SECTION A: A REVIEW OF THE EPIDEMIOLOGY, PATHOPHYSIOLOGY AND MANAGEMENT OF MELIOIDOSIS

In this section, the current understanding of our knowledge of melioidosis is reviewed, together with audits of Australian epidemiology and clinical management.

First, a literature review details our current understanding of the epidemiology of the disease, the nature of bacterial virulence and host defence and current management strategies. This review in chapter 1 has been published in *Clinical Microbiology Reviews*.

The epidemiology of melioidosis in Australia in the 2001/02 season was reviewed in chapter 2, the first time this has been performed in this country using notifiable disease data. This was reported in the *Communicable Diseases Intelligence* [1]. The observation that post-cyclonic flooding was linked to a case-cluster is developed further in chapter 12.

Granulocyte colony stimulating factor (G-CSF) and meropenem had been used at the Royal Darwin Hospital since 1998. Chapters 3 and 4 review the clinical experience with these agents at the Royal Darwin Hospital. It was noted that the use of these agents was associated with a large fall in mortality; possible confounders to this effect were explored. Both these chapters have been published in *Clinical Infectious Disease* [2] and *Antimicrobial Agents and Chemotherapy* [3] respectively. In this thesis, all chapters except 10 and 13 have been published and only the introductions and referencing style have been altered to avoid repetition.

References

1. Cheng AC, Hanna JN, Norton R, et al. Melioidosis in northern Australia, 2001-02. Commun Dis Intell 2003;27:272-7

2. Cheng AC, Stephens DP and Currie BJ. Granulocyte colony stimulating factor (G-CSF) as an adjunct to antibiotics in the treatment of pneumonia in adults. Cochrane Database Syst Rev 2003:CD004400

3. Cheng AC, Fisher DA, Anstey NM, Stephens DP, Jacups SP and Currie BJ. Outcomes of patients with melioidosis treated with meropenem. Antimicrob Agents Chemother 2004;48:1763-5

1. Review of the literature

1.1. Background and History

The pathologist Alfred Whitmore and his assistant C. S. Krisnaswami first described melioidosis as a "glanders-like" disease among morphia addicts in Rangoon, Burma in 1911 (1, 2). They recognized a new organism that fulfilled Koch's postulates for causation of disease. This bacterium, that could be isolated from autopsy specimens on peptone agar and potato slopes, could be distinguished from the organism causing glanders by its relatively rapid growth, its motility and the lack of the Strauss reaction when injected into guinea pigs. Based on these characteristics, "… sufficiently peculiar to distinguish it from all pathogenic bacteria previously known to us"(1), they correctly surmised that this new bacterium was closely related to that which caused glanders, a finding that has only recently been confirmed by molecular studies (3-5).

This disease, now termed melioidosis, was named from the Greek μηλις (*melis*; distemper of asses) and ειδος (*eidos*; resemblance) by Stanton and Fletcher in 1921 (6). During the last century this gram negative environmental bacterium has been variously known as *Bacillus pseudomallei, Bacillus whitmorii* (or *Bacille de Whitmore*), *Malleomyces pseudomallei, Pseudomonas pseudomallei* and since 1992, *Burkholderia pseudomallei* (7).

In the latter half of the 20th century, melioidosis has emerged as an infectious disease of major public health importance in South East Asia and northern Australia. In Ubon Ratchathani, Thailand, *B. pseudomallei* accounts for up to approximately 20% of community-acquired bacteraemias (8). At the Royal Darwin Hospital, Australia, it has been the commonest cause of fatal community-acquired bacteraemic pneumonia (9, 10).

Significant improvements have been made in defining the optimal antibiotic therapy for melioidosis, largely due to clinical trials in Thailand. However, the choice of antibiotic regimen has not been shown to impact on mortality within the first 48

hours of admission (11) and severe melioidosis in Thailand is still associated with a case fatality rate of approximately 50% (12). In Australia, the mortality rate is still significant and approaches 20% among all patients with melioidosis (13).

1.2. Epidemiology

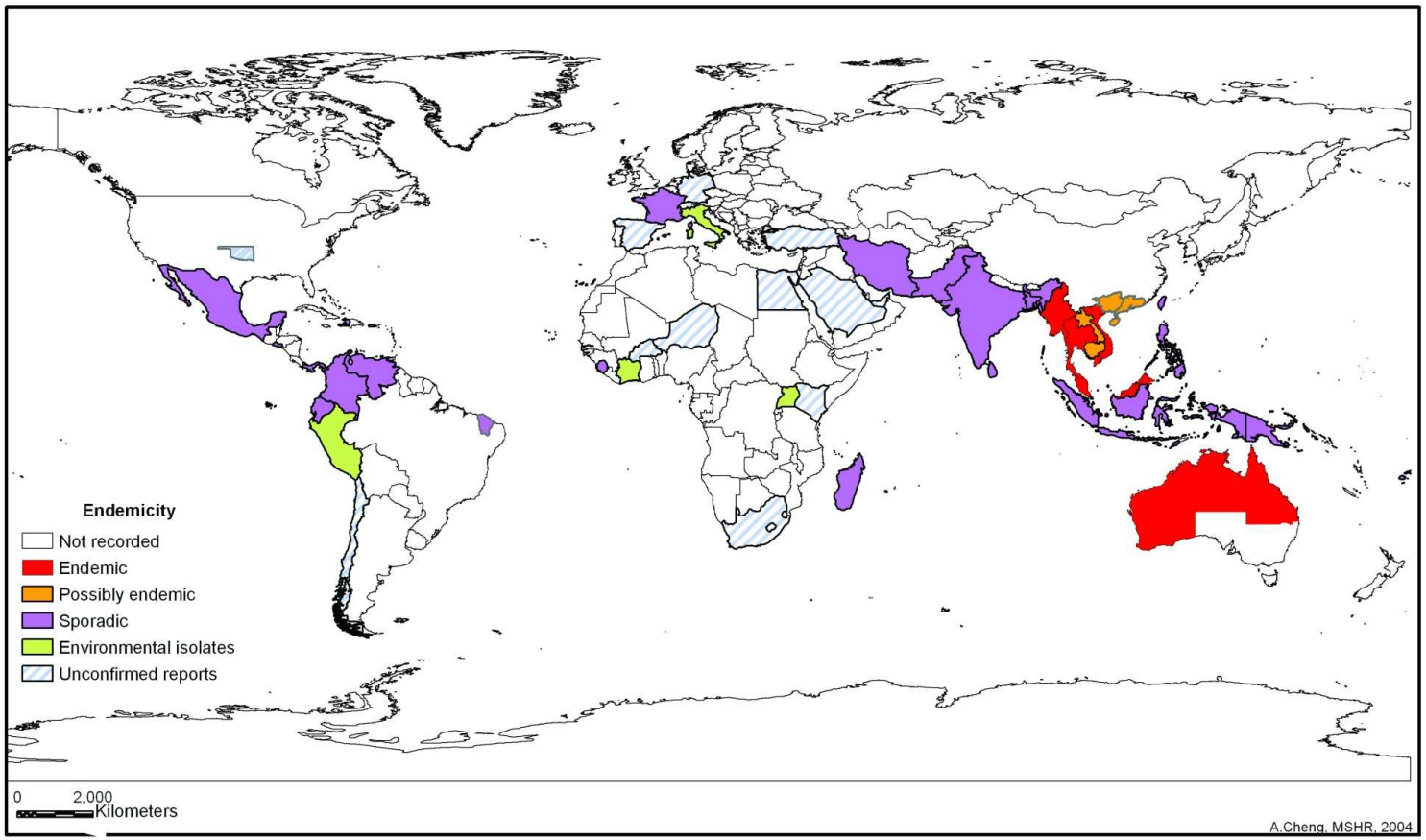
Melioidosis is regarded as endemic to south east Asia and northern Australia, approximately corresponding to the tropical latitudes between 20°N and 20°S. The worldwide epidemiology of melioidosis has been comprehensively reviewed by David Dance (14, 15); data from these and other more recent reports are summarized in table 1-1 and figure 1-1

Table 1-1: Worldwide distribution of melioidosis based on reported cases

Level of evidence	Country
Endemic; multiple case series described	Northern Australia (13, 16), Thailand (8, 17), Singapore (18-20), Malaysia (21), Burma (1), Vietnam (22, 23)
Possibly endemic; multiple cases, significant numbers of exported cases	Southern China (24, 25), Hong Kong SAR (3, 26, 27), Brunei (15), PDR Laos (28) (Newton P, personal communication), Cambodia (29, 30), Taiwan (31-35), India (36-39) (40, 41)
Epidemic; limited outbreak	Aruba (Netherlands Antilles) (42), France (15), Brisbane River Valley (Queensland, Australia) (43, 44),
Sporadic case reports	***Asia*** Indonesia (45-48), Bangladesh (36, 49-52), Philippines (48), Pakistan (36), Sri Lanka (53) ***Americas and Caribbean*** Guadeloupe (54), Martinique (55), Puerto Rico (56, 57), Ecuador (58), Panama (59), El Salvador (15, 58), Haiti (15), Brazil (60, 61), Costa Rica, Colombia (Dance D, personal communication), Venezuela (Redondo MC, Abstr. 11th Int Conf Infect Dis, Abstr 58.026, 2004)

	Pacific Guam (62), Fiji (63)(Currie BJ, Lowe M, unpublished data), Papua New Guinea (9, 64-67), New Caledonia (Hello S, personal communication) ***Africa and Middle East*** Iran (15, 68), Uganda (15), Sierra Leone (15), The Gambia (15), Madagascar (15), Kenya (69)
Unconfirmed identification, uncertain travel history or serological evidence only	***Asia-Pacific*** Korea (15), Hawaii (70), East Timor (Armstrong P, personal communication), Japan (71) ***Europe*** Spain (15), Germany (15) ***Africa and Middle East*** United Arab Emirates (14), Saudi Arabia (14), Egypt (15), South Africa (72), Turkey (15), Egypt (15), Niger (73), Burkina Faso (Upper Volta) (15) ***Americas*** Mexico (74), United States (75-79).
Isolates from environment only	Italy (80), Peru (15), Côte d'Ivoire (15), Reunion Island (15), Haiti (15)

Figure 1-1: Worldwide distribution of melioidosis



Writing over 10 years ago, Dance noted that published case reports and series are likely to only represent the "tip of the iceberg" as culture facilities are not available in most of the rural tropics where the infection is likely to be prevalent. This is also evident in the apparent changing epidemiology of the infection; despite Krishnaswamy documenting melioidosis in 5% of all autopsy deaths in 1917, the only reported cases from Burma since 1945 have been in travelers (12, 35, 81). Similarly, Thailand and Australia, where the highest rates of disease are currently noted, did not record cases until 1947 and 1950 respectively (15, 82).

Other anomalies that may be related to incomplete ascertainment include the high serological prevalence (7%) of melioidosis in returning American troops stationed in Vietnam (22) but a low rate of disease in the indigenous population (83, 84). In addition, the high prevalence of melioidosis in the Issan region of northeastern Thailand contrasts with the low prevalence in PDR Laos to the east of the Mekong River and Cambodia further south (28-30).

A caveat to this paradox is the uncertainty associated with the seropositivity rates in south east Asia that may represent exposure to the less pathogenic *B. thailandensis* (12). The worldwide distribution of *B. thailandensis* is yet to be clearly defined, but it is clear that it constitutes the commonest soil isolates in north-east Thailand (85) but has not been found in Australia (86).

1.2.1. Melioidosis in the Australia – Pacific region

Melioidosis was first recognized within Australia from an outbreak in sheep in 1949 in Winton, northern Queensland (87). The first human case described was a diabetic who died from septicaemic melioidosis in Townsville in 1950 (82) and the first case reported from the Northern Territory was from 1960 (88). This apparently late emergence of such an important infectious disease in northern Australia led to suggestions that *B. pseudomallei* may have colonized Australia from south east Asia

(89), although the molecular diversity of isolates contrasts with foci in non-endemic areas (90).

Although the endemic area of melioidosis has generally been regarded as restricted to the latitudes 20^{o}S and 20^{o}N in southeast Asia and northern Asia (91), large outbreaks have occurred outside this area in Australia, including the first case in Winton (22^{o}S) and 159 cases of melioidosis in pigs over 3 years in the Burnett River region (25.5^{o}S) attributed to a contaminated water supply (92). Autochthonous cases have also occurred outside this area in south west Western Australia (WA) (90, 93), the Brisbane River Valley in Queensland (27 ^{o}S) (43, 44), Alice Springs and Mackay.

Epidemiological studies have defined an annual incidence rate in the Top End of the Northern Territory as 16.5/100,000 between 1989 and 1999 (9), with rates as high as 41.7 per 100,000 in 1998 associated with two severe weather events and high annual rainfall (Currie BJ, Trop Med Int Health, 2004 in press). There have been few other population-based rates described previously in Australia, but in a geographically-restricted area within the Torres Strait in northern Queensland in the 2000/2 seasons (94) a rate of 40 cases per 100,000 was documented. In contrast to many other endemic countries, most patients are from remote locations but are transported to referral hospitals in the Top End region of the Northern Territory, the Kimberly region of West Australia and Far North Queensland and the Torres Strait for management.

Environmental sampling has revealed widespread isolation of the organism from soil, mud and pooled surface water in northern Australia, including Queensland (95), around Darwin (96, 97) and remote communities in the Northern Territory and West Australia (90, 98). Two outbreaks have been linked to contamination of the drinking water supply, where disease control measures including such as cleaning of the water supply and pipes led to a cessation of the outbreaks (99-101).

Although serological tests have been demonstrated to have poor sensitivity and specificity in clinical situations, seroprevalance is likely to reflect background exposure to *B. pseudomallei* on a population basis. Serosurveys of populations in

northern Australia have demonstrated relatively low rates of seropositivity, compared to the rates seen in northeastern Thailand. This was reflected in a study in Queensland, where seroposivitity in urban populations (up to 5%) was lower than in patients residing in rural locations or of Aboriginal or South Pacific origin (up to 10%) (16), similar to those found in the primarily indigenous population of Arnhem Land in the Northern Territory (12.8%) (9). These seroprevalences contrast with the much higher rates in immigrants from south east Asia to Queensland (29%) (16).

At least six cases of melioidosis have been reported from Port Moresby in Papua New Guinea (64, 65, 102, 103) as well as one additional case in an ex-serviceman living in Brisbane (67) for which the place of exposure was not clear. Small serological surveys in the Port Moresby region have not demonstrated antibodies to *B. pseudomallei* (65, 104). However, a series of clinical cases in Balimo, Western Province has led to the suggestion that melioidosis may occur elsewhere in the country (9, 66).

1.2.2. Distribution of melioidosis in Asia

1.2.2.1. THAILAND

The high rates documented in northern Australia compares to the annual incidence of 4.4 cases per 100,000 from Ubon Ratchathani province in northeast Thailand (105). Other centres in north east Thailand, such as Khon Kaen, Nakhon Ratchasima, Buri Ram and Udon Thani also see large numbers of patients. In a national survey, 30 of the 125 hospitals did not have microbiological facilities, and during 1994-5, the annual number of isolates was over 1,100; this probably represents a conservative estimate of the number of cases of melioidosis in Thailand (17).

In Thailand, *B. pseudomallei* is widely distributed in soil and more particularly pooled surface water such as in rice paddies (106-108). However, the rate of the closely-related but less virulent *B. thailandensis* which had previously been recognized as *B. pseudomallei* may account for the variation in disease

throughout the country (85); the ratio of *B. pseudomallei* to *B. thailandensis* found in soil, highest in the northeast, matches rates of clinical *B. pseudomallei* isolation throughout the Kingdom (17, 109).

These findings and the possibility of the existence of other less virulent strains of *B. pseudomallei* may also account for the much higher rates of seropositivity seen in Thailand (110), compared to the endemic areas of northern Australia (16).

1.2.2.2. PDR LAOS

Despite the highest rates in Thailand documented in the northeast Issan region, relatively few cases have been reported in adjacent Laos (28). Mahosot Hospital in Vientiane recognized a handful of cases each year constituting 2% of blood culture isolates at this referral centre between 2000 and 2002 (Newton P, personal communication), despite recovery of *B. pseudomallei* from soil isolates in the Vientiane region (Wuthiekanun V, J Clin Microbiol, in press). Outside of Vientiane, microbiological facilities are limited and the epidemiology is undefined.

1.2.2.3. VIETNAM

Melioidosis was first noted in what is now southern Vietnam by Pons and Advier (111), and the first descriptions of the saprophytic niche of *B. pseudomallei* was made by Vaucel in Hanoi and Chambon in Saigon (now Ho Chi Minh City) (15, 112).

With the large numbers of French and later American troops based in Vietnam with exposure to environmental *B. pseudomallei* and access to modern clinical and laboratory services, large numbers of cases were described from the 1940's to the 1970's (15). Cases have continued to be reported in returning servicemen for up to 29 years following exposure (113-116), as well as sporadic cases in Vietnamese emigrants and returned travelers to other countries (117, 118).

However, recent attempts at systematic surveillance have not found significant proportions of *B. pseudomallei* in blood culture isolates or soil around Ho

Chi Minh City (84), although it is likely to be found elsewhere in the country. This may be analogous to the situation in Papua New Guinea, with the patchy distribution of *B. pseudomallei* in the environment associated with clinical cases.

1.2.2.4. MALAYSIA AND INDONESIA

Stanton and Fletcher noted animal cases at the Institute of Medical Research of the Federated Malay States as far back as 1913, and published these and subsequent human and animal cases in 1932 (119).

Cases have continued to be described from both peninsular and East Malaysia, and most recently, Puthucheary reviewed 50 septicaemic cases of melioidosis in 1992 at a single referral centre in Kuala Lumpur and noted a total of 85 cases from June 1976 to June 1991 (120). A serosurvey conducted in Malaysia in 1964-6 revealed a 7.3% seropositivity (IHA titres >1:40), with the highest rates in recruits from Kedah (peninsular Malaysia) and Sabah (east Malaysia)(121).

Sporadic cases of melioidosis from Indonesia have been reported in the Dutch literature for many years (46, 122) in addition to more recent exported cases to Australia (123) the United Kingdom (48) and the United States (47).

1.2.2.5. SINGAPORE

In Singapore, melioidosis has been a notifiable disease since 1989; an annual rate of 1.7 cases of melioidosis was documented between 1989 and 1996 with the majority (89%; 337 cases) culture-confirmed cases (19). More recently, higher numbers have been noted since 1998 when 104 patients were reported (124). During early 2004, 57 cases have been reported with an unusually higher case-fatality rate (40%) and attributed to abnormally heavy rains and flooding (Lim OP, Abstr 4th World Melioidosis Congress, Abstr. 3, 2004). The case-fatality rate for patients with severe melioidosis otherwise appears in line with more developed countries (125).

Serosurveys have consistently demonstrated a low rate of seropositivity in Singapore (0.2% in the general population and 1.6% in construction workers), except in immigrants from Thailand or Malaysia (18, 19). Isolation of *B.*

pseudomallei from surface water appears to be less common than in the 1960s (126) and than from other countries in the region. Unusually, a seasonal pattern or an association with rainfall has not been noted in Singapore, in contrast to most other series (19).

1.2.2.6. CHINA, HONG KONG SAR AND TAIWAN

Small numbers of cases of locally acquired melioidosis have been described in Hong Kong (26, 27, 127, 128) and a seroprevalence of 14% was demonstrated using IHA in a tuberculosis sanatorium (129). An ongoing outbreak has been described in marine mammals in an ocean park (130). A case series and several sporadic cases, mostly autochthonous, have been described in Taiwan (31-35, 131).

On mainland China, *B. pseudomallei* has been isolated from 4.2% of soil and water specimens in Hainan Island and adjoining coastal provinces as north as 25°N (132), confirmed by human cases and seroprevalences (IHA>1:40) of up to 34% in farmers in the region (24, 25). In the 7 isolates tested from culture-positive cases, a high rate of ceftazidime resistance was observed (57%) (25).

1.2.2.7. OTHER PARTS OF ASIA

Multiple cases have been reported from disparate regions of India but have been largely restricted to a few large medical centers presumably where identification is possible (39, 40, 133-136), some, including an apparent outbreak of a bubonic plague-like illness ascribed to *B. pseudomallei* (38) have been disputed (37, 137). Cases have been reported in returning travelers from the Indian subcontinent to Europe, suggesting poor ascertainment of cases locally (36, 138). In addition, one serosurvey revealed a seroprevalence of 7% in a rural rice-growing area near Vellore (40).

Although few cases have been described in Sri Lanka (53), a report described a case that was believed to have been acquired in that country (139). Sporadic cases of melioidosis have also been reported from travelers returning from Bangladesh (36, 49, 52), including a series of three patients presenting with septic arthritis following travel to Sylhet (51). One case has been described from within the

country (50). Although reported in up to 10% of autopsy deaths in Rangoon, Burma in the original series, since 1945 the only case reported was in a Dutch traveler (81), and a second possible exported case in a Taiwanese traveler (35).

