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MELIOIDOSIS IN THE AMERICAS

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Abstract. Melioidosis is a potentially severe bacterial infection caused by *Burkholderia pseudomallei*. There has been growing awareness of the disease in the Americas, particularly since the Vietnam conflict when it was diagnosed in returning service personnel. Accidental laboratory exposure indicates the difficulty making a culture-based diagnosis when melioidosis has not been considered in the differential diagnosis. Melioidosis is most likely underdiagnosed in tropical Central and South America where conditions are more suited to persistence of *B. pseudomallei* in the environment. Recent melioidosis case clusters in northeastern Brazil highlight the threat posed to rural populations located far from specialist services. Increased clinical awareness of the disease and improvements in laboratory diagnostic methods are likely to bring wider recognition of melioidosis in the Americas.

INTRODUCTION

Melioidosis is a potentially fatal bacterial infection of the tropical and subtropical zone between 20°′S and 20°N.[1,2] Some endemic cases occur both north and south of the tropics.[3,4] The recent detection of melioidosis in northeastern Brazil highlights the extent of its distribution in the Americas and underlines the need for improved diagnostic methods.[5]

Melioidosis is a disease of environmental exposure that is normally acquired through occupational or recreational encounter with moist soil or surface water containing *Burkholderia pseudomallei*, the infective agent. Infection results from inhalation, inoculation, and possibly ingestion of this organism.[6] There is debate over the relative importance of these routes of infection. High risk groups include rice farmers, laborers, indigenous groups, and adventure travelers.[1] The peak risk period for acute infection is during the wet season, particularly within a week of the onset of heavy rain.[7] Melioidosis has an unusually wide range of disease presentation, varying from septicemia with pneumonia and rapid deterioration to multiple organ systems failure through subacute disease with focal suppuration or abscess formation to asymptomatic exposure with no clinical evidence of infection until late-onset acute disease.[1,6] The longest disease-free interval between exposure and culture positive melioidosis is 63 years.[8] Those at most risk of septicemic or other acute manifestations of melioidosis have underlying medical conditions such as diabetes, chronic renal failure, alcoholic liver disease, or chronic respiratory pathology[9] (Table 1).

Although *B. pseudomallei* is not difficult to culture in sterile fluid samples from patients with acute melioidosis, there are a series of diagnostic pitfalls that cause difficulties for the diagnostician and the clinical pathologist, even in melioidosis-endemic areas where the diagnosis is anticipated (Table 1). These problems include a lack of distinctive features of acute infection, potential involvement of almost any body site by subacute suppurative disease, unexpected late-onset acute disease months or years after exposure, the finding of more common bacterial pathogens in bacteriologic specimens from non-sterile sites, atypical bacterial colony appearance in primary cultures, misleading bacterial identification results from proprietary laboratory tests, and *B. pseudomallei* polymerase chain reaction (PCR) inhibitors in tissues affected by suppurative subacute infection. Serologic tests, such as the widely used indirect hemagglutination assay, often give borderline positive or negative results in the early stages of acute septicemic disease.[10] Single high titers may be obtained from asymptomatic persons and false-positive reactions may occur. An increasing antibody titer is more specific for recent infection, but is not definitive proof of disease.

A proportion of acute infections will be fatal, despite the best efforts of the attending physician. Those at highest risk of fatal infection are patients with *B. pseudomallei* meningoencephalitis. Approximately one-fourth of septicemic patients will relapse despite receiving intravenous antibiotics, often at approximately 10–14 days after onset of acute infection (Table 1). The initial treatment regimens currently recommended are ceftazidime plus cotrimoxazole, or meropenem for 2–4 weeks. Concerns about early relapse and late onset disease have led many to follow this first phase of therapy with a second, eradication phase with a combination of oral agents such as cotrimoxazole and doxycycline for at least 12 weeks.[11]

