The Lancet Infectious Diseases

Melioidosis is an opportunistic infectious disease: the 30-year Darwin Prospective Melioidosis Study

--Manuscript Draft--

Manuscript Number:	THELANCETID-D-20-04750R2
Article Type:	Article (Original Research)
Keywords:	Melioidosis; Burkholderia pseudomallei; Opportunistic infection; select agent; community acquired pneumonia; tropical diseases
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Abstract:	Background: The global distribution of melioidosis is under considerable scrutiny, with both unmasking of endemic disease in African and Pacific nations and evidence of more recent dispersal in the Americas. Tropical northern Australia has high incidence rates and The Darwin Prospective Melioidosis Study commenced in October 1989. We present epidemiology, clinical features, outcomes and bacterial genomics from this 30-year study, highlighting changes in the last decade. Methods: Prospective study of epidemiological, clinical and laboratory data for all culture-confirmed melioidosis cases from the tropical Northern Territory of Australia from October 1 st 1989 until September 30 th 2019. Multivariable analysis determined predictors of clinical presentations and outcome. Incidence, survival and cluster analyses were facilitated by population and rainfall data and genotyping of Burkholderia pseudomallei including multilocus sequence typing and whole-genome sequencing.

	Findings: There were 1148 individuals with culture-confirmed melioidosis of whom 133 (11.6%) died. Median age was 50 years, 48 were children ≤ 14 years old (4.2%), 721 (63%) were male and 600 (52%) Indigenous Australians. All but 186 (16%) had clinical risk factors; diabetes 513 (45%), hazardous alcohol use 455 (40%); only 3/133 (2.3%) fatalities had no identified risk. Pneumonia was the commonest presentation (595; 52%), bacteraemia occurred in 633/1135 (56%), septic shock in 240 (21%) and 180 (16%) required mechanical ventilation. Cases correlated with rainfall with 80% occurring in the "wet" season (November through April). Median annual incidence rate was 20.5/100,000 people; the highest annual incidence in Indigenous Australians was 103.6/100,000 in 2011-2012. Over the 30 years, annual incidences increased as did the proportion with diabetes, while mortality decreased to 17/278 (6%) over the last 5 years. Genotyping of B. pseudomallei confirmed case clusters linked to environmental sources and defined evolving and new sequence types. Interpretation: Melioidosis is an opportunistic infection , but with early diagnosis, specific antimicrobial therapy and state-of-the-art intensive care, mortality can be reduced to below 10%. However, mortality remains much higher in the many endemic regions where health resources remain limited. Genotyping of B. pseudomallei informs evolving local and global epidemiology. Funding: The Australian National Health and Medical Research Council.

Title Page

Melioidosis is an opportunistic infectious disease: the 30-year Darwin Prospective Melioidosis Study

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ABSTRACT

Background: The global distribution of melioidosis is under considerable scrutiny, with both unmasking of endemic disease in African and Pacific nations and evidence of more recent dispersal in the Americas. Tropical northern Australia has high incidence rates and The Darwin Prospective Melioidosis Study commenced in October 1989. We present epidemiology, clinical features, outcomes and bacterial genomics from this 30-year study, highlighting changes in the last decade.

Methods: Prospective study of epidemiological, clinical and laboratory data for all culture-confirmed melioidosis cases from the tropical Northern Territory of Australia from October 1st 1989 until September 30th 2019. Multivariable analysis determined predictors of clinical presentations and outcome. Incidence, survival and cluster analyses were facilitated by population and rainfall data and genotyping of *Burkholderia pseudomallei* including multilocus sequence typing and whole-genome sequencing.

Findings: There were 1148 individuals with culture-confirmed melioidosis of whom 133 (11.6%) died. Median age was 50 years, 48 were children ≤ 14 years old (4.2%), 721 (63%) were male and 600 (52%) Indigenous Australians. All but 186 (16%) had clinical risk factors; diabetes 513 (45%), hazardous alcohol use 455 (40%); only 3/133 (2.3%) fatalities had no identified risk. Pneumonia was the commonest presentation (595; 52%), bacteraemia occurred in 633/1135 (56%), septic shock in 240 (21%) and 180 (16%) required mechanical ventilation. Cases correlated with

rainfall with 80% occurring in the “wet” season (November through April). Median annual incidence rate was 20.5/100,000 people; the highest annual incidence in Indigenous Australians was 103.6/100,000 in 2011-2012. Over the 30 years, annual incidences increased as did the proportion with diabetes, while mortality decreased to 17/278 (6%) over the last 5 years. Genotyping of *B. pseudomallei* confirmed case clusters linked to environmental sources and defined evolving and new sequence types.

Interpretation: Melioidosis is an opportunistic infection, but with early diagnosis, specific antimicrobial therapy and state-of-the-art intensive care, mortality can be reduced to below 10%. However, mortality remains much higher in the many endemic regions where health resources remain limited. Genotyping of *B. pseudomallei* informs evolving local and global epidemiology.

Funding: The Australian National Health and Medical Research Council.

Research in context

Evidence before the study

For almost a century after its first description in Myanmar, melioidosis remained an enigmatic tropical disease from Southeast Asia described in military conflicts and the occasional returned traveller. Diagnosis requires culture of *Burkholderia pseudomallei* and that necessitates laboratory resources that remain unavailable in many tropical locations globally. Clinical descriptions were often of chronic pulmonary infection or of presumptive latency with activation years after infection. Recognition of the large and increasing burden of severe sepsis from melioidosis in endemic regions only became evident following studies in northeast Thailand supported by quality laboratory microbiological capability. Subsequently sequential randomized trials of antimicrobial therapy from Thailand have defined current therapy. In parallel to improved laboratory diagnostic facilities unmasking endemic locations across the globe, genotyping of *B. pseudomallei* has informed the epidemiology of melioidosis by tracking the source and mode of individual infections as well as supporting that intercontinental spread of this sapronotic pathogen has occurred over millennia.

Added value of this study

The 30-year Darwin Prospective Melioidosis Study began in 1989, the year of publication of the initial trial from Thailand that showed mortality halving with the introduction of ceftazidime. The 30-year data from 1148 consecutive cases describe distinct infecting scenarios and clarify the incubation period of acute disease, proportions with chronic melioidosis and the rarity of activation from latency. Demographics, risk factors and correlations with outcomes are quantified as is the diverse clinical spectrum of melioidosis. Genotyping of *B. pseudomallei* from cases

and the environment together with weather data support the importance of inhalational melioidosis during monsoonal events, as well as showing dynamic local bacterial dispersal which interplays with an evolving global story of dissemination of melioidosis, most recently in the Americas. With adequate laboratory and intensive care resources mortality under 10% has been achieved, with deaths only in those with clinical risk factors; highlighting the recognition of melioidosis as an opportunistic infectious disease.

