



Experience with *Mycobacterium haemophilum* in Australia, 1977-2003

Introduction

Mycobacterium haemophilum is a fastidious microorganism that requires X-factor (haemoglobin or haemin) or ferric ammonium citrate (FAC) and only grows around 30-32°C. Since it was first described in 1978¹ there have been 104 isolations in Australia up to 2003 and the Australian Mycobacterium Reference Laboratory Network (AMRLN) have collected strains and associated epidemiological information. This review looks at this information and laboratory isolation media and practices used to recover *M. haemophilum* in Australia. Some important recommendations are made.

During the period 1977 to 2003, *M. haemophilum* was isolated from a wide variety of sites, more often from non-immunocompromised individuals than from immunocompromised ones, when HIV data were excluded (Figures 1 & 2). The demographic profiles of the cases are given in Table 1.

Disease occurred across all ages from 1-92 but had a peak in the 1-2 year old age group that was associated mainly with lymphatic disease. The male to female ratio overall was roughly equal but varied when the data were stratified. Amongst the known HIV cases, there were 12 males and one female, the average age was 42 years (range 17-53) and the main tissues involved were blood (n=2), skin (n=5), soft tissue (bursa, bone, joint; n=3) and sputum (n=3). In the immunosuppressed (non-HIV) group, there were 12 males and 22 females, the average age was 56 (range 3-92) and the main tissues involved were blood/ bone marrow (n=2), skin (n=27), soft tissue (tendon; synovium; n=3), sputum (n=4) and lymph node (n=2). The main underlying conditions were renal or bone marrow transplantation, malignancy or steroid involvement.

CF (Frank) Haverkort

Mycobacterium Reference Laboratory
Western Australian Centre for Pathology &
Medical Research (PathCentre)
Division of Microbiology &
Infectious Diseases
Diagnostic Bacteriology & Mycology
Locked Bag 2009
Nedlands, WA 6909
Tel: (08) 9346 2162
Fax: (08) 9346 3354
E-mail: frank.haverkort@health.wa.gov.au

In the 'healthy' group, there were 21 males and 20 females, the average age was 23 years (range 1-82) and the tissue types associated were skin (n=17), tendon (n=1) and lymph nodes (n=25). When we stratified the data for the lymphatic cases, the average age was 11 years (range 1-57) and for the skin/soft tissue cases the average age was 40 (range 1-82). Some patients had isolations from more than one site. No information was available for 5% of the data on site of infection, and 15% of the data on immunological status.

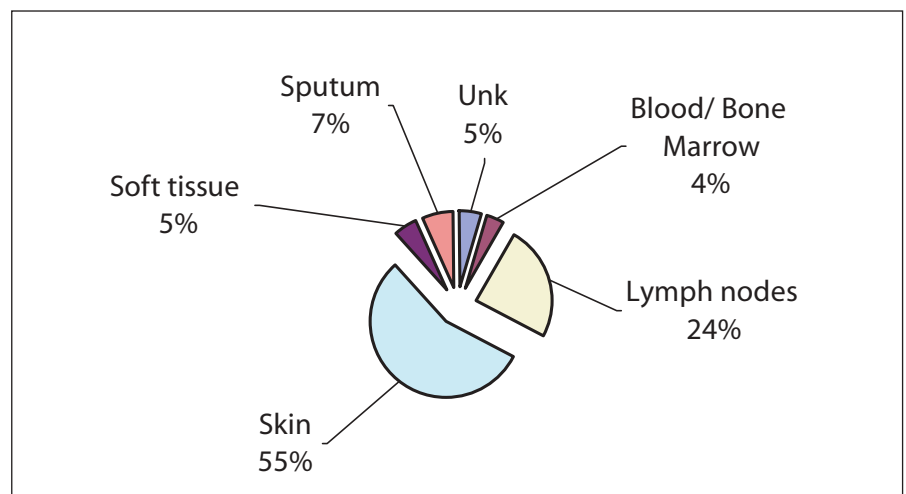


Figure 1. *M. haemophilum* in Australia 1977-2003 (n=104), by site of isolation. In some cases, isolations occurred from more than one site.