Imported cases into the United Kingdom probably reflect the prevalence of melioidosis within the countries of origin, distorted by the magnitude of immigration from those countries. Human and animal cases have been imported from Bangladesh, Pakistan, India, Indonesia and the Philippines (35, 36, 47, 48). Serological studies in East Timor suggest exposure to *B. pseudomallei* but no culture-confirmed cases have been reported (Armstrong P, personal communication)

1.2.3. Areas outside of Asia

Most cases reported outside south east Asia are from travelers to endemic areas, requiring an awareness of this disease by clinicians worldwide (36, 139-143). However, sporadic autochronous cases have been reported throughout the world including west and east Africa, the Caribbean, central and south America and the Middle East (14, 15).

Much debate has focused on the question of indigenous *B. pseudomallei* in the Americas. In North America, early reported cases were associated with travel (76), had poorly documented travel histories (77, 78) or are disputed (79).

Possibly the most intensely studied organism was the “Oklahoma isolate” from a soil-contaminated wound infection following a farming accident (75), identified by the authors as *B. pseudomallei*, but possessing atypical characteristics felt to put this identification in doubt (15). Subsequent molecular studies have been conflicting (144) with phylogenetic analysis placing this isolate in a distinct group apart from both *B. thailandensis* and *B. pseudomallei* (3).

1.2.4. Animals and melioidosis

A wide variety of animal species have been shown to be susceptible to melioidosis, including camels, horses, sheep, cattle, goats, pigs, kangaroos (92, 145-148), koalas (149), alpacas (Janmaat A, Aust Vet J, in press), deers, cats, dogs (150), and captive marine animals (130). Cattle, water buffalo and crocodiles are considered relatively resistant to melioidosis despite their constant exposure to mud (151) (150). Birds are also considered relatively resistant to melioidosis (152), although cases have been reported (153, 154).

A variety of animals have been used in experimental models including inbred mouse strains (155), chickens (156), rats and guinea pigs (2). Most recently, the susceptible Balb/c and more resistant C57BL/6 inbred mouse strains have been used extensively in studies of host responses to *B. pseudomallei* (157).

Epizoonotic outbreaks have also been reported from imported animals from endemic areas. A cluster of infections is sheep, goats and pigs living on Aruba (Dutch Antilles) was ascribed to *B. pseudomallei* in 1957 (42). An outbreak in a Paris zoo in the 1970s resulted in the spread to other zoos and equestrian clubs throughout France and the deaths of at least two humans and a number of animals. “L'affaire du Jardin des Plantes” was thought to be either due the importation of horses from Iran or an infected panda donated by Mao Zedong (12, 15).

1.3. Bacteriology and Pathogenesis

1.3.1. General bacteriology

B. pseudomallei is visualized as a gram-negative bacillus with bipolar staining, and is vacuolated, slender and has rounded ends; it is often described as having a “safety-pin” appearance. It is oxidase positive and can be distinguished from the closely related but less pathogenic *B. thailandensis* by its ability to assimilate arabinose (158, 159). Whitmore distinguished it from *B. mallei* by its motility on hanging drop but in semisolid media this finding is less reliable (106).

On culture, it demonstrates differing colonial morphology, with mostly smooth colonies initially, and subsequent dry or wrinkled colonies on further incubation.

The clinical significance of the various colony types, including small colony variants, is being prospectively investigated in Thailand (Chantratita N, personal communication).

1.3.2. Environmental microbiology and epidemiology

B. pseudomallei is a resilient organism capable of surviving hostile environmental conditions, including prolonged nutrient deficiency (160) (of durations of up to 10 years; V. Wuthiekanun, personal communication), antiseptic and detergent solutions (161) (150, 162), acidic environments (pH 4.5 for up to 70 days) (163) and a wide natural temperature range (24^{o} to 32^{o}C), dehydration (soil water content <10% for up to 70 days) (164, 165) but not exposure to ultraviolet light (164). It is likely that harsh environmental conditions may confer a selective advantage for the growth of *B. pseudomallei.*

The saprophytic nature of *B. pseudomallei* was first recognized in 1955 in French Indo-China (112). Some early studies implicated the aerosolization of dry dusts as a route of acquisition for American servicemen in Vietnam based on the high incidence in helicopter crews (23). However, further studies have demonstrated highest yields from moist soils and pooled surface water (107, 108, 166).

The association between surface water and melioidosis is supported by the strong association with monsoonal rains (8, 9, 91, 167) and with occupational and recreational exposure to surface water and mud (8, 9, 91) particularly with flooding of the rice paddies and planting at the commencement of the monsoonal season (105). The finding that higher rainfall is significantly associated with sepsis and pneumonia may suggest that environmental conditions during the monsoonal season may be associated with inhalation rather than inoculation as the primary mode of acquisition (167).

In particular, moist clay soils seem to be favoured by the organism (95) and populations residing on these soil types in Darwin have a higher rate of disease (168). Sampling studies in Australia have suggested that bacterial counts increase to

a depth of 60-90 cm (95, 108), but the finding that dry, shallower soils may be culture negative yet PCR positive has led to the suggestion that the organism may persist in a viable but non-culturable state (VBNC) (96). Although cleared, irrigated areas have been shown to be favoured by the organism in Malaysia and Thailand (107, 108, 166), these are not found in the Top End region of Australia; a sampling study of the site of an aborted attempt to grow rice in the Northern Territory at Fogg Dam failed to recover *B. pseudomallei* (Currie B, unpublished data).

In a West Australian outbreak, both *B. pseudomallei* and the parasite *Acanthamoeba* spp were isolated from a potable water source and the environment. Subsequent studies suggested that *B. pseudomallei* may infect *Acanthamoeba* trophozoites by coiling phagocytosis, a process described with other pathogens such as *Legionella pneumophila* (169). The significance of this interaction and those with plant-associated organisms as a reservoir for persistent environmental contamination is being assessed (Levy A, Inglis T, personal communication).

Contamination of drinking water supplies, rather than soil, has also been implicated in other outbreaks in Australia (92, 99). Chlorination of the water supply was associated with the termination of one of these outbreaks and appears to be effective against *B. pseudomallei in vitro* (Thomas AD, unpublished data). However, sensitive techniques using flow cytometry do demonstrate the possible presence of viable bacteria in small numbers despite free chlorine concentrations of up to 1000 parts per million (170). A study examining the impact of chlorinators on community rates of melioidosis in the Top End of the Northern Territory is detailed in chapter 11.

Factors that may influence the distribution of *B. pseudomallei* in the environment may include physical factors such as rainfall, humidity, ultraviolet radiation and temperature, and chemical factors such as soil composition, other vegetation and the use of fertilizers and recent soil disturbance such as excavation and ploughing (98). The implications of global climate change for the epidemiology of melioidosis is as yet unknown (171).

There has been some interest in the interaction between species in the *Burkholderia* genus (172, 173). As *B. cepacia* can degrade toxic compounds in pesticides and is

active against many soil-borne pathogens, there has been interest in its use as a crop biological control agent (174). However, numerous insertion sequences within *B. cepacia*, including for some strains sequences identical to *B. pseudomallei* insertion sequences (175) and transposable genetic elements in *B. pseudomallei* have been identified (176). This justifies concerns that widespread agricultural use of *B. cepacia* may be a hazard to human health, with the potential for more virulent *B. cepacia* bacteria to emerge following horizontal transmission of genetic elements (174).

1.3.3. Bacterial virulence factors

Like many saprophytic organisms, *B. pseudomallei* is a resilient bacterium that can survive in a variety of hostile conditions, including nutrient deficiency, acid and alkaline pH, in disinfectant and antiseptic solutions including detergents and chlorine, exposure to many antibiotics and at extremes of temperature. *B. pseudomallei* is also well adapted to its many hosts, producing proteases, lipases, lechithinase, catalase, peroxidase, superoxide dismutase, haemolysins, a cytotoxic exolipid and a siderophore. It is resistant to complement lysosomal defensins and cationic peptidases and can survive within many eukaryotic cell lines including professional phagocytes such as neutrophils and macrophages (12).

B. pseudomallei produces a glycocalyx polysaccharide capsule that is probably an important virulence determinant (177). This capsule (biofilm, or "slime") allows for the formation of microcolonies in a protective environment in which the organism is phenotypically altered, resulting in significant antibiotic resistance (178). In other bacteria such as *B. cepacia*, it is believed that biofilm formation is stimulated by bacterial quorum-sensing mediators such as N-acylhomoserine lactones (179); early work has defined putative signaling pathways in *B. pseudomallei* that may be virulence factors (Valede E and Song Y, Abstr. 4th World Melioidosis Congress, Abstr 30, 31, 2004).

Altered phenotypes such as slow-growing small colony variants can be observed on primary plates from clinical specimens (Wuthiekanun V, personal communication) or induced by passaging studies *in vivo* or *in vitro* and are also associated with significant antibiotic resistance. They may subsequently revert spontaneously to

their normal morphology and antibiotic susceptibility (180). The significance of other mutant forms of the organism, such as cell wall-deficient L-forms that can be only induced *in vitro* by passage through rabbit alveolar cells (181), remains uncertain. This may suggest that unusual mechanisms may mediate the survival of *B. pseudomallei* within the body such as the "globi" observed within macrophages and giant cells in autopsy specimens (182).

Ultrastructural studies using electron microscopy have observed multiplication within the vacuoles of phagocytes following internalization and subsequent endosome lysis (183). When *Acanthamoeba* trophozoites are infected with *B. pseudomallei,* bacterial escape from vacuoles is mediated by coiling phagocytosis, a process described with other pathogens such as *Legionella pneumophila* as discussed earlier (169). Movement of the bacterium towards one pole of the cell occurs by means of continuous actin polymerization into a "comet-tail" formation similar to that observed with other pathogens such as *Listeria* (184-186). Direct cell-to-cell spread is thought to occur by the induction of these cellular protrusions and fusion of cell membranes to form multinucleated giant cells (184, 185, 187) which have also been observed in human tissue (182).

1.3.3.1. SECRETORY ANTIGENS

The role of secreted antigens is unclear. Many, including proteases (188) (189), phospholipase C (190), haemolysin, lecithinase and lipase (191) are probably secreted via the general secretory pathway (type II secretion system) (192). Transposon mutations in the general secretory pathway, resulting in a failure to secrete protease, lipase or lecithinase, does not appear to result in an attenuation of virulence in an animal model(151). However, the finding that the relationship between the density of bacteraemia and mortality is similar in melioidosis compared to other gram negative bacteraemias suggests that exotoxins do not play a significant role in determining outcome (12).

However, a number of type III secretion systems (TTSS) have been described (193). TTSS in other organisms such as *Salmonella enterica* are activated under specific

conditions to allow delivery of effector molecules to host cells in order to facilitate invasion and survival in phagosomes (194, 195). This presumed function in *B. pseudomallei* is supported by the finding of homology between the SPI-1 pathogenicity island of *Salmonella enterica* (Inv/Spa/Prg) and TTSS3 of *B. pseudomallei* (185, 193, 196). Bacterial products secreted by this TTSS (termed Bsa, or *Burkholderia* secretion apparatus) including BopE, are thought to result in cytoskeletal rearrangements facilitating host cell invasion (196). *B. pseudomallei* with mutations in the Bsa and BopE system are also confined to the endosome and unable to gain access to cell actin, suggesting that this system is also important in mediating endosomal membrane lysis (185).

In addition, less virulent *B. thailandensis* do not contain some TTSS (193) and *B. pseudomallei* with mutations involving the TTSS translocator BipE and putative effectors have attenuated virulence (197). Microarray studies have determined that growth of *B. thailandensis* in the presence of arabinose results in downregulation of the TTSS3 via the putative positive regulator *bsaN*, suggesting that the loss of the ability to assimilate arabinose is linked to the increased virulence of *B. pseudomallei* in humans and animals (198).

1.3.3.2. CELL-ASSOCIATED ANTIGENS

A number of cell associated antigens have been demonstrated to be immunogenic in patients with melioidosis, including capsular polysaccharide (CPS), lipopolysaccharide (LPS; formerly O-PS II) (199) and flagellin proteins (200-203).

Antibodies to LPS have been shown to be protective against severe disease in humans (203) and in animals (204). The important role of LPS is supported by studies examining laboratory-induced mutations in the gene coding for LPS that demonstrate a susceptibility to alternative complement pathway (205, 206) and an attenuation in virulence in a mouse diabetic model (205, 207).

Capsular polysaccharide appears to have a role in environmental protection (208), immune system evasion (209) and attachment to epithelial cells (210). Capsular

polysaccharide appears to provide protection within the phagosomal environment (211, 212) and mutants of this antigen are less virulent than wild type strains (213). In addition, passive immunization against an exopolysaccharide provided protection against high-dose challenge in a mouse model (214).

Antigenic differences in CPS or other surface proteins may account for the lack of epithelial attachment and pathogenicity of *B. thailandensis* (215, 216). The ability of *B. pseudomallei* to attach and invade epithelial cell lines appears to be growth phase- and temperature-dependent; the mechanisms underlying this *in vitro* phenomenon and its clinical relevance is yet to be determined (217). A number of genes for different CPS have recently been described, termed CPS I – IV. CPS I (previously thought to represent a component of LPS and known as O-PS I) is found only in *B. pseudomallei* and is a virulence determinant; CPS-II is downregulated *in vivo* and is thought to be involved in environmental survival (Reckseilder-Zenteno S, Abstr. 4th World Melioidosis Congress, Abstr. 37, 2004).

Outer membrane proteins, such as a protein tyrosine phosphatase, have been defined (218). Although bacteria do not contain the substrate tyrosine phosphate, analogous enzymes in other bacteria such as *Yersinia* have alternative substrates that are believed to be important in signal transduction (208). However, acid phosphatases do not appear to be a major virulence determinant as strains with mutations of *AcpA*, resulting in loss of phosphatase activity, retain their virulence (219). Other outer membrane proteins, of MW 70kD, 38kD, 31kD, 24kD and 17kD have also been identified and used in diagnostic tests; the 38kD peptidoglycan associated protein appears to form aggregates and function as a porin (220, 221).

Other cell-associated antigens include type I pili (*fimA, fimC* and *fimD)*, a putative type IV pili gene complex and other antigens with a strong homology to the *pilB, pilC* and *pilD* of *Pseudomonas aeruginosa* have been defined. Flagellin proteins may be important in pathogenesis; flagellin-specific antiserum passively protected diabetic infant rats against *B. pseudomallei* challenge (222). However, conflicting results have been reported using non-motile *fliC* mutants which was less virulent in Balb/c mice (223) but not in Syrian hamsters or diabetic mice (200).

A largely unexplored area has been the role of iron metabolism in determining virulence. It is known that a siderophore, malleobactin, is elaborated by *B. pseudomallei* that is efficient at acquiring iron at acidic pH (224). This siderophore is regulated by the *fur* gene that also regulates superoxide dismutase and peroxidase (225). In other organisms such as *Vibrio parahaemolyticus*, iron-restricted conditions results in the induction of siderophore production which parallels increase virulence (226). The virulence of *B. pseudomallei* in a mouse model appears to be attenuated by iron-enriched media (227). However, clinical conditions with iron overload, such as haemosiderosis and thalassemia, appear to be associated with increased rates of melioidosis (228, 229). Like infections with *Vibrio* and *Salmonella* spp, this may suggest that iron plays a more important role in pathogenesis than has been recognized.

It is important to note that these putative virulence factors are mechanisms developed (in evolutionary terms) by the organism to survive in its as-yet-unidentified ecological niche(s). They also happen to allow the bacterium to avoid the host immune responses of humans and animals. However, infection of these hosts is accidental and is not likely to provide an evolutionary advantage for an otherwise environmental organism. This fact is reflected in the poor characterization of bacterial products as being truly virulent in animal studies and its primary affinity for hosts with impaired immunity. In addition *B. pseudomallei* generally has low disease-causing potential in healthy hosts despite its ubiquity in the environment. This stands in contrast to other organisms whose ecological niche is in humans and animals, such as *Staphylococcus aureus*, which can affect otherwise healthy individuals and where virulence factors such as the Panton-Valentine leukocidin correlate with severe disseminated disease.

1.3.4. Molecular epidemiology

A variety of molecular tools have been used to infer genetic relatedness between isolates of *B. pseudomallei*. These have included pulsed field gel electrophoresis (PFGE), random amplified polymorphic DNA (RAPD) and ribotyping. These studies have demonstrated that environmental isolates can be identical to

epidemiologically-related human or animal strains (90, 99, 230, 231), that recurrent infection is usually due to relapse with the same strain rather than reinfection with a different strain (232) (233, 234) and that outbreaks of infection may be clonal (90, 99).

These and other studies have demonstrated considerable diversity in isolates (235). This suggests that introduction of *B. pseudomallei* to these regions is not a recent event, in contrast to clonal outbreaks in non-endemic regions (90). Comparisons of typing methods demonstrate that RAPD analysis and PFGE are more discriminatory than ribotyping (231, 236, 237). Multilocus sequence typing (MLST) appears to have a similar discriminatory ability as PFGE (3).

The finding that the antigenically similar *B. thailandensis* was relatively avirulent led to a suggestion that different strains of *B. pseudomallei* may have differing virulence. Molecular typing of Australian isolates by PFGE and ribotyping have shown three clusters, termed A, B and C (90, 227, 231). There was stability in the levels of virulence within two of these clone types, but many isolates in this study did not fall into defined groups (227).

Two other studies have suggested that clinical presentation or outcome may depend on strain type. Certain ribotypes appeared to be associated with a higher mortality or risk of relapse in one study (235). A second small study (n=18) using multilocus enzyme electrophoresis (MLEE) and RAPD suggested that soft tissue infections were restricted to one cluster and respiratory and neurological infections were seen in another cluster (238).

More recently, a sequence-based successor to MLEE, MLST, has been developed for *B. pseudomallei*. This technique, based on allelic differences present at seven housekeeping genes, is ideal for genetic analysis beyond the outbreak situation due to its reproducibility and the slow rate of genetic change in the allelic sites and allows for comparisons between strains typed at different laboratories though an internet-based database (http://www.mlst.net/). This study confirmed the diversity seen in isolates worldwide and places *B. mallei* within this wider *B. pseudomallei* group, both distinct from *B. thailandensis* (3).

A study comparing MLST with *SpeI*-based PFGE, together with inferences regarding the global epidemiology of melioidosis is detailed in chapter 14.

1.3.5. Genome sequence of B. pseudomallei and comparison with B. mallei

A major recent advance has the completion of sequencing and the annotation of both the genomes of *B. mallei* (strain ATCC 23344) and *B. pseudomallei* (clinical strain K92643) (4, 5). The genome of *B. pseudomallei* is composed of two large chromosomes (of 4.07 and 3.17 megabases respectively) that demonstrate functional partitioning (4). The larger chromosome codes for many genes required for cell metabolism and growth and the smaller tends to code for genes required for survival and virulence factors. A summary of some of the key genes identified is listed in Table 1-2. The finding that the gene order is conserved on the larger chromosome and has a greater number of orthologous matches when compared to other bacteria suggests that they may not have common ancestry. A striking finding was the demonstration of genomic islands, identified by anomalies in GC content or dinucleotide signatures together with accompanying coding sequences resembling those of mobile genetic elements. These are likely to represent DNA that has been recently horizontally acquired and are variably found in clinical and environmental strains of *B. pseudomallei.*

In contrast, *B. mallei* does not contain any of these genomic islands, suggesting that horizontal acquisition of DNA is not an important source of genetic variation as in *B. pseudomallei* (5). Gene deletion, probably as a result of mammalian host restriction and a consequent reduction in selective pressure, is suggested by the absence of many of the genes required for environmental survival in *B. pseudomallei.* Rather, numerous insertion sequences and simple sequence repeats point to an alternative mechanism for genetic variability in *B. mallei*.

Table 1-2: Genes associated with survival and virulence identified in the B. pseudomallei genome (4)

Survival	**Virulence**
Secondary metabolism; possible antibiotic, surfactant, siderophore biosynthetic pathways	**Secretion**; Type I, II, III and V secretion systems, including three type III secretion systems
Drug resistance; Ambler class A, B, D beta-lactamases, multidrug efflux pumps, aminoglycoside acetyltransferase	**Surface components**; lipopolysaccharide, capsular polysaccharide and potential surface polysaccharide biosynthesis
Intracellular stress; superoxide and nitric oxide detoxification enzymes	**Exoproteins**; phospholipase C, metalloprotease A and other proteases, collegenase
Motility and chemotaxis; flagella system, chemotaxis-associated proteins	**Adhesins**; surface proteins that may modulate host-cell interaction
	Fimbriae and pili; type I and IV pili and *tad*-type pili.

