CHAPTER II

REVIEW OF LITERATURE

1. Historical Background

Pseudomonas pseudomallei was first recognized in April 1911 at Pathological laboratory, Rangoon, Burma by Whitmore and Krishnaswami[5], when a Burmese "morphia" addict aged 10 years succumbed from pneumonitis. Postmortem findings of the first reported case revealed peculiar cheesy consolidation of the lung and several subcutaneous abscesses on thighs. The slender, irregularly staining, gram negative bacillus found in the lesions resembled the glander bacillus in morphology but not in motility. After this discovery, an additional 37 cases were found in the Rangoon area within 1 year[1]. This infectious disease was highly fatal and formed multiple abscesses in numerous organs. They demonstrated that the organism was pathogenic for guinea pigs by inoculation and ingestion. They did not characterize many of its growth and staining properties, but suggested the name *Bacillus pseudomallei* because of its close resemblance to *Bacillus mallei*, the etiologic agent of glanders.

In 1925, Stanton and Fletcher[21] observed similar human infection in Malaysia and gave the name of "melioidosis" to the disease and "*Bacillus whitmori*" to the causative organism. The name "melioidosis" is derived from the Greek word "melis", meaning

"a distemper of asses" and "eidos", meaning "resemblance". This name was given because melioidosis clinically and pathogenically resembles glanders, a chronic and debilitating disease of equines which is due to *Bacillus mallei*. After this initial description, physicians began to diagnose the illness in humans and historical sequence of events; outlined by Thin and associates[22].

Subsequently physicians became familiar with the various clinical features of melioidosis and laboratory personnel were alerted to the organism. The disease is discovered more frequently in endemic tropical countries, Southeast Asia, with the greatest concentration of reported cases especially Thailand[4].

The first case of melioidosis in Thai people was reported by Chittvej et al[6] in 1955. Later there were many cases reported by several hospitals. The National Workshop has shown an increasing number of patients[4] as follows: 28 cases of melioidosis from Nakornrajsrima Hospital (1983-1985), 112 cases from Ubolrajthani Hospital (1983-1985), 222 cases from Khon Kaen Hospital (1982-1985), 20 cases from Songkla Nakarin Hospital (1983-1985), 61 cases from Chiengmai (1979-1985), 19 cases from Chulalongkorn Hospital (1980-1985), 30 cases from Siriraj Hospital (1975-1985) and 47 cases from Ramathibodi Hospital (1978-1985). There were a large number of patients found in every part of Thailand, especially in the Northeastern and the Southern parts.[40] Sixty to ninety percent of the patients are farmers and workers who are continually being exposed to soil and water in rice fields and also rubber plantations[4].
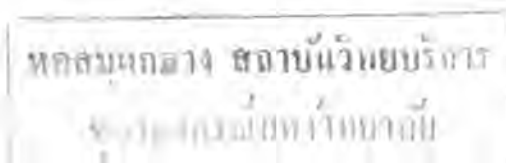
2. Bacteriology

2.1 Taxonomically, *Bacillus pseudomallei* and *Bacillus mallei* were closely related. They caused rather similar disease and had some antigenic affinity with each other[21]. They were classified together and used to be placed successively in the genera *Actinobacillus*, *Pfeifferella*, *Malleomyces*, *Loefflerella* and *Acinetobacter*. The resemblance of *Whitmore's bacillus* to *Pseudomonas aeruginosa* was recognized at least 50 years ago, and was strengthened by the observation that it was oxidase positive[23] and had polar flagella[24] Numerous other biochemical characters were common to Whitmore's bacillus and other pseudomonads were subsequently discovered. However, the glanders bacillus grew poorly on ordinary laboratory media; it was non-motile, and was a strict animal parasite. It seemed almost unthinkable to include it in a genus of culturally unexacting, motile, free-living organisms. Re-examination of the evidence[25] left little doubt, however, that the glanders bacillus closely resembles Whitmore's bacillus in metabolic activities and biochemical characters, and that both are pseudomonads. Additional evidence indicates(1) that these two organisms are closely related by the successful hybridization of their DNA,[28] and(2) that they are pseudomonads by their G+C content of 69 percent for *P. pseudomallei*, 69.5 percent for *P. mallei*, which is similar to that of other members of the group (58-71 percent). *P. pseudomallei* is classified in RNA group II (pseudomallei group) of aerobic pseudomonads on the basis of rRNA/DNA hybridization, used for dividing the pseudomonads into various RNA homology groups which represent natural genetic arrangement[26,27].