Implications of all the available evidence

The continuing high mortality in many melioidosis endemic regions is a stark reflection of global resource disparities, with basic microbiology laboratory facilities, access to the required antibiotics and quality intensive care management for severe sepsis often unavailable. While considerable funding is being directed at *B. pseudomallei* as a potential biothreat agent, recognition of these clear and persisting inequities is arguably a greater priority.

Introduction

In 1912 melioidosis was first described in Burma as a newly recognised glanders-like disease of humans. Reports from other Southeast Asian countries soon followed.(1, 2) The genomics era has provided a fascinating but still incomplete picture of the global presence and spread of *Burkholderia pseudomallei,* the bacterium which resides in complex ecosystems in the soil and water of endemic regions and causes melioidosis in exposed humans and animals. Although glanders was described by Hippocrates, whole genome sequencing has shown that *Burkholderia mallei*, the bacterium causing glanders, is a derivative clone of *B. pseudomallei,* having evolved through gene loss and selection to be a horse-adapted pathogen that can no longer survive in the environment.(3) Glanders has been used in biological warfare and both *B. mallei* and *B. pseudomallei* are listed as Tier 1 select agents. Rapidly progressive sepsis with high mortality from aerosol inhalation of *B. pseudomallei* is the concern that funds the current diagnostic, therapeutic and vaccine initiatives for melioidosis.(4)

Genomic analyses suggest that *B. pseudomallei* evolved in the environment of Australia, subsequently spreading to Southeast Asia during the last ice age.(5) From Asia *B. pseudomallei* spread to Madagascar and Africa(6) and more recently from Africa to the Americas. Global modelling predicted an estimated 165,000 human cases annually, with 89,000 deaths.(7) Substantial improvements in surveillance and laboratory resources are needed to verify these predictions and confirm the burden of disease in comparison to currently-designated neglected tropical diseases.(8)

Geospatial modelling predicted many regions globally, including the southern USA to be receptive to, if not already endemic for, *B. pseudomallei*.(7) Past investigations

linked cases of melioidosis in the USA to either travel or prior residence in overseas endemic countries or probable infection from unidentified contaminated products imported from Asia.(9) Recent bacterial genotyping of one recent and another historical autochthonous case supports the hypothesis that melioidosis is now endemic in Texas, linking to known endemic regions in the Americas.(10)

The Darwin Prospective Melioidosis Study (DPMS) began in 1989, documenting all cases of melioidosis in the tropical "Top End" of the Northern Territory of Australia (Fig. 1). We described 252 cases after 10 years(11) then 540 total cases after 20 years.(12) With an additional 610 cases over the last 10 years, we now present the findings from 1148 individuals with melioidosis collected prospectively over 30 years and describe dynamic changes in epidemiology, clinical parameters and bacterial genomics occurring over the 3 decades.

Methods

We included and prospectively followed all patients diagnosed with culture-confirmed melioidosis in the Top End over 30 years from October 1, 1989 until September 30, 2019. Culture-confirmed melioidosis is a laboratory-notifiable disease in the Northern Territory and blood, sputum, urine and pus cultures from both primary care clinics and hospitals are routinely processed to include identification of *B. pseudomallei*. Once *B. pseudomallei* was confirmed, all patients were managed by the Royal Darwin Hospital Infectious Diseases Department, with the vast majority admitted to Royal Darwin Hospital for assessment and commencement of intravenous therapy. Positive serology in symptomatic patients directed clinicians to provide

157 further cultures, with a confirmed diagnosis of melioidosis and inclusion in the study
158 only for those culture-positive.
159
160 The definitions and descriptions used for clinical and demographic risk factors and for
161 clinical illness parameters remained constant throughout the 30-years as described
162 previously, (11, 12), and are summarised in Supplementary Document 1. First
163 episode melioidosis was categorised as: acute (defined as symptoms present for < 2
164 months); chronic (symptoms present ≥ 2 months); or activation from latency.
165 Activation of disease from a latent focal infection was a clinician-directed
166 categorisation based on two scenarios with culture-confirmed new clinical illness
167 occurring in individuals with no prior culture-confirmed melioidosis; long-standing
168 positive melioidosis serology with usually a very high titre and no recent exposure
169 events; or individuals with long-standing pulmonary radiological abnormalities with
170 new pneumonia specifically involving the area of radiological abnormality.
171
172 Over the 30 years Darwin and international melioidosis treatment guidelines have
173 been informed by randomized comparative studies of antimicrobial therapy from
174 Thailand.(13, 14) DPMS treatment includes ≥14 days of intravenous ceftazidime or
175 meropenem (meropenem being mostly restricted to those in intensive care), followed
176 by eradication therapy with sulfamethoxazole/trimethoprim, usually for 3 months.(15)
177 Therapy for all patients is directed by the Infectious Diseases Department, based on
178 the Darwin treatment guidelines, which have evolved over the 30 years as
179 described.(15) Once discharged from hospital on oral eradication therapy, patients are
180 seen in clinic initially weekly then monthly, with follow up continued ideally until at
181 least 6 months following completion of therapy. However, in reality around half of all

patients do not complete eradication therapy.(15) For complex clinical scenarios decisions are by Department consensus, following formal case discussions (moderated usually by BJC).

Recurrent melioidosis was defined as re-presentation with *B. pseudomallei* culture-positive clinical disease occurring after the time designated for treatment completion (both intravenous and oral phases) for the previous episode, irrespective of whether the patient was adherent to the therapy or initially lost to follow up. Recurrent melioidosis was determined to be relapse or new infection based on epidemiology and comparative genomics using multilocus sequence typing and whole genome sequencing. Primary study outcome was death from melioidosis, either during initial or recurrent melioidosis episode. Death from other causes was also recorded up to December 31, 2019.