The bacterial cause of melioidosis is a gram-negative bacillus from the beta-proteobacteria group known as *B. pseudomallei*. The species was previously known as *Pseudomonas pseudomallei* and was grouped with other members of the *Pseudomonas* group until related bacteria such *Pseudomonas cepacia* were allocated the separate genus of *Burkholderia*. More commonly encountered Pseudomonads such as *P. aeruginosa* can be distinguished by their easily recognized phenotypic features such as pigment production, in addition to major genotypic differences. *Burkholderia pseudomallei* is oxidase positive, resistant to a wide range of antibiotics including gentamicin, polymyxin, and the second-generation cephalosporins, and can survive for long periods in a wide range of environments including distilled water, moist soil, and inside mammalian cells.[12–14] It is closely related to the other human pathogens in the genus (*B. mallei* and *B. cenocepacia*) and to non-pathogenic near-neighbors such as *B. thailandensis*.[15]

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TABLE 1
Presentation and diagnosis of melioidosis*

Epidemiologic and clinical setting	Diagnostic pitfalls	Laboratory diagnostic criteria
History of travel to tropical zone	Melioidosis not yet recognized as endemic in many tropical locations; distribution patchy and seasonal	
Exposure to moist soil or surface water	Delayed onset may be months–years after exposure	
Rural work outdoors Adventure or eco-tourism Military service		
During wet season or extreme weather	Peak risk may be two weeks after heavy rainfall	
Underlying diabetes, chronic renal failure, alcoholic liver disease or chronic lung disease	Co-morbidities cause higher risk of septicemic disease, rapid progression, and fatality	
Febrile illness	Commonest acute presentations are nonspecific	*Burkholderia pseudomallei* isolated from blood culture, CSF, respiratory secretions, or other sample
Septicemia	Common intravenous antibiotics (e.g., gentamicin, ceftriaxone) are ineffective and unsuitable for trial of presumptive therapy. Relapse in up to 25%.	*B. pseudomallei* isolated from blood culture
Pneumonia	Small numbers of *B. pseudomallei* in respiratory secretions outnumbered by commensal bacteria	Growth of *B. pseudomallei* on selective agar media
Pneumonia with septicemia		*B. pseudomallei* grown from blood culture or respiratory secretions
Respiratory distress	Primary septic cause may no longer be evident	
Multiple organ systems failure		
Meningoencephalitis	Unusual in some locations endemic for melioidosis	*B. pseudomallei* isloated from CSF
Focal suppuration as abscess, cellulitis or ulcer	Undisclosed abscess may be source of bacteremia	
Skin, soft tissues Face including orbits Osteomyelitis, septic arthritis Prostate	Etiologic diagnosis may be missed if identification of bacteria from non-sterile site depends on substrate-utilization tests	
Asymptomatic seroconversion	Single high titer does not establish timing of exposure. False-positive and false-negative results occur.	Indirect hemagglutination assay rising titer of single high titer in absence of other evidence of melioidosis is only supportive of diagnosis

* CSF = cerebrospinal fluid.

NORTH AMERICA

Imported disease in Vietnam veterans. Most cases of melioidosis in the United States have been in veterans of the Vietnam conflict. It was realized in the late 1960s that melioidosis could be observed as acute febrile illness years in soldiers who returned home from the Indochinese theater of operations when the propensity of this disease to present without warning after a disease-free interval was noted by infectious disease specialists.[16,17] A bizarre record was kept for the longest interval to delayed onset acute disease. The longest interval reported in a Vietnam veteran is 29 years.[18] The longest interval, 63 years, was recently reported for a veteran of the Pacific war of 1941–1945.[8] Although the disease was not widely recognized until long after the Second World War, there are reports of melioidosis in Far East prisoners of war.[19] This unpredictability continues to cause significant diagnostic difficulty for the attending physician and the clinical laboratory.[20,21]