Taxonomy

Family *Psedomonaceae*

Genus *Pseudomonas*

Species *Pseudomallei*

2.2 Morphology

P. pseudomallei is a small slender gram negative rod, 0.3-0.5 μm in width and 1.5 in length. Most cells in young culture at 35 °C will show rod shape with a clear center and darkly stained ends (bipolar staining) which resemble a close safety pin as shown in Fig 1. 48 hrs cultures, show that most cells are oval to round, and only the periphery stains; such cell may be mistaken for endospores. When grown in suitable medium, the rod which contains poly beta-hydroxybutyrate (PHB) may be seen by phase contrast microscopy or in films stained with Sudan black. The organisms show dark granules of PHB. It is a nonspore - forming bacillus, not acid fast and has no capsule. It can be motile by a polar tuff of three or more flagela (multitrichous flagella) which can be observed by light microscope after staining with the tannic acid fuchsin method described by Leifson[28].

2.3 Cultural character

P. pseudomallei grows well at 37 °C and pH 7 on ordinary bacteriological media and mineral base medium, including eosin methylene blue agar (EMB) or Mac Conkey agar. The colonies vary from mucoid and smooth to rough and wrinkled in texture, and from bright

orange to cream in color. After overnight incubation on blood agar at 37 °C, the colonies appear smooth, translucent, convex and entire with a diameter of 0.2-0.4 mm. 48 hrs later, they show characteristic white opaque growth and produce alpha hemolysis as shown in Fig 2. If incubation on blood agar exceeds 48 hrs, the colonies are usually 3-4 mm. in diameter and become umbonate with an uneven, wrinkled surface and produce beta hemolysis as shown in Fig 3. Frequently it has a characteristic musty, earthy odor which is produced by fresh culture.

In broth culture, *P. pseudomallei* produces a smooth growth overlaid with a pellicle like *P. aeruginosa*.

Fig 1 Gram stain of *P. pseudomallei* showing typical bipolar staining
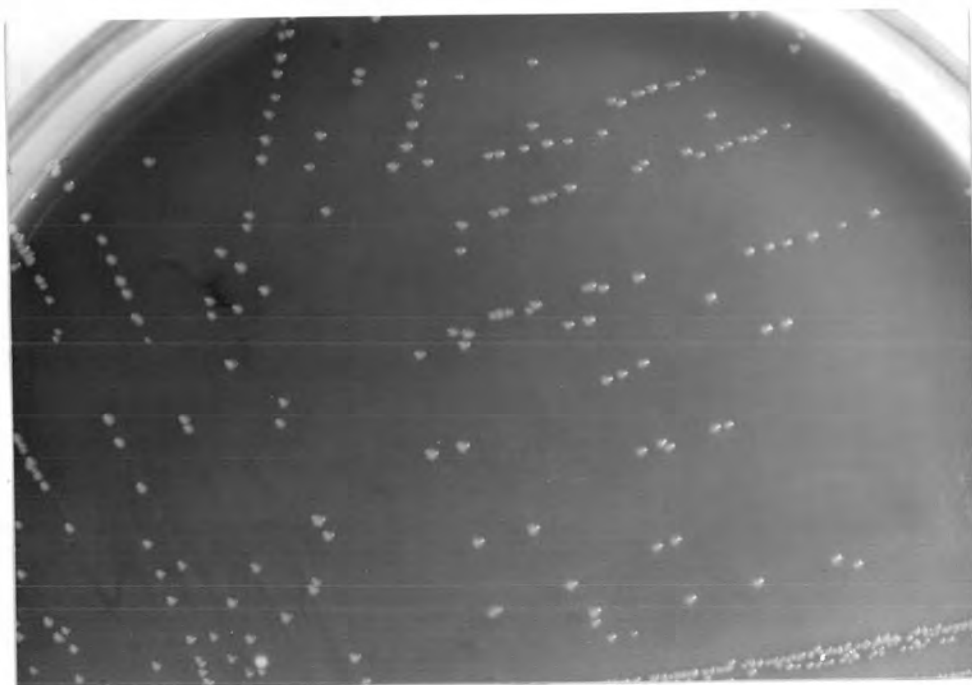
Fig 2 Characteristic opacity and alpha hemolysis of 24 hrs colonies
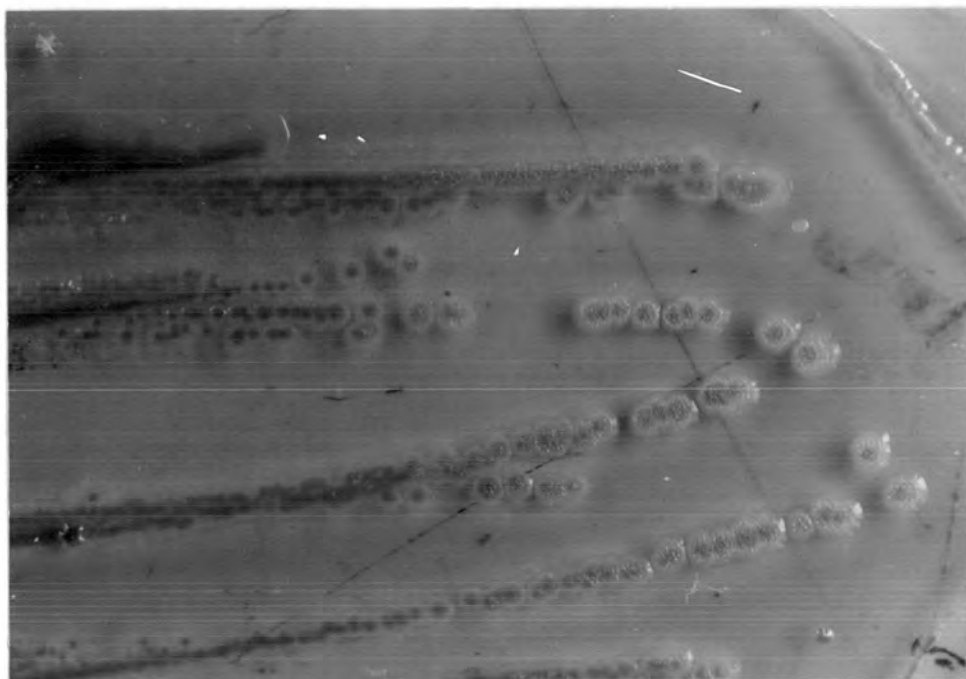
of *P. pseudomallei*