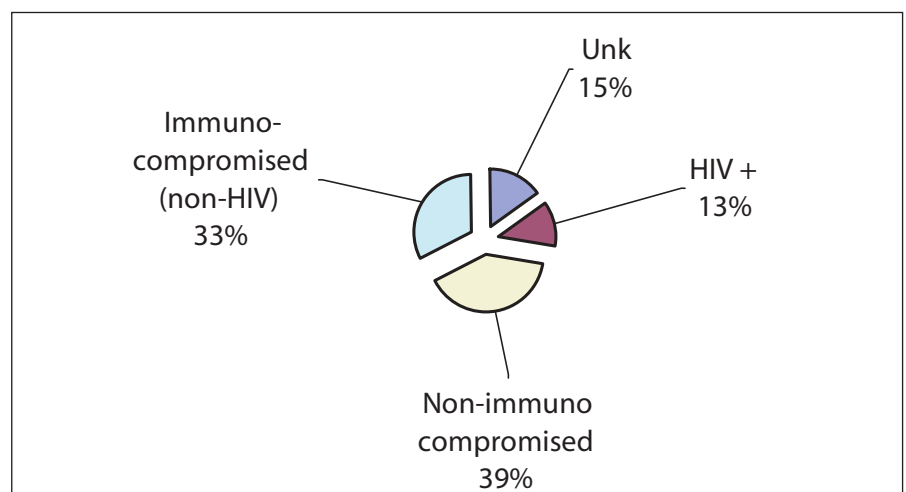


Figure 2. *M. haemophilum* infection in Australia 1977-2003 (n=104), and relationship to immunological status.

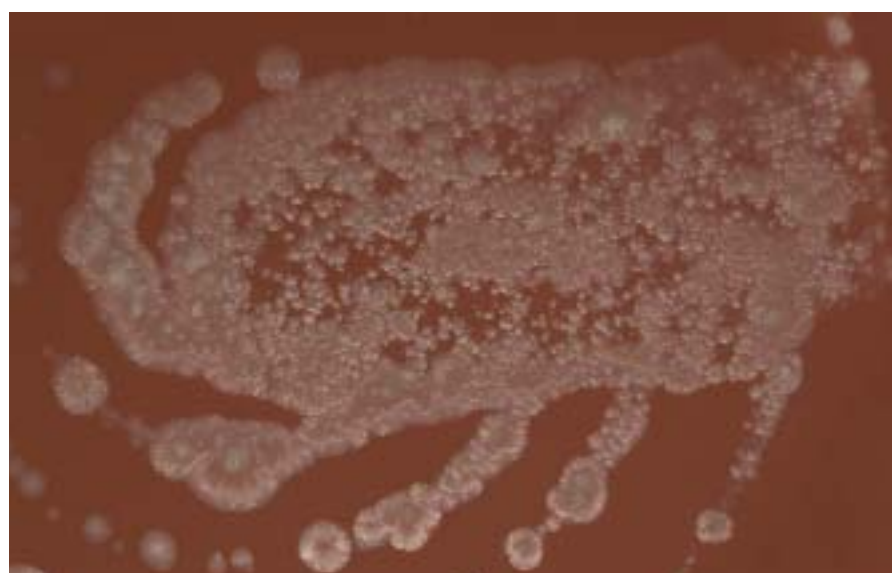


Table 1. Demographic analysis of infections caused by *M. haemophilum* for which the diagnostic history was available, Australia, 1977-2003 (n=88).

	HIV n=13	Immuno- compromised (non-HIV) n=34	Non immuno- compromised n=41
M:F ratio	12:1	12:22	21:20
Ave age (y) (range)	42 (17-53)	56 (3-92)	23 (1-82) for lymphatic: 11 (1-57) for skin/soft t: 40 (1-82)
Blood/ BM	2	2	–
Skin	5	27	17
Synovial	1	2	–
Tendon	–	1	1
Bursa	1	–	–
Bone	1	–	–
Sputum	3	4	–
Lymph node	–	2	25

Some isolations were made from more than one site.

* A retrospective clinical and outcomes review of 52 Western Australia (WA) cases was performed in 2004 by Dr Astrid Arellano, Infectious Diseases and Microbiology Registrar, Women's and Children's Health Service, Princess Margaret Hospital, Perth, WA. Patterns of disease as well as therapeutic outcomes are examined. A manuscript is in preparation.



M. haemophilum on chocolate agar plate.

Because the data also highlighted the fact that over half the isolations occurred in WA, we investigated the primary isolation media used to recover *M. haemophilum*. In WA, 50% of isolations (when the primary isolation medium was known) were recovered on Kovács B83 medium² and very few on Lowenstein Jensen (LJ) with FAC, a medium used widely in other States.

We were concerned there may be some peculiarity of geography or growth requirement amongst the WA strains, so we undertook a trial of all in-use primary isolation media in Australia (and New Zealand) against stored isolates.