The extent of exposure to *B. pseudomallei* in high-risk populations is difficult to gauge from occasional case reports. Sero-epidemiologic studies have not been sufficiently comprehensive nor the methods used sensitive enough to provide an accurate picture of subclinical disease burden, but they do provide evidence for undetected exposure to *B. pseudomallei* in servicemen.[22] The burden of hidden disease in the ageing veteran population is not known. Military field hospitals are said to have seen hundreds of cases of melioidosis in Vietnam. It is interesting to note that clinical melioidosis was only occasionally reported in British troops serving in Borneo during the Malayan emergency despite serologic studies showing evidence of seroconversion.[23] Hypothetical reasons include differences in the virulence of *B. pseudomallei*, the type of vegetation (including the pattern of rice cultivation), and exposure to soil and water experienced by combat troops.[24] It is notable that the Mekong delta carries silt from a river system that drains parts of northeast Thailand now known to be highly endemic for melioidosis.[25,26]

Endemic disease. There has been a long-running argument over endemic melioidosis in North America fuelled by one case contracted after a farming accident in Oklahoma.[27] The infection had features consistent with subacute melioidosis, but the clinical isolate had some phenotypic features that placed it apart from other clinical *B. pseudomallei* isolates and it was originally thought to be another, unidentified bacterial species.[28] DNA hybridization studies and other molecular

analyses including multilocus sequence typing subsequently placed this isolate in a separate clade of *B. pseudomallei*.[15] The Oklahoma isolate is clearly separate from other members of the species and may represent a distinct bacterial species. In this particular case, there was no evidence of exposure to soil or muddy water outside the United States. There was one other reported instance of melioidosis believed to have been contracted in the United States that involved a young adult who sustained severe facial injuries during a motor vehicle accident.[29] Eight weeks after orbital enucleation, he developed *B. pseudomallei* infection in the remaining tissues of the enucleated orbit. There was no history of overseas travel to provide a history of environmental exposure in a recognized endemic location. There have been several cases of melioidosis contracted after direct person-to-person transmission from people who contracted the disease in a recognized endemic zone. One of the best documented cases was a case of probable sexual transmission from a war veteran to his sex partners.[30]

LATIN AMERICA

The first properly documented case of melioidosis in South America was reported from Ecuador in the 1960s.[31] The authors of this report asserted that earlier claims of melioidosis cases from the Western Hemisphere may have been premature.[32–34] In two cases, the isolates were not available for independent laboratory confirmation and in the third case the isolate was probably misidentified. The first of these possible cases was diagnosed in the United States in 1945 but the putative exposure could have been outside the United States while the patient was working in the Panama Canal Zone in 1927–1928.[32] The report of Beigeleisen and others also mentioned two probable melioidosis cases from Panama.[31] There was an unsuccessful attempt to demonstrate the presence of *B. pseudomallei* in rice fields near São Paulo, Brazil.[35] There have been other reports of possible melioidosis from Brazil, including isolation of *B. pseudomallei* from burns patients, but these lacked detail on disease presentation, exposure, or corroborating evidence.[36] There have been reports of sporadic human melioidosis from other parts of Central America.[37–40] More recently, there have been occasional reports of melioidosis diagnosed unexpectedly after presumed exposure in parts of South or Central America not previously known to be endemic for the disease.[41] (Champagne J, unpublished data). It is possible that the lack of advanced diagnostic laboratory support in many of these countries, particularly in rural settings where melioidosis is more common, has led to under-diagnosis of the disease.[42] Refugee health programs and unusual climate events provide opportunities to test the hypothesis. Refugees from Central America have recently been found to have melioidosis during immigration health screening (Champagne J, unpublished data). Melioidosis has also been reported following flooding.[43] It has yet to be seen whether tropical storms cause cases of melioidosis in the region in the same way that cases of the disease were caused after the Indian Ocean tsunami of 2004.[44]