1.3.6. Role of host immune responses

Although many studies have observed and defined immune responses believed to be important in pathogenesis, several points require explanation by any comprehensive model of pathogenesis;

- The specific co-morbidities present in patients susceptible to melioidosis, particularly patients with diabetes, thalassaemia, alcoholism and renal impairment;

- That repeated exposure, sufficient in some patients to provoke an antibody response to a highly conserved lipopolysaccaride antigen, is insufficient protection against infection, although there is some evidence that high titre antibodies against LPS may be protective against severe infection;

- That although interferon-γ appears to be vital in resistance, as might be expected for an intracellular bacterium, the role of adaptive CD4-mediated immunity remains uncertain, particularly as human immunodeficiency virus infection does not seem to be a risk factor for disease;

- The contribution of the various bacterial products to infection, and more specifically, the relative contribution of endotoxin and exotoxins to pathogenesis and evasion of the immune system; the finding that the relation between bacterial counts in blood and mortality is similar to that with other Gram negative organisms suggests that exotoxins do not contribute directly to outcome.

- The site from which latent infection may reactivate, as relapse after apparently successful treatment and extended incubation period after exposure suggest a dormant state similar to that seen in tuberculosis.

1.3.6.1. INNATE IMMUNITY

B. pseudomallei appears to be resistant to serum bactericidal components (239); although the alternative complement pathway is activated resulting in phagocytosis, it is resistant to the effects of the terminal complement membrane attack complex (240). Phagocytosis was increased by the addition of specific antibodies and complement (241).

Studies of the role of phagocytes in melioidosis have demonstrated conflicting results. It is established that *B. pseudomallei* can survive and multiply within professional phagocytes including macrophage/monocyte and neutrophil cell lines (211, 242). It appears to be able to evade phagosome-lysosome fusion and destroy

the phagosome membrane as soon as 15 minutes after ingestion (183). This is consistent with functional studies demonstrating poor early bactericidal activity in most (199, 240, 243) but not all studies (244).

The co-morbidities recognized as risk factors for melioidosis also may be operating by impairing neutrophil function. Diabetes mellitus has been demonstrated to result in impaired chemotaxis, phagocytosis, oxidative burst and killing activity (245-252). Similar defects have been described with alcohol (253-262), chronic renal failure (263-267) and thalassemia (268). Reports of patients with chronic granulomatous disease (56, 269) support the role of neutrophils in resistance to melioidosis.

Of further interest is the possibility of reversing these functional defects with granulocyte colony stimulating factor (G-CSF). Multiple studies of G-CSF in non-neutropenic animal models have demonstrated improvements in markers of clinical outcome (259, 270-273). In human studies, improvements are seen in laboratory markers following G-CSF use (274-276) and diabetic foot infection may benefit from the use of G-CSF (277, 278). However, large multicentre clinical trials have failed to demonstrate benefits in pneumonia and severe sepsis possibly due to late administration of G-CSF in the course of the illness (279-281)
A review of the use of G-CSF in septic shock at the Royal Darwin Hospital in Australia is detailed in chapter 3; further progress toward a clinical trial forms one basis of this thesis.

1.3.6.2. ROLE OF MACROPHAGES IN IMMUNITY

As mentioned previously, *B. pseudomallei* appears to be able to survive and multiply within professional phagocytes, including those of the macrophage/monocyte lineage (183, 242). Although this is assumed to play a role in the site of latent infection, little is known regarding precise localization of latent intracellular *B. pseudomallei* after early host-bacteria interaction and the bactericidal ability of macrophages.

Macrophages exposed to *B. pseudomallei* do not appear to respond in the same way as to other pathogens; in one study, lower levels and slower production of iNOS and TNF-α production was seen in a macrophage cell line exposed to *B. pseudomallei* when compared to *E. coli* and *Salmonella typhi* (282, 283). However, the responsiveness of these cells, as well as their bactericidal activity, was increased by priming with IFN-γ, providing an explanation for the important role of this cytokine in mediating resistance (282). Similarly, the low levels of IFN-β production by macrophages may also mediate poor intracellular control by iNOS-dependant mechanisms (284).

In addition, ultrastructural studies of macrophage-*B. pseudomallei* interactions have compared responses in patients with melioidosis and healthy controls. They suggest that there is less early phagolysosome fusion in macrophages from melioidosis patients, resulting in higher intracellular bacterial concentrations (Puthucheary S Abstr 4th World Melioidosis Congress Abstr. 40, 2004)

TNF-α, produced primarily by macrophages but also B cells, T cells and fibroblasts, is an early and potent proinflammatory cytokine. High systemic levels are associated with septic shock, including in patients with melioidosis where high TNF-α levels were associated with mortality. However, TNF-α is required for the containment of infection; neutralization of TNF-α in a mouse model increased susceptibility to melioidosis (285).

The *TNF2* allele, representing a stable mutation in the TNF-α promoter, (substitution of G→A at base –308) is associated with increased production of TNF-α. It is associated with increased susceptibility to mucocutaneous leishmaniasis, cerebral malaria and meningococcal purpura fulminans (286). In 109 of 123 Thai patients with melioidosis, the presence of *TNF2,* present in 9% of the study population, was associated with an increased risk of death, septicaemia, multiple and single organ involvement. When matched to 74 seronegative controls, the presence of *TNF2* was associated with a statistically significant relative risk of melioidosis of 2.3 (287).

It has been speculated that the intracellular niche of this organism and its interaction with hosts with specific co-morbidities, such as diabetes, suggests that the innate immune response plays a prime role in the control of this organism (13). Severe impairment of specific cellular immunity, such as with advanced HIV infection, does not appear to be a risk factor for infection. Similarly, disease may be present even with high antibody titres indicating that natural humoral immunity does not appear to be protective. The relatively minor role of specific immunity relative to innate immunity in this infection may explain the capacity of this organism for latency as well as the lack of protection against disease despite repeated exposure to the organism.

1.3.6.3. ROLE OF T CELLS IN IMMUNITY

A classic framework to view specific host responses is the type1/type 2 dichotomy where cell mediated responses are usually inversely proportional to the levels of antibody response. Type 1 responses are generally adaptive against intracellular pathogens; C57BL mice are more resistant to *Leishmania major* and IL-$10^{-/-}$ mice to *Listeria monocytogenes* and their resistance is ascribed to their tendency to type 1 responses (288).

In melioidosis, this appeared to be supported by the demonstration that C57BL6 mice, with a propensity to type 1 responses, were relatively resistant to *B. pseudomallei* compared to Balb/c mice (157, 289), and the demonstration of the key role of interferon-γ, a key Th1 cytokine that stimulates phagocytosis, the oxidative burst and intracellular bacterial killing (285, 290) and its correlation with the severity of illness in humans (291, 292).

Similarly, the *sine qua non* of Th1 polarization, IL-12, can be demonstrated in patients with melioidosis and in *ex vivo* blood simulated with heat-killed *B. pseudomallei*, and neutralization of IL-12 can be demonstrated to result in impaired IFN-γ production (291) and increased susceptibility in mice (285). In addition, the

ratio of IgG1 to IgG2 in humans (293) and mice (157) also suggests that a type 1 response may be protective.

However, there is evidence that anti-inflammatory Th2 responses are necessary to balance pro-inflammatory Th1 responses; severe sepsis may represent an unregulated Th1 response. In septic patients, as in melioidosis, poor outcomes are associated with high levels of both IL-6 (a Th1 cytokine) and IL-10 (a Th2 cytokine) (294). This may suggest that the intensity of both pro- and anti-inflammatory responses merely reflect the magnitude of the inflammatory insult. More extensive cytokine characterization of Th1 and Th2 responses in mice strains with differential susceptibility has now demonstrated a mixed pattern; levels of mRNA for Th1 cytokines (IFN-γ, IL-6 and IL-12)and Th2 cytokines (IL-10) increased in both susceptible BALB/c and resistant C57BL/6 mice (295).

Other studies have implicated effector cells as cytotoxic T lymphocytes and NK cells responding to IFN-γ via the CXC chemokines IP-10 and Mig (296). These cells may act to induce apoptosis in infected cells via granzymes (GrA and GrB)(297). NK cells and, unexpectedly, $CD8^+$ T cells, have been demonstrated to be a source of IFN-γ early in the response to infection with *B. pseudomallei* (298). This process appears to be a "bystander" phenomenon mediated by IL-12 and IL-18 rather than engagement of the T cell receptor.

The demonstration of a specific cell-mediated immune response in survivors of acute melioidosis may suggest that this may be important in the long-term control and prevention of relapse (299, 300). However, most puzzling is the absence of evidence to suggest that HIV infection is a risk factor for melioidosis despite its prevalence in Thailand (301).

1.3.6.4. ANTIBODY RESPONSES

As discussed above, many *B. pseudomallei* components have been demonstrated to be immunogenic in patients with melioidosis, including capsular polysaccharide

(CPS), lipopolysaccharide (formerly O-PS II) (199), flagellin proteins (200-203) and other cell wall proteins (302). Of these, antibodies against the LPS and possibly exopolysaccharide and flagellin components have been demonstrated to be protective (203, 214, 222).

Antibodies of all classes against a culture filtrate antigen were demonstrated in patients with prior melioidosis and levels were highest for IgG, particularly the IgG1 and IgG2 subtypes (303) and antibodies could persist variably for over 3 years (293). The description of a case with a persistently high IHA titre (>1:5120) for many years who presented with reactivation in association with staphylococcal endocarditis may suggest that persistently high titres may define a group that require close follow up (304). Evidently, the antibody response resulting from repeated natural exposure to *B. pseudomallei* and *B. thailandensis* is insufficient to provoke a protective response for primary infection or relapse.

1.3.6.5. OTHER HOST FACTORS

A possible association with HLA-DRB1*1602 and severe melioidosis, independent of possible confounders such as diabetes mellitus, was described (305), but this has not been borne out by other studies, placing susceptibility genes between the HLA-Cw and HLA-DQ loci of the HLA-B58 haplotype (306)

Studies examining the role of polymorphisms in Toll-like receptor alleles and mannose-binding lectin genes and their correlation with the severity of disease are currently underway (Wiersinga WJ, personal communication). It is likely that recognition of bacterial endotoxin or other bacterial components play a significant role in adaptive and maladaptive responses in melioidosis as with other septic states.

A promising therapy is the use of the use of the bacterial genomic sequences in the form of unmethylated CpG oligodeoxynucleotide (CpG oDN), either as an immunoprotective agent or as a DNA vaccine adjuvant. In a mouse model, CpG was shown to be strongly protective when administered prior to exposure to *B. pseudomallei*, possibly by increasing bacterial phagocytosis by macrophages (307,

308). Although no significant adverse reactions have been noted in human studies to date, theoretical concerns focus on the possibility of DNA integration into host cells (although CpG itself does not contain a promoter), the induction of adverse cytokine profiles and the possibility of triggering autoimmune phenomena in susceptible patients (309).

1.3.7. Prospects for vaccine

A Canadian group has been exploring potential vaccination strategies which have been reviewed recently (310). Despite recent work demonstrating some protection in animal models following vaccination with *B. thailandensis* and other attenuated strains (311, 312), it is unlikely that this strategy alone will provide sufficient protection given the extensive exposure histories of patients to both *B. thailandensis* and *B. pseudomallei*. Other approaches that have been investigated include the use of DNA vaccines to the *fliC* flagellin structural gene and the use of attenuated *B. pseudomallei* mutants. Attenuated strains would have to be demonstrated to be avirulent; a case report suggesting that even ara^{+} *B. thailandensis* can cause clinical infection has important implications for vaccine research (159).

A *B. mallei* candidate conjugate vaccine has been synthesized by linking the capsular and O-PS lipopolysaccharide antigens to exotoxin A of *Pseudomonas aeruginosa.* This vaccine is being investigated in a horse model of glanders (151, 313). Given the cost of such vaccines, the relatively low incidence rate of the disease, the uncertain duration of protection and the resource-constrained regions in which such a vaccine might be used, it is unlikely that a vaccine for *B. mallei* or *B. pseudomallei* would find use outside of military settings.

Although protective footwear is often recommended for patients at high risk of melioidosis, the efficacy of this advice has not been evaluated. The relationship between extreme weather events and cases of melioidosis is noted in chapter 2 and analyzed further in chapter 12.

1.4. Clinical features

1.4.1. Risk factors

A number of risk factors for developing melioidosis have been defined in several studies and are summarized in Table 1-3. Patients with diabetes mellitus, in particular, have a high incidence of melioidosis with up to 60% of patients with pre-existing or newly diagnosed type 2 diabetes (13, 97, 105, 314). Although it was suggested that insulin may have a direct effect on *B. pseudomallei* (207), the high incidence of type 2 diabetes, rather than type 1 diabetes, points away from this as the mechanism of action (315, 316). Subsequent studies have attributed the inhibitory effect to a preservative used with insulin (317).

Studies have examined risk factors in patients with melioidosis were compared with septic and non-septic hospital controls to estimate a relative risk. In a Thai study, diabetes, thalassaemia, renal disease (defined as renal calculi or renal failure), and occupational exposure to surface water were all associated with an increased risk of melioidosis (314). A population-based study in Australia defined adjusted relative risks of 4.0 (3.2-5.1) for those aged ≥45 years, 2.4 (1.9-3.0) for males, 3.0 (2.3-4.0) for Aboriginal Australians, 13.1 (9.4-18.1) for diabetics, 2.1 (1.6-2.6) for those with excess alcohol consumption, 4.3 (3.4-5.5) for chronic lung disease and 3.2 (2.2-4.8) for chronic renal disease. The reason for these specific risk factors is not clear, but many have implicated the effect of these co-morbidities on neutrophil function (13, 105), known to be important in the pathogenesis of melioidosis (242).

The use of steroids is associated with an increased risk of melioidosis; this includes steroid-containing herbal remedies ("yaa chud") in Thailand, documented in up to 10% of Thai patients (314). In the Australian series, chronic obstructive pulmonary disease and the consumption of kava and alcohol have also been implicated (13).

Despite cell mediated immunity being implicated as a mechanism of resistance to melioidosis (299, 300), infection with the human immunodeficiency virus (HIV) does not appear to be a major risk factor (301).

A number of case studies have noted an intriguing association with chronic granulomatous disease that may highlight the under-recognized role of neutrophil defects in pathogenesis (56, 269). Similarly, reports of patients with haemosiderosis may suggest that impairment of phagocytic cells may be important (228, 229). Case reports of melioidosis and previous or subsequent mycobacterial infection (*M. tuberculosis, M. terrae, M. leprae*) may reflect a common host susceptibility to these intracellular pathogens (65, 105, 318, 319).

Table 1-3: Clinical risk factors for melioidosis

Risk factor	Level of evidence
Diabetes mellitus	Between 37-60% of patients are diabetic, mainly type 2. Case-control and population-based studies in Australia and Thailand; estimated RR 5.9 – 13.1 (13, 97, 105) (314)(Currie BJ, Trop Med Int Health, 2004 in press)
Thalassaemia	α-thalassemia trait common in Thailand (44%) but disease less common (8%); case-control studies in Thailand estimate RR 10.2 (105) (314, 320)
Aboriginality	Population-based study in Australia estimate RR 2.7-8.1; assumed to relate to exposure to soil/water (13, 97) (Currie BJ, Trop Med Int Health, 2004 in press).
Male gender	All series in Australia, Thailand, Malaysia and Singapore demonstrate male preponderance (19, 97, 105) (Currie BJ, Trop Med Int Health, 2004 in press)
Soil/water exposure	Rice farmers constitute 81% of patients in Thailand, relative risk in case-control study estimated at 3.3 (314) (105)
Renal disease	Patients with renal impairment or failure comprise 10% of

	Australian series (13) with relative risk of 3.2 (Currie BJ, Trop Med Int Health, 2004 in press). Renal disease (renal failure and calculi) associated with increased risk of melioidosis (OR 2.9) (105) (314)
Alcohol	Conflicting evidence; excessive alcohol use documented in 39% of Australian patients with RR 2.1-6.7 in case-control and population-based study (13, 97)(Currie BJ, Trop Med Int Health, 2004 in press). Less prevalent in Thai patients (12%)(105)
Kava use	Use of *Piper methysticum* root documented in 8% of Australian series (13) but not associated with pneumonia in case-control study (321)
Chronic lung disease	Present in 27% of Australian patients(13) with relative risk of 4.3 (Currie BJ, Trop Med Int Health, 2004 in press)
Splenectomy	Case studies, often related to thalassemia (320, 322, 323)
Aplastic anaemia, febrile neutropenia	Case reports only (105, 324).
Chronic granulomatous disease	Two case reports (56, 269)
Mycobacterial disease	Case reports of patients with atypical mycobacteria, *M. tuberculosis* or *M. leprae* infection may suggest common host susceptibility (318) (65, 319) (105)
Dengue haemorrhagic fever	Five of 18 paediatric patients in Thailand (323)
Neutropenia	Case report (325)
Renal transplantation	Case report; patient also diabetic (41)
Systemic lupus erythematosus or steroid use	Case reports; also associated with immunosuppressives (27, 326, 327) (105). Steroid-containing herbal remedies documented in up to 10% of Thai patients (314).
Glucose-6-phosphatase deficiency	Case reports (105)

Haemosiderosis	Case reports (228, 229). One unreported case of pulmonary haemosiderosis secondary to mitral valve disease (Currie B, personal communication)
Cystic fibrosis	Reports from travelers to endemic areas (36, 142, 328, 329)
Porphyria cutanea tarda	Subsequent to episode of melioidosis; likely to be an adverse event to medication (330).

1.4.2. Clinical syndromes

In all series, pneumonia is the most common presentation of melioidosis and is involved in approximately half of all cases. It is conventionally thought that lung involvement arises after haematogenous spread following inoculation based on cases of pneumonia arising following a history of inoculation and the finding that radiographic assessment of pneumonia often lags behind the patient's clinical status. However, early reports implicating inhalation in helicopter crews based in Vietnam (23) and the presence of a marked association of the rates of pneumonia with rainfall (167) may suggest that inhalation may be more important than had been previously appreciated.

Important clinical differences have been seen between patients in Australia and Thailand (Table 1-4); the high incidence of genitourinary infection in Australia with prostatic abscesses occurring in 18% of males, the absence of suppurative parotitis in Australia in contrast to a rate of 30-40% in Thai children (331), and the distinct but uncommon encephalomyelitis syndrome seen in tropical Australia.

Table 1-4: Variation in clinical pattern of melioidosis worldwide

Clinical presentation	Royal Darwin Hospital series (1989-99; n=252) (13)	Singapore series (1989-1996; n=331[†]) (19)	Kuala Lumpur series (1976 – 1991; n=50)* (120)	Infectious Diseases Association of Thailand series ‡ (n=686) (332)	Sappasithiprasong Hospital series (1986-7; n=63)* (8)
Pneumonia/pleural effusion	58%	NR	58%	45%	23%
Genitourinary infection	19%	NR	10%	7%	8%
Skin /soft tissue infection	17%	NR	24%	13%	13%
Neurological melioidosis/brain abscess	4%	NR	6%	3%	NR
Splenic abscess	4%	NR	2%	2%	NR
Liver abscess	2%	NR	4%	7%	NR

Other intra-abdominal	3%	NR	4%	5%	NR
Prostatic abscess	18% (of males)	NR	NR	0.3%	NR
Parotid abscess	0%	NR	NR	2%	NR
Bone/joint	4%	NR	12%	5%	4%
Pericardial effusion	1%	NR	2%	3%	NR
No clinical focus	10%	NR	NR	NR	51%
Septic shock	20%	NR	16%	NR	30%
Bacteraemia	46%	43%	100%*	58%	100%*
Mortality	19%	39%	65%	38-61%	68%

NR: not recorded; * bacteraemic cases only; † Culture-confirmed cases only ‡Summary of reported cases presented in 1985 from Khon Kaen Hospital (1982-5), Ubon Ratchathani (1982-5), Srinagarind Hospital (1978-85), Nakhon Ratchsima (1983-5), Chulalongkorn Hospital Bangkok (1980-5) and Nontaburi (1983-5)

Encephalomyelitis, characterized by brainstem encephalitis and flaccid paralysis, is seen in 4% of melioidosis presentations in northern Australia and is associated with considerable morbidity and mortality (333). Small numbers of children with a similar syndrome have been recognized in Thailand (334). Cultures of cerebrospinal fluid were only positive in one of seven cases with monocytic pleocytosis the most common finding (335). This should be distinguished from more focal suppurative infections involving the central nervous system which have been well-described (336-341). Some of these may represent direct spread from contiguous sites, such as facial sinuses (341) or orbital cellulitis (334). Primary meningitis has been described in Thailand (334) but more often results from ruptured cerebral abscesses (12). Neurological involvement has also been described in animals (148, 152, 342).

Acute suppurative parotiditis accounts for up to 40% of paediatric cases but only small numbers of adult cases in Thailand (331, 343). It seems to arise in patients with no defined risk factors and is generally associated with a good prognosis. It may be bilateral in 10% and may be complicated by rupture or permanent facial nerve palsy. It has only been reported once in Australia (94).

