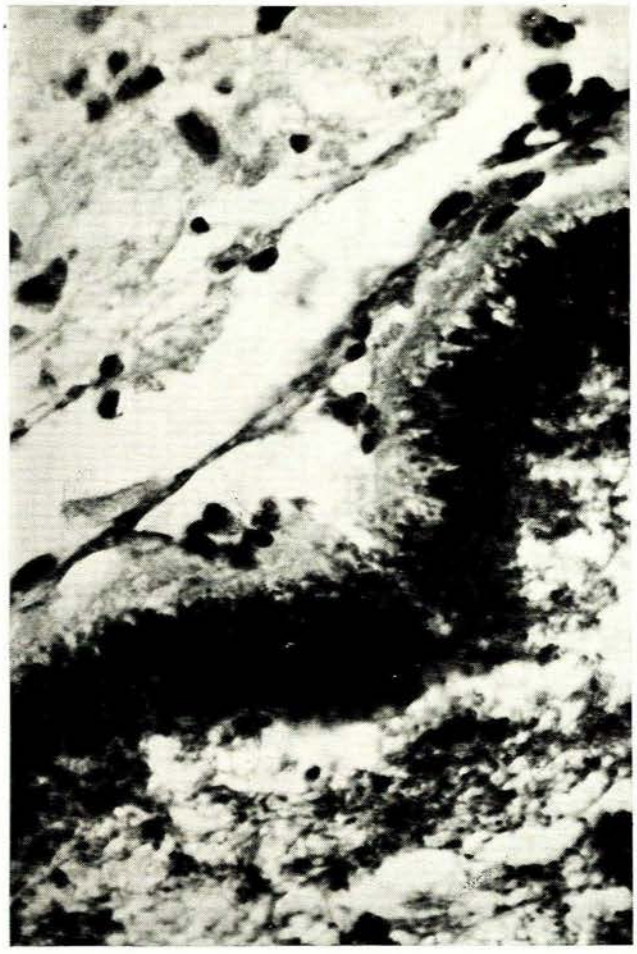

Fig. 5. Magnification (× 400) of a section showing a portion of a granule on the lower half of the picture. The granule is surrounded by a homogeneous eosinophilic area. The dense outer filament layer has a radially toothed appearance.

He was then treated with ampicillin and dapsone for 7 weeks, and dapsone alone for a further 8 weeks. The lesions appeared to be totally healed, and the patient walked without discomfort. The X-ray appearance also showed great improvement, although there were still some round osteolytic lesions and the cortex outline was still slightly disturbed.

We propose to treat the patient with dapsone for at least one year to forestall a recurrence of this disease. For a summary of the treatment, see Table I.

Grains

The grains discharged from the sinuses were of two sizes: small (1 - 2 mm diameter) and large (2 - 5 mm diameter) (Fig. 6). Both types were yellowish-white and lobulated. The grains were found to be soft and could easily be crushed into a paste under a coverslip. The smaller grains had possibly developed more recently. Clusters of 4 - 6 grains of either size were often observed discharging from the sinuses.

Microscopy of the fresh grains showed Gram-positive, delicate, unsegmented branching filaments. No fragmentation, spore or chlamydospore formation was observed. The organism was non-acid-fast.

Culture

Fresh grains were inoculated directly onto Sabouraud dextrose agar (with chloramphenicol and cycloheximide), Löwenstein-Jensen medium and glycerol agar. Growth was slow on Sabouraud agar at 27°C. After 28 days small, shiny, white- to cream-coloured colonies were noticed. They became wrinkled and folded and after 6 weeks some colonies began to produce a red pigment. Growth at 27°C on glycerol agar appeared sooner (10 - 18 days), the colonies appearing much the same as on Sabouraud agar. They were slightly sunken into the medium. The best growth at 27°C was obtained on Löwenstein-Jensen medium. The colonies were bigger, crust-like and wrinkled,

TABLE I. SUMMARY OF THERAPY GIVEN

	Therapy	Dosage per day	Duration of therapy	Result
In hospital	Streptomycin	1 g	10 weeks	Rapid improvement
	Penicillin G	400 000 E		
	Trimethoprim/ sulphamethoxazole (Bactrim)	(Trimeth. 80 mg, Sulph. 400 mg.) 4 tablets		
	Mist. Pot. Iod.	1,5 g KI		
At home	Trimethoprim/ sulphamethoxazole (Bactrim)	(Trimeth. 80 mg, sulph. 400 mg.) 4 tablets	3 weeks	Worse
	Mist. Pot. Iod.	1,5 g KI		
In hospital (2nd admission)	Ampicillin	1 g	7 weeks	Progressive improvement
	Dapsone	50 mg	7 weeks	
		100 mg	8 weeks	

with irregular edges. They soon displayed various shades of red.