The first laboratory-confirmed case of human melioidosis in Brazil was in February 2003.[5,45] There was only one survivor from a family group of four children who contracted the disease. A gram-negative bacillus was grown and presumptively identified as *B pseudomallei* five days after death of the third child.[5] Although cultures from the one survivor remained negative for *B. pseudomallei*, she did seroconvert by *B. pseudomallei* indirect hemagglutination assay during her convalescence. In 2004, a small cluster of febrile illness occurred in a rural area approximately 165 km from the first case cluster (Figure 1).[45] One adult patient with culture-confirmed *B. pseudomallei* septicemia died. In early 2005, a small group of adults developed a febrile illness after an incident in which their car left the road and plunged into a river. One patient progressed to pneumonia with respiratory failure and died. That patient had blood cultures positive for *B. pseudomallei*. Environmental investigations led to the recovery of *B. pseudomallei* from river water and riverside mud collected during investigation of the second case cluster. A Dutch visitor to the coastal edge of the state developed a febrile illness during his travels in Ceará in 2003 and returned home to The Netherlands where he subsequently died of culture-confirmed melioidosis.[46] A recurring theme in melioidosis investigations has been the difficulty of arriving at an etiologic diagnosis when laboratory resources and experience with *B. pseudomallei* are limited. The first Brazil outbreak isolate was confirmed in Rio de Janeiro by phenotypic methods,[5] independently confirmed by molecular methods, and subtyped by DNA macrorestriction and *Eco* RI ribotyping.[45] It has been possible to develop a working case definition so that state health authorities can monitor the emergence of melioidosis in Ceará and neighboring parts of Brazil.[11] Preliminary seroepidemiologic studies suggest that asymptomatic exposure has occurred more widely than suggested by the children affected by the first case cluster.[45] In this rural part of northeastern Brazil, females were more likely to be seropositive. The pattern of seropositivity needs confirmation in larger studies because it is different from that in southeast Asia where melioidosis is common in rice farmers.[47,48] Molecular epidemiologic methods (DNA macrorestriction and automated *Eco* RI ribotyping) demonstrated *B. pseudomallei* strain diversity in northeastern Brazil, and suggest a possible link with melioidosis in northern Australia.[45]

Diagnostic laboratory incidents. Lack of professional familiarity with endemic melioidosis, combined with the sporadic occurrence of travel-related disease, pose a special problem for diagnostic laboratories in the United States. There are too few culture-positive cases to sustain expertise in hospital laboratories where blood culture and other clinical *B. pseudomallei* isolates are most likely to be recovered. Consequently, the first scientist to encounter a new isolate may not recognize *B. pseudomallei* until the combination of unusual Gram stain features, an antibiotic resistance pattern, and an earthy smell suggest its identity. Recent experience indicates that this kind of exposure can even occur in a well-equipped university center laboratory, and not just in smaller hospital laboratories.[49] The most common type of exposure is likely to be touching live cultures with ungloved hands. This is unlikely to pose a significant infection risk unless the laboratory worker has uncovered skin lesions. Aerosol generation is another theoretical risk that can be minimized by handling all such procedures in a biologic safety cabinet. Sniffing presumptive *B. pseudomallei* cultures to detect the characteristic earthy smell is common practice in disease-endemic areas, but there are no reports of melioidosis contracted as a result of this practice. Laboratory-acquired infection with *B. pseudomallei* has been