Patient demographic, epidemiological, clinical and laboratory details were stored in MariaDB v10.2.31 (Oracle, California) and analysed using Stata v15.1 (Stata, Texas). Details of the statistical methods used for patient data analysis, including multivariable logistic regression analyses to identify associations with clinical presentations, bacteraemia and a fatal outcome from melioidosis are provided in Supplementary Document 1. Population numbers by region and ethnicity were extrapolated from Australian census data. Methods for analysis of incidence rates and rainfall over the 30 years are in Supplementary Document 1, with incidence rate trends over time and correlations with season, region, ethnicity and rainfall calculated using generalized additive models. Estimates of survival at 60 days and 5 years after melioidosis diagnosis were obtained using Kaplan-Meier analyses in Graph Pad Prism

v7.04 (Graph Pad, San Diego, CA, USA). The survival curves with 95% confidence intervals were compared using the log-rank test. For full 5 years survival data, only cases of melioidosis until September 30th, 2014 were included. Multilocus sequence typing (MLST) was performed on *B. pseudomallei* isolates as summarised in Supplementary Document 1, with sequence types (STs) deposited on the *B. pseudomallei* MLST website (https://pubmlst.org/bpseudomallei/).(16)

This study was approved by the Ethics Committee of the Northern Territory Department of Health and Menzies School of Health Research (02/38).

Role of the funding source

The sponsor of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. All authors have full access to all the data in the study and the corresponding author had final responsibility for the decision to submit for publication.

Results

Two of the patients included in the 20-year cohort(12) were removed from analysis because they were from Central Australia and not treated at Royal Darwin Hospital. The final numbers were 252 cases in the first decade, 286 in the second and 610 in the third. There were 1212 episodes of melioidosis in 1148 individuals over 30 years, with 133 (11.6%) deaths attributable to melioidosis, 127 occurring from the initial infection and 6 following recurrent melioidosis (Fig. 2). Overall, sixty (5.2%) individuals had one or more recurrences of melioidosis, of which 44 were relapse and 20 new infection (Fig. 2). Of the 1015 individuals who survived melioidosis as of

December 31, 2019, 37 (3.6%) were lost to follow-up and 336 (33%) had died from other causes, predominantly comorbidities.

Epidemiology

Median age was 50 years (range 7 months-97 years), with only 48 (4.2%) children ≤14 years old (Table 1). As previously identified, the commonest risk factors in those diagnosed with melioidosis were diabetes (45%) and hazardous alcohol use (40%)(11, 12), but neither were individually predictive of mortality (Table 1). The absence of any clinical risk factor was strongly predictive of survival; of the 186 (16%) patients with no identified clinical risk factor only 3 (1.6%) died from melioidosis. Patients with at least one clinical risk factor were 8.4 times (95% CI: 2.7–26) more likely to die from melioidosis than patients without risk factors.

Occupational exposure to soil/surface-water was documented for 187 (16%) and recreational exposure for 892 (78%). A potential infecting event was documented for 255 (22%) (Supplementary Table 1). For 70 patients with a suspected inoculating event on a known date, incubation period was 1-21 days (median 4 days; IQR 3-7 days). Common scenarios included recreational and occupational activities related to gardening and outdoor maintenance, such as cutaneous exposure through cuts and trauma, and presumptive aerosol exposure from lawn mowing, weed-whacking and high-pressure hosing (Supplementary Table 1).

Yearly case numbers tracked with rainfall (Fig. 3A) and severe weather events were linked to regional clusters.(17) Examples are included in Supplementary Document 1. During the wet season in Darwin, the monthly incidence rate per 100,000 people

increased on average 14% (95% CI 8.5–19.0) for every 100mm increase in total monthly rainfall while accounting for ethnicity and annual trends (see Supplementary Document 1 for methods).

Genotyping of *B. pseudomallei* confirmed contamination of unchlorinated water supplies was associated with one cluster of nine cases (four fatal) in a remote Aboriginal community(18) and a separate cluster of two cases on a rural property.(19)

923 first presentations (80%) were in the "wet-season" (November through April) (Supplementary Fig. 1). Annual incidence rates were 4.8–51.2 (median 20.5) cases/100,000 people (Fig. 3A). 2011-2012 had the highest overall incidence and for that 12-month period which included a particularly high-rainfall wet season, the incidence in Indigenous Australians across the Top End was 103.6/100,000 and for Indigenous Australians > 14 years of age residing in urban Darwin or surrounds the incidence was 315.4/100,000 (Supplementary Fig. 2). Accounting for trends over time, the monthly incidence rate for the Indigenous population in Darwin in the four high-rainfall months of December through March was on average 4.2 times higher (95% CI 3.5-5.1) than the non-Indigenous population in Darwin and 2.2 times higher (95% CI 2.0-2.5) than the Indigenous population in remote Top End regions (Fig 3B) (see Supplementary Document 1 for methods).

Clinical Presentation

Of 1148 primary melioidosis presentations, 1013 (88.2%) were acute, 106 (9.2%) chronic and 29 (2.5%) were considered activation from latency (Fig. 2). Presentations with chronic melioidosis were predominantly with sub-acute pulmonary disease, often

mimicking tuberculosis (n = 44), or non-healing skin infections (n = 36). In comparison with those presenting with acute melioidosis, patients with chronic melioidosis were 6.5 times (95% CI 1.6-26) less likely to die from melioidosis (2/106 died compared with 125/1013).

Blood cultures were positive for *B. pseudomallei* in 633/1135 (56%), septic shock occurred in 240 (21%), usually on presentation or within 24 hours, 278 (24%) were managed in the intensive care unit, 180 (16%) required mechanical ventilation and 100 (9%) required renal replacement therapy.

Pneumonia was the primary diagnosis in 595 (52%) patients, skin abscesses in 149 (13%), of whom only five were bacteraemic and none died, and genitourinary infection in 140 (12%), of whom 103 (74%) were males with prostatic abscesses. Bacteraemia with no evident focus of infection was the presentation in 130 (11%), commonly immunocompromised patients with acute febrile illness. Children were 5.3 (95% CI 4.0-7.1) times more likely than adults to present with skin abscesses (28/48 compared with 121/1100) and 3.9 (95% CI 2.0-7.8) times less likely than adults to be bacteraemic (7/48 compared with 626/1087).

In multivariable analysis, presentation with pneumonia was independently associated with diabetes, chronic lung disease, rheumatic heart disease or cardiac failure, female sex, Indigenous ethnicity and presentation in the four high-rainfall months of December through March (Supplementary Table 2A). Presentation with skin abscesses reflected younger, healthier people (Supplementary Table 2B). Independent predictors of bacteraemia were age ≥50 years, diabetes, hazardous alcohol use,

chronic kidney disease, immunosuppression, malignancy, Indigenous ethnicity and presentation in the four high-rainfall months (Supplementary Table 2C).