Fig 3 Characteristic wrinkled surface (daisy-head appearance) and

beta hemolysis of seven days old colonies of *P.pseudomallei* on

sheep blood agar

2.4   Metabolic and biochemical character

P. pseudomallei is an obligatory aerobe and uses denitrification as an alternative mechanism for respiration under anaerobic condition. It can grow anaerobically in the presence of nitrate, however, it does form gaseous nitrogen in nitrate broth that incubates aerobically. Therefore, the amount of gas formed in the reduction of nitrate may be small or demonstrated only at 25°C. It can grow at 42°C but the range of temperature over which it will grow is rather narrower than that of P. aeruginosa. It grows poorly at temperature as high as 21°C. It can form acid from various sugars in Hugh and Leifson's or ammonium salts media, and can produce many enzymes such as cytochrome oxidase, catalase, arginine dihydrolase and extra cellular hydrolytic enzymes that attack poly-beta-hydroxybutyrate, gelatin, Tween 80 and starch etc. Like P. aeruginosa, it forms a heat stable alkaline phosphatase[29] and hemolysin[30]. It is usually KCN resistant and does not attack malonate.

P. stutzeri may be confused with P. pseudomallei. However, P. stutzeri usually develops a slightly yellow growth pigment, does not oxidize lactose, and is rarely proteolytic and motile by a single polar flagellum. The other organism that may be confused with P. pseudomallei when gas is not formed readily from nitrate is P. cepacia. P. cepacia produces lysine decarboxylase enzyme and negative for arginine dihydrolase enzyme. The properties that are useful for the differentiation of P. pseudomallei from other species of pseudomonads are described by Gilardi et al [31] as shown in Table 1.

Table 1 Characters used in identification of *Pseudomonas* species[31]