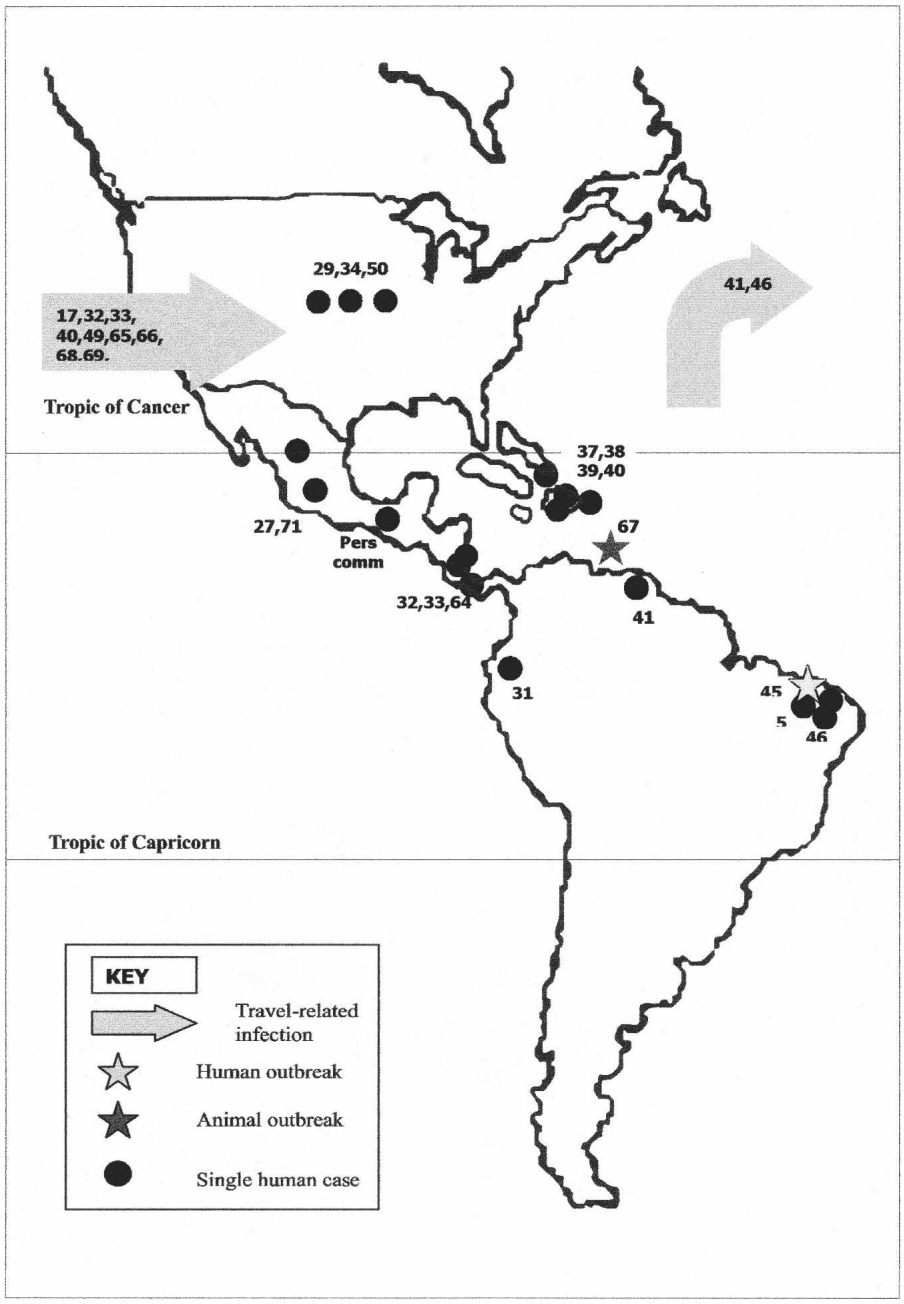

FIGURE 1. Distribution of melioidosis in the Americas. Numbers indicates references cited in this paper.

reported after exposure during a laboratory accident in which direct inoculation of live cultures is believed to have occurred.[50] Contemporary standards of laboratory practice should prevent these types of *B. pseudomallei* exposures. Delayed recognition of *B. pseudomallei* through over-reliance on substrate use tests has been implicated as a potential laboratory safety issue.[51] Rapid diagnostic tests have been developed to resolve this problem but are still not in widespread use outside Australia and southeast Asia.[52]