Secondary foci were identified up to 3 weeks after admission: secondary pneumonia in 107/553 (19%) without primary pneumonia, secondary prostatic abscesses in 40, osteomyelitis in 36, septic arthritis in 29 and secondary skin lesions usually as multiple pustules in 21 (19 bacteraemic).

Since 1995 all patients had imaging for internal abscesses, with abdomen/pelvis CT scan or abdominal ultrasound. Supplementary Table 3 lists organ abscesses and other infection foci. Notably 99 patients showed CT-scan evidence of mediastinal lymphadenopathy/inflammatory-masses and 10 an inflammatory gastrointestinal mass. Over the 30 years only 2 patients (0.2%) had parotid abscess, both adults.

Outcomes

For the 60 patients with one or more recurrence, median time from the date of initial admission to first relapse was 8.2 (range 3.4-54) months, compared to 49 (range 10-225) months between initial admission and new infection. Correlates with relapse included diabetes (25/42 (60%) in relapse cases vs 430/979 (44%) in those with no relapse; $p = 0.046$), and chronic renal disease (11/42 (26%) in relapse cases vs 108/979 (11%) in those with no relapse; $p = 0.0027$).

Of the 133 deaths from melioidosis, 127 died during the initial melioidosis episode (Fig. 2); median time from admission to death of 4 days (range 0-481) (Supplementary Fig. 3A-C). Eight patients were dead before arrival at hospital and 11

died on the day of admission. Five patients died on relapse and 1 from a new melioidosis infection (Fig. 2). Three children died, all with risk factors.(20) For the remaining 130 deaths, median age was 55 (range 20-93) years, with primary diagnosis including pneumonia 90 (68%), bacteraemic sepsis without focus 18 (14%), genitourinary sepsis 9 (7%) and neurological melioidosis 4 (3%). Three of the 133 deaths occurred in patients without identified clinical risk factors and 97 (73%) had diabetes and/or hazardous alcohol use. In multivariable analysis independent predictors of mortality were age ≥50 years, rheumatic heart disease or cardiac failure, malignancy and presentation during the four high-rainfall months (Supplementary Table 2D).

The dynamic nature of epidemiology and outcomes over 30 years

Three major trends were identified: an increase in the incidence of melioidosis (Fig. 3A); a rising proportion of cases from urban Darwin and surrounds (Fig. 3B and Supplementary Fig. 4); and a decrease in mortality (Supplementary Table 4). During the first 5 years mortality from melioidosis was 31% (27/88), but this fell to 6% (17/278) in the last 5 years ($p < 0.0001$). This is unlikely to reflect improved case ascertainment capturing less severe disease. Over the 3 decades, there was no trend to increase in rates of bacteraemia or septic shock (Supplementary Table 4). Furthermore, there was a significant trend over the 3 decades of increase in the proportion aged ≥50 years, females and the proportions with diabetes, malignancy and immunosuppression, while the proportion with no risk factors significantly decreased (Supplementary Table 4, Supplementary Figure 4). The only significant change in clinical presentations was a trend of decreasing presentation with neurological melioidosis (Supplementary Table 4).

The dynamic nature of B. pseudomallei genotypes over 30 years

MLST of the 1108 (97%) available isolates from 1148 primary presentations revealed 349 *B. pseudomallei* STs of which 243 were found only in a single patient. *B. pseudomallei STs* from DPMS patients from Darwin and surrounding rural hamlets were analysed over the 30 years and contrasted with those STs in rural towns and remote Indigenous Top End communities. There was a large diversity of STs which was especially evident in rural and remote Top End regions (Supplementary Fig. 5). The number of different STs each year increased when rainfall and case numbers increased, but in urban Darwin and surrounds there was a trend over time to fewer ST numbers per case numbers (Supplementary Fig. 6). New STs continued to emerge each year, but at a diminishing rate, with significantly lower ST diversity in years 21-30 in comparison to years 1-10 (Supplementary Fig. 6).

The relative proportions of the common urban STs 36, 109 and 132 remained constant (Supplementary Fig. 5). ST553 was rare in earlier years but increased to become the commonest ST over the last 5 years. Environmental sampling revealed a suburban hotspot for ST553, with ST553 cases clustering in this area.(21) The first case of melioidosis due to ST562 occurred in urban Darwin in 2005(22), and since then the proportion of cases due to this ST has increased as this presumptively introduced Asian ST has become more widely established.

Discussion

Northeast Thailand and the Top End of Australia are the two locations where melioidosis has been consistently documented as a major cause of community-acquired sepsis and in particular community-acquired pneumonia,(23, 24) both having annual incidence rates of around 20 cases/100,000, with year-to-year variability linked to rainfall and with incidence rates increasing over time.(25, 26)

The Darwin prospective melioidosis study highlights the concept that *B. pseudomallei* is an opportunistic pathogen.(27) With rapid diagnosis, availability of appropriate specific antimicrobial therapy (ceftazidime or meropenem) and access to state-of-the-art intensive care, overall mortality of melioidosis can be <10%, with death a very rare outcome for healthy hosts. The relative contributions to the decrease in mortality in the Darwin study remains uncertain but it has been noted that the reduction in mortality coincided with the introduction of an intensivist-led model of care and the empiric use of meropenem for critically ill patients.(28) That mortality remains 40% or higher in many melioidosis-endemic regions reflects delays in presentation, limited laboratory resources and access to therapy and intensive care.(25, 29, 30) Indigenous Australians had higher incidences of melioidosis throughout the study, likely reflecting higher rates of both clinical risk factors and environmental exposure. Overall mortality was not higher for Indigenous Australians and Indigenous ethnicity was not an independent risk factor for death from melioidosis.