Test, substrate, or morphology	P. aeruginosa (84 strains)		P. fluorescens (194 strains)		P. putida (273 strains)		P. stutzeri (164 strains)		P. mendocina (6 strains)		P. pseudomallei (7 strains)		P. mallei (6 strains)		P. cepacia (901 strains)		P. maltophilia (527 strains)	
	Sign	% Positive	Sign	% Positive	Sign	% Positive	Sign	% Positive	Sign	% Positive	Sign	% Positive	Sign	% Positive	Sign	% Positive	Sign	% Positive
Acid:																		
Glucose, 1% (OFBM)	+	98	+	100	+	100	+	100	+	100	+	100	+	100	+	100	+	100
Fructose	+ or −	89	+	99	+	99	+	94	+	100	+	100	+ or (+)	100	+	100	+	99
Galactose	+ or −	80	+	97	+	99	+	91	−	100	+	100	+	100	+	100	+	25
Mannose	+ or −	77	+	99	+	99	− or +	88	−	100	+	71	+ or (+)	100	+	0	−	98
Rhamnose	− or +	20	− or +	48	− or +	32	− or +	24	+	100	+ or −	86	−	0	+	99	−	54
Xylose	− or +	85	+	97	+	99	+	94	−	0	+	86	−	0	+	98	+	88
Lactose	−	0	− or +	12	− or +	14	−	0	+ or −	0	+	100	(+) or +	100	+ or −	81	+	92
Sucrose	−	0	− or +	47	−	8	−	98	−	0	−	86	−	0	+	97	+	100
Maltose	− or +	11	− or +	36	− or +	21	+ or −	98	−	0	+	100	(+)	100	+	100	−	0
Mannitol	− or +	68	+ or −	93	− or +	18	+	69	−	0	+	100	(+) or −	83	−	97	−	0
Lactose, 10% (PAB)	− or +	14	+ or −	62	− or +	42	−	0	−	0	−	0	−	NT	+ or −	78	+	95
ONPG	−	4	−	1	+ or −	1	−	0	−	0	−	0	−	NT	−	0	−	0
Proverdin	+ or −	71	+	95	+ or −	84	−	0	−	0	−	0	−	0	−	0	−	0
Hydrogen sulfide (KIA)	−	0	− or +	0	−	0	−	0	−	0	+ or −	86	−	NT	−	37	+ or −	40
Nitrate reduction	+ or −	75	− or +	19	−	0	+	100	+	100	+	100	−	0	− or +	0	−	0
Gas from nitrate	+ or −	61	−	5	−	0	+	100	+	100	+	100	−	0	−	0	−	2
Indophenol oxidase	+	100	+	100	+	100	+	100	+	100	+ or +(w)	100	+(w) or −	67	−	92	−	0
Arginine dihydrolase (DBM)	+	99	+	99	+	99	+	0	+	0	−	100	+ or (+)	100	+	90	−	99
Lysine decarboxylase	−	0	−	0	−	0	−	0	−	0	−	0	−	0	+ or −	65	+	0
Ornithine decarboxylase	−	8	−	0	−	0	+ or −	51	+ or −	50	−	0	−	NT	−	2	−	0
Phenylalanine deaminase	−	7	−	3	−	0	−	0	−	0	−	0	−	0	−	0	−	0
Hydrolysis:																		
Urea	+ or −	67	− or +	41	− or +	43	− or +	15	+ or −	50	− or +	43	− or +	17	− or +	43	−	0
Esculin	−	0	−	0	−	0	−	0	−	0	+ or −	57	−	NT	+ or −	65	+	100
Tween 80	+ or −	71	+ or −	63	+ or +	18	+	97	+ or −	83	+ or −	86	−	NT	+	100	+	100
Starch	+	7	− or +	19	+	18	+	93	−	0	−	0	−	NT	+	1	−	0
DNA	− or +	10	+	90	−	0	−	0	−	8	+ or −	86	−	NT	+ or −	50	+	100
Lecithin	+	8	+	100	−	0	−	1	−	0	+	100	−	0	+ or −	74	+	100
Gelatin	+ or −	50	+	100	−	1	−	0	−	0	+	0	−	NT	+ or −	60	−	0
Acetamide	− or +	39	− or +	14	−	4	−	0	−	0	− or +	43	−	NT	−	3	−	0
Growth on:																		
SS	+ or −	88	+	97	+	98	+ or −	82	+ or −	83	+	100	+ or −	67	+	93	+	100
MacConkey	+	99	+	100	+	10	+	100	+	100	+	0	−	NT	−	0	−	0
6.5% NaCl	+	7	−	2	− or +	92	−	2	+	100	+	0	−	NT	+ or −	68	−	0
Cetrimide (PA)	+	91	+	96	+	100	−	100	+	100	+	100	+	100	+	99	−	2
MBM + acetate	+	96	+	100	+	0	+ or −	89	+	100	+	100	+	100	+ or −	58	− or +	48
Growth at 42°C	+	100	−	0	−	0	+	99	+	100	−	0	−	NT	+	0	+	93
Polymyxin susceptible	+	98	+	97	+	99	+	100	+	100	−	100	−	NT	−	99	+	100
Motile	+	97	+	100	+	100	+	100	+	100	+		−		+		+	
No. of flagella	1		>1		>1		1		1		>1				>1		>1	

* Except where indicated, cultures were incubated at 30°C. All strains were indole negative. *P. aeruginosa* strains did not produce pyocyanin. Abbreviations for all tables: OFBM, OF basal medium; PAB, purple agar base; DBM, decarboxylase base, Moeller; SS, salmonella-shigella agar; PA, Pseudosel agar; MBM, mineral base medium. Signs: +, 90% or more positive within 2 days; −, no reaction (90% or more); (+), reactions delayed 3 or more days; − or +, most cultures positive, some strains negative; − or +, most strains negative, some cultures positive; 1, polar monotrichous flagella; and >1, polar multitrichous flagella.

b Data from Hugh (21).

c Signs: +, 90% or more positive within 2 days; −, no reaction (90% or more); (+), reactions delayed 3 or more days; (−) or −, most reactions delayed 3 or more days, some occur within 2 days; + or (+), most reactions occur within 2 days, some delayed 3 or more days; +, (−), or −, most reactions occur within 2 days, some delayed 3 or more days, some negative; (+) or −, most reactions delayed 3 or more days, some negative; + or −, most cultures positive, some strains negative; − or +, most strains negative, some cultures positive; (w), weakly reactive; 1, polar monotrichous flagella; >1, polar multitrichous flagella.

d NT, Not tested.

e Not all strains were examined; results are based on the fraction of strains tested.