Biopreparedness. Concerns about the deliberate release of biologic weapon agents by terrorist groups have stimulated interest in a small group of uncommon bacterial infections including melioidosis.[53] The inclusion of melioidosis and glanders in Centers for Disease Control and Prevention–listed group B select agents probably relates more to their development and occasional use as biologic weapons by European nations than to any current concern about their utility as agents of bioterrorism. Although *B. pseudomallei* can survive in the inanimate environment for prolonged periods, it is not directly transmissible from human to human and therefore does not pose a serious epidemic threat. Moreover, its tendency to cause overwhelming septicemic disease in adults with underlying medical conditions is consistent with an opportunist pathogen. Uncertainty about the final means of exposure, technical difficulties producing bulk cultures and reducing them to an easily dispersed form, and the limited number of laboratories with *B. pseudomallei* in their culture collections all reduce the suitability of this agent for terrorist

TABLE 2
Chronology of melioidosis in the Americas*

Year	Presenting complaint	Complications	Outcome	Culture results	Diagnosed	Identity confirmed	Travel	Exposure	Occupation	Reference
1947	Buttock abscess	Sinus	Discharge	*M. pseudomallei*	USA	None	Panama	Fall on buttock	Machinist	32
1948	Retroperitoneal abscess	Septicemia	Died	*M. pseudomallei*	USA	None	Panama		Marine Corps	33
1951	Inguinal abscess	Sinus	Discharge	*M. pseudomallei*	USA	None	Unknown		Stockyard	34
1960	Optic and peripheral neuritis	CHL toxicity	Discharge	*P. pseudomallei*	USA	Yes	Unknown	Panama	Soldier	64
1964	Necrotic foot ulcer	Delirium	Died	*P. pseudomallei*	USA	Yes	Unknown	Ecuador, rice field	Farmer	31
1965–1969	72 cases, various	Unknown	14 died	Unknown	62 in Vietnam 10 in USA	Unknown	Vietnam	Unknown	Servicemen	65
1967	9 cases, pneumonitis		Survived	*P. pseudomallei*	USA	Unknown	Vietnam	Unknown, no combat wounds	Soldiers	66
1977	Pelvic abscess		Discharged	*P. pseudomallei*†	USA	Yes	Mexico	Soil contaminated crush injury	Farmer	27
1978	Short of breath	Tibial tenderness	Survived	*P. pseudomallei*	USA	Yes, CDC	Vietnam, Asia	Unknown	Marine veteran	17
1980	Eye socket after enucleation		Not recorded	*P. pseudomallei*	USA		None outside USA	Motor vehicle accident	Waste disposal	29
1981	Calf abscess Lung nodules		Discharge	*P. pseudomallei*	USA	Yes, CDC	None to endemic region	Unknown	Laboratory worker	50
1983	Osteomyelitis	Septicemia	Died	*P. pseudomallei*	USA	None	Vietnam	Shrapnel wound	Marine veteran	68
1984	Meningitis	Third nerve palsy	Survived	None	USA	Serology, CDC	Vietnam	13 delay	Veteran	69
1986	Swollen knee	Pneumonia	Died	*P. pseudomallei*	USA		Mexico		Clerk	70
1986	Septicemia meningitis		Died	*P. pseudomallei*	Puerto Rico	Yes, CDC	Puerto Rico	Unknown	Diabetes, SLE, cirrhosis	37
1991	Burn wounds	Not recorded		*P. pseudomallei*	Unknown	Unknown				36
1995	Septicemia		Survived	*B. pseudomallei*	Martinique	Yes, Institut Pasteur	Martinique	Unknown	Unknown	38
1997	Fever, cough, neck pain	Hilar lymph nodes	Died during therapy	*B. pseudomallei*	USA	Yes, CDC	Puerto Rico	Unknown	Child with CGD	40
1997	Fever	Pleural effusion	Survived	*B. pseudomallei*	Guadeloupe	No	Guadeloupe	Unknown	Child	39
2001	Headache and fits	Cerebral abscess	Survived	*B. pseudomallei*	USA	No	El Salvador	Unknown	Refugee	Unpublished data
2002	Fever, hoarseness	Arterial aneurysm	Survived	*B. pseudomallei*	USA	Unknown	Borneo	Unknown	Management consultant	71
2003	Renal abscess pneumonia		Survived	*B. pseudomallei*	Portugal	Unknown	Resident in Venezuela			41
2004	Septicemia		Died	*B. pseudomallei*‡		Yes, Australia	Recent heavy rainfall		Child of goat farmer	5
2005	Respiratory distress septicemia	Blood culture of *B. pseudomallei*	5 deaths, 1 survivor	Outbreak: 2003, 3 deaths, 1 survivor. 2 additional cases; 2 deaths, 2 other locations	Brazil	Yes	Outbreak, recent heavy rainfall	Outbreak, swimming in irrigation dam; others: sitting in river water, immersion in river	Outbreak: children of goat farmer; others = adults	45
2005	Pneumonia		Died	*B. pseudomallei*	The Netherlands	Yes	Brazil		Eco-tourist	46
2005	Exposure, plus index case		No disease, survived	No *B. pseudomallei*	USA USA	NA Yes	Clinical laboratory Australia	Culture exposure Unknown	Laboratory staff, tourist	49