The high proportion of patients with prostatic infection in Australia contrasts with the higher proportions of patients with liver and spleen abscesses seen in Thailand (344). In Australia, the prevalence of prostatic infection (18% of male patients) mandates routine imaging, with drainage commonly required (13, 345). This contrasts with other internal abscesses that may respond to medical therapy alone (13).

Bone and joint infections are uncommon and may be difficult to differentiate from other causes of infection, except that the systemic features of the illness may be more prominent. Surgical drainage is often required, together with long courses of intravenous antibiotics (346).

Skin and soft tissue infections are a common manifestation of melioidosis, and may be the source of systemic infection or result from haematogenous spread. Presentations may be rapidly progressive similar to necrotizing fasciitis from other

organisms (347). Infections involving many other sites have been described, including mycotic aneurysms, mediastinal infection, thyroid and scrotal abscesses (13). Corneal ulcers were described in a series of three Thai patients following corneal trauma. Extensive ulcers, subconjunctival abscesses and hypopyon were managed with topical and intravenous ceftazidime with good outcomes (348). Other ocular manifestations include orbital cellulitis and with contiguous spread to the sinuses (349). Cardiac involvement is rare; pyopericardium is probably the most common manifestation but myocardial abscesses (350) and endocarditis are said to occur (332).

Markers of organ dysfunction, including leucopenia, particularly lymphopenia, hepatic dysfunction (raised AST, ALT and bilirubin), renal dysfunction (raised urea and creatinine) and metabolic derangements (hypoglycemia and acidosis) on admission appear to predict mortality (8, 345). The development and validation of a scoring system for mortality based on markers of organ dysfunction is detailed in chapters 9 and 10.

The response to therapy is often poor, with the mean duration of fever of 9 days documented (351). Treatment failure, when ascribed to antibiotic therapy alone (rather than undrained sites of infection or resistance), has been defined in studies as fever exceeding 14 days or bacteraemia exceeding 7 days (351). Persistently positive cultures from other sites and radiological abnormalities are not uncommon and do not necessarily portend a poorer prognosis (13, 352).

Markers of inflammation such as C-reactive protein (CRP) are often performed as markers of infection and clinical response; a small study demonstrated that CRP levels were elevated in 46 patients with melioidosis, that the CRP level responded within two days in patients with uncomplicated treatment courses and that persistent elevation in four patients was attributed to undiagnosed sites of infection or inadequate treatment. In addition, elevations in CRP predicted relapse, even in the absence of fever or leukocytosis (353). A review of the use of CRP demonstrating its lack of utility is detailed in chapter 8.

The clinical use of procalcitonin has also been assessed in Thailand; although the level of procalcitonin reflected the clinical severity of illness and the response to therapy, this was not specific to melioidosis. In addition, there was a wide variation in response, with a significant mortality even in patients with low procalcitonin levels (354).

The state of asymptomatic carriage has been mooted (233), but in 1000 hospitalized children and 4545 adults (1011 patients with melioidosis and 3524 healthy subjects) in Thailand, the positive predictive value of a throat swab for clinical disease was 100% (110, 355). In goats, asymptomatic carriage has been reported (147).

Sporadic patients, particularly those with cystic fibrosis, have been described with long term carriage without signs of overt disease (142), raising the possibility that these strains are behaving more like *B. cepacia* in this group of patients.

Relapsed disease after apparently successful treatment is well described and is associated with a similar mortality to the initial episode. It occurs in 13-23% of cases and patients represent a median of 6-8 months (but up to many years) later (233) (356). Factors associated with a higher risk of relapse included poor adherence to therapy, the use of doxycycline monotherapy or amoxicillin-clavulanate in the eradication phase, severe disease (RR 4.7 compared to localized disease) the use of ampicillin-clavulanate or the 4-drug “conventional therapy” in the intensive phase (RR 2 compared to ceftazidime) and eradication therapy of less than eight weeks (RR 2.5) (233, 356). In the Royal Darwin Hospital series, only one patient of >60 treated with TMP/SMX eradication relapsed (233); improved compliance with this simple eradication regimen, in addition to close follow-up, is likely to be an important factor. Differences are also noted in the dosing of TMP/SMX; in Australia, a higher dose is given (8/40mg/kg bd) compared to Thailand (5/25mg/kg bd).

In the majority of cases, relapse is due to reactivation of the original infecting strain (demonstrated by restriction fragment length polymorphism or pulsed field gel electrophoresis). Infection with a different strain was demonstrated in between 4%

and 7% of cases in Thailand and Australia (232, 233) and in 1 of 5 recurrent cases in Malaysia. (234).

1.4.3. Modes of acquisition and incubation period

Three modes of acquisition are recognized for *B. pseudomallei*, inhalation, ingestion and inoculation, but the relative contributions of each are yet to be determined. As with other infectious diseases, it is likely that these factors, as well as the size of inoculum, are responsible for the pattern and severity of disease. Situations likely to be associated with a high inoculum, such as near drownings, are associated with a short incubation period even less than 24 hours (32, 105).

Inhalation was initially thought to be the primary mode of acquisition, based on studies from US soldiers in Vietnam where it was noted that helicopter crews seemed to have a high incidence of the disease (23). This, and its long incubation period, resulted in melioidosis acquiring the sobriquet “the Vietnamese time bomb” (23); although sporadic cases have continued to surface in the United States from remote exposure (115, 116), the feared epidemic failed to materialize. Work done in the 1950’s (357) and more recently, driven by biodefence (358), has defined infectious doses via this mechanism in mice and other animals. The finding that periods of heavy rainfall are associated not only with higher numbers of cases but also pneumonic presentations and cases of increased severity may suggest a shift to inhalation during extreme weather events (167). This is supported by the recent observation that a number of patients in Singapore that presented during a period of heavy rainfall were elderly, non-ambulant patients with no history of exposure to soil or surface water (Lim OP, unpublished data).

It is now believed that inoculation is the major mode of acquisition. Minor wounds to the feet of rice farmers is common during the planting and harvesting seasons where farmers spend most of the working day wading in mud and surface water; inoculation at the time of a snake bite has also been described (8). In the Darwin series, 25% of patients give a history of an inoculation injury prior to presentation; in this subgroup of patients, an incubation period of 1-21 days has been defined (9).

It is also clear that more chronic presentations are not uncommon, with 13% having symptoms exceeding 2 months at presentation (9). In addition, incubation periods as long as 24 to 29 years in ex-servicemen to Papua New Guinea and Vietnam have been described (67, 115, 359).

Ingestion has been suggested as a mode of infection (23, 360). In animals, this route has been implicated by findings of infected gastro-hepatic nodes in pigs (92). These findings have also been noted on occasion in humans with an Australian having microabscesses of the gastric wall with seeding of the peritoneum from a ruptured gastric ulcer (9). However, the contribution of this route of infection is undefined; although contamination of potable water has been implicated as the point source in two outbreaks (99, 100), this may not necessarily reflect ingestion as the primary mode of transmission.

Two laboratory-acquired cases have been described; one associated with sonication outside a safety hood (361), the other after organisms were spilled during centrifugation (362) highlighting the need for biosafety precautions (363, 364).

Other unusual modes of transmission include person-to-person, both in a sibling of a child with cystic fibrosis (328) and a possible sexual transmission from a returned serviceman to his partner (365) although the latter was based on serological evidence only. Although prostatic infection is common in Australia and sexual transmission has been suggested (366), no confirmed cases of sexual transmission are known to have occurred from these cases (Currie BJ, unpublished data).

Reports of neonatal cases suggest perinatal transmission (367, 368) (332) and one case has been attributed to culture-positive breast milk (Ralph A, Pediatr Infect Dis J, in press). Vertical transmission has only been proven on one occasion (369) in humans and transplacental spread has been documented in goats (150).

Possible epizoonotic human infections have been implicated in at least three cases in humans in Australia (150). Nosocomial transmission to four animals who had attended a single veterinary practice was ascribed to contamination of a multidose

injectable solution (150). Two cases of suspected nosocomial infection were reported from Srinagarind Hospital in Thailand associated with contamination of chlorhexidine/cetramide (Savlon) (332). Contaminated detergent has also been implicated in a cluster of cases in a small remote Australian community (162). In the original description of melioidosis, intravenous use of illicit opiates was described as a risk factor but has not been implicated since (2).

Reactivation of melioidosis has been described following influenza infection (114), but this probably not a significant determinant of the season variation in Australia as the influenza season comes after the melioidosis season (233). Cases have been noted of reactivation associated with other illnesses such as staphylococcal endocarditis (304). Malaria and seasonal dietary factors have also been proposed as triggers (105) but these are obviously not applicable in Australia. It is more likely that seasonal agricultural practices and exposure to surface water are more important determinants of the seasonal pattern of disease.

1.4.4. Melioidosis as a potential bioweapon

Almost a century before the "anthrax letters" incident in the United States, it has been suggested that Sir Arthur Conan-Doyle recognized the potential of this infectious agent as a potential bioweapon in his Sherlock Holmes story "The Dying Detective". In this story, Holmes is sent a box designed to inoculate the recipient with "Tapanuli fever" on opening, and it is thought by many to represent melioidosis (370, 371).

More recently, *B. pseudomallei* has been considered an important potential bioweapon with increasing funding overseas for research into virulence factors and vaccine development (310, 358). It is believed that biological weapons research using *B. pseudomallei* occurred in the former USSR, although the extent of this effort and the possibility of engineered antibiotic resistant strains, remains unknown (372, 373). Other countries with a military interest in *B. pseudomallei* included the United States and possibly Egypt (374, 375).

Non-primate animal models of inhalational melioidosis were defined in the 1950s (357) as well as more recently (358), but apart from laboratory accidents (361, 362), no other cases are believed to have occurred outside the natural environment. One study has demonstrated the value of prophylactic or immediate ciprofloxacin or doxycycline (for 5 days following exposure) in increasing the median lethal dose in an animal model of peritoneal inoculation (376).

The potential risk posed by *B. pseudomallei* as a bioweapon is uncertain. Melioidosis carries a potentially high mortality rate and its causative agent has intrinsic antibiotic resistance and a wide host range. However, weaponization has not been known to have been performed, it does not spread from person to person and the susceptibility of immunocompetent individuals after inhalation is not clear.

In contrast, the closely related bacterium *B. mallei* was believed to have been used in the First World War by the Central Powers to infect Russian horses with glanders on the Eastern Front, with significant effect. Subsequently, deliberate infection of *B. mallei* of human prisoners of war and animals took place at the Pinfang (China) Institute during the Second World War by the Japanese (374). Further development with *B. mallei* was also undertaken in the United States, the former Soviet Union and Egypt, (375, 377) and glanders has possibly been used in the Afghanistan conflict between 1982 and 1984 (372).

1.5. Diagnosis and management of melioidosis

1.5.1. Diagnosis

Isolation of *B. pseudomallei* from bodily fluids of patients remains the gold standard in diagnosis, and requires the use of selective media for non-sterile specimens. Gram's staining and other histopathological stains are not specific for the organism. A number of techniques have been employed to attempt to reduce the time required to achieve a diagnosis including antigen detection on specimens or on culture supernatant, antibody detection, molecular techniques and rapid culture techniques (Table 1-5). Although many of these rapid tests have been developed, few have been

extensively tested in the field and only the indirect haemagglutination assay (IHA), latex agglutination and immunofluorescence are currently used clinically.

Table 1-5: Sensitivity and specificity of diagnostic tests for culture-confirmed melioidosis

		Sensitivity	Specificity	Reference
Antibody detection				
Complement fixation test	Unknown (n=47)	NR	100%	(378)
Serum IHA	Thailand (n=150)	71%	75%	(379)
Serum IHA	Thailand n=184	77%	92%	(380)
Serum IHA (purified antigens)	Thailand n=101	46-94%	34-82%	(302)
Serum IHA	Thailand n=130	61.9%	79.9%	(381)
Serum IHA	Thailand n=148	64%	93%	(382)
Serum IHA	Thailand (n=299)	72%	68%	(383)
Serum IHA	Australia (n=298)	80%	91%	(384)
Serum IHA (acute serum only)	Australia (n=191)	85%	100%	(385)
Serum IFA-IgG (acute serum only)	Australia (n=191)	86%	99%	(385)
Serum ELISA (IgG; acute serum only)	Australia (n=191)	79%	99%	(385)
Serum ELISA (purified glycolipid antigen)	Japan/Vietnam/ Thailand n=416	100%	97.8%	(386)
Serum ELISA IgG (immunoaffinity-purified antigen)	Thailand (n=150)	88%	83%	(379)
Serum ELISA IgM	Thailand (n=150)	87.8%	81.8%	(379)
Serum – ELISA (various antigens)	Thailand (n=101)	74-82%	75-80%	(302)
Serum ELISA (immunoaffinity-purified antigen)	Thailand n=130	71.4%	86.2%	(381)
Serum ELISA (culture-filtrated antigen)	Thailand n=148	93%	97%	(382)
Serum DOT (culture-filtrate antigen)	Thailand n=101	72%	64%	(302)
Serum DOT (culture-filtrated antigen)	Thailand n=130	85.7%	85.3%	(381)
Serum DOT (culture-filtrated antigen)	Thailand n=148	94.1%	99.2%	(382)

Immunochromogenic card test – IgG	Australia (n=298)	88%	90%	(384)
Immunochromogenic card test – IgM	Australia (n=298)	77%	69%	(384)
Immunochromogenic card test – IgG	Thailand (n=299)	79%	90%	(383)
Immunochromogenic card test – IgM	Thailand (n=299)	67%	80%	(383)
Serum – Western blot	Thailand n=101	70%	91%	(302)
Serum (Various antibody targets)	Thailand n=101	23-59%	56-95%	(302)
Serum IFA (whole cell antigen)	India n=22	45-63%		(387)
Antigen detection				
Specimen - Immunofluoresence	Thailand n=272	73%	99%	(388),
Serum ELISA (19.5kD antigen)	Thailand n=147	82%	96%	(389)
Direct specimen - ELISA (MAb 5F8)	Thailand n=114	75%	98%	(390)
Urine IFA	Thailand	81%	96%	(391)
Molecular detection				
Blood PCR (LPS1, LPS2)	Thailand n=130	95.2%	91.7%	(381)
Blood PCR (16S rRNA)	Australia n=52	100%	67%	(392)
Blood PCR (various primers)	Thailand	31-41%	47-100%	(393)
Blood PCR (16S rRNA)	Thailand n=29	47%	100%	(394)
Blood PCR	Thailand n=7	100%	100%	(395)
Blood culture supernatant				
MAb-LA	Thailand n=1369	95.1%	99.7%	(396)
MAb-LA	Thailand	100%	86%	(397)
MAb-LA	Thailand	100%	96%	(397)
MAb-LA	Thailand (n=88)	100%	100%	(398)

1.5.1.1. CULTURE-BASED METHODS

Selective media have long been used for the isolation of *B. pseudomallei* from non-sterile fluids and environmental samples, utilizing the broad antibiotic resistance of the organism (399, 400). Ashdown tested his eponymous medium, with tryptase soy agar with glycerol, crystal violet, neutral red and gentamicin (4mg/L), in 8,000 clinical specimens in Townsville in 1979. *B. pseudomallei* was isolated in 8 specimens, with *Klebsiella* spp (n=73), *Pseudomonas aeruginosa* (n=57), *Enterococcus faecalis* (n=23), *B. cepacia* (n=17) and *Serratia marcescens* (n=14) the most common contaminants (401). A modified Ashdown's broth, with colistin, is now also used (402), particularly in cultures from non-sterile sites such as throat, rectal and wound swabs (403). The role of a recently described *B. pseudomallei* selective agar (BPSA) (404) and the more available *B. cepacia* selective agar are currently being evaluated in Thailand (Peacock S, personal communication).

Time to blood culture positivity, reflecting the density of bacteraemia, correlated with mortality in Thailand; 73.7% of patients died if blood cultures became positive with 24 hours, compared to 40.9% of those with a time to detection of >24 hours (405). In this study using the automatic BacT/Alert system, 62% of positive cultures were detected in the first 24 hours and more than 90% in within 48 hours (405).

Alternative blood culture methods could decrease the time to obtain a positive culture, but at the cost of reduced sensitivity. Compared with conventional broth-based blood culture (median time to positivity 61.8 hours; n=42), the Isolator lysis centrifugation had 81% sensitivity with time to positivity 39.3 hours, and the pour plate method had 61% sensitivity with median time to positivity 45.5 hours (406). Cultures of bone marrow have the same sensitivity as blood culture (407).

There are conflicting opinions as to the reliability of the API 20NE test panel; two studies have reported good results with this manual system (402) as with the API 20E system (408). However, another study found that 6 of 50 *B pseudomallei* strains at a West Australian laboratory were misidentified, mostly commonly as *Chromobacterium violaceum,* and a further 4 strains gave indeterminate results (409). The Vitek automated system is widely used; the Vitek 1 system, but not the

Vitek 2 system appeared to identify *B. pseudomallei* reliably (408). These findings have significant implications for laboratories in non-endemic areas where the organism is only occasionally encountered. Colonial morphology on Ashdown's medium and where available latex agglutination (396), and immunofluoresence (388, 410) are practical ways to identify *B. pseudomallei* in endemic areas (15).

1.5.1.2. ANTIGEN DETECTION

Although a variety of antigen detection methods have been studied, none are yet commercially available. Antigen tests have been developed for use on direct specimens or in blood culture supernatant; of these, latex agglutination for culture identification and direct immunofluoresence from direct specimens (such as sputum, urine or pus) have been used in research labs in Thailand.

Antigen tests for exotoxin and cell components have shown reasonable sensitivity and specificity in studies but most have not been field-tested. These include two enzyme-linked immunosorbent assay (ELISA) for exotoxin in culture supernatant (411) and a 40kD secreted protein (412, 413) and monoclonal antibodies for cell wall components including LPS (398) and a 30kD protein (397) and an exopolysaccharide (414). A fluorescent urinary antigen system has been developed; in initial trials, a sensitivity of 81% and specificity of 96% was defined (391), but a subsequent evaluation gave poorer results (302).

Although not commercially available, the only test finding widespread use in Thailand currently is a monoclonal latex agglutination test against the 200kD protein that was evaluated in 12 centres in Thailand. It was shown to agglutinate blood culture fluid positive for *B. pseudomallei*, including strains with atypical LPS patterns, with a sensitivity of 95%. The test was also highly specific and did not agglutinate ara^+ *B. thailandensis* strains with a specificity of 99.7% (396).

Immunofluoresence from direct specimen (including sputum, urine and pus) is the most promising way to reduce the time-to-diagnosis in endemic areas; a result can

be obtained within an hour, but only where specialized microscopy facilities are available. Reagents are based on antibodies to lipopolysaccharide and protein fractions of *B. pseudomallei* but are not currently commercially available (388, 415). An evaluation of this technique and potential improvements to even further simplify the methods are currently underway (Wuthiekanun V, personal communication). Immunofluoresence, as with latex agglutination, may also be useful in identifying *B. pseudomallei* from cultures (410) as well as distinguishing *B. pseudomallei* from *B. thailandensis* (416).

1.5.1.3. ANTIBODY DETECTION

Indirect haemagglutination (IHA) remains the most widely used test despite its poor sensitivity and specificity. It was first described in 1965 (417) and has been used extensively in serosurveys (418, 419). An older method had been the complement fixation test (378).

The use of the IHA is problematic in endemic areas, particularly Thailand where rates of background seropositivity may be up to 30-47% in various populations (420), presumably due to subclinical exposure to *B. thailandensis* or *B. pseudomallei* early in life (110, 421). Background seropositivity appears to be less common in Australia except in immigrants from south east Asia (16); the lower cut-off titre in Australia reflects this (1:40 compared to 1:160 in Thailand (415, 422)). Although IgM antibodies should be more specific (420, 423), field tests of IgM antibody detection have not reflected this promise (379).

In addition, the sensitivity of IHA is limited in patients with acute septic illnesses (380). Furthermore, there is some heterogeneity between strains in LPS, a major component in the crude antigen used in the IHA; antibodies against atypical LPS may not cross react against the IHA antigens, depending on which organisms are used to prepare the IHA assay reagent (424).

Efforts have been made to refine the antigen targets including refinement of a 30kD exotoxin and 19.5kD, 40kD and 200kD proteins. Enzyme linked immunosorbant assays (ELISA) based on LPS and 30kD and 200kD proteins has been validated in a

clinical context; IgG, but not IgM appears to be more sensitive (74-82%) and specific (75-80%) than the IHA, but still lack the performance necessary for clinical use (302). Other tests for detection of antibody have had similar results and none have performed sufficiently well to replace the IHA (381).

Longitudinal studies of antibody titre have revealed an unpredictable response, with IgG, IgM and IgA to a culture filtrate antigen responses falling, rising or persisting over periods of 1-6 years in seven patients (293). Similarly, IHA titres, reflecting total antibody levels, persisted over 2 years in 20 of 23 patients (423).