At 37°C, the strain grew faster (6 - 10 days) on all the abovementioned media. No aerial mycelium was observed and no diffusible pigment was noted. At 37°C, the strain hydrolysed casein after 4 - 6 days, gelatin was liquefied and litmus milk peptonised and coagulated after 20 - 30 days. In 0,4% gelatin, small, round, white-pinkish colonies

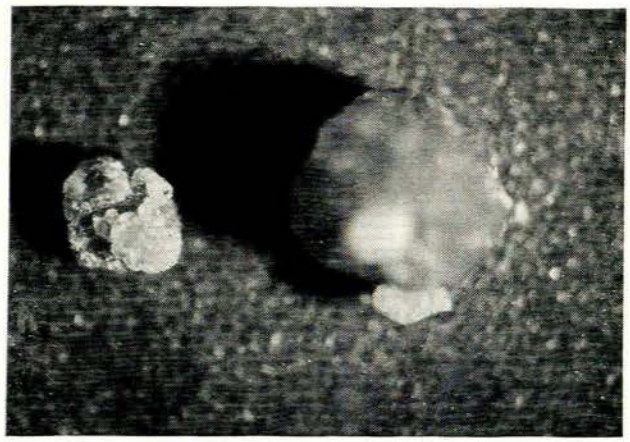

Fig. 6. Granules: small — 1,5 mm diameter (left), **and large — 4 mm diameter** (right). **Both types were yellowish-white and lobulated.**

grew (37°C) after 14 - 20 days. Survival at 50°C was not tested. Microscopy of the culture showed the same features as were seen in the fresh grains—delicate, branching, Gram-positive filaments without fragmentation or acid-fastness. By comparing our isolate with a type strain of *A. madurae* (CBS 254.58), by observing the strikingly large soft, yellow-white grains produced, and by noting the

TABLE II. PHYSIOLOGICAL SIMILARITIES AND DIFFERENCES OF *A. PELLETIERI* AND *A. MADURAE* AS ISOLATED BY US IN THE TRANSVAAL. SUMMARY OF GEOGRAPHIC DIFFERENCES

Physiology	*A. madurae*	*A. pelletieri*
Fragmentation of filaments	−	−
Acid fastness	−	−
Casein hydrolysis	+	+
Liquefaction of gelatin	+	+
Growth in 0,4% gelatin	+	−
Litmus milk	C*	C*

Grains	*A. madurae*	*A. pelletieri*
Size	2 mm diam.	1 mm diam.
Colour	White-yellow	Red
Consistency	Soft	Very hard

Geographical distribution		
Africa (apparently unique areas italicised)	*Algeria*, Mauritania Senegal, Mali, Upper Volta, Niger, Nigeria, Chad, Sudan, *Zaire*, *Uganda*, Somalia, *Affars* + *Isas* South Africa (1 case)	Mauritania, Senegal, Mali, Upper Volta, Niger, Nigeria, Chad, Sudan, *Tanzania*, *Kenya*, Somalia. South Africa (3 cases)
Elsewhere	South and Central America Madagascar (1 case)	South America (few cases) Madagascar (few cases)

* Peptonised and coagulated.

microscopy, the proteolytic and amylolytic properties,[9] we felt confident that we were dealing with a genuine example of *A. madurae*.

DISCUSSION

Both species of mycetoma pathogens, *A. madurae* and *A. pelletieri*, which have been isolated from river water in South Africa, have failed to grow at 37°C. The pathogenicity of these wild, free-living strains is therefore doubtful. It is uncertain how these strains in river water must change before they can cause mycetoma.[9]

The pathogenic strains of *A. madurae* and *A. pelletieri* are closely related (Table II). *A. madurae* can grow in 0,4% gelatin, but not so *A. pelletieri*. The grains they produce in tissues differ in size, colour and consistency. Case reports of *A. pelletieri* are mainly confined to Africa while *A. madurae* cases show a wider distribution (Table II). Moreover, there are no reports of any transitional or seemingly mixed infections. This demonstrates that the two species differ in spite of some close physiological resemblances.

Countries with a warm, dry climate seem to produce more mycetoma cases,[7] and the pathogens are found mainly in the soil. It seems unlikely that water plays an important role as a source of the infection. If water is a natural habitat of the organisms, selection for the pathogens can take place in irrigated soil.

The identification of the causative agent in mycetoma cases is valuable because it suggests conservative treatment and saves the patient an amputation. Treatment in cases of actinomycotic mycetoma readily fails if discontinued too early as the patients require supervised treatment for as long or longer than any case of tuberculosis. For prolonged treatment sulphones and trimethoprim/sulphamethoxazole combinations are indicated, provided the side-effects of these drugs are borne in mind.

This work was undertaken with the support of the South African Medical Research Council.

REFERENCES

1. Vincent, M. H. (1894): Ann. Inst. Pasteur, **8**, 129.
2. Gordon, Ruth E. (1966): J. Gen. Microbiol., **45**, 355.
3. Lechevalier, Mary P. (1968): J. Lab. Clin. Med., **71**, 934.
4. Lechevalier, Mary P. and Lechevalier, H. A. (1970): *The Actinomycetales*, p. 311. Jena: Gustav Fischer.
5. Lechevalier, H. A. and Lechevalier, Mary P. (1970): *Op. cit.*,[4] p. 393.
6. Wilson, A. M. M. (1965): East Afr. Med. J., **42**, 182.
7. Lynch, J. B. and Moghraby, I. (1961): Trans. Roy. Soc. Trop. Med. Hyg., **55**, 446.
8. Mariat, F. (1963): Bull. Soc. Path. exot., **56**, 35.
9. Lawson, Ellen N. and Davey, Lorna M. (1972): J. Appl. Bact., **35**, 389.
10. Findlay, G. H. and Roux, H. F. (1971): Brit. J. Derm., **85**, suppl. 7, p. 85.