2.5 Resistance

Unlike *P. aeruginosa*, *P. pseudomallei* can not grow on 0.1% centrimide agar. However, it is resistant to various dyes such as crystal violet. Ashdown[32] recommends a selective medium containing 1 in 200,000 crystal violet, glycerol neutral red and gentamycin in trypticase soy agar (TSA), on which *P. pseudomallei* forms pink, wrinkled colonies. *P. pseudomallei* survives at least a month in water, faeces and dried soil and for a week in putrefying carcasses[23].

2.6 Phage

Specific bacteriophages which have been isolated selectively infect only *P. pseudomallei* and *P. mallei* [33], but other pseudomonads are not susceptible. The presence of these phages, free in the environment, may be taken as an evidence for free living character at least one of these hosts.

3. Antigenic structure

The antigenic composition of *P. pseudomallei* is as complex as that of other gram negative bacteria. The four antigens have been delinated in *P. pseudomallei* as follows: envelope(K)antigen may be a slime like substance though not a capsule, and said to be associated with virulences (although the evidence for this matter is not clear); flagella(H) antigen; mucous substance(M) antigen and somatic(O) antigen. The antigens of the latter type are presumably responsible

in the part for cross reactivity with some strains of Enterobacteriaceae in indirect haemagglutination test. The H and M antigens are heat labile and sensitive to alcohol and 0.5% formalin. The O and K antigens are heat stable[34,35].

P. pseudomallei has an antigenically homogenous group which relates to *P. mallei* [34], however, there is doubt whether they constitute one serotype or not [36]. In 1970, Duodin et al[35] applied the gel diffusion precipitation (GDP) and immunoelectrophoresis to study the complex antigens of *P. pseudomallei* from various sources in the Southeast Asia and Australia. It early distinguished *P. pseudomallei* at least two serotypes; the serotype I was found in Asia and Australia; the serotype II seemed to be rare outside Australia. Their human, animal and soil origins had no effect upon the distribution of the strains into two serotypes.

4. Toxins

In 1957, Liu et al[30] showed that *P. pseudomallei* produced two extracellular toxic materials in filtrate from broth culture. They were both lethal and dermanecrotic. In 1958, Heckly and Nigg[37] seperated two heat labile toxic constituents, both of which killed mice when injected intraperitoneally. One toxin was lethal and necrotizing, whereas the other toxin was lethal but nonnecrotizing. The production of exotoxin was enhanced by the presence of mucin in the growth medium. Certainly these toxins, precipitated with antiserum to culture filtrate, were strain specific. In 1960, Rapaport et al [38] reported that the aqueous extract of

P. pseudomallei contained water-soluble-heat-stable substances which demonstrated the endotoxin activity. In 1987, Ismail et al[39] purified *P. pseudomallei* exotoxin (MW 31,000) which showed lethal effect in mice and demonstrated that exotoxin production in culture was enhanced in the presence of glycerol and developed monoclonal antibody. ELISA technique was developed to detect the specific antigens as a research tool in screening for nontoxigenic strains or mutant, of *P. pseudomallei*. These discoveries showed that *P. pseudomallei* has two toxins, an endotoxin and exotoxin.

5. Experimental infection

Small laboratory animals may be infected with *P. pseudomallei* by feeding, inhalation and scarification of the skin or infection into the tissues. *P. pseudomallei* frequently changes in virulence in the laboratory. Virulence often falls on prolonged culture but may be restored by animal passage. The low virulence may produce non fatal subacute or chronic infection[23].

Considering the LD50 for the subcutaneous or intraperitoneal injection of *P. pseudomallei* virulent strain, the hamster is the most susceptible animal[23]. Somewhat larger doses are required to kill guinea pigs.

Intraperitoneal injection of *P. pseudomallei* usually causes death within 1-2 weeks. There is a fibrinous and later nodular peritonitis together with the characteristic of "Straus reaction" in male animal. The testiles swell in 2-3 days and later becomes

greatly enlarged.

