MELIOIDOSIS

D N FRAZER, FIMLS, ART(Canada)

Cornwall General Hospital, Cornwall, Ontario
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SUMMARY: The author presents his personal laboratory experience of melioidosis and reviews the history, epidemiology, salient clinical features and bacteriology including the serological diagnosis of this dangerous tropical disease.

Introduction

Melioidosis is a glanders-like disease of man. The acute disease associated with septicaemia may have a mortality rate close to ninety percent but is still a relatively rare occurrence in man. Sub-acute and chronic pulmonary infections, as well as latent and sub-clinical infections, are also recognised. Serological surveys have shown that up to ten percent of indigenous populations and visitors to endemic regions may suffer from clinically inapparent or unrecognised disease¹⁻⁴. Thin *et al* stressed the importance of early diagnosis of chronic latent and sub-acute melioidosis to prevent progression to acute disease which still has a very poor prognosis⁵.

My particular interest in melioidosis began during service in the Royal Army Medical Corps in Malaysia from 1964 to 1966. I was associated with the laboratory diagnosis of two patients who succumbed to melioidosis i.e. the first two cases reported by Thin *et al*³. Interest was further aroused during a year I spent as Chief Technologist in the Department of Microbiology, Makerere University, Kampala, Uganda 1971-72. In addition to more routine functions in the University and Mulago hospital laboratories, I was involved in a research project screening for melioidosis antibodies in Uganda. This paper is a review of my laboratory experiences in the diagnosis of melioidosis.

History and geographical distribution

The disease is caused by the gram negative bacillus *Pseudomonas pseudomallei*. Originally described by Whitmore in 1911 as *Bacillus pseudomallei*, the causative organism has been documented under many pseudonyms; *Bacillus whitmori*, *Pfeifferella whitmori*, *Loefflerella pseudomallei*, *Malleomyces pseudomallei*, *Flavobacterium pseudomallei* and *Actinobacillus pseudomallei*⁵⁻⁷. However Cowan and Steel showed the organism to possess flagella and proposed it be classified within the genus *Pseudomonas*⁸.

Whitmore's first report, published in 1912, suggested the disease was an affliction of vagabonds and morphine addicts in Rangoon, Burma^{7,9}. In the following year, Fletcher recognised a glanders-like disease occurring amongst laboratory animals in Kuala Lumpur, Malaysia, and in 1917 Stanton described the first infection in a human patient in the same city. Stanton also showed the isolated organism from the human patient to be the same as those described by Whitmore and Fletcher in 1912 and 1913^{10,11}. Altogether in the following 15 years, Stanton

and Fletcher reported a total of 39 patients suffering from melioidosis in this region of the Malay peninsula¹¹.

The disease was next reported from Vietnam in 1925 and the first report of an infection in a European was recorded by Denny and Nicholls from Ceylon in 1927. Infections also occurred in both Allied and Japanese soldiers during the second World War and in the post war period, reports of the disease were received from many parts of the world^{8, 12-15}. More recently melioidosis posed a considerable military health problem to American forces in Vietnam in the sixties and seventies^{5, 16, 17, 27, 29}. Indeed, the frequency and commonly fatal results of the acute form of the disease within the American Military during this period, prompted Time Magazine to refer to it as the "Vietnamese Time Bomb"¹⁸.

Fig. 1. Known endemic geographical distribution of melioidosis¹⁵. Each spot indicates an area from which at least one bacteriologically diagnosed case of the disease has been reported.

Epidemiology and Source in Nature

For many years it was assumed the primary reservoir of infection was wild rats, although the disease itself was not apparently widespread amongst the rat population of endemic areas. The outbreak in laboratory animals reported by Fletcher in 1913 was thought to be the result of contamination of animal foodstuff with the excreta of wild rats. More recent studies by Strauss and his co-workers in Malaysia show the organism to be a saprophyte of soil and surface water^{19,20}. Survival times of *Pseudomonas pseudomallei* are shown.