The results of that trial showed two main features. Firstly, haemin-containing media (chocolate agar – regardless of manufacturer) and Kovács B83 developed visible colonies within 5-7 days, 1 or 2 days earlier than the best of the LJ-pyruvate-FAC media, although there was little difference by Day 13. Secondly, some strains did not grow well on all of the LJ formulations and there was wide variation in the concentrations of FAC used (the best contained FAC at 0.085%).

The trial also emphasised how easily *M. haemophilum* could be recovered in a routine bacteriology laboratory simply by incubating chocolate agar up to 10 days at 30-32°C (the strains grew far more poorly on horse blood agar). Strains grew very rapidly in Mycobacteria Growth Indicator Tubes (MGIT; BD Biosciences, USA) with added X-factor.

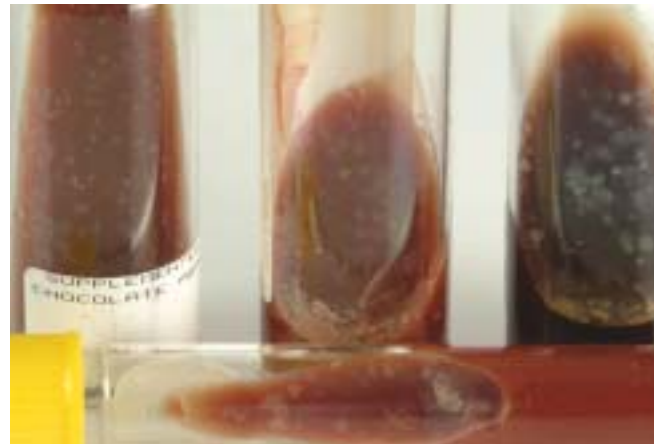
Thus Kovács B83 medium, per se, demonstrated no nutritional advantage, although it does contain penicillin as a helpful selective agent. The LJ + 0.085% FAC formulation was the most successful, and its formulae, and that of Kovács B83 medium, are available on request.

Discussion

M. haemophilum has been a rarely encountered pathogen, but should be seen more frequently as laboratories re-examine their isolation practices.



M. haemophilum on various FAC slopes.



M. haemophilum on chocolate agar and B83 slopes.

Unfortunately, the Australian clinical data presented here are skewed, with half the isolates coming from WA and several deficiencies in isolation media and techniques noted.

Appropriate *M. haemophilum* media, set up at 30-32°C, should be used for most tissues types: blood, bone marrow, skin, tendons, joints, bone, bursa, lymphatic &

the like. In immunocompromised individuals, it requires a specific 30-32°C culture of pulmonary samples. Recovery media requires rigorous quality assurance and users should be encouraged to use standardised formulations.

The AMRLN will continue to review the *M. haemophilum* story for Australia and a better demographic picture will emerge

of this most interesting *Mycobacterium* species. (The author would be grateful for strains and details of future cases).

References

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2. Cousins DV, Francis BR & Gow BL. Advantages of a new agar medium in the primary isolation of *Mycobacterium bovis*. *Vet Micro* 1989; 20:89-95.

Diagnosis of mycobacteria in the routine diagnostic laboratory

Introduction

Non-tuberculous mycobacteria (NTM) have become increasingly important over the last 20 years. These mycobacteria are classified as 'rapid growers' (growth in 7 days or less) or slow growers (growth of isolated colonies in more than 7 days).

The rapidly growing mycobacteria (RGM) are the group of mycobacteria which are being recognised in cultures performed in routine diagnostic microbiology laboratories. The increase in notification rate of RGM is partially due to a heightened alertness for mycobacteria by clinicians and laboratory scientists and technicians. However, diagnostic delays remain relatively common, with

Chris Gilpin

Queensland Mycobacterium
Reference Laboratory
Queensland Health Pathology Services
Prince Charles Hospital,
Rode Road, Chermside, Qld 4032

mycobacteria being considered only following failed treatment with empiric therapy.

In immunocompetent patients, the most commonly encountered rapidly growing mycobacteria include *Mycobacterium chelonae*, *M. abscessus*, and the *M. fortuitum* complex which are often

implicated in skin and soft tissue infections associated with penetrating injuries, catheter associated or iatrogenic infections. *M. mucogenicum*, which is resistant to the activity of many disinfectants, is seen in catheter associated infections. In immunocompromised patients or patients with underlying lung pathology, e.g. cystic fibrosis disease, RGM can cause severe disseminated pulmonary disease.

M. abscessus is the most predominantly isolated pathogen among the latter patient group. Other clinically significant mycobacteria which can be isolated in the routine bacteriology laboratory include *M. haemophilum* and *M. marinum*. It is