6. Epidemiology

P. pseudomallei is a free living bacterium and is found widely in the soil and water surface of wet rice fields, fields of newly planted oil palm, monsoon drains, gardens and playing fields in the Southeast Asia[21,40]. Ellison[41] found 2.8 percent of 1,120 water and 1.8 percent of 1,078 soil samples collected from Kualalumpur, Malaysia, positive for P. pseudomallei. Finkelstein et al[42] used hamster inoculation to study the geographic distribution of P. pseudomallei in Thailand and found that it was widely distributed, particularly in the Southern peninsula with the isolation rates ranging from 30 to 50 percent of soil and water sample tested. These data were recently confirmed[4], Patamasucon was able to isolate the organism from 30% of samples collected from soil surface and deep fresh water. The organism was not found in the central and northern region and was rarely isolated in the northeasthern region of Thailand. The organism was isolated from surface water with a wide range of pH values from a low of pH 2 to a high of pH 9. Isolation rates appeared higher during rainy season and is in still rather than flowing water. Highest isolation rates were obtained from the shaded and moist ground in the fields[43].

Thomas et al[44,45] found that the organism could survive up to 30 months in soil that has been held on a shaded shelf or underground clay. When exposed to direct sunlight or ultraviolet, viable count decreases rapidly. In nature, bacterial growth requires

reasonably high temperatures, increased humidity and rainfall to produce stagnant pools and muddy water courses. A selective medium development by Ashdown[32] is suitable for screening in area where gentamycin resistant microorganism is not commonly encountered in culture specimens.

Reported cases are most commonly from endemic areas as well as many other countries. Those cases occurring in the later are believed to have acquired initial infection in the Southeast Asia and North Australia[7]. Areas or countries where cases of melioidosis were reported are summarized in Table 2[48].

Of all countries, Thailand has by far the greatest number of reported cases[4]; case reports had been accumulated for over 800 cases by 1986 though only three cases of active melioidosis have been recorded before 1965. This is due to the renaisence of interest in the melioidosis initiated by the Seato Medical Research Study on melioidosis in Thailand during the Vietnam War and by the Infectious Diseases Association of Thailand.

7. Mode of transmission

Disease in human is mostly acquired from contamination through a pre-existing skin abrasion or ulcer with infectious soil and water or by inhalation of infectious dust particles[47]. It was formerly believed that direct human to human infection did not occur, but in 1977 veneral transmission was documented[46]. There are at least two studies showing that melioidosis is able to occur in

Table 2 Countries or cities where active cases of melioidosis were reported. (Names in parentheses represent areas from where the reported cases were believed to have acquired *Psudomonas pseudomallei*) [46].

Africa - Gambia

Australia

- Northern Territory

- Queensland

- Townsville district

- Torres Strait island

- New South Wales

- Melbourne (Queensland)

Burma

Cambodia

Chad

Canada (Cambodia)

Denmark (Kenya)

England

Ecuador

France (Cambodia)

Guam

Hong Kong

Indonesia

India

Iran

Korea

Laos

Madagascar

Malaysia

Mexico

Papua New Guinea

Panama

Russia

Singapore

Sri Lanka

Thailand

Philippines

Turkey

United states [San Francisco], Hawaii, Georgia Oklahoma, California

United States [Vietnam, Laos, Thailand, Mexico]

Vietnam

West Indies

neonates who perhaps acquired the pathogen birth canal, infected through the amniotic fluid or respiratory equipment[48]. However vagina swab was consistently negative for *P. pseudomallei* in suspected index cases except in one aborigine[50]. Recent studies revealed the infection can be acquired inside a hospital or laboratory room through contaminated antiseptic or intraveneous fluid therapy, bronchoscopy, urethral catheterization or inhalation during laboratory work[51].

Incubation period can be as long as 26 years[52]. Evidence for this derives from the reports that travellers or soldiers found to contact melioidosis from endemic areas, became ill several weeks to years after arriving home[7].

The infection is continually present in endemic area through the year with a preponderance of rainy or wet season[53].

8. Pathogenesis

P. pseudomallei which causes melioidosis occurs sporadically in wide range of mammalian species including man, pig, sheep, goat, monkey, lamb, dolphin, cattle, galah, horse, rat, kangaroo and several rodents from endemic area[53,54,55,56].

The pathogenesis of *P. pseudomallei* in man depends on numerous aspects of the host pathogen relationship as well as age, occupation, immunologic competence and perhaps nutritional status of the host[7]. Little can be said regarding to the role of strain

difference in the variability of clinical illness observed. Thermolabile lethal exotoxin and proteolytic enzyme are believed to contribute the lethality in humans. Mechanisms intracellulary survival in elements of the reticuloendothelial system have not been studied. This ability, which is unique for this pathology, renders melioidosis quiescent inside the infected persons for many years and may contribute to abscess formation in spleen and liver when dormancy is reactivated[57].