A rapid immunochromogenic test (PanBio Ltd, Australia) for IgM and IgG appeared to perform well in a series of 121 patients. However the high sensitivity (IgG 100%, IgM 93%) and specificity (both assays 95%) reported were compared against IHA, rather than culture, as a gold standard (425). Similar results were reported from the Northern Territory, demonstrating a good correlation with IHA (426).

Recent work in Thailand suggested that the IgG immunochromogenic test (PanBio Ltd, Queensland, Australia) may be moderately sensitive (79%) but more specific (90%) than IHA (383); this, and positive predictive value remains to be validated prospectively. However, a parallel study performed in Darwin demonstrated that IgG performed similarly to IHA, whereas IgM had poor specificity when compared to culture (384). Nevertheless, a commercial test kit using standardized antigens would be useful for non-endemic areas where the positive predictive value of a serological test is much higher.

1.5.1.4. MOLECULAR METHODS

Many tests based on molecular detection of *B. pseudomallei* have been described but few have been field-tested. Primers have been evaluated targeting regions in the 23S ribosomal RNA, 16S RNA and the 16S and 23S RNA junction (96, 302, 393-395, 427, 428). Use of primers to detect a region of the 16S RNA demonstrated a sensitivity of 100% but a low specificity in a small clinical study (392). More recent

studies are examining the role of a PCR for the type III secretion system (429) in clinical and environmental specimens (Gal D, unpublished data) as well as other 16S-specific primers (430).

16S sequencing has long been used for the identification of bacterial species; this method has been used for phylogeny (431, 432) as well as clinical identification of *Burkholderia* spp (142). Sequencing of the groEL gene may also be useful, but may not reliably differentiate *B. mallei* from *B. pseudomallei* (433, 434).

1.5.2. Antibiotic resistance

B. pseudomallei exhibits resistance to diverse groups of antibiotics, including cephalosporins (except ceftazidime), penicillins, rifamycins and aminoglycosides. In addition, its relative resistance to quinolones and macrolides limit therapeutic options for the treatment of melioidosis. The *in vitro* susceptibility of *B. pseudomallei* against antibiotics is summarized in Table 1-6

Table 1-6: In vitro activity of selected antibiotics against B. pseudomallei

Agent	Source Number of isolates	MIC_{50} (mg/L)	MIC_{90} (mg/L)	Range (mg/L)	Comments
Amikacin	UK collection (n=12)	NR	64	32-64	(435)
Amoxicillin	Australian clinical isolates (n=100)	>64	>64	>64	(436)
Amoxicillin-clavulanate	Australian clinical (N=170)	NR	NR	2/0.5 – 4/1	(437)
Amoxicillin-clavulanate	UK collection (Unknown number)	8	8	4-8	(438)
Amoxicillin-clavulanate	UK collection (n=12)	NR	8	4-8	(435)
Amoxicillin-clavulanate	Thailand; consecutive clinical isolates (n=100)	4	4	0.5-8	(439)
Amoxicillin-clavulanate	Thailand; selected resistant isolates (n=24)	256	>512	1->512	(439)
Amoxicillin-clavulanate	Australian clinical isolates (N=100)	2	4	2->64	(436)
Amoxycillin	Thailand, Hong Kong collection (N=27)	64	128	32-256	(440)
Amoxycillin-clavulanate	Thailand, Hong Kong collection (N=27)	4	8	4-8	A:C ratio 2:1 (440)
Ampicillin	Thai (Khon Kaen) clinical isolates (N=199)	32	32	0.25->256	(441)
Ampicillin	UK collection Unknown number	64	>64	32->64	(438)
Ampicillin-sulbactam	Thai (Khon Kaen) clinical isolates (N=199)	4	8	0.25-128	(441)
Azithromycin	UK collection Unknown number	64	>64	1->64	(438)

Azlocillin	Australian clinical isolates (N=100)	2	4	2-16	(436)
Aztreonam	Thai (Khon Kaen) clinical isolates (N=199)	16	32	8->256	(441)
Aztreonam	Clinical Thai isolates (N=97)	25	25	6.25-50	(442)
Aztreonam	Thailand, Hong Kong collection (N=27)	8	16	8-16	(440)
Aztreonam	Australian clinical isolates (N=100)	4	8	2-16	(436)
Biapenem	Thailand; consecutive clinical isolates (n=100)	0.25	0.25	0.06-1	(439)
Biapenem[a]	Thailand; selected resistant isolates (n=24)	0.5	2	0.12-2	(439)
Carbenicillin	Canadian – source unknown (n=20)	NR	100	50-200	(443)
Carumonam	Thai (Khon Kaen) clinical isolates (N=199)	2	4	0.5-256	(441)
Carumonam	UK collection (n=12)	NR	8	4-16	(435)
Cefamandole	Australian clinical isolates (N=100)	>64	>64	>64	(444)
Cefazolin	Australian clinical isolates (N=100)	>64	>64	>64	(444)
Cefepime	Clinical Thai isolates (N=97)	12.5	12.5	3.13-50	(442)
Cefixime	Thai (Khon Kaen) clinical isolates (N=199)	2	4	1-16	(441)
Cefoperazone	Thailand, Hong Kong collection (N=27)	8	16	8-16	(440)
Cefoperazone-sulbactam	Malaysia (N=50)	NR	4	NR	(445)
Cefotaxime	Thailand, Hong Kong collection (N=27)	2	8	2-8	(440)

Cefotaxime	Clinical Thai isolates (N=97)	3.13	3.13	0.78-12.5	(442)
Cefotaxime	Australian clinical isolates (N=100)	4	8	2-8	(444)
Cefotaxime	Australian clinical isolates (N=100)	4	8	2-8	(436)
Cefoxitin	Australian clinical isolates (N=100)	>64	>64	>64	(444)
Ceftazidime	Australian clinical (N=170)			1-3	(437)
Ceftazidime	Clinical Thai isolates (N=97)	0.78	1.56	0.39-3.13	(442)
Ceftazidime	UK collection Unknown number	2	>64	2->64	(438)
Ceftazidime	Malaysian collection	NR	2	NR	(120)
Ceftazidime	Thai (Khon Kaen) clinical isolates (N=199)	1	2	0.126-16	(441)
Ceftazidime	Thailand; consecutive clinical isolates (n=100)	1	2	0.25-32	(439)
Ceftazidime	Thailand; selected resistant isolates (n=24)	16	64	1-256	(439)
Ceftazidime	Thailand, Hong Kong collection (N=27)	1	1	1-2	(440)
Ceftazidime	Australian clinical isolates (N=100)	2	4	1-8	(436)
Ceftazidime[a]	Australian clinical (N=170)	NR	NR	1.0 4.0	(437)
Ceftriaxone	Thailand, Hong Kong collection (N=27)	2	4	2-4	(440)
Ceftriaxone	Australian clinical isolates (N=100)	4	8	2-8	(436)
Cefuroxime	Thailand, Hong Kong collection (N=27)	16	32	16-32	(440)

Cefuroxime	UK collection Unknown number	64	>64	32->64	(438)
Cefuroxime	Australian clinical isolates (N=100)	16	64	8-64	(444)
Cefuzonam	Clinical Thai isolates (N=97)	3.13	6.25	1.56-25	(442)
Cephalexin	Australian clinical isolates (N=100)	>64	>64	>64	(444)
Cephalothin	Australian clinical isolates (N=100)	>64	>64	>64	(444)
Chloramphenicol	Australian clinical (N=170)	NR	NR	8	(437)
Chloramphenicol	Clinical Thai isolates (N=97)	12.5	25	6.25->200	(442)
Chloramphenicol	UK collection Unknown number	8	16	4-16	(438)
Chloramphenicol	Malaysian collection	NR	32	NR	(120)
Chloramphenicol	Thailand, Hong Kong collection (N=27)	8	8	4-32	(440)
Chloramphenicol	United States NIH collection (N=51)	16	32	4-32	(446)
Chloramphenicol	(N=64)	6.3	6.3	1.6-12.5	(447)
Ciprofloxacin	Thailand, Hong Kong collection (N=27)	2	8	1-8	(440)
Ciprofloxacin	Thai (Khon Kaen) clinical isolates (N=199)	4	8	0.125-16	(441)
Ciprofloxacin	Clinical Thai isolates (N=97)	3.13	3.13	0.78-6.25	(442)
Ciprofloxacin	UK collection Unknown number	2	4	0.5-4.0	(438)

Ciprofloxacin	Australian clinical isolates (N=100)	2	8	0.5-16	(436)
Cycloserine	United States NIH collection (N=51)	32	42	8-64	(446)
Doxycycline	Australian clinical (N=170)	NR	NR	1-4	(437)
Doxycycline	UK collection Unknown number	0.5	1.0	0.5-1.0	(438)
Doxycycline	United States NIH collection (N=51)	4	8	1-16	(446)
Doxycycline	Lab strains – source not specified (N=56)	0.8	3.1	0.8-3.1	(447)
Enoxacin	Clinical Thai isolates (N=97)	6.25	6.25	3.13-25	(442)
Erythromycin	United States NIH collection (N=51)	128	128	16-128	(446)
Fosfomycin	Clinical Thai isolates (N=97)	>200	>200	>200	(442)
Gentamicin	UK collection Unknown number	32	64	0.125 - 64	(438)
Gentamicin	UK collection (n=12)	NR	64	16-64	(435)
Gentamicin	United States NIH collection (N=51)	8	64	0.5 - >128	(446)
Imipenem	Clinical Thai isolates (N=97)	0.39	0.78	0.2-1.56	(442)
Imipenem	Australian clinical (N=170)	NR	NR	0.5-1.5	(437)
Imipenem	UK collection Unknown number	0.5	1	0.5-1.0	(438)
Imipenem	Thai (Khon Kaen) clinical isolates (N=199)	0.5	0.5	0.06-4	(441)
Imipenem	Thailand; consecutive clinical isolates (n=100)	0.5	0.5	0.12-1	(439)

Imipenem[a]	Thailand; selected resistant isolates (n=24)	1	4	0.25-8	(439)
Kanamycin	United States NIH collection (N=51)	8	32	1-32	(446)
Lomefloxacin	Clinical Thai isolates (N=97)	6.25	6.25	3.13-25	(442)
Meropenem	Australian clinical (N=170)	NR	NR	0.5 – 1.5	(437)
Meropenem	Clinical Thai isolates (N=97)	0.78	0.78	0.39- - 3.13	(442)
Meropenem	Thailand; consecutive clinical isolates (n=100)	1	1	0.25-1	(439)
Meropenem[a]	Thailand; selected resistant isolates (n=24)	1	4	1-4	(439)
Minocycline	Clinical Thai isolates (N=97)	1.56	3.13	0.78-3.13	(442)
Moxalactam	Australian clinical isolates (N=100)	8	16	4-16	(444)
Nalidixic acid	Clinical Thai isolates (N=97)	25	50	3.13->200	(442)
Neomycin	United States NIH collection (N=51)	64	128	8 - >128	(446)
Netilmicin	UK collection (n=12)	NR	128	16-128	(435)
Norfloxacin	Clinical Thai isolates (N=97)	12.5	12.5	1.56-50	(442)
Norfloxacin	Australian clinical isolates (N=100)	4	8	1-32	(436)
Novobiocin	United States NIH collection (N=51)	8	16	4-32	(446)
Ofloxacin	Thailand, Hong Kong collection (N=27)	2	8	2-8	(440)

Ofloxacin	Clinical Thai isolates (N=97)	6.25	6.25	0.78-12.5	(442)
Ofloxacin	UK collection Unknown number	4	8	1-8	(438)
Panipenem	Thailand; consecutive clinical isolates (n=100)	0.5	1	0.06-2	(439)
Panipenem[a]	Thailand; selected resistant isolates (n=24)	1	16	0.5-16	(439)
Piperacillin	Thai (Khon Kaen) clinical isolates (N=199)	1	2	0.25-16	(441)
Piperacillin	Australian clinical (N=170)	NR	NR	0.5-2.0	(437)
Piperacillin	UK collection Unknown number	4	4	2-4	(438)
Piperacillin	UK collection (n=12)	NR	8	4-16	(435)
Piperacillin	Thailand, Hong Kong collection (N=27)	1	2	1-2	(440)
Piperacillin-tazobactam	Thai (Khon Kaen) clinical isolates (N=199)	1	1	0.25-8	(441)
Pipericillin	Clinical Thai isolates (N=97)	1.56	1.56	0.39-3.13	(442)
Pipericillin	Australian clinical isolates (N=100)	1	2	1-4	(436)
Rifampicin	Clinical Thai isolates (N=97)	25	25	3.13-25	(442)
Rifampicin	UK collection Unknown number	8	16	4-16	(438)
Rifampicin	Thailand, Hong Kong collection (N=27)	8	16	8-16	(440)
Rifampicin	United States NIH collection (N=51)	32	64	16-128	(446)
Sulphamethoxazole	UK collection Unknown number	16	>64	16->64	(438)

Sulphamethoxazole	Clinical Thai isolates (N=97)	50	50	12.5-100	Agar dilution (442)
Temafloxacin	Thai (Khon Kaen) clinical isolates (N=199)	8	16	0.25-32	(441)
Temafloxacin	Clinical Thai isolates (N=97)	3.13	6.25	0.78-12.5	(442)
Tetracycline	Clinical Thai isolates (N=97)	6.25	12.5	0.78-12.5	(442)
Tetracycline	Malaysian collection	NR	16	NR	(120)
Tetracycline	Thailand, Hong Kong collection (N=27)	4	8	4-16	(440)
Tetracycline	United States NIH collection (N=51)	4	8	1-16	(446)
Ticarcillin	Thai (Khon Kaen) clinical isolates (N=199)	>256	>256	64->256	(441)
Ticarcillin	Thailand, Hong Kong collection (N=27)	128	256	128-256	(440)
Ticarcillin	Australian clinical isolates (N=100)	>64	>64	>64	(436)
Ticarcillin-clavulanate	Thai (Khon Kaen) clinical isolates (N=199)	4	4	1->256	(441)
Ticarcillin-clavulanate	Thailand, Hong Kong collection (N=27)	16	16	8-16	T:C ratio 14.5:1 (440)
Ticarcillin-clavulanic acid	Australian clinical isolates (N=100)	16	16	4-32	(436)
Trimethoprim	Clinical Thai isolates (N=97)	25	25	1.56-25	(442)
Trimethoprim	UK collection Unknown number	16	>64	4 - >64	(438)
TMP/SMX	Clinical Thai isolates (N=97)	12.5	12.5	0.78-25	Agar dilution (442)
TMP/SMX	Malaysian collection	NR	>32	NR	(120)

TMP-SMX	Thailand, Hong Kong collection (N=27)	16	16	8-32	(440)
TMP/SMX	Australian clinical (N=170)			0.25/4.75 – 1/19	(437)
TMP/SMX	Australian clinical and collection; (N=80)	5/25	5/25	0.625/3.125 – 20/100	Agar dilution (448)
TMP/SMX	Australian clinical and collection; (N=80)	1.0/19	2/38	0.19/3.61 – 2.0/38	E-test (448)
TMP/SMX	UK collection Unknown number	16	>64	4 - >64	Microbroth dilution (438)
Tosufloxacin	Clinical Thai isolates (N=97)	1.56	3.13	0.39-6.25	(442)

[a] 24 isolates resistant to ceftazidime or amoxicillin-clavulanate; NR: not reported

Ceftazidime, the carbapenem antibiotics (imipenem and meropenem) and to a lesser degree amoxicillin-clavulanate remain the backbone of current initial treatment. Primary resistance to these agents was not observed in 170 isolates from the Darwin prospective study and ceftazidime resistance only emerged on therapy in one patient (437). Carbapenem antibiotics appear to be useful even for isolates exhibiting extended beta-lactamase activity (439).

Within the *B. pseudomallei* genome, seven genes encoding Ambler class A, B and D beta-lactamases have been identified (4). Functionally, the most important of these is the Bush-Jacoby-Medeiros class 2e beta-lactamase BPS-1, encoded by the gene *BlaA* (or *PenA*; Ambler class A) that hydrolyses most cephalosporins but is readily inhibited by clavulanate (449, 450). Acquired resistance to beta-lactam antibiotics while on treatment with a beta-lactam/beta-lactamase inhibitor combination or ceftazidime resulted from three distinct phenotypic changes; de-repression of the chromosomal enzyme, an insensitivity to inhibition by beta-lactamase inhibitors and a beta-lactamase specific for ceftazidime (451). These were associated with mutations in the *BlaA* gene (452). Overexpression of the class D beta lactamases, OXA-42 and OXA-43, may also be responsible for ceftazidime resistance in some isolates (453). Although a class C beta-lactamase was initially identified from the genome sequence (453), it is likely that this represents a class B metallo beta-lactamase (4); the clinical significance of this beta-lactamase is not yet known as resistance to carbapenems remains uncommon (439).

Antibiotics with borderline MIC against *B. pseudomallei* do not appear to be clinically useful in the intensive phase; these include the tetracyclines, chloramphenicol, the quinolones and ceftriaxone (11, 454, 455). The significance of other *in vitro* phenomena such as post-antibiotic effect (456) and time-kill studies (439) are not known but may confer carbapenems with a theoretical advantage.

The oral antibiotic TMP-SMX, with or without doxycycline and chloramphenicol, is used for the prolonged eradication phase. They have been demonstrated to be of little activity in the acute phase (11) and are bacteriostatic *in vitro* (442, 457, 458).

Primary resistance to chloramphenicol and doxycycline occurs infrequently (6% approximately) (351, 442).

Testing for TMP-SMX resistance is problematic, with disc diffusion methods probably over-estimating the extent of resistance. Methods that determine the minimum inhibitory concentration (E-tests, broth microdilution or agar dilution) are recommended and demonstrate much lower rates of primary resistance (3%-10%)(448). However, resistance rates appear to be higher in Thailand (459); the clinical significance of this is unknown.

Acquired resistance to doxycycline has been observed when doxycycline has been used as monotherapy (437), and much less frequently with TMP-SMX monotherapy (13, 437). Acquired resistance to ceftazidime while on therapy is an uncommon cause of treatment failure but acquired resistance more frequently occurs with chloramphenicol (458). Relapse attributable to resistance may occur with either oral or intravenous agents used in treatment (437, 460).

The use of combination therapy in the initial intensive phase is routine in the Northern Territory and parts of Thailand with the rationale of protection against the emergence of resistant strains during therapy and the improved intracellular penetration of TMP-SMX (13, 458, 461). However, *in vitro* studies suggest antagonism (462); clinical evidence for this is currently lacking.

B. pseudomallei is susceptible to kanamycin, although this antibiotic is no longer used in the treatment of clinical melioidosis. Transposon mutation analysis has revealed that the efflux system AmrAB-OprA (coded by the gene *amr*; aminoglycoside-macrolide resistance) confers resistance. Although efflux systems conferring macrolide resistance have been described, this system is a unique mechanism for high level aminoglycoside resistance (206, 463).

The use of *in vivo* models to test antibiotic combinations remains a research tool. Inglis *et al* used synergy testing in *Acanthamoeba* trophozoites to guide antibiotic therapy in a patient failing treatment (464). Although successful in this case, the

applicability of this remains uncertain and is unlikely to gain widespread acceptance. Similarly, the Balb/c inbred mouse model has also been employed to determine candidate interventions with similar caveats (465, 466).

More recent attention has focussed on the role of biofilms in protecting bacteria against antibiotics. In one such study, *B. pseudomallei* was incubated on a silastic surface for 16 hours. Electron microscopy demonstrated that cells in biofilm were still viable after 24 hours of exposure with up to 200 times the MIC of the planktonic cells (800μg/mL ceftazidime and 8000 μg/mL TMP/SXT) (178). Antibiotics combinations active against *B. pseudomallei* in biofilm were shown to be ciprofloxacin/clarithromycin, ciprofloxacin/azithromycin, and imipenem/ azithromycin; the clinical relevance of these findings is not known (467).

1.5.3. Antibiotic treatment

Six published and one unpublished randomized controlled trials have examined intensive phase interventions in severe melioidosis and are the basis of the ceftazidime-based regimens used currently (Table 1-7). For eradication phase therapy (also pessimistically known as “maintenance” therapy), three published and one unpublished trial have examined the role of oral antibiotics in preventing relapse (Table 1-8). Recommended intensive and eradication antibiotic regimens used in Thailand and Australia differ significantly; currently recommended regimens are detailed in Table 1-9 and Table 1-10 respectively. These interventions were the subject of a systematic review (468).