Support for recognition of melioidosis as an opportunistic infection is reflected in the findings from the Darwin study that clinical illness (melioidosis) and severe disease and death after exposure to *B. pseudomallei* are determined predominantly by host clinical risk factors. Each wet season weekend thousands of children and healthy

406 adults undertake recreational activities in often muddy sport fields, gardens and
407 various tropical Darwin environments, where previous studies isolated *B.*
408 *pseudomallei* from 7/10 popular sport fields.(31) Despite this exposure and the
409 substantial increase in case numbers over 30 years, the number of children with
410 melioidosis remains at 0-3 each year. The commonest presentation in children was
411 skin abscesses, mostly single, without fever and with no bacteraemia or evidence for
412 infection elsewhere, likely reflecting robust innate and adaptive immunity controlling
413 the inoculated bacteria at the skin and preventing bacteraemic spread.
414
415 There remain important gaps in understanding the epidemiology of infection with and
416 disease from *B. pseudomallei*. This is reflected in the paradox that, despite similar
417 melioidosis incidence rates, seropositivity for *B. pseudomallei* in northeast Thailand is
418 as high as 50%(32), while in northern Australia seropositivity is under 5%.(33)
419 Substantial funding of research on melioidosis diagnostics, pathogenesis, therapeutics
420 and vaccines has been driven by the concern that aerosolized *B. pseudomallei* poses a
421 serious potential biothreat.(4) While inhalation of aerosols was postulated as the mode
422 of infection in helicopter crews with melioidosis pneumonia during the Vietnam war,
423 the relative contributions in endemic areas of inhalation, percutaneous inoculation and
424 ingestion of *B. pseudomallei* remain unclear, as do the range of clinical scenarios
425 following each mode of infection. It has been postulated that the far higher rates of
426 parotid and liver abscesses in Thailand and elsewhere in southeast Asia than seen in
427 Australia may be attributed to a higher rate of ingestion as the mode of infection in
428 southeast Asia, where water supplies are often unchlorinated in rural regions. (27, 34)
429

While the spectrum of presentations of melioidosis is diverse, over half of cases in this and other studies present with pneumonia.(23, 24, 35) While the severity and outcome of melioidosis are predominantly driven by the predisposing host clinical risk factors, the contribution of mode of infection impacts particularly on melioidosis pneumonia.(4, 27) Aerosol inhalation of *B. pseudomallei* during severe weather events is supported by epidemiological analysis, aerosol sampling studies and the common radiological findings of mediastinal involvement. In Singapore proportionally more presentations were with pneumonia during heavy monsoonal seasons(35), consistent with our findings demonstrating strong correlations of high-rainfall months with pneumonia, bacteraemia and mortality. Air sampling in Taiwan during typhoons linked detection of *B. pseudomallei* with a surge of melioidosis downwind in an urban environment.(36) Whole-genome sequencing allowed us to match *B. pseudomallei* from air sampling to clinical *B. pseudomallei* isolates from a patient with pneumonia and mediastinal melioidosis.(37)

The global footprint of environmental *B. pseudomallei* remains unknown but is informed by improved environmental sampling and melioidosis case detection. Recent reports from the Americas suggest both unmasking of previously unrecognized endemic melioidosis in South America and a dynamic situation potentially with spread of *B. pseudomallei* northwards in the Caribbean and Central America.(38, 39) The report of two possibly endemic cases of melioidosis in Texas,(10) necessitates targeted environmental sampling in the southern United States to establish whether *B. pseudomallei* is endemic. Such confirmation would have major implications for current biosecurity rulings around the laboratory handling and transport of *B. pseudomallei* as a listed Tier 1 select agent.

Genotyping of recent clinical and environmental isolates of *B. pseudomallei* from Puerto Rico supports possible dispersal through severe weather events such as hurricanes.(39) Genotyping of 30 years of DPMS isolates shows a large genomic diversity reflecting the ancient origins of *B. pseudomallei* on the Australian continent, but also the dynamic nature of *B. pseudomallei* in a high-incidence endemic setting. While several dominant genotypes have persisted throughout the 30 years, two *B. pseudomallei* genotypes have emerged with each reflecting a different epidemiology. ST553 has increased to become the dominant genotype in the Darwin region, having proliferated during a period of intense urban construction. ST562 emerged in 2005 as a new genotype for urban Darwin. It is phylogenetically clearly an ST of Asian origin, with ST562 also reported from Hainan Island, China and Taiwan.(22) The lack of diversity amongst Australian ST562 strains suggests a recent point source introduction to northern Australia from Asia, but how and when this occurred remains unknown.

There are some limitations of this study. Over the 30 years we continued to use the original definitions for risk factors and septic shock(11), without adding contemporary pneumonia and sepsis severity scores developed since this prospective study commenced. Limiting severity analysis to bacteraemia, septic shock and death precluded a more in-depth analysis of pathogenesis. Phylogeographical analysis of *B. pseudomallei* genotypes is dependent on accurate location information and even with prospective collection of real-time patient history, attribution of location of infection will sometimes be incorrect for those who work and travel across the regions of the Top End. Our assessment of the extent of internal organ infection from disseminated

melioidosis is likely to be an underestimate, as routine imaging only commenced for all patients in 1995 and even with routine imaging less extensive organ involvement may be missed.

In conclusion, the 30-year prospective study of melioidosis cases in the tropical Northern Territory confirms the importance of diabetes as the major risk factor for melioidosis and defines the spectrum of presentations and disease in a well-resourced environment. Early diagnosis, specific antimicrobial therapy and state-of-the-art intensive care therapy have reduced the mortality from melioidosis to under 10% overall and to zero in healthy individuals. Genotyping of *B. pseudomallei* informs both local molecular epidemiology and the evolving global melioidosis story.

Contributors

Conceptualization (BJC); Patient data collection, management and follow up (BJC, EMM, RWB, RNP, CSM, APR, ES, JD, SHE, SJ, SL, PM, VLK, NMA); Project administration (BJC, MM); Supervision (BJC, MM, VLK, NMA); Laboratory procedures (RWB, MM, MK, JRW); Data curation (LMW, MM, JRW); Data accessed and verified (BJC, LMW, EMM). Formal analysis including genomics and visualization (LMW, MK, EMM, JRW, CW, PM, MM, BJC); Original draft preparation (BJC); Review and editing (all); Funding acquisition (BJC, MM, MK, NMA, EMM, VLK).