9. Clinical manifestation

Spectrum of melioidosis in man varies from subclinical to protean overwhelming and resembles other acute and chronic bacterial infections. Thus, melioidosis has been termed "The great imitator"[9] for every infectious disease, and virtually every organ can be affected. Its chronic form has been mistaken for anaerobic[10], tuberculosis fungal infection[11,12], and pyogenic form for Staphylococcus aureus or other acute bacterial infections. Histopathologic study also illustrated abscess in acute infection and granuloma in chronic form[58]. The majority of infected persons are probably asymtomatic. Illness can be delayed for years due to unique ability of P. pseudomallei to remian quiscent inside the infected persons who are at risk of developing acute exacerbation[58,59]. The factors that influence reactivation of latent bacteria are not well understood but probably include enviromental variables, stress or immunity status of host.

Common serious infections reported include acute pneumonitis

with variable radiographic findings and [58,80] septicemia. Less severe forms are infection localized to skin, joint, pleural space, lymph node, liver, spleen, kidney, pericardium, urogenital organ and muscle[61,62,63,64,65,] as shown in Table 3. Genito-urinary infections are not uncommon in Australian Aboriginals[86]. It is not uncommon for melioidosis to manifest as pyrexia of unknown origin in endemic area where abscess in abdominal organs is not intensively searched for.

Since mortality rate and therapeutic effect of an antibiotic depend heavily on severity of disease and to facilitate a comparison of clinical data or efficacy of antibiotic used. In 1985, the Infectious Disease Association of Thailand worked out a clinical classification based on 595 reported cases[4] as follows:

(1) Disseminated septicemic melioidosis; *P. pseudomallei* is cultured from blood. The infected persons are symtomatic and the infection spreads, to many organs such as skin, lung and etc.

(2) Non-disseminated septicemic melioidosis;the organism is cultured from blood and the infection is localized to some organs, and does not spread.

(3) Benign bacteremic melioidosis; the organism can be cultured from blood, and the infected persons are asymptomatic.

(4) Localized melioidosis; the organism can not be cultured from blood, however it can be cultured from some organs such as a liver abscess

(5) Probable melioidosis; the organism can not be cultured from infected persons who are symptomatic, but the serological test gives a positive result.

Table 3 Clinical manifestation of melioidosis[46].

organ system	common
Respiratory	pneumonitis Lung abscess Pleural effusion Empyema
Skin and soft tissue	Cellulitis Subcutaneous abscess Infected wound Chronic granuloma
Skeletal	Septic arthritis (knee,ankle,elbow joints) Osteomyelitis
Hepatobiliary	Liver abscess Splenic abscess
Genitourinary	Urinary tract infection (pyelonephritis) Prostatitis or Prostatic abscess
Lymphadenopathy	Cervical lymphadenitis or abscess
Cardiovascular	Pericarditis Pericardial effusion
Others	Prolonged pyrexia without identified source Septicemia

(6) Subclinical melioidosis; the organism can not be cultured and the infected persons are asymptomatic, but the serological test gives a positive result.

This classification would allow physicians to compare the beneficial effect of antimicrobials at the same level of severity which are more self-limited. Clinical trials with conventional and new antiboitics are underway in Thailand for therapeutic efficacy study in septicemic melioidosis.

10. Diagnosis

The broad spectrum of clinical manifestations of melioidosis demands that it be considered in an endless number of diseases. Melioidosis should be included in differential diagnosis of septicemia, pulmonary infection or unknown febrile illness in patients who have lived in an endemic area. The clinical manifestations in melioidosis are not diagnostic, therefore, the bacteriologic diagnosis is essential. The protocol for melioidosis diagnosis should be as follows:

1. Gram stain of pus can be a presumptive diagnosis if the characteristic bipolar staining of gram negative bacilli was seen.

2. Culture of blood and appropriate sources such as synovial fluid, plural fluid, peritoneal fluid and pus should be done. The bone marrow and the buffy coat from peripheral blood are the

additional source for bacteriologic diagnosis. The organism is readily cultivated in usual solid and broth media. Colonies vary from smooth to rough and often wrinkle after a few days incubation. This character is non specific, but a preliminary clue for *P. pseudomallei* identification. The distinctive, musty odor which once experienced can be recognized.

Useful clues are the dry wrinkled colony appearance, bipolar staining, positive oxidase reaction, nitrate reduction to nitrite and production of acid slant with neutral bottom in triple sugar iron agar after 24 hrs incubation and subsequent change to acid pH in the bottom without gas after 72 hrs incubation.

Agglutination with specific antiserum in 1:10 dilution is a rapid confirmatory test[32]. A selective medium consists of Mac Conkey agar with gentamycin, vancomycin, colistin and nystatin (Mac-GM-VCN) has been proved to increase the recovery rate of *P. pseudomallei* from clinical specimens.