Table 1-7: Clinical trials of intensive phase intravenous antibiotics in severe melioidosis (adapted from (12))

Intervention		Number of patients		Outcome measures	
Regimen	Dose (mg/kg/d) and duration	Enrolled	Culture-confirmed	Treatment failure	Mortality
(11) • Ceftazidime vs • Chloramphenicol and doxycycline and TMP/SMX	120 100 4 10/50 All at least 7 days	161	34 31		37% 74%
(461) • Ceftazidime and TMP/SMX vs • Chloramphenicol and doxycycline and TMP/SMX	100 8/40 100 4 8/40 10-14 days	136	27 34		18.5% 47%
(352) • Ceftazidime vs • Amoxicillin-clavulanate	120 120/40 At least 7 days	379	106 105	39% 51%	47% 47%
(351) • Ceftazidime vs • Imipenem	120 50 At least 10 days	296	106 108	41% 20%	38% 36%
(469) • Ceftazidime and cotrimoxazole • Cefoperazone/sulbactam and cotrimoxazole	25 8/40 100 8/40	84	20 20		21% 16%

(470) • Ceftazidime and TMP/SMX vs • Cefoperazone-sulbactam	100 8/40 25/25 14 days	219	51 51		14% 18%
Chierakul *et al* (in press) • Ceftazidime vs • Ceftazidime and TMP/SMX	120 120 10/50 At least 10 days	449	118 123		No significant difference

Table 1-8: Clinical trials of eradication phase oral antibiotics in treatment of melioidosis (Adapted from (12))

Trial	Dose (mg/kg/day)	Duration	Number of patients	Relapse rate
(471) • Amoxicillin-clavulanate vs • Chloramphenicol and Doxycycline and TMP/SMX	 60/15mg/kg/d 40 mg/kg/d 4 mg/kg/d 10/50 mg/kg/d	 20 weeks 20	 49 52	 16% 4%
(472) • Doxycycline vs • Chloramphenicol and Doxycycline and TMP/SMX	 4 mg/kg/d 40 mg/kg/d 4 mg/kg/d 10/50 mg/kg/d	 20 20	 58 58	 26% 1%
(454) • Azithromycin and Ciprofloxacin vs • Doxycycline and TMP/SMX	 8 mg/kg/d 8 mg/kg/d 4 mg/kg/d 10/50 mg/kg/d	 12 20	 36 29	 22% 3%
Chaowagul *et al* (in press) • Doxycycline and TMP/SMX vs • Chloramphenicol and Doxycycline and TMP/SMX	 4 mg/kg/d 10/50 mg/kg/d 40 mg/kg/d 4 mg/kg/d 10/50 mg/kg/d	 12-20 12-20	 89 91	 No significant differences

Table 1-9: Intensive phase antibiotic regimens used for patients with normal renal function

	Royal Darwin Hospital (13)	Other recommended regimens (12)
Intensive phase	• TMP/SMX 8/40mg/kg (up to 320/1600mg) 12 hourly AND • (Ceftazidime 50mg/kg (up to 2g) intravenously, 6 hourly OR • Meropenem 25mg/kg (up to 1g) intravenously, 8 hourly) AND • Granulocyte colony stimulating factor (G-CSF; filgrastim) 300mcg IV for 10 days if patient has septic shock Duration of therapy at least 14 days, longer (4-8 weeks) for deep-seated infection, osteomyelitis or septic arthritis. Patients may be discharged for outpatient administration of ceftazidime if clinically stable	• Ceftazidime 40mg/kg intravenously 8 hourly [a] OR • Ceftazidime 19mg/kg intravenously bolus followed by 3.5mg/kg per hour continuous infusion OR • Imipenem 20mg/kg intravenously 8 hourly [b] OR • Ampicillin-clavulanate 20/4mg/kg intravenously every 4 hours[c] Duration of therapy at least 10 days or until clear clinical improvement

[a] Without TMP/SMX; first-line regimen at Sappasithiprasong Hospital, Ubon Ratchathani

[b] Second line regimen for treatment failure with ceftazidime at Sappasithiprasong Hospital

[c] Second-line regimen for empiric treatment of melioidosis at Sappasithiprasong Hospital

Table 1-10: Eradication phase antibiotic regimens used for patients with normal renal function

	Royal Darwin Hospital (13)	Other recommended regimens (12)
Eradication phase	• TMP/SMX 8/40mg/kg (up to 320/1600mg) 12 hourly Duration of therapy at least 3-6 months; close follow-up and monitoring of adherence important	• Chloramphenicol 10mg/kg orally qid for 4 weeks AND • Doxycycline 2mg/kg orally bd for at least 20 weeks AND • TMP/SMX 5/25mg/kg orally bd for at least 20 weeks[d] OR • Amoxicillin-clavulanate 30/15mg/kg orally tds for 20 weeks AND • Amoxicillin 30mg/kg orally tds for 20 weeks[e]

[d] First line regimen at Sappasithiprasong Hospital

[e] Second line regimen in adults and first line regimen for children (<14 years) and pregnant women at Sappasithiprasong Hospital

The only treatment to demonstrate a mortality benefit is ceftazidime: in a sequential open-label randomized trial of ceftazidime (120mg/kg/d) against chloramphenicol/ doxycycline/ TMP-SMX (known as "conventional" therapy) in severe disease. In 161 Thai adults, of whom 65 were culture-confirmed and 51 bacteraemic, the use of ceftazidime was associated with a 50% reduction in mortality, from 74% to 37%. Survival curves suggested that the benefit was seen after 48 hours, suggesting irreversible severe disease in those that died (11). There results were replicated in another Thai centre, Khon Kaen, where a fall in mortality from 47% to 19% was observed in association with ceftazidime with TMP-SMX in 136 patients, of which 61 had culture-confirmed melioidosis (461). In this study, the group that received conventional therapy had more severe disease that may have partially accounted for the mortality difference observed.

Ceftazidime-based regimens have been used as the control arm for studies of the intensive phase; studies since have failed to demonstrate improved outcomes with amoxicillin-clavulanate, cefoperazone-sulbactam or imipenem. An important issue in these studies is the wide variation in the baseline mortality rate; ranging from 14% to 47% in ceftazidime-based arms. This is likely to represent differences in the severity of illness due to differences in inclusion criteria. In addition, the timing of enrolment, reflecting in part the use of rapid diagnostics such as immunofluorescence, is likely to impact on observed mortality rates as a lower mortality is seen in patients who survive the first 24-48 hours of admission. The systematic review highlighted the need for a severity of illness scoring system (468).

Intravenous amoxicillin-clavulanate is not available in Australia, but is widely used in Thailand for the treatment of empiric sepsis and melioidosis. Pharmacokinetic considerations dictate that an increased frequency of dosing (four-hourly) to ensure adequate trough levels of clavulanic acid (473). A clinical trial of this agent in intensive therapy demonstrated a similar mortality, but a higher treatment failure rate requiring a change in antibiotic regimen (25% vs 5%) (352). In maintenance therapy, use of oral amoxicillin-clavulanate (with supplemental amoxicillin) was associated with a higher relapse rate than conventional TMP-SMX, doxycycline and chloramphenicol (16% vs 4%); however, only 50% of patients were adherent to the

full 20 week duration of therapy and this appeared to be the most important factor in determining relapse (471).

Imipenem-cilistatin was compared with ceftazidime in 214 patients; this trial was terminated prior to planned enrolment due to the withdrawal of pharmaceutical company support. Mortality was not different overall, even when adjusting for known prognostic factors. Use of imipenem was associated with lower rates of treatment failure in patients surviving >48 hours (20% vs 41%) than ceftazidime; however, this may have been a potentially subjective endpoint in this open-label trial (351). This may explain the high rate of treatment failure in the ceftazidime arm of this trial compared to the ceftazidime arm in the amoxicillin-clavulanate trial conducted at the same centre (352) despite similar definitions of treatment failure.

Current studies include a trial of ceftazidime alone compared with ceftazidime with TMP-SMX where no significant differences in mortality were demonstrated (Anunnatsiri S, Abstr 4th World Melioidosis Congress, Abstr 10, 2004). Future studies of meropenem against ceftazidime, particularly in light of the incomplete carbapenem study, will be of interest particularly in the treatment of severe sepsis where lower MIC, a more favourable time-kill profile and lower endotoxin release may be of a theoretical advantage. A retrospective review of meropenem use at the Royal Darwin Hospital is presented in chapter 4.

Although in treatment of uncomplicated melioidosis, it is usual for blood cultures to become negative within a few days, defevescence may take somewhat longer. In one trial, the median duration of fever following commencement of antibiotics was 9 days, with the interquartile range 4.5 to 15 days (range up to 39 days)(351) similar to that seen with another study (10-11 days) (470).

Although patients that survived had lower rates of bacteraemia (50-63%) than non-survivors, 10-18% of surviving patients remained bacteraemic after 4 days of treatment and 8-21% of surviving patients were bacteraemic after 7 days of imipenem or ceftazidime respectively (351). Patients with a fatal outcome often had cultures persistently positive until death (13). In addition, sputum cultures in uncomplicated pneumonia may take more than a week to become negative (13);

persistent cultures from sites other than blood do not necessarily portend a poorer prognosis (352).

A method to reduce the cost of antibiotics required for treatment using continuous infusions of ceftazidime was explored in a pharmacokinetic study. In this study a bolus dose of 12 mg/kg intravenously was followed by a constant infusion of 4 mg/kg/hr was found to provide an adequate dose while allowing for a total lower dose to be delivered than with traditional 8 hourly bolus dosing (474). These data support the use of elastomeric infusion devices (Baxter, Sydney, Australia) through a peripherally inserted central catheter (PICC) to complete intensive phase antibiotic therapy for melioidosis in Australia (13, 475). However, this technology is currently expensive, with infusers and antibiotics costing approximately US$100 per day excluding staffing costs (475).

The clinical utility of serum antibiotic levels by means of a bioassay was assessed in a Thai study (476). Pre or post-dose serum bactericidal and inhibitory titres did not appear to correlate with outcome in 195 adult patients, reflecting similar studies in other groups of patients with other infections (477).

Fewer studies have been performed examining eradication therapy. In Thailand, the four-drug regimen (chloramphenicol, doxycycline and TMP-SMX) is commonly used with a relapse rate of approximately 10% (471). Lower rates have been reported in Australia using TMP-SMX alone (13). Adherence to therapy (only 50% completing the 20 week course of therapy in the Thai study) is the most important factor predicting relapse; in addition, duration of therapy of 8 weeks was associated with high rates of relapse (471, 478).

Doxycycline alone for eradication therapy is associated with unacceptable rates of relapse (26%) and treatment failure (16%) compared with the conventional regimen of TMP-SMX, doxycyline and chloramphenicol. However, the latter is associated with high rate of adverse events (32% in this trial) that may limit adherence (472). Similarly, short course (8 week) ciprofloxacin-azithromycin and longer course (up to 20 weeks) quinolone monotherapy was also associated with high relapse rates of >20% (454, 479). A trial comparing the 4-drug regimen with TMP-SMX and

doxycycline has recently been completed in Thailand suggesting that TMP-SMX with doxycycline is associated with equivalent relapse rates as the 4-drug regimen (Chaowagul W, Abstr 4th World Melioidosis Congress, Abstr. 6, 2004). An analysis of this study suggested that the failure to complete at least 12 weeks of therapy remained the most important determinant of relapse, reinforcing the necessity for adherence to therapy and the need to define a better tolerated regimen. A future trial is planned comparing the role of TMP-SMX alone compared with TMP-SMX and doxycycline.

Ceftriaxone has borderline activity against *B. pseudomallei in vitro*, but whether it is active *in vivo* is debated. In a retrospective review the initial use of ceftriaxone and cefotaxime were associated with a higher mortality compared to patients treated empirically (455). However, no attempt was made to control for severity of infection or time to administration of antibiotics. The use of high dose ceftriaxone (2g IV daily) for the empiric treatment of community acquired sepsis is widespread in Australia and in Thailand.

A systematic Cochrane review published in 2001 did not identify any further unpublished clinical trials. It supported the use of a ceftazidime or imipenem containing intensive therapy phase followed by a long oral eradication phase. It found little data available on the appropriate therapy for mild disease and for eradication therapy. Criticisms of trials identified included the failure to conceal allocation, the failure to use an intent-to-treat analysis and poor standardization of markers of severity. (468).

Animal models of prophylactic antibiotic regimens have been prompted by the potential for intentional release, but may also be important when considering accidental laboratory exposure (376). In one study, prophylactic ciprofloxacin or doxycycline was demonstrated to raise the median lethal dose of *B. pseudomallei* when inoculated intraperitoneally. TMP-SMX would also be theoretically effective but its use has not been assessed.

Future issues yet to be addressed include the definition of the minimum duration and regimen required for eradication, whether oral therapy without an initial intensive intravenous phase can be used for mild disease, and the optimal regimens in children where relapse appears to be uncommon.

1.5.4. Management of sepsis syndrome

A detailed review of the management of sepsis is beyond the scope of this review and has been summarized in recent consensus guidelines (480). It should be noted that few of the interventions discussed are supported by evidence (Table 1-11) and none have included patients with melioidosis.

Table 1-11:Interventions demonstrated to reduce mortality in critically ill patients (supported by clinical trials evidence)

Intervention	Evidence
Goal directed therapy	Early goal directed therapy, based on fluid and blood product infusions, inotropes and ventilation with invasive monitoring was associated with a fall in mortality in a clinical trial (31% vs 47%) (481)
Insulin infusion	Intensive glycaemic control (4.4 to 6.1 mmol/L) by means of an insulin infusion was associated with a fall in mortality in surgical intensive care patients in a clinical trial (8% vs 5%) (482)
Sedation protocol	Use of sedation protocols associated with a reduced length of stay and duration of ventilation (483)
Polyclonal immunoglobulin	Use of polyclonal immunoglobulin associated with mortality benefit in a meta-analysis (RR 0.64) (484); concerns about the quality of the pooled studies
Activated protein C	Use of drotrecogin alfa in patients with severe sepsis was associated with a fall in mortality in a large clinical trial (31% vs 25%)

Limiting tidal volumes	Ventilation with lower tidal volumes (6mL/kg) in patients with acute lung injury/acute respiratory distress syndrome was associated with a fall in mortality (40% vs 31%)(485); other smaller studies show conflicting results (480).
Physiological dose steroids	Low-dose hydrocortisone and fludrocortisone in patients with relative adrenal insufficiency due to severe sepsis was associated with a fall in mortality in a clinical trial (63% vs 53%) (486)
Deep venous thrombosis prophylaxis	Multiple large studies of heparin and/or mechanical devices in general ICU populations (480).
Stress ulcer prophylaxis	Multiple large studies of proton pump inhibitors or histamine receptor antagonists (480).

Much recent interest has focused on the use of drotrecogin alfa (activated protein C) in severe sepsis (487) where a small but significant mortality benefit has been demonstrated. Although its high cost makes it of little relevance to the majority of patients with severe melioidosis worldwide, it has been used in a small number of patients at the Royal Darwin Hospital (Stephens DP, personal communication). Activated protein C levels have been demonstrated to be low in severe melioidosis (488); there is no evidence of any pathophysiological differences between patients with severe sepsis due to *B. pseudomallei* and other Gram negative organisms.

A negative trial that did include patients with melioidosis involved lexipafant, a platelet-activating factor receptor antagonist. In a randomized placebo-controlled trial in Thailand involving 131 patients, no significant difference in mortality was observed in patients with severe sepsis, including 36 patients with melioidosis (489).

Although exogenous administration of G-CSF has not been associated with improved outcomes in patients with pneumonia and/or severe sepsis in large clinical

trials (279-281), melioidosis may differ from infections due to other organisms due to the key role of neutrophils in patients with recognized risk factors for neutrophil defects. G-CSF may reverse these defects (259), act to counter inflammatory cytokines (490, 491) and increase intracellular concentrations of antibiotics (492-494). A review of G-CSF use, suggesting a large mortality benefit is detailed in chapter 3; further progress toward a clinical trial is a theme of this thesis.

Intensive glycaemic control has been associated with improved outcomes in surgical intensive care patients in Belgium (482). Given the prevalence of diabetes and acute hyperglycaemia in patients with melioidosis, and its putative role in inducing neutrophil dysfunction, a clinical trial examining the role of insulin infusions for tight glycaemic control would be of interest.

1.6. Conclusions

Melioidosis is a disease of public health importance in south east Asia and northern Australia that has the potential for epidemic spread to non-endemic areas. Sporadic case reports elsewhere in the world suggest that as-yet-unrecognized foci of infection may exist. Environmental determinants of this infection, apart from a close association with rainfall, are yet to be elucidated.

Identification of virulence factors has been accelerated by the completion of genome sequencing of a strain of *B. pseudomallei*, but these are yet to be translated into accurate, practical and commercially available diagnostic tests. The presence of specific risk factors such as diabetes suggest that functional neutrophil defects are important in the pathogenesis of melioidosis. Other studies have defined virulence factors that allow evasion of killing mechanisms by macrophages, and a possible role for cell-mediated immunity. Whether a vaccine could prevent infection or severe disease due to *B. pseudomallei* remains to be seen. Economic constraints may make vaccination an unrealistic option for many endemic regions.

Despite improvements in antibiotic therapy, melioidosis is still associated with a significant mortality attributable to severe sepsis and its complications. Studies exploring the role of preventative measures, earlier clinical identification and better management of severe sepsis are required to reduce the burden of this disease.

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2. Melioidosis in Northern Australia, 2001-02

2.1. Introduction

Various case series have documented the epidemiology of melioidosis in northern Australia in separate geographical locations, including the Top End [1] and the Torres Strait Islands in Queensland [2].

Melioidosis has only been a notifiable disease in the endemic areas of northern Australia recently, and a comprehensive review of the epidemiology has not previously been attempted. This report, using notification data, describes the epidemiology of melioidosis in the 2001-02 season in northern Australia.

2.2. Methods

We collated data from the following sources: the Menzies School of Health Research, Darwin, the Tropical Public Health Unit, Queensland Health, and the Department of Health and PathCentre, Perth. Rainfall data was obtained from the Bureau of Meteorology [3]. Population statistics, derived from the 2001 national census, were obtained from the Australia Bureau of Statistics [4]. Locations of towns were taken from the Gazetteer of Australia, 2001 [5]. We included cases within the northern region of Australia. The endemic region is generally regarded as the area north of 20°S. All cases were from within this region, except one north Queensland case from Mackay (21°10'S) where autochthonous cases had been seen in previous years.

Melioidosis is a notifiable disease in Queensland, Northern Territory and Western Australia. A case was included if cultures from any body site were positive for *B. pseudomallei* and the patient presented with a illness consistent with melioidosis during the period between 1 November, 2001 and 31 October, 2002. Location was taken from the patient's place of residence; for travellers, the place of presentation. Serological diagnoses were not included as previous work has suggested that positive serology is neither sensitive or specific in the diagnosis of melioidosis [6, 7].

The timing of the wet season varies in northern Australia; in Western Australia and Northern Territory, it is defined as the six month period between 1 November and 30 April, in north Queensland, from 1 December to 31 May.

2.3. Results

2.3.1. Epidemiology

In the 12 months to 31 October, 2002 there were 47 cases of melioidosis in the northern areas of Western Australia (1 case), Northern Territory (23 cases) and Queensland (23 cases). Epidemiological features of these cases are summarized in Table 1.

In addition, a number of patients were notified but excluded; this included three patients who had been notified previously and re-presented with relapsed disease. *B. pseudomallei* was also isolated from the sputum of a 14-year-old boy with cystic fibrosis; because he was otherwise asymptomatic, it was considered that the isolation indicated colonization, and the case was not included. One patient presented to a hospital in Perth (31°S) with an exposure history from an overseas endemic area. We are also aware of two cases elsewhere in southern Australia where culture-confirmed melioidosis was epidemiologically linked to travel to the Northern Territory.

Table 2-1: Cases of melioidosis by State/Territory; 1 November, 2001 to 31 October, 2002

	WA	**NT**	**QLD**	**Total**
Number of cases	1	23	23	47
Mortality	1	4 (17%)	7 (30%)	12 (26)
• Attributed to illness	1	3	6	10
• Other cause	0	1	1	2
Wet season cases*	1	18 (78%)	21 (95%)	40 (87%)
Median age (range) years	22	51 (3-79)	56 (30-87)	52 (3-87)
Male	1	15 (65%)	17 (74%)	33 (70%)
Total population	41,969	148,641	596,498	809,334

Rate (per 100,000/yr)	2.4	15.5	3.9	5.8
Indigenous	0	13 (57%)	12 (52%)	25 (53%)
Indigenous rate (per 100,000 yr)	0	42.0	22.3	25.5
Paediatric (<15 yrs)	0	1	0	1

** Wet season: Northern Territory/Western Australia: November-April, Queensland: December-May; date of onset not evident for one Queensland case*

The median age was 52 (range 3 to 87) years and 33 (70%) were male. There was one child (3 years of age) with melioidosis during this time. There were 25 infections involving Indigenous Australians. The rate of melioidosis was 5.8 per 100,000 overall and 25.5 per 100,000 in Indigenous Australians.

In the Northern Territory, most cases were seen around Darwin. Ten cases were from the Darwin urban region and four from the rural areas surrounding Darwin. One patient presented to Tennant Creek Hospital (19º39'S); although locally acquired cases have been seen there previously, this patient had recently travelled from further north in the endemic area. Six patients developed their illness in remote Aboriginal communities and were transferred to Royal Darwin Hospital for further management.