Acknowledgements

We thank the numerous medical, nursing, laboratory and public health colleagues at Royal Darwin Hospital, the Northern Territory Department of Health and Menzies

505 School of Health Research, who over the 30 years of the study have assisted with
506 patient diagnosis and treatment, data collection and *B. pseudomallei* identification and
507 genotyping. We are grateful to Erin Price and Derek Sarovich (University of the
508 Sunshine Coast), who established and led the Menzies *B. pseudomallei* genomics
509 program for seven years. We also thank Vanessa Rigas and Glenda Harrington who
510 continue to curate the Menzies *B. pseudomallei* collection. Population estimates were
511 kindly provided by Steve Guthridge. Continuing inspiration is provided by many
512 colleagues from around the world who share their experiences through the
513 International Melioidosis Network
514 (https://groups.google.com/forum/#!forum/melioidosis).
515
516 **Funding**
517 The research was funded under the Australian National Health and Medical Research
518 Council grant numbers 1046812, 1098337, 1142011, 1135820 and 1131932 (The
519 HOT NORTH initiative).
520
521 **Data sharing**
522 Individual patient data will not be available, but the data dictionary and bacterial
523 genomic data can be provided on request.
524
525 **Conflicts of interest**
526 We declare no conflicts of interest.
527 **References**
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Tables

TABLE 1

EPIDEMIOLOGY, CLINICAL RISK FACTORS AND OUTCOMES FOR 1148 PATIENTS WITH MELIOIDOSIS

		Patients		Died from melioidosis						
		n	% of total	*n*	Mortality					
Epidemiology	Age <15y	48	4.2%	3	6.3%					
	Age 15-49y	524	46%	45	8.6%					
	Age 50+	576	50%	85	15%					
	Male	721	63%	86	12%					
	Female	427	37%	47	11%					
	Indigenous Australian	600	52%	72	12%					
	Non-Indigenous	548	48%	61	11%					
Regions	Darwin urban	632	55%	81	13%					
	Darwin rural hamlets	154	13%	12	7.8%					
	Regional & remote Top End	349	30%	37	11%					
	Outside Top End	13	1.1%	3	23%					
Clinical Risk factors						***p*[3]**	**RR**	**95% CI**		
	Diabetes	513	45%	62	12%	0.72	1.1	0.78	-	1.5
	Hazardous alcohol use	455	40%	56	12%	0.72	1.1	0.80	-	1.5
	Chronic lung disease	312	27%	45	14%	0.13	1.4	0.98	-	1.9
	Chronic renal disease	140	12%	24	17%	0.10	1.6	1.06	-	2.4
	Malignancy	111	9.7%	20	18%	0.10	1.7	1.07	-	2.6
	Immunosuppressive therapy and other immunosuppression[1]	106	9.2%	18	17%	0.13	1.5	0.98	-	2.4
	Rheumatic heart disease and/or congestive cardiac failure	102	8.9%	19	19%	0.10	1.7	1.10	-	2.7
	Kava use	39	3.4%	5	13%	0.80	1.1	0.48	-	2.6
	Other[2]	39	3.4%	7	18%	0.29	1.6	0.79	-	3.2
	No clinical risk factors	186	16%	3	2.3%	<0.0001	0.12	0.04	-	0.37

[1]Clinical risk factor parameters as defined in refs 11 and 12 and Supplementary Figure 1

[2]Includes the only 4 patients with HIV infection (0.3%); a rate similar to that seen at Royal Darwin Hospital for sepsis in general.

[3]P values adjusted for multiple testing using the False Discovery Rate (FDR) method.