TABLE I

Medium	Survival time in days	Medium	Survival time in days
Dry earth	27	Urine	17
Tap water	44	Pus	120
Faeces	27	Putrid carcasses	7

Route of infection occurs through broken skin, inhalation of contaminated dust particles or possibly from ingestion of contaminated food. Clinical histories by Thin *et al*, in their account of 10 human cases of melioidosis in Malaysia, clearly showed all patients had a recent history of skin abrasions from sports and other outdoor activities³. In a survey of 150 cases from Vietnam, one third were helicopter crewmen who were, of course, exposed to inhalation of dust disturbed by helicopter rotors¹⁵.

Serological studies show the organism to be endemic throughout Southeast Asia. In 1963, Nigg provided ample evidence of the presence of sub-clinical infection in native Thais. Using both complement fixation tests and haemagglutination reactions, she demonstrated antibodies to *Pseudomonas pseudomallei* in eight per cent and 29 per cent of 405 sera tested by both methods²¹. Antibodies have also been detected in the sera of apparently healthy and symptomless servicemen returning from overseas duty in Vietnam and Malaysia^{4,22,23}.

There are no substantial reports in published literature of transmission of melioidosis from animal to man and there is but one report of human to human transmission²⁴. McCormick and his associates described a war veteran who presented with prostatitis two years after his return from Vietnam. *Pseudomonas pseudomallei* was isolated from prostatic secretions and he also had a positive haemagglutination titre of greater than 1/640. Cultures of vaginal swabs from his wife were negative for the organism, however she did reveal an equally high haemagglutination titre. In 1968, Green and Tuffnell reported a case of laboratory acquired infection and suggested special care be directed to handling live cultures in the laboratory. Indeed, some workers also recommend isolation procedures for the patient suspected of suffering from melioidosis²⁵. It is interesting to point out here that the American Type Culture Collection considers *Pseudomonas pseudomallei* highly infectious and hazardous to public health and requires permits from both the U.S. Department of Agriculture and the U.S. Public Health Service before undertaking shipment of a stock culture (personal communication). *Pseudomonas pseudomallei* falls into Category B1 of the Department of Health and Social Security Code of Practice for the Prevention of Infection in Clinical Laboratories and Post-mortem Rooms.

Diagnosis

Acute forms of the disease are of sudden onset, with associated fever, confusion and prostration. It is commonly progressive with overwhelming septicaemia. Malnutrition or debilitation from other diseases such as diabetes mellitus, carcinoma and cirrhosis may only serve to confuse the clinical picture or may even precipitate active disease states from sub-clinical and latent infections. *Pseudomonas pseudomallei* can be isolated from blood and sputum, or from pustular lesions which may be present on the arms, legs and main body trunk^{3,16,26}. In fatal cases the organism has been isolated from autopsy material from most major organs of the body except the intestinal tract¹⁵. Thin suggested three additional classifications of melioidosis, namely, sub-acute, chronic and sub-clinical. Within these divisions, symptoms may be even more obscure and resemble nothing more than mild

or chronic respiratory ailments^{5, 12, 17, 22, 27, 28, 29}. In other instances, latent infections with recrudescence up to 26 years following initial exposure have been reported³⁰. Initial growth of the organism is slow, and after only 24 hours incubation it may be missed amongst normal flora of sputum cultures. A good clinical history, a suspicious physician and a well informed laboratory will prompt prolongation of sputum cultures to 48 hours and beyond, in order to ensure retrieval and isolation of the causative organism².

Pseudomonas pseudomallei is a gram negative bi-polar staining bacillus. Bi-polar staining characteristics are more apparent when stained by a Romanowsky technique⁵. It is non-capsulated, non-acid fast, non-sporing and is motile by possession of polar flagella. The organism grows well on simple media, including nutrient, blood, McConkey and Sabouraud's dextrose agars but does not produce growth on desoxycholate citrate and Salmonella/Shigella (SS) agars. In endemic areas, Thin recommended the use of a selective medium, crystal violet glycerol agar, for screening pus and sputa specimens. It contains three percent glycerol and 1/200,000 crystal violet in a nutrient agar base³.