In Queensland, there were three main geographical foci of cases. Seven cases (30% of the Queensland total) were from the one Gulf community, six (26%) were from Townsville and adjacent suburbs, four (17%) were from the Torres Strait and the Northern Peninsula Area, and the remaining six (26%) were from other areas. Of note, only one case was acquired in Cairns.

2.3.2. Rainfall and incident cases

The majority (87%) of cases were seen during the wet season months (see Figure 1). During this time, rainfall across the Top End of the Northern Territory was between 150-600mm less than the 1961-1990 30-year median rainfall. On the western side of Cape York, rainfall was between 75-300mm greater than the median rainfall. The location of cases together with average annual rainfall is detailed in Figure 1.

In the Northern Territory, the number of cases was low compared to previous years; in the preceding 12 months there were 33 cases and in the 1997-8 season there were 48 cases. In addition, there were no cases in November for the first time since 1989; this was attributed to the lower than average rainfall. Most cases were in the Northern Territory wet season (n=18), with 12 in January and February coinciding with the relatively late onset of the monsoon rain. The monthly distribution of cases are detailed in figure 2.

Figure 2-1: Geographic distribution of cases of melioidosis and average annual rainfall in Australia

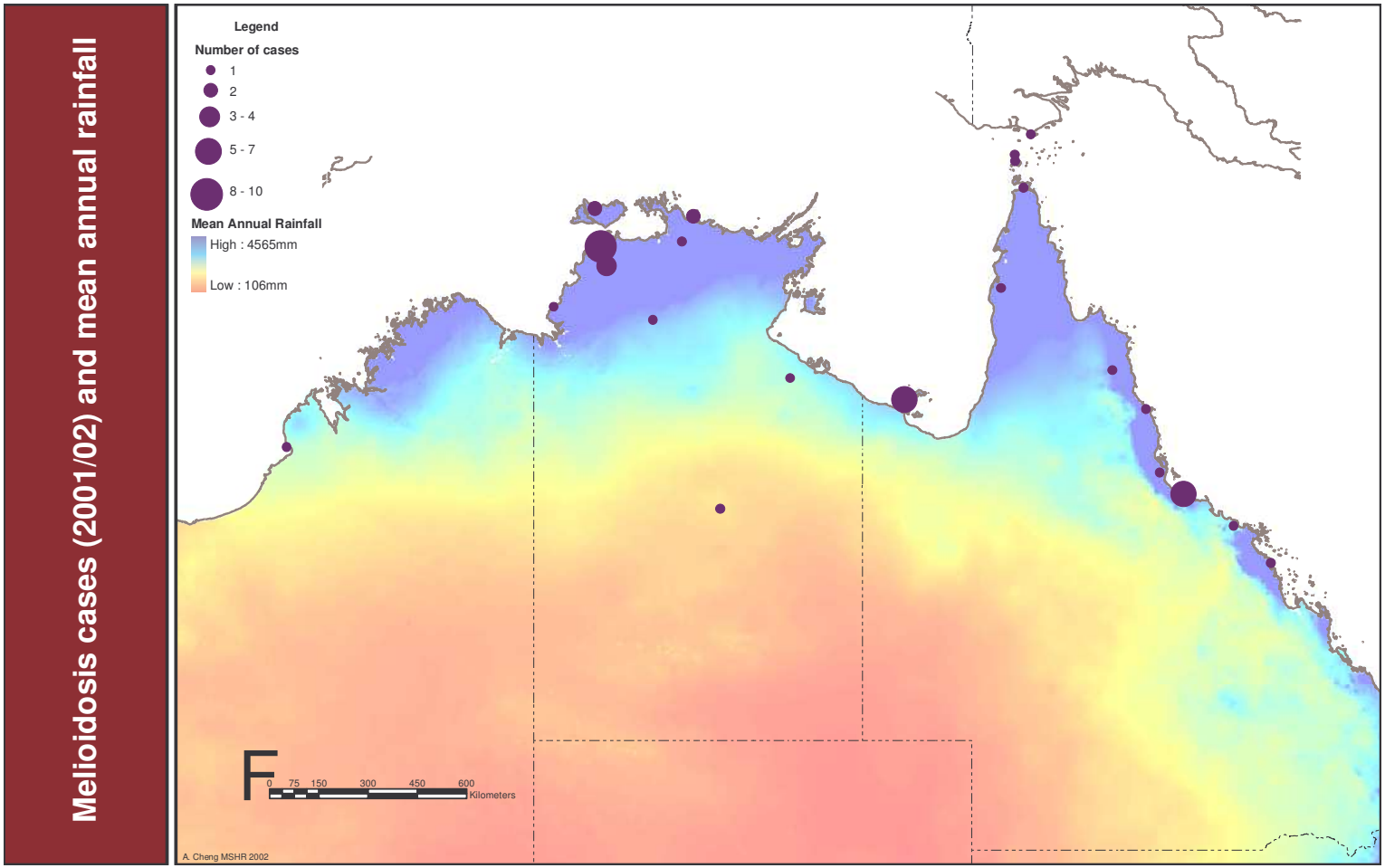
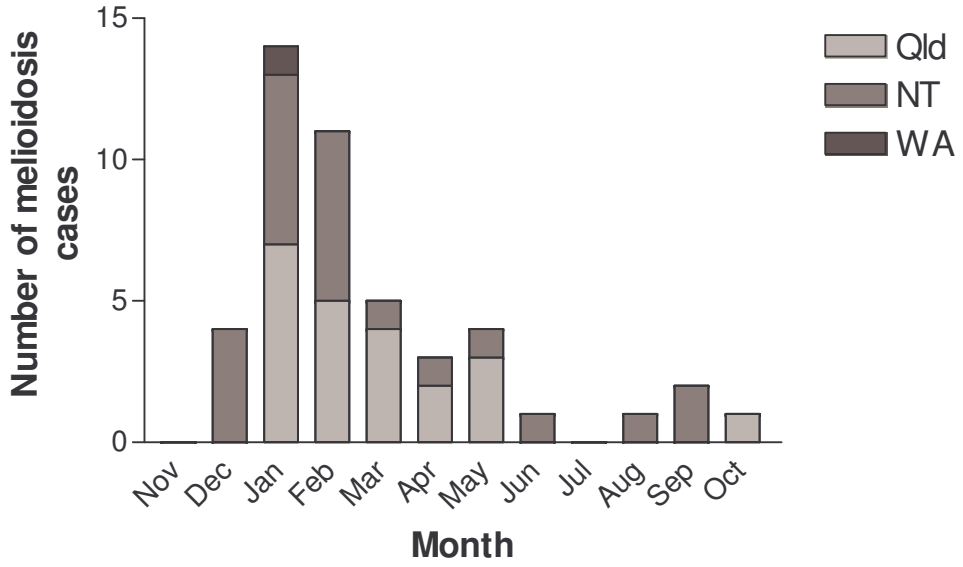


*Figure 2-2: Monthly number of cases in northern Australia (November 2001 – October 2002)**



*Date of onset not evident for one Queensland case

In Queensland, the seasonal peak started in January (see Figure 2). However, five of the seven cases in January, and seven (30%) of the total number of cases, were from the same community in the Gulf country north of Mt Isa. The first two cases at the community became unwell within 3 days of a tropical cyclone that passed over the community, leading to extensive flooding, in early January. Similarly, two of the three cases in Townsville in February occurred very soon after heavy rainfall in the city.

2.3.3. Clinical features

Risk factors for infection included diabetes (n=20; 43%), alcohol-related problems (n=14; 30%), renal disease (n=9: 19%), chronic obstructive airway disease (n=8, 17%), immunosuppression (n=4; 8.5%) and malignancy (n=2; 4.2%). Only seven patients (15%) did not have obvious medical risk factors; only two of these did not have a history of occupational or recreational exposure to mud/water.

Most cases had pneumonia (n=24; 51%) with other infections involving bone/joint (n=3; 6.3%), prostate (n=5; 11%), skin/soft tissue (n=3; 6.3%), gastrointestinal tract (n=1), spleen (n=1) and the central nervous system (n=1).

Overall, 12 patients (25%) died; 5 (41%) from the overwhelming acute infection. Two deaths were felt to be attributable to other causes (underlying end stage renal disease and malignancy). Two patients died prior to or on admission to hospital. The case fatality rate in Indigenous Australians was higher than in other patients, but this difference was not statistically significant (33% vs 17%, Fisher's exact: p=0.3).

Excluding two patients who died prior to or during admission in Queensland, and two patients with mild pneumonia who did not require admission, the remaining 43 patients spent a total of 1,182 days in hospital. The median duration of hospital stay was 18 days (range 1 to 114).

2.4. Discussion

Melioidosis is endemic in northern Australia. The average annual rate in the Top End of the Northern Territory is 16.5 per 100,000 with a rate of 34.5 per 100,000 in the 1997/98 season [1]. In the Torres Strait communities in northern Queensland between 1995 and 2000, the annual rate was 42.7 per 100,000 [2]. These rates are much higher than those documented in northeast Thailand (3.5-5.5 per 100,000) and Singapore (1.7 per 100,000) [8, 9].

Rainfall during the 2001-02 wet season varied from previous seasons, with later and lower than average precipitation in the Top End of the Northern Territory. This was reflected in the lower number of cases in the Northern Territory, with no cases seen in November for the first time since records commenced in 1989. However, the higher than average rainfall around Cape York was not associated with increased numbers of cases in this area possibly indicating the influence of as yet undefined factors other than rainfall *per se*. Ongoing studies are examining the role of other environmental factors, such as rainfall rate, soil type and physical properties of drinking and surface water, in the epidemiology of melioidosis.

As previously noted, Indigenous Australians are over-represented in the melioidosis cases [10]. In the defined area of northern Australia, it was estimated that 12.4% of the total 2001 population at risk were Aboriginal and Torres Strait Islander people, whereas 53% of the cases in this report occurred in Indigenous people. Although this may partly be related to exposure, risk factors such as diabetes and renal disease are also more common in this population. Other important risk factors in this and the wider population include high alcohol intake, and occupational and recreational exposures.

The prevalence of risk factors, namely diabetes, alcohol-related problems, chronic lung disease and chronic renal disease is similar to that described previously in Australia [1]. Similarly, the clinical features of this disease, with pneumonia present at presentation in half the cases, with smaller percentages of patients with skin and soft tissue infections, osteomyelitis and genitourinary infection reflect patterns noted previously [10]. The clinical pattern of disease varies from the Thai series, where many of the cases have no obvious clinical focus [11]. In addition, paediatric disease is much less common in Australia in comparison to Thailand [12].

The diversity of presentations with melioidosis is illustrated by a number of the cases during this year. A 51 year old man, presented to his local medical officer with impotence following a flu-like illness, and a prostatic abscess was subsequently diagnosed. A 3 year old child presented with ataxia and brainstem encephalitis following a culture-positive scalp boil. Two patients identified as having had previous mycobacterial infections, one with *M. leprae* and another with *M. terrae*; isolated case reports have noted this association that may reflect a common host susceptibility to these intracellular pathogens [13-15]. Additionally, the presentation of non-acute melioidosis may mimic that of tuberculosis. A number of patients in previous years had been treated for presumed tuberculosis, but subsequent cultures were negative for *M. tuberculosis* and positive for *B. pseudomallei* (unpublished data).

The only culture-confirmed case of melioidosis presenting in the endemic region of Western Australia during the 12 month period was a tourist, who presented following recent travel from the Northern Territory. Despite appropriate antibiotics and intensive supportive therapy he had a rapidly fatal septic course. The occurrence

of melioidosis in this and other travellers, although uncommon, reinforces the need for clinicians throughout Australia to be mindful of this disease in patients that have been in endemic areas.

The mortality from melioidosis in the Northern Territory has halved over the past decade. Historically, most deaths have been attributable to the complications of severe sepsis due to overwhelming infection [10]. With improvements in recognition of melioidosis, the earlier commencement of therapy, and improved intensive care management of patients with severe sepsis, an increasing proportion of deaths are attributable to causes other than the sepsis syndrome, such as the complications of the prolonged treatment course and underlying disease.

There is considerable economic cost associated with melioidosis. Hospital admission, including the need for intensive care, is likely to represent only a fraction of the cost associated with this disease. Treatment of melioidosis often requires outpatient administration of expensive antibiotics and extensive follow-up, and may involve patients in remote settings.

Further studies aimed at determining the environmental factors important in the development of melioidosis are detailed later in this thesis. Efforts are also continuing to improve the awareness of melioidosis in communities to reduce exposure to this organism in high-risk individuals during the wet season.

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3. Adjunctive granulocyte colony stimulating factor is associated with improved survival in septic shock due to melioidosis

3.1. Introduction

Melioidosis is associated with a significant mortality both in Australia and Thailand. The majority of this mortality is attributable to fulminant sepsis and its complications [1]; at the Royal Darwin Hospital it is the commonest cause of septic shock [2]. Despite improvements in antibiotic regimens, no interventions have been demonstrated to improve early mortality within the first 48 hours of admission [3].

Granulocyte colony stimulating factor (G-CSF) is a naturally occurring cytokine that primarily increases neutrophil production. It has demonstrable effects on neutrophil function, including chemotaxis, superoxide production and intracellular killing [4]. Other studies have demonstrated effects on anti-inflammatory cytokines [5] and intracellular concentrations of antibiotics [6]. Although subsequent studies have been negative [7, 8], in 1998 a clinical trial was published that suggested that a subgroup of patients with severe pneumonia may benefit from the administration of recombinant human G-CSF (filgrastim) [9]. The animal and human literature about the use of G-CSF in sepsis were examined and discussed by the intensivist and infectious diseases specialists and it was decided that G-CSF would be added to the treatment of septic shock specifically in an attempt to reduce the almost universal mortality (95%) associated with septic shock due to melioidosis.

The experience with the use of G-CSF in septic shock generally has previously been reported [2]. In that study, six patients in the G-CSF group but no patients in the control group had melioidosis. Thus, conclusions could not be made regarding the use of G-CSF in melioidosis due to small numbers accrued at that stage. Thus, with the benefit of further experience, the use of G-CSF in the treatment of melioidosis was audited and possible confounders for the reduction in mortality observed was explored.

The empiric management of community acquired sepsis in the Top End of the Northern Territory (where melioidosis is endemic) includes the use of ceftriaxone 2g IV prior to transfer to hospital based on its partial activity against *B. pseudomallei in vitro* [10] and its long half life [11]. In hospital, empiric ward management is with ceftriaxone for patients not suspected of having melioidosis, or ceftazidime if melioidosis is suspected based on risk factors and exposure. In January 1998, meropenem was introduced as empiric management of community-acquired sepsis in the wet season in patients admitted to intensive care. All patients admitted to ICU with sepsis in the wet season receive meropenem as initial therapy until cultures exclude melioidosis.

3.2. Methods

The Royal Darwin Hospital is the referral center for all patients in the Top End extending across to north Western Australia, an area of 516,945 km^2 with approximately 150,000 inhabitants. In this area, there are only two urban centers with populations over 5000; Darwin (~90,000) and Katherine (~8000).

G-CSF was adopted by a consensus decision of the intensive care and infectious diseases specialists at Royal Darwin Hospital in November 1998 in an attempt to reduce the almost universal mortality from septic shock due to melioidosis seen prior to that time. Recombinant human G-CSF (filgrastim, Neupogen, Amgen) was administered to all patients admitted to the intensive care unit (ICU) with septic shock, including those with melioidosis, usually within an hour of meeting the criteria for septic shock [12]. There was no delay in administration, as the microbiological diagnosis was not required in order to meet the criteria for treatment with G-CSF. G-CSF was administered at a dose of 300mcg IV daily and was continued for 10 days, or longer if the patient continued to meet the definition of septic shock. The course was terminated earlier if the patient was discharged from intensive care or if total neutrophil count exceeded 75,000 cells/ml.

Standard treatment for septic shock in intensive care at the Royal Darwin Hospital includes aggressive fluid management, vasopressor support with noradrenaline, early continuous venovenous haemofiltration for acute renal failure or severe

acidosis and early intervention with mechanical ventilation as required.

An intensive care specialist was first appointed in the Northern Territory in March 1998. The ICU was administered prior to that time by specialist anaesthesists in conjunction with internists. The appointment of an intensivist resulted in many changes to management protocols. Changes introduced in 1998 included the introduction of a closed intensive care unit model, the use of early and aggressive enteral feeding, the use of protective ventilation strategies [13] and the more aggressive use of haemodynamic monitoring. Other protocols have been introduced between 1998 and 2002 including a sedation protocol [14], a protocol for the use of physiological steroids in septic shock [15] and an infection control protocol that has resulted in a fall in nosocomial infection rates.

A prospective database has stored clinical details of patients with melioidosis since 1989. We included patients that had been admitted to the intensive care unit between August 1989 and September 2002 with culture-confirmed melioidosis that met the definition of septic shock. Clinical details of each case were abstracted onto standardized data forms. The use of G-CSF and possible confounding factors were analyzed in two time periods; patients given G-CSF (subsequent to December 1998; G-CSF group) and those not given G-CSF (prior to December, 1998; historical control group). We defined mortality as a death occurring during the hospital admission. White cell counts were assessed on the day of ICU admission, with the subsequent highest count in the following 14 days also noted.

In order to estimate the cumulative effect of the appointment of an intensivist and the subsequent change in ICU patient management, we examined ICU mortality before and after March 1998 excluding the patient population under examination. For this analysis, we examined all admissions to the ICU during the period March 1, 1992 to July 17, 2001. As G-CSF was used in the treatment of patients with septic shock other than melioidosis and has previously been reviewed [2], we considered the mortality of patients that did not have the diagnosis of sepsis, pneumonia or melioidosis - the conditions most commonly associated with septic shock.

Ethical approval for this review was obtained from the Human Research Ethical

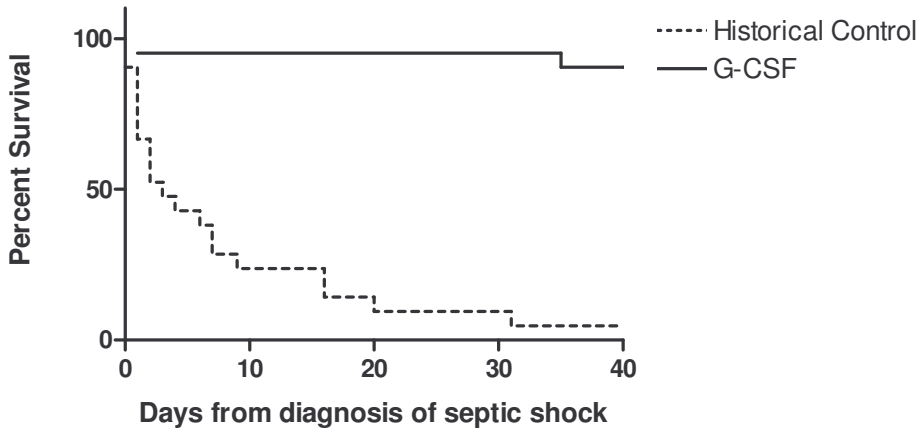
Committee of the Department of Human Services and the Menzies School of Health Research. Statistical analysis was conducted using Intercooled Stata for Windows 7.0 (Stata Corp, College Station, TX), using Fisher's exact test and the Mann Whitney U test for comparison of non-parametric data, except where indicated in the text. Statistical significance was deemed to be present if $p<0.05$.

3.3. Results

During the period August 1989 to September 2002, 341 patients were admitted with culture-confirmed melioidosis, of which 42 patients had septic shock requiring admission to intensive care. Higher numbers of patients with septic shock were seen in 1998-9 (n=9) and 2000-1 (n=13) mostly attributed to heavy monsoonal rainfall.

During the period December 1998 – September 2002, 21 patients were administered G-CSF for septic shock due to *B. pseudomallei* (G-CSF group) with two deaths (mortality 9.5%). In contrast, 21 patients were admitted to the Intensive Care Unit between August 1989 and November 1998 with septic shock due to *B. pseudomallei* (historical control), with a single survivor (mortality 95.2%) (Fisher's exact; $p<0.001$). The timing of deaths is illustrated in figure 1 (logrank test; HR=18.3, $p<0.0001$). A summary of results is provided in Table 3-1.

Figure 3-1: Kaplan Meier survival curve from day of admission for G-CSF and historical control groups



Patients had a similar median age (G-CSF group 49 years; historical control 50 years; not statistically significant (NS)) with lower proportions of females (24% vs 43%) and of Aboriginal ethnicity (62% vs 81%; NS) in the G-CSF group, although two patients in the G-CSF group were indigenous New Zealanders. There were no significant differences in the median duration of illness prior to presentation (G-CSF group 3 days; historical control 4 days; NS). Similar proportions of patients were from the urban centers of Darwin or Katherine compared to remote communities (G-CSF group 42%, historical control 38%; NS).