Figure 1

Click here to access/download;Figure;FIGURE 1.tif

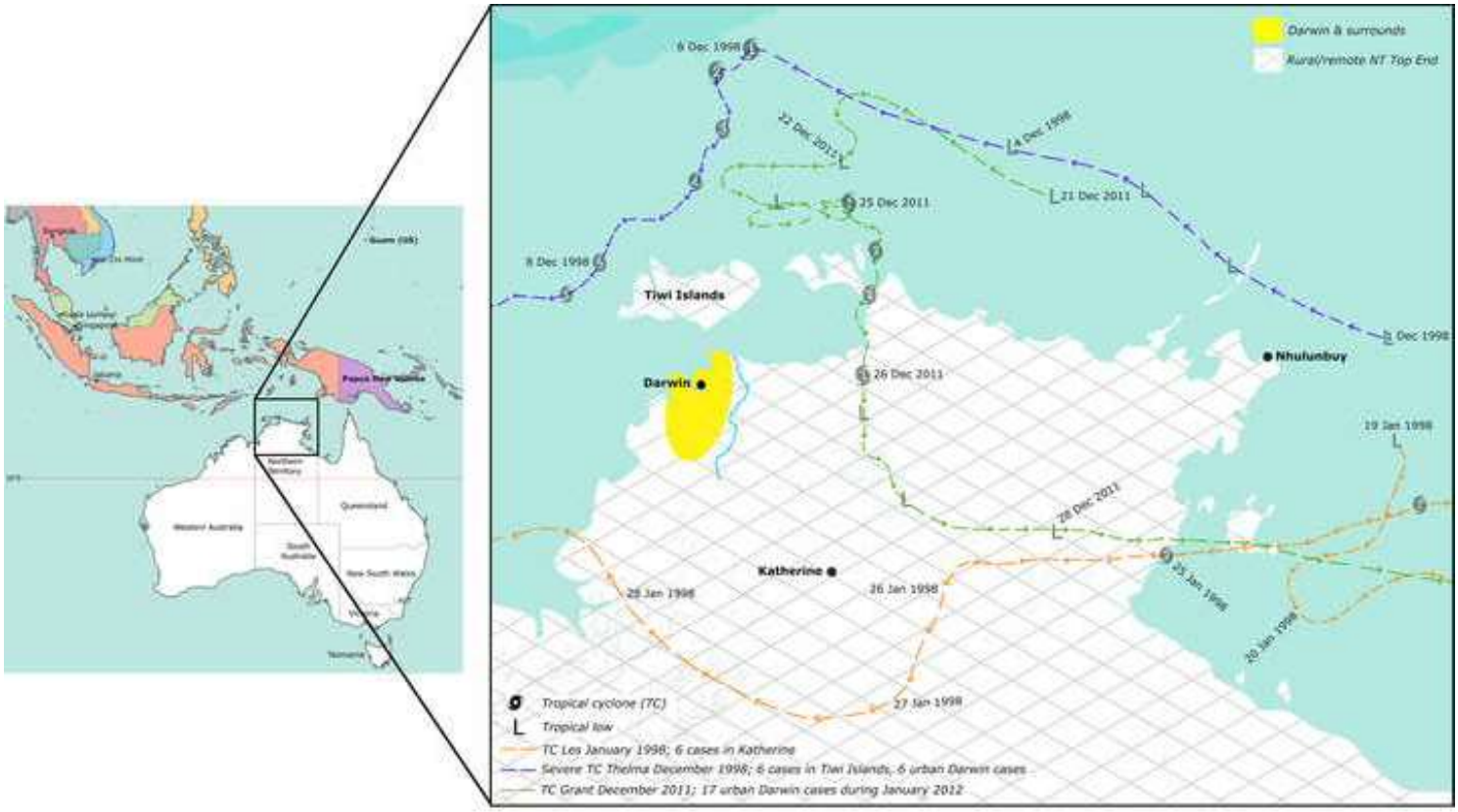

Figure 2

Click here to access/download;Figure;FIGURE 2.tiff

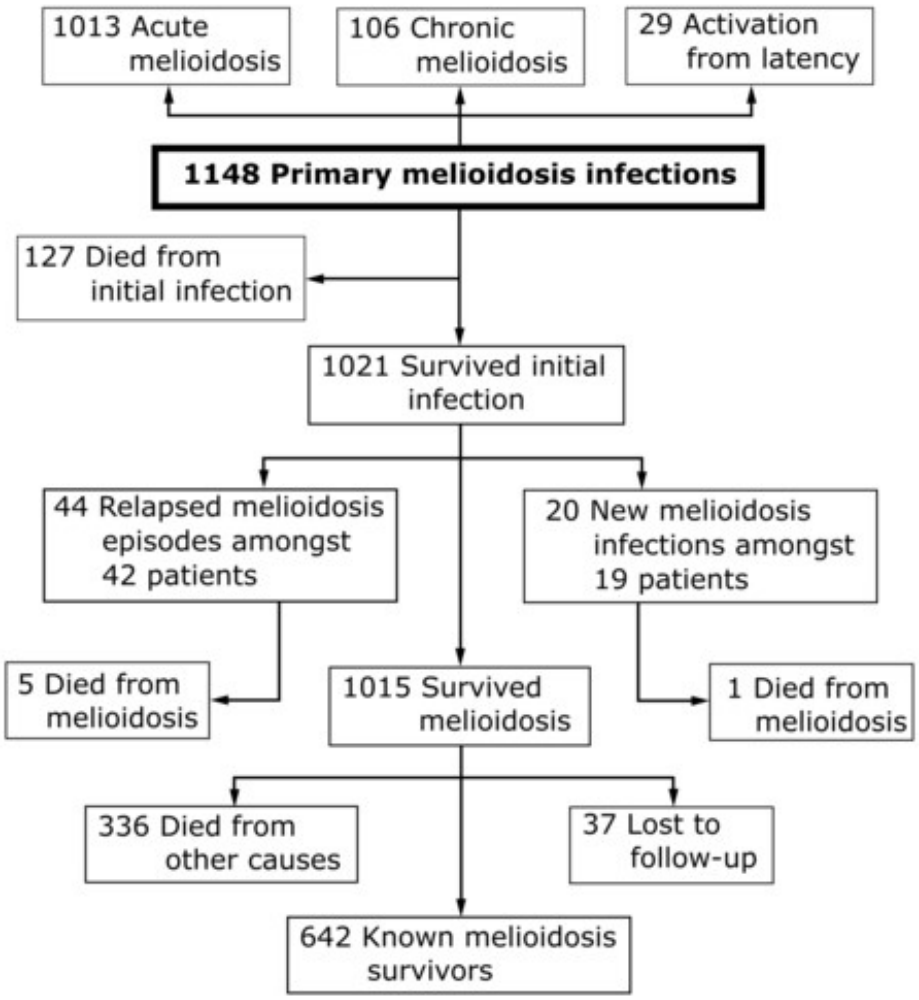

Figure 3A

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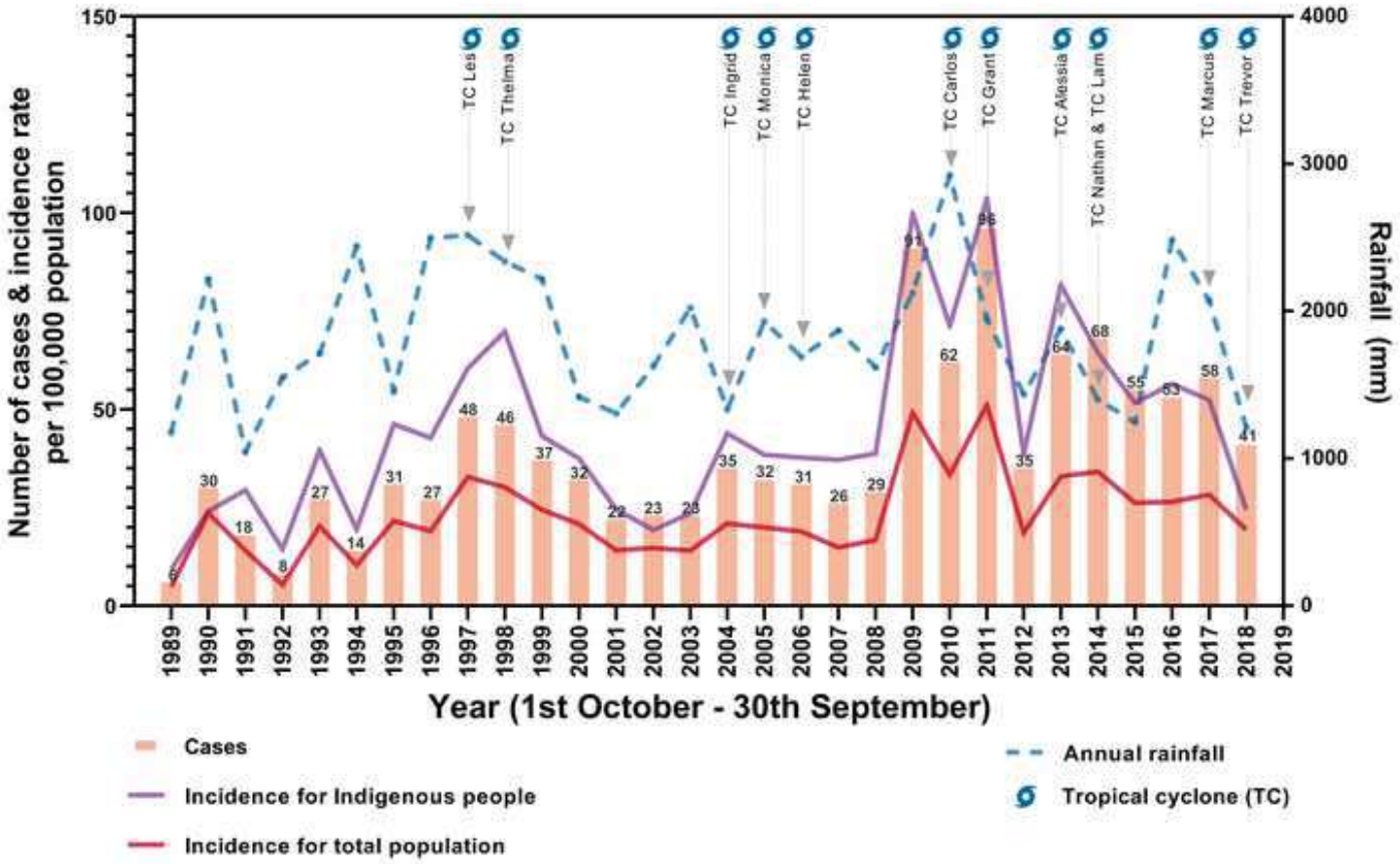