The organism is strictly aerobic and grows well at 37°C. After 24 hours, the colony is round, entire, dome shaped, slightly grey and no more than 1 mm in diameter. In 48 to 72 hours the colony becomes rough, highly wrinkled, cream to yellow in colour and is from 5 mm to 8 mm in diameter. There is a characteristic earthy or musty odour, and alpha haemolysis is present when grown on sheep blood agar. Further incubation for 7 to 14 days at 37°C produces beta haemolysis, an extremely corrugated colony with regular radial striations and a deep brown insoluble pigment. On McConkey agar, colonies are initially pink with a suggestion of a metallic sheen, later turning deep pink or red with oxidation of lactose. Growth in nutrient broth and other liquid media produces a heavy surface scum and pellicle. Occasional but rare, smooth or non-haemolytic strains of the organism have also been reported^{5, 31}.

Biochemically, *Pseudomonas pseudomallei* is catalase and oxidase positive and is actively motile by both semi-solid agar and hanging drop methods. On Kligler

Table II
Biochemical reactions to *Pseudomonas pseudomallei*

Substrate or test	% Positive in less than 2 days	% Positive in greater than 3 days	Substrate or test	% Positive in less than 2 days	% Positive in greater than 3 days
Nitrates to nitrites	100		Maltose	96	4
Nitrates to gas	85		Mannitol	100	0
Urease	35		Selicin	12	8
Citrate	96		Sucrose	42	0
Arginine dihydrolase	100		Xylose	27	73
Lysine decarboxylase	0		Sorbitol	77	23
Ornithine			Adonitol	31	58
decarboxylase	0		Arabinose	81	19
Glucose	100	0	Rhamnose	0	4
Lactose	100	0	Raffinose	12	0

Table III
Important differential characteristics of *Pseudomonas pseudomallei*³².

Character	<i>Pseudomonas aeruginosa</i>	<i>Pseudomonas putida</i>	<i>Pseudomonas maltophilia</i>	<i>Pseudomonas stutzeri</i>	<i>Pseudomonas cepacia</i>	<i>Pseudomonas pseudomallei</i>
Wrinkled colony	0	0	0	100	0	83
Polymyxin susceptibility	100	100	86	100	0	0
Lactose oxidation	0	28	0	0	100	100
ONPG	0	0	95	0	90	0
Nitrates reduction	60	0	0	100	0	100
Gluconate	100	74	0	2	12	0
Lysine decarboxylase	0	0	20	0	93	0
Arginine dihydrolase	96	97	0	0	0	100

Key: 0 = Negative. Figure = Percent Positive of tested strains.

Iron agar, the butt and slant are alkaline after 24 hours, the butt becomes acid after 48 hours and hydrogen sulphide is not produced. Gelatin stabs are uniformly liquefied after 48 hours⁶ and the organism attacks carbohydrates by oxidation. Table II illustrates variations in biochemical activity^{5, 6, 8, 31, 32} and Table III indicates important characters and biochemical reactions which will assist differentiation of *Pseudomonas pseudomallei* from other more common members of the genus³².

Positive identification can be confirmed with animal inoculation. Sub-cutaneous or intra-peritoneal injection of *Pseudomonas pseudomallei* into a male guinea pig produces a typical Strauss reaction. Enlarged and swollen testicles are evident in two to five days with subsequent death in seven to ten days. The organism can be retrieved from heart blood cultures at post mortem or from localised abscesses present in the liver, spleen or kidneys of the guinea pig²⁰.

Serology

In the excellent review *Pseudomonas pseudomallei*, Howe, Sampath and Spotnitz described four associated antigens of the organism; soluble antigen, flagellar antigen, somatic O antigen and an envelope antigen, K. The somatic O antigen is probably responsible for cross reactivity with members of the Enterobacteriaceae, whilst the K envelope antigen has been reported to be associated with virulence, although in the latter case, evidence is not conclusive. Sonic disruption or aqueous extraction of the soluble antigen and complement fixation or haemagglutination tests are recommended for serological confirmation or diagnosis of melioidosis¹⁵.

Strauss favoured an indirect haemagglutination technique²³, a modification of one originally described by Ileri in 1965³³. Using rabbit produced antisera, he reported good specificity against 14 strains of *Pseudomonas pseudomallei*, three strains of *Actinobacillus* and one strain each of *Pseudomonas aeruginosa*, *Pasteurella tularensis*, *Pasteurella pestis*, *Listeria monocytogenes*, *Brucella abortus*,

Acinetobacter species and *Leptospira* species. With six diagnosed human cases and 18 suspected cases of melioidosis, he substantiated sensitivity of the method in detecting antibodies from both chronic and acute phases of the disease. He suggested a titre of 1/40 or greater to be indicative of mild or sub-clinical infections. Considerably higher titres of 1/640 and greater have been found in bacteriologically proven cases of the disease^{2,24}.