Table 3-1: Summary of results comparing G-CSF and historical control groups

	G-CSF group	Historical control	Significance
Number of patients	21	21	
Mortality	2 (9.5%)	20 (95.2%)	P<0.001
Australian Aboriginal	13 (62%)	17 (81%)	NS
Age (median, range; years)	49 (28-64)	50 (11-74)	NS
Male	16 (76%)	12 (57%)	NS
Duration of illness prior to presentation (median, range; days)	3 (1, 8)	4 (1, 90)	NS
Patient from urban setting	9 (42%)	8 (38%)	NS
Pneumonia	19 (90%)	21 (100%)	NS
Other site of infection	10 (48%)	6 (28%)	NS
Diabetic	13 (57%)	12 (62%)	NS
APACHE 2 score (mean, range)	24.8 (13, 33)	25.6 (13, 44)	NS
Use of carbapenem	19 (90%)	6 (28%)	P<0.001
Time to receipt of ceftazidime or carbapenem (median, range; days) [a]	0 (-15,0)	0 (-2, 5)	P=0.007

Use of ceftriaxone as initial therapy	8 (38%)	18 (86%)	P=0.004
Time to receipt of ceftriaxone, ceftazidime or cerbepenem (median, range; days) [a]	0 (-15,0)	0 (-5, 2)	NS
White cell count on admission to ICU (Median, range; cells/µl)	12400 (4900-40200)	9100 (2000 – 36900)	NS
Peak white cell count (Median, range)	38500 (16300-90900)	Not analyzed	

[a] relative to day of septic shock (negative indicates antibiotic received prior to diagnosis of septic shock); 4 patients in historical control group did not receive ceftazidime or carbapenem.

3.3.1. Site and severity of illness

Pneumonia was the most common site of infection and was present in all patients except two in the G-CSF group. Ten patients (48%) in the G-CSF group and six patients (28%) in the historical control group had infection of other sites (NS). Risk factors were similar in each group with 12 patients (57%) in the historical control group and 13 (62%) in the G-CSF group having diabetes (NS). The mean APACHE 2 score was 24.8 in the G-CSF group and 25.6 in the historical control group (NS).

3.3.2. Management

There was increasing use of carbapenems (imipenem or meropenem) in the G-CSF group (90% of the G-CSF group vs 29% of the historical control group; $p<0.001$). A higher proportion of the historical control group had received ceftriaxone as initial therapy (86% vs 38%; $p<0.01$). Three patients in the historical control group did not receive either ceftazidime or a carbapenem as they died within 48 hours and prior to a diagnosis of melioidosis; all three patients received ceftriaxone. All patients in the G-CSF group and 15 patients in the historical control group received ceftazidime or a carbapenem prior to or within 24 hours of the diagnosis of septic shock.

3.3.3. Safety

Of the 21 patients in the G-CSF group, three (14%) had white cell counts exceeding 75,000 cells/mL. Three patients in the G-CSF group had ECG and/or biochemical evidence of myocardial damage; white cell counts at this time were 10,400, 18,000 and 58,000 cells/mL respectively; none of these cardiac events were fatal and one had evidence of myocardial damage on presentation to hospital. One patient in the historical control group had a myocardial infarction.

White cell counts were similar in both groups on admission (G-CSF group: median 12400 cells/ml, range 4900-40200 cells/ml; historical control: median 9100 cells/ml, range 2000-36900 cells/ml; NS) It was not possible to determine peak white cell counts in the historical control group due to their short survival; patients in the G-CSF group had a variable rise in white cells (median peak count 38500 cells/ml, range 16300-90900 cells/ml) after a median of 6 days (range 1-11 days).

3.3.4. Analysis of all ICU admissions and mortality

During the period March 1, 1992 and August 14, 2001, data were available for 3147 patients admitted to intensive care, of which 2647 did not have melioidosis, sepsis or pneumonia. Significant diagnoses (>2%), apart from sepsis, melioidosis and pneumonia, were multiple trauma (9.3%) and head trauma (6.8%), chronic obstructive airways disease and asthma (5.5%), intracranial haemorrhage (5.4%), cardiac failure and cardiogenic shock (5.2%), neurological diseases (4.6%), cardiac arrest (4.2%), gastrointestinal perforation and obstruction (3.1%), drug overdose (2.7%) and seizures (2.3%).

In the time prior to March 1, 1998 (n=1669), the mean APACHE 2 score was 16.6 and observed mortality 23.7%. In the period following March 1, 1998 (n=978), the mean APACHE 2 score was 17.8 and the observed mortality 21.3%. In a Poisson regression, the APACHE 2-adjusted mortality ratio was 0.77 when comparing the period after March 1, 1998 to the period prior to that date ($p<0.002$).

3.4. Discussion

The rationale for introducing G-CSF for the treatment of septic shock due to melioidosis was based on the following data available at the time:

1. *Burkholderia pseudomallei* has been shown to survive and multiply within cells, including neutrophils [16];
2. Co-morbid conditions associated with the mortality from and development of melioidosis [1], including diabetes, chronic renal failure and hazardous alcohol use, are also associated with functional neutrophil defects [17-19];
3. G-CSF has been shown to improve outcomes from sepsis in animal models and improve neutrophil function *in vitro* [19, 20];
4. Evidence available at that time suggested that G-CSF may be of benefit in patients with multilobar pneumonia, although no benefit was seen overall [9]. In addition, patients had improvements in diabetic foot ulcers associated with increased neutrophil function [21].
5. G-CSF is generally well-tolerated with an extensive history of use in the treatment of neutropenia [4].

The subsequent experience, with an associated reduction in mortality from 95% to 10% due to this condition has been in sharp contrast to the large published studies of G-CSF in non-neutropenic infection. In recent studies, no benefit was attributed to the use of G-CSF in patients with community-acquired multilobar pneumonia [7] or severe pneumonia and severe sepsis [8, 22]. In the final trial, investigators suggested that delays in administering G-CSF might have contributed to its negative result. The ICU protocol in this study dictated that G-CSF was to be administered very shortly after admission to ICU and the diagnosis of septic shock.

In performing this retrospective review of the experience with G-CSF, several potential confounders were considered but it was felt that each would be unlikely to result in such a large reduction in mortality.

Could the appointment of an intensivist reduce mortality to this extent? Clearly, the appointment of an intensivist has been associated with modest improvement in mortality at this institution, with a fall in mortality in both septic shock (where mortality is confounded by routine G-CSF use) [2] and in critically ill patients with other diagnoses. Although other studies examining the effect of intensivists on mortality are likely to suffer from a significant publication bias, the magnitude of the effect at RDH (36% reduction in mortality adjusted for severity of illness), is in line

with published studies [23-26]. Thus, it is unlikely that this factor is entirely responsible for the 90% reduction in mortality in the melioidosis and septic shock subgroup.

Could earlier diagnosis and earlier administration of antibiotics result in this effect? Ceftriaxone was used more often as initial therapy in the historical control group and the G-CSF group received ceftazidime or a carbapenem antibiotic earlier. A retrospective review in Thailand suggested poorer outcomes in patients treated initially with ceftriaxone or cefotaxime compared to ceftazidime or carbapenems [27]. However, this study did not control for time to receipt of antibiotics or severity of illness, and patients treated with a third generation cephalosporin were more likely to be bacteraemic (78% vs 62%). Patients treated empirically with ceftazidime or a carbapenem had a lower mortality compared with those treated with a third generation cephalosporin and changed to ceftazidime or a carbapenem (42% vs 61%). This difference, a relative rate reduction of 31%, is unlikely to fully account for the difference in mortality seen in this series; however it is acknowledged as potentially represents a significant confounding factor. In the absence of data from clinical trials, the use of ceftriaxone (2 grams IV, higher than the conventional dose used in Thailand, 20mg/kg IV) is advocated in the empirical management of adult community acquired sepsis in the Top End region. Ceftriaxone has *in vitro* activity against *B. pseudomallei* as well as other common bacteria causing community acquired sepsis, is available in remote settings and has a long half-life, important when considering delays in medical evacuation from remote settings [10, 11].

No significant differences were found in the geographical location of patients between the two groups, as delays in treatment due to transport may have impacted on the course of the illness. In support of this, there were no significant differences in the severity of illness on admission to intensive care, as such delays prior to admission would be expected to result in more severely unwell patients.

Could other changes in management account for this effect? Although meropenem is yet to be tested in a clinical trial, another carbapenem antibiotic, imipenem, was tested in Thailand [28]. Although this trial was underpowered due to the withdrawal of funding, no difference in mortality was seen between groups after enrolment of

214 patients with culture-confirmed melioidosis and an overall mortality of 36.9%. This suggests that if such a difference exists, it would likely be small. Of the other changes introduced into management protocols around this time, only protective ventilation strategies [13] and the use of aggressive monitoring with early goal directed resuscitation [29] have been demonstrated to have an impact on mortality.

Could changes in admission criteria for intensive care select for patients more likely to survive? There have not been any changes to ICU admission policies with respect to patient selection over this time. G-CSF and historical control groups had a similar severity of illness measured by APACHE 2 scores. Every patient in this study with melioidosis had the presence of co-morbidities recognized as risk factors for melioidosis.

Are other clinical features and in vivo models consistent with a beneficial effect? Such features may have included a quicker resolution of fever and shorter duration of blood culture positivity; however, such comparisons would not be meaningful due to the short survival of patients prior to the use of G-CSF. A group has recently studied G-CSF as an adjunct to ceftazidime in a Balb/c mouse model of acute melioidosis [30]. In these studies, there was no benefit associated with the use of G-CSF in addition to ceftazidime. However, given that previous animal work using other pathogens has failed to translate to humans in clinical trials, such animal models may not be indicative of benefit associated with this therapy in humans.

A previous concern has been that G-CSF may increase the incidence and severity of sepsis-induced acute respiratory distress syndrome [31]. It was found that ARDS could not be readily distinguished from pneumonia in this retrospective study, but in the four clinical trials of G-CSF in pneumonia [7-9, 22] there was no significant increase in the incidence of ARDS, organ dysfunction or serious adverse events in all studies.

Within the limitations of the study design, we have observed a fall in mortality from 95% to 10%, associated with the use of G-CSF. Although we cannot exclude the possibility that this may be due to a convergence of confounding factors, including the presence of an intensivist and the earlier use of effective antibiotics, the fall in

mortality is in excess of that which might be ascribed to these factors. It is hypothesized that the prompt use of G-CSF in this patient group with co-morbid conditions associated with neutrophil dysfunction may have contributed, at least in part, to the reduction in mortality associated with the intracellular pathogen *B. pseudomallei*. These results deserve further scrutiny; the progress toward a placebo-controlled trial in conjunction with colleagues in Thailand is discussed in this thesis.

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4. Outcomes of patients with melioidosis treated with meropenem

4.1. Introduction

Despite excellent *in vitro* activity, there is little published clinical data regarding the use of meropenem in the treatment of melioidosis. A clinical trial compared the use of the related carbapenem, imipenem, to ceftazidime in patients with confirmed severe melioidosis in Thailand in 214 patients [1]. However, despite a reduction in the treatment failure in the imipenem arm, a possibly subjective endpoint in this open-label trial, there were no significant differences in mortality. However, this trial was underpowered due to the withdrawal of pharmaceutical support.

Carbapenem antibiotics have some theoretical benefits over ceftazidime, in that they are more active *in vitro* [2-5], demonstrate a post-antibiotic effect [6] and are associated with decreased endotoxin release [7]. Thus, carbapenem antibiotics may be of benefit in patients with critical illness with melioidosis, a group with a historically high mortality. Furthermore, their broad spectrum is attractive for empiric coverage of common causes of community-acquired sepsis at the Royal Darwin Hospital.

Based on these considerations, meropenem has been used for the treatment of selected patients with melioidosis, including critically ill patients with severe sepsis, since 1997. The association between the use of granulocyte colony stimulating factor (G-CSF) and a dramatic fall in mortality from septic shock due to melioidosis was discussed in the previous chapter. However, it was acknowledged that the use of meropenem may have confounded this analysis; in this series, the experience with meropenem is reviewed, comparing outcomes with contemporaneous patients treated with ceftazidime.

4.2. Methods

The Royal Darwin Hospital is a 300-bed referral centre based in Darwin, Australia and receives patients from the Top End of the Northern Territory, an area endemic for melioidosis. Since 1989, we have prospectively documented clinical details on all cases of melioidosis seen in the Top End [8].

Meropenem has been used in selected cases of melioidosis at the Royal Darwin Hospital since August, 1997. We advocate the use of meropenem 25mg/kg (up to 1g) 8 hourly with trimethoprim/sulfamethoxazole (8/40mg/kg or up to 320/1600mg 12 hourly) with a total duration of intensive intravenous therapy of at least 14 days, followed by an extended course of oral eradication therapy. For patients with impaired renal function (except for patients on haemofiltration [9]), the dose of meropenem is adjusted by altering both dose and interval according to established guidelines [10].
For this study, clinical details were reviewed for patients treated for melioidosis since August 1997. For this study we defined two groups; the meropenem group comprised patients that received meropenem as part of their therapy for melioidosis (including patients switched from ceftazidime). The ceftazidime-only group received ceftazidime as treatment and excluded patients that received carbapenems during their therapy course.

Indications for the use of meropenem include patients with critical illness (including severe sepsis) admitted to intensive care for management, clinical failure or intolerance to ceftazidime and relapse following previous therapy with ceftazidime. Other patients received ceftazidime plus trimethoprim/sulphamethoxazole as initial therapy. Details of possible adverse events were specifically sought, treatment failure requiring a change in therapy and disease relapse.

At the Royal Darwin Hospital, patients were referred to the intensive care unit for management of severe sepsis, respiratory failure due to poor gas exchange or poor conscious state or renal replacement therapy for acute and/or chronic renal failure. Standard management of patients with severe sepsis, defined by standard criteria [11], including melioidosis included the routine use of G-CSF (since 1998) [12], early goal directed resuscitation strategies similar to those previously published [13], early enteral feeding, the use of sedation protocols [14](all since 1998) and physiological dose steroids [15](since 2001).

Statistical tests were performed using Intercooled Stata 7.0 (College Station, Texas, United States). For comparisons of proportions, Fisher's exact test was used. For

comparisons of non-parametric distributions, the Mann-Whitney U test was used. Statistical differences were deemed significant at the 0.05 level.

Approval to review data for this study was given by the Human Research Ethics Committee of the Department of Human Services and the Menzies School of Health Research.

4.3. Results

During the period August 1, 1997 to July 31, 2003, 217 patients were treated for melioidosis at the Royal Darwin Hospital. Meropenem was administered in 63 patients; 5 patients relapsed and were retreated giving a total of 68 admissions. In the patients treated with meropenem, 19% died (8 attributable to melioidosis, 4 due to unrelated causes; suicide, non-melioidosis sepsis, heart failure and metastatic carcinomatosis).

In comparison, ceftazidime was used exclusively in 154 patients (including 11 patients with readmission due to relapse; total 165 admissions). In these patients, the mortality rate was 18% (16 attributable to melioidosis, 12 due to underlying disease). Characteristics of patients are detailed in table 1. Higher proportions of patients treated with meropenem had severe sepsis and bacteraemia ($p<0.001$), reflecting our selection criteria for the use of this antibiotic. In the subgroup with severe sepsis, the use of meropenem was associated with a lower mortality compared to ceftazidime (25% vs 76%, $p<0.001$).

Table 4-1: Characteristics of meropenem- and ceftazidime-treated patients

	Meropenem-treated patients	Ceftazidime-treated patients	Odds ratio (95% CI)	p-value
Number of patients	63	154		
Age[a]	50 (6mths – 73 years)	49 (2-78)		NS
Male gender[a]	46 (73%)	114 (74%)		NS
Mortality due to infection	8 (13%)	16 (10%)	1.3 (0.52, 3.0)	NS
All cause mortality[a]	12 (19%)	28 (18%)	1.1 (0.51, 2.2)	NS
Diabetic[a]	30 (48%)	53 (34%)	1.7 (0.95, 3.1)	0.09
Renal failure[a]	6 (10%)	12 (8%)	1.2 (0.46, 3.4)	NS
Number of episodes	68	165		
Pneumonia[b]	40 (59%)	74 (44%)	1.8 (0.99, 3.1)	0.06
Genitourinary infection[b]	6 (9%)	29 (18%)	0.45 (0.18, 1.1)	0.11
CNS infection[b]	4 (6%)	2 (1.2%)	5.1 [d]	0.06
Severe sepsis[b]	28 (41%)	21 (13%)	4.8 (2.5, 9.2)	<0.001
Mortality in severe sepsis	7 (25%)	16 (76%)	0.10 (0.03, 0.38)	<0.001
Received G-CSF[c]	21 (31%)	0	[d]	<0.001
Received physiological dose corticosteroids[c]	5 (7%)	0	[d]	0.002
Bacteraemic[b]	51 (75%)	65 (39%)	4.6 (2.5, 8.6)	<0.001
Mortality in bacteraemic	10 (20%)	16 (25%)	1.6 (0.70, 3.7)	NS

[a]as proportion of total number of patients; [b]as proportion of total number of episodes (including readmissions for relapse); [c]received G-CSF (commenced 1998) and corticosteroids (commenced 2001) for septic shock; [d] 95% confidence intervals not calculable

Of the 68 episodes where meropenem was used, the majority were commenced on meropenem as initial therapy (n=48). Reasons for the initial choice of meropenem were severe sepsis including septic shock (n=28), central nervous system infection (n=4), relapsed disease following apparently successful treatment with ceftazidime (n=8) and other clinical reasons (n=8), including a single patient from whom a ceftazidime-resistant strain was isolated. In addition, ceftazidime was used initially in 20 admissions where there were subsequent changes to meropenem; 17 for worsening clinical condition on treatment and three for suspected adverse reactions (rash 2; thrombocytopenia 1). Ceftazidime was used subsequent to the course of meropenem in 12 episodes, in 9 cases once the patient's clinical status had stabilized and to facilitate discharge for home therapy with continuous ceftazidime infusion via elastomeric pump. One patient was treated initially with imipenem and later changed to meropenem.

There was one probable and three possible adverse events associated with the use of meropenem. One patient had ongoing fever and neutropenia that resolved once therapy had been changed to a ceftazidime-based regimen. One patient had seizures associated with intracranial infection that occurred both prior to and subsequent to the commencement of meropenem treatment. One patient had a rash while on meropenem and trimethoprim/sulfamethoxazole that resolved after antibiotics were changed to chloramphenicol and doxycycline. One further patient had thrombocytopenia which persisted following cessation of meropenem; therapy with meropenem was later recommenced once his thrombocytopenia had resolved without incident. No patients required a change in therapy due to abnormal liver function with meropenem.

No strains were isolated that had primary resistance to meropenem. A thoracotomy and change in therapy from meropenem to ceftazidime in one patient was prompted by an increasing MIC to meropenem (0.75 to 4 mg/L), but this strain remained sensitive.

This series includes 21 patients treated with G-CSF, 19 of which have been reported in chapter 2.

4.4. Discussion

Although meropenem has been used for the treatment of melioidosis in isolated case reports [16-18], this observational study is the first to demonstrate positive outcomes from melioidosis using meropenem. Although benefits are not quantifiable in this observational study, the overall mortality rate observed is similar to that in ceftazidime-treated patients despite a deliberate selection bias toward patients with more severe infection receiving meropenem. More specifically, the mortality in those with severe sepsis was lower with meropenem.

Despite its expense, meropenem provides practical and theoretical advantages over both ceftazidime and imipenem. Unlike imipenem, meropenem is not associated with seizures, a particular concern in patients with renal failure and in patients with intracerebral infection. In addition, meropenem has a more favourable dosing schedule (three vs four times/day) compared with imipenem. Compared to ceftazidime, carbapenem antibiotics have a lower minimum inhibitory concentration for *B. pseudomallei* [3-5] and a faster time-kill profile [2], including resistant isolates [3]. Additionally carbapenems demonstrate a post-antibiotic effect not seen with ceftazidime [6] and are associated with decreased endotoxin release [7]. These factors may be important in critically unwell patients requiring more rapid control of high bacterial loads and pro-inflammatory dysregulation. Although a previous clinical trial where imipenem was compared to ceftazidime failed to find any mortality benefit [1], a significant reduction in mortality in patients with severe sepsis was observed. This may reflect an interaction between other intensive care interventions in this developed world context and the use of meropenem.

Many other factors are likely to contribute to the low mortality observed in this study compared to those published previously [1, 19, 20], including early diagnosis and treatment, the availability of resources for intensive care management, the use of G-CSF and evidence-based protocols for severely septic patients. Our evidence also supports the use of ceftazidime in milder infections, and to complete the intensive phase in an outpatient setting, not possible with meropenem due to its poor stability.

In this study, it is noted that meropenem is associated with outcomes at least as good as with ceftazidime. Theoretical considerations support this data which suggest that

its use may be associated with improved outcomes in patients with severe sepsis in a setting where other intensive care interventions have been optimized. This hypothesis deserves further scrutiny in appropriately-powered randomized controlled trials. Meanwhile, this work supports Australian guidelines listing meropenem as an alternative first-line agent in the treatment of severe melioidosis [8, 10].

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