J. Hyg., Camb. (1970), **68**, 97
Printed in Great Britain

Isolation of *Mycobacterium xenopei* from water taps

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(*Received* 19 *August* 1969)

SUMMARY

An increase in the number of isolations of *Mycobacterium xenopei* from sputum suggested environmental contamination. The organism was recovered from water taps in 61 of 111 pairs of hot and cold water taps in one hospital, 20 of the 74 pairs in another hospital, but from only 3 of 61 pairs of taps in a third hospital and two of 34 pairs in private houses. Scotochromogens were recovered from taps in the first two hospitals only, and *M. kansasii* was isolated twice from the same tap in the first hospital. No mycobacteria were isolated from pigeon droppings or viscera, nor from patients' tooth brushes, tooth powder or razor debris. The source of contamination is not known.

INTRODUCTION

During 1967 there was an increase in the number of isolations of *Mycobacterium xenopei* from specimens of sputum examined at the Epsom Laboratory. Of 435 sputa examined, 16, all from different patients, yielded scanty but pure growths of *M. xenopei*, whereas in the previous year this organism had been isolated once only. Thirteen of these patients were known cases of pulmonary tuberculosis and further sputa from these yielded neither *M. xenopei* nor *M. tuberculosis*. The remaining three patients had left the hospital and could not be traced.

Ten of these patients were in one ward at Hospital A. Contamination from an environmental source was suspected and it was decided to investigate likely sites in this ward and in the laboratory. Subsequently, after the isolation of *M. xenopei* from water taps in both ward and laboratory, the survey of taps was extended to other wards at Hospital A, to Hospitals B and C and to 10 private houses, all supplied by the same water company. Hospitals A and B had water towers housing storage tanks which were not completely protected from dust and bird droppings. Hospital C had water tanks in the roof space; this was not normally accessible to birds, although they had entered it in the past.

METHODS

Collection of material

Swabs were taken from hot and cold water taps and cultures were made from the tooth powder, tooth brushes and electric razors used by the patients. No water

carafes were used in these wards. Droppings from pigeons and from seagulls which congregated during the winter months on the grass surrounding the ward were collected.

The laboratory reagents and apparatus used in the culture of tubercle bacilli were tested.

Bacteriological methods

Sterile cotton wool swabs were used to sample the water taps and centrifuge buckets; each swab was agitated in 1 ml. of quarter-strength Ringer's solution. The fluid was treated by a modified Petroff's method (Cruickshank, 1965) with both incubation at 37° C. and centrifugation at 4° C. for 15 min.; two Lowenstein–Jenson slopes were inoculated and incubated for 8 weeks. At first one swab was used for each pair of hot and cold taps, but later in the survey one swab was used for each tap.

Tooth powder, tooth brushes and razor debris were mixed with a small quantity of quarter-strength Ringer's solution. Pigeon and seagull excreta were suspended in quarter-strength Ringer's solution and allowed to stand for 30 min. The supernatant was concentrated by centrifugation and inoculated on Lowenstein–Jenson slopes after treatment by the modified Petroff's method.

A control consisting of 1 ml. of quarter-strength Ringer's solution in a universal container was included with each batch of tests. This was treated in the same way as the test specimens.

Identification of mycobacteria

Cultures showing acid fast organisms were examined by the methods described by Collins (1967) except that sensitivity tests were restricted to isoniazid and ethionamide.

RESULTS

The isolation rates of *M. xenopei* in pairs of hot and cold taps in Hospitals A and B (55 % and 27 % respectively) were very much higher than at Hospital C (5 %) (Table 1). *M. xenopei* was isolated from the taps of only one of the ten houses swabbed.

Table 1. *Isolation of mycobacteria from water taps*

Building studied	Total no. of pairs of taps sampled (hot and cold)	Mycobacteria isolated		
		M. xenopei	Scoto-chromogens	*M. kansasii*
Hospital A	111	61 (55)	11 (10)	1 (1)
Hospital B	74	20 (27)	37 (50)	—
Hospital C	61	3 (5)	— —	—
Ten private houses	34	2*(6)	— —	—

* These isolations were from one house. Numbers in parentheses indicate percentages.

https://doi.org/10.1017/S0022172400028540 Published online by Cambridge University Press

M. kansasii was isolated from two of five swabs from one tap in hospital A over a period of 9 months. The identity of this strain was confirmed by the Tuberculosis Reference Laboratory, Cardiff.