During my research project in Uganda in 1971/72 the population group under study comprised adult Africans from the capital region of Kampala and from rural areas throughout the country. Caucasians, from a sizeable ex-patriate community, and ethnic Asians present in the country at the time were excluded from the study. An indirect haemagglutination technique as described by Strauss, Alexander and Rapmund²³ was used to detect serum antibodies to *Pseudomonas pseudomallei*. Table IV shows preliminary findings from the survey. I must emphasise the data is elementary and incomplete. Prevailing political circumstances in Uganda in late 1972 precipitated a premature end to my employment contract and of course to the research project. The study was less than half completed and much of a parallel study of a 'normal' population was lost. Perhaps more important, communication and travel within the country was difficult, and the one individual with a very high titre of antibodies (1/2560) could not be traced for follow up investigations. I quote these preliminary findings purely to provoke thought and discussion and to suggest that *Pseudomonas pseudomallei* may indeed be present in this part of the African continent in spite of the lack of reported isolations.

Table IV
Preliminary findings — Antibodies to *Pseudomonas pseudomallei* in Uganda

	Negative or non-specific reactions		Mild or sub-clinical infections					Highly suggestive of active disease	Total tested
Ant. Titre 1:	10	20	40	80	160	320	640	1280	2560
Kampala City Council workers	37								37
Mulago — Kampala suburbs	115	12	5	5					137
Kilembi mines — West Uganda	117	18	4	2	1			1	143
Soroti — Central Uganda	3		1						4
Kasingati — Northern Uganda	88	11	4	1	1				105
TOTALS	360	41	14	8	2			1	426

Note: 25 of 426 (5.9%) individuals showed antibodies to *Pseudomonas pseudomallei* at a titre of 1:40 or greater.

Chemotherapy

Thin and his associates, in their review of ten human cases of melioidosis, used combined and vigorous treatment with tetracycline and chloramphenicol. They accepted that Fournier and Chambon, in 1958, demonstrated in vitro syner-

gism between the two antibiotics. They also emphasised the importance of monitoring vital functions with prompt use of necessary supportive measures in attempts to reduce the high mortality associated with acute disease³.

Pseudomonas pseudomallei has been consistently susceptible to novobiocin with disk diffusion tests in the laboratory^{15,31}, however therapeutic effectiveness of the drug is not yet conclusive. Zimmerman successfully treated an acute septicaemic melioidosis with gentamycin²⁶ and more recently, trimethoprim/sulphamethoxazole has proved adequate in treatment of chronic pulmonary melioidosis²⁸. In spite of these latter reports, a review of published literature indicates distinct preference for therapy with tetracycline alone or in combination with chloramphenicol. Prolonged treatment with high doses of the drugs is essential to ensure complete elimination of the causative organism^{2,3,15,16,27,31}.

Conclusions

Extensive epidemiological studies show *Pseudomonas pseudomallei* to be widespread in soil and surface water throughout Southeast Asia^{19,20,21} with endemic foci also present in the Malagasy Republic (Madagascar), Guam and the north Queensland coast of Australia^{14,15}. There is some evidence from reported case histories (Figure 1), to suggest the organism may be more widely distributed in nature between latitudes of 20 degrees north and south around the world¹⁷.

Considerably more than three millions, American and Commonwealth servicemen and their dependants, have been exposed to the causative organism in Indochina and Malaysia alone and serological surveys indicate as few as one per cent and as many as 10 per cent of these may suffer from clinically inapparent or latent disease. Recrudescence infections, many years after initial exposure to *Pseudomonas pseudomallei*, are not uncommon. As present military generations retire, and return to civilian life, and with the current popularity of international travel, it seems likely melioidosis will present in a number of unforeseen places. Successful diagnosis demands a high index of suspicion by an astute physician who will inform the laboratory of the possibility of the disease. In an editorial, the Lancet stated: "Melioidosis should be borne in mind in the differential diagnosis of septicaemia, chest infection or other febrile illness, in any patient who is in, or has visited, an endemic area"¹⁷.

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