* *M.* = *Malleomyces;* CHL = chloramphenicol; CDC = Centers for Disease Control and Prevention; SLE = systemic lupus erythematosus; CGD = chronic granulomatous disease; NA = not applicable.

use. Nevertheless, recent advances in laboratory biopreparedness have improved knowledge of the pathobiology of melioidosis pathogenesis. Work on the role of *B. pseudomallei* capsular polysaccharide, the genetic determinants of secreted exoproducts, the genetic relatedness of *B. pseudomallei* and *B. mallei*, and laboratory models of melioidosis[54–58] are important steps towards vaccine development. However, useful progress has been made on application of PCR protocols to *B. pseudomallei* identification. Several PCR protocols have been validated for identification of *B. pseudomallei* based on specific gene targets. These include a portion of the sequence between the 16S and 23S encoding sequences,[59] genes encoding a type-three secretion system,[60] a synonymous single nucleotide polymorphism,[61] and a specific fatty acid synthase.[62] We recently proposed a laboratory discovery pathway that integrates preliminary diagnostic laboratory steps with PCR-based methods.[63] A selection of specific genetic targets have been combined in a molecular typing system known as multilocus locus typing to give a reproducible measure of genetic relatedness between *B. pseudomallei* strains.[15] These molecular diagnostic and typing methods are available only in larger reference and research laboratories. Commercially available diagnostics suitable for use outside melioidosis-endemic regions are likely to take even longer to become available.

LESSONS FROM THE AMERICAS

The changing epidemiology of melioidosis in the Americas provides useful insight into this emerging infectious disease (Table 2). In North America, melioidosis is generally a travel-related disease affecting people who visit disease-endemic locations for pleasure, business, or military reasons.[16–19,22,23,30,64–66] Sporadic cases of melioidosis in adventure travelers and military veterans will continue to puzzle physicians for the foreseeable future, and the history of potential exposure will often be established after laboratory diagnosis.[20,21,46] In Latin America, melioidosis is a disease of mainly rural and remote communities and probably goes undetected most of the time.[5,32,33,45,66,67] Molecular typing of clinical isolates from recent cases in northeastern Brazil does not support recent incursion and rapid spread of one strain.[45] The impression that the disease may be spreading in Latin America can be explained by gradual improvement in diagnostic laboratory methods combined with increasing awareness of the disease. If melioidosis has been present in northeastern Brazil for some time, subacute disease and some septicemic cases may have been missed and smoldering subacute infections occur more widely in the region. A better grasp of the epidemiology of melioidosis in the Americas will shed more light on its regional origins, the critical exposure required to establish infection, and how *B. pseudomallei* adapts to such a variety of environmental habitats. The current research focus on biosecurity will accelerate improvements in diagnosis, therapy, and prevention of this naturally occurring emergent disease.

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