Although bacteriologic study is the mainstay of diagnosis in acute and subacute forms, serological tests are more useful in latent and asymptomatic forms of the disease. There are several tests for the determination of *P. pseudomallei* antibody as follows:

1/ Agglutination test[67,88]

2/ Indirect haemagglutination test (IHA)[13,14,15,18]

3/ Complement fixation test (CF)[14,18,89]

4/ Indirect fluorescent antibody staining (IFA)[70,71,72]

5/ Enzyme linked immunosorbent assay (ELISA)[73]

Agglutination test is not much valuable because of unreliable result[88]. Previously the indirect haemagglutination test, [Alexander et al[14]] used a heat stable and presumably polysaccharide antigen, absorbed on erythrocytes. Nigg[16] used a phenol water extract of bacteria, but most other workers used a mallein like material (melioidin) obtained by boiling the supernatant of old broth culture.

In complement fixation test, Nigg and Johnson[68] used an unheated crude aqueous extract of disintegrated bacteria as the antigen.

In acute melioidosis in humans, both types of antibody will appear towards the end of the first week of illness. From the second to the fifth week, about 90 percent of serum specimens are positive by both of the IHA and CF test[14]. Neither tests distinguish glanders from melioidosis, however, with the exception of specificity, the IHA test is more specific than the CF test, but the ELISA test is even more specific than both[73].

The diagnostic titer values for interpretating the positive reactions are as follows: 1:80 or greater for IHA test, 1:10 or greater for CF test and 1:1,600 or greater for ELISA test, respectively.

Ashdown et al[71] described the indirect immunofluorescence test for specific IgM which was the most reliable mean of detecting active cases of melioidosis serologically. This IFA test is more specific and sensitive than IHA,CF and ELISA test. The

problem with IFA is that the equipments are very expensive. The IHA
test is rather popular routine test in Thailand. However, There are
still some drawbacks to all the above serological tests since
significant level of antibody can usually be found in an endermic
population. Therefore, every laboratory should find out and set up
the level of antibody titers for itself in each part of Thailand.
The other difficulty is that sometimes the physicians can not make
any decision on the patients who have significant antibody titers,but
must waiting for rising titers which takes time. In some patients
with septicemia,although the clinical manifestations increase rapidly
the levels of remain low and would be insignificant for the diagnosis
of the diseases. The ambiguities of arising from such serological
testing are now looked at more critically.

11. Antimicrobial susceptibility of *P. pseudomallei*

Characteristically,*P. pseudomallei* is resistant to penicillin,
ampicillin, first generation and second generation cephalosporins,
streptomycin, gentamicin, tobramycin, netilmicin and polymyxin. In
vitro study with 23 strains recently isolated, Aswapokee N et al[74]
showed the MIC_{90} values of chloramphenicol, tetracyclines and
kanamycin remained within therapeutic ranges and then were unable to
demonstrate the bactericidal effect of chloramphenicol and
tetracycline for *P. pseudomallei*. Co-trimoxazole,[75,76] but
tetracycline[77] and doxycycline are effective and appear useful for
long-term oral administration in particular, for therapy of
prostatitis[78,79]. Hobby et al[80] found that rifampicin is 100
times more active than chloramphenicol against one strain. Novobiocin

is uniformly active against *P. pseudomallei* in vitro but clinical experience is hampered by its toxicity, rapid development of bacterial resistance and limited availability.

Among the newer bactericidal antibiotics, the third-generation cephalosporins except cefsulodin were moderately active in vitro especially, ceftazidine[81,82]. The broad-spectrum penicillin, piperacillin, is by far most effective and bactericidal as it exhibited lowest MIC_{90} for this organism from various studies[74,83,84]. Since piperacillin is relatively unstable to beta-lactamase compared with ceftazidine, its efficacy may be attributed to either better penetration through outer cell wall or higher affinity for essential pencillin binding proteins compared to ceftazidine. However, the MICs of these beta-lactam antibiotics increase several fold with inoculum size of *P. pseudomallei* greater than 10^8 CFU/ml. Quinolone compounds such as ciprofloxacin and ofloxacin have values for MIC_{90} at 4-8 mg/L and are considered inappropriate for clinical use[82,84] as amikacin.

So far, all strains tested with chromogenic cephalosporin method exhibits more or less beta-lactamase activity, most of which is located intracellularly.[82] When 20 strains of *P. pseudomallei* were exposed to sub MIC of penicillin and cefoxitin for four consecutive days, they failed to increase the MIC values of ampicillin and piperacillin. The evidence was suggestive of constitutive betalactamase production mediated by either plasmid or chromosome.

Using checkerboard titration to study the effect of

antimicrobial combination in agar. Vorachit[81] et al was able to show synergistic effect with chloramphenicol-tetracycline combination and indifferent effect with either chloramphenicol-kanamycin or tetracycline-kanamycin combinations. Eickhoff[85] et al reported definite antagonistic effects in vitro, for combinations of sulfadiazine-kanamycin. However, the clinical significance of these effects is largely unknown.