Scotochromogens (47 strains) were isolated from taps in Hospitals A and B only.

Once *M. xenopei* had been isolated from water taps, hot and cold taps were sampled separately. *M. xenopei* was isolated from 36 hot taps but from only 11 corresponding cold taps.

Hospitals A and B both had water towers which had been contaminated with pigeon droppings. Twenty-seven samples of pigeon droppings from the vicinity of the water tanks, and livers and spleens from eight dead pigeons, were examined over a period of 7 months with negative results. Eleven samples of seagull droppings were also negative.

M. xenopei was not isolated from tooth powder, tooth brushes or electric razors used by the patients in a ward at Hospital A.

M. xenopei was present in most of the water taps in the laboratory and was occasionally isolated from the centrifuge buckets. However, it was not isolated from any of the reagents used, nor from the controls which were included with each of the 28 batches of specimens. During the period of study 187 samples of sputum were cultivated for mycobacteria and all were negative for *M. xenopei*. It seemed highly unlikely, therefore, that laboratory contamination could account for the isolation of *M. xenopei* from taps; (28 % of all taps tested were positive).

DISCUSSION

Mycobacterium xenopei was described by Schwabacher (1959), who isolated it from a skin lesion in a toad (*Xenopus laevis*) in a pregnancy diagnosis laboratory. The role of this organism as an opportunist pathogen is discussed by Marks & Schwabacher (1965) and by Marks (1968). *M. xenopei* is frequently isolated from pathological material but in a high proportion of cases it appears to have no significance. Marks & Schwabacher reported 24 non-significant isolations (including six from urine) amongst 50 cases studied. In the three years, June 1966 to June 1969, 68 of 103 cultures identified as *M. xenopei* at The Regional Centre for Tuberculosis Bacteriology in London were single isolations and probably not significant. Eleven of these were from urine, three from gastric contents, two from endometrium and the remainder from sputum.

Most isolations reported in the United Kingdom are from London and South-Eastern England (Marks & Schwabacher, 1965). The normal habitat of *M. xenopei* is not known, but in view of the high incidence in coastal areas in England and Europe (Marks, 1964) and the high optimal growth temperatures (42°–44° C.), it is possible that sea-birds might be infected. Pigeons are also very common in the Epsom area but our attempts to isolate *M. xenopei* from seagull and pigeon droppings and from dead pigeons were not successful.

M. xenopei was isolated from 300 patients in a single hospital district in Le Havre by Lelieur (1968). The organism was obtained once only from 268 of the 300 patients and environmental contamination was suspected. This mycobacterium

was not, however, recovered from dust, air, pigeon droppings or water, but the water taps were not examined.

At Epsom it seems likely that the organisms entered the water tanks at some time, survived heating in the calorifiers (the temperature of the tap water in the three hospitals varies between 54° and 64° C.) and infected the slime in the water taps. *M. xenopei* is 'thermophilic' (42°–44° C.) and may survive higher temperatures for short periods when the taps are in use. It does not grow appreciably at 25° C. in 3 weeks and this temperature preference may account for the higher rate of isolation from hot taps.

The results of this investigation suggest that strains of *M. xenopei* isolated on one occasion only should not be regarded as significant and that if the organism is encountered several times in the same laboratory over a short period contamination should be suspected.

The isolation of *M. kansasii* is of interest. The natural habitat of this organism is not known and attempts to recover it from the environment in areas where there is a high incidence of *M. kansasii* infection have been unsuccessful (J. Marks, personal communication; Spencer Jones, 1969).

The distribution of scotochromogens was unexpected; only Hospitals A and B yielded these organisms. No scotochromogens were cultured from sputum specimens during the period of study, but in general sputum receives more prolonged treatment with sodium hydroxide than that given to the swabs from water taps, and species of mycobacteria are known to differ in susceptibility to this reagent.

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