Figure 3B

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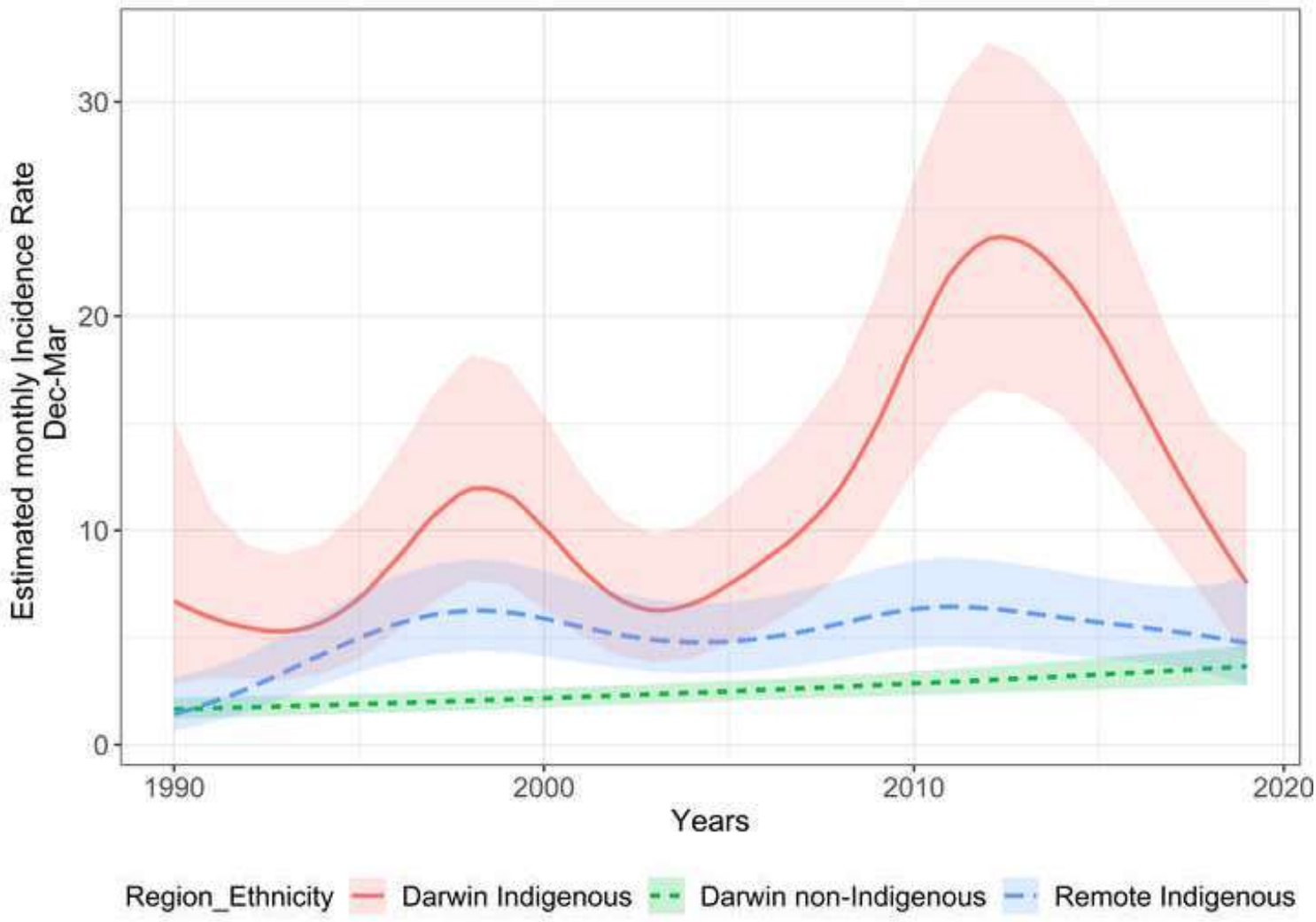


SUPPLEMENTARY TABLE 1 PRESUMPTIVE INFECTION EVENTS IN 255 PATIENTS

Nature of Event	Number of cases	Event examples
Cut skin documented	93 (36%)	15 gardening, 8 stick injury, 6 hunting, 5 fishing, 5 playing sports, 4 whipper-snipper/weed-wacker injury, 3 splinter, 2 swimming, 1 puncture wound, 1 pitchfork in duck yard
Injury/accident with soil/water exposure	14 (5%)	5 motor vehicle accident, 2 ingrown toenail resection, 1 crush injury, 1 fall from bike, 1 fall from flying fox, 1 fall from ladder, 1 fall from tree, 1 fall onto bitumen/asphalt, 1 potting mix in mouth post dental work
Flood water exposure	22 (9%)	17 cut skin
Rainstorm exposure	35 (14%)	6 cyclone exposures, 6 exposed under shelter, 1 fishing
Gardening	18 (7%)	4 mowing lawn, 2 mulching
High pressure hosing	8 (3%)	
Ingestion	2 (1%)	1 eating muddy sandwich, 1 mastitis breast milk ingestion
Bite/sting	8 (3%)	4 dog, 2 insect, 1 crocodile, 1 jellyfish
Burns	4 (2%)	
Children playing in muddy yard	5 (2%)	
Other surface water/soil/mud exposure - non-occupational	21 (8%)	3 aerosolized soil exposures, 3 car washing/cleaning, 2 walking in yard, 2 digging trench, 1 hunting, 1 clearing drains
Other surface water/soil/mud exposure - occupational	18 (7%)	4 earthmovings, 3 digging trench, 3 drilling, 1 fencing, 1 jackhammering, 1 cleaning pumps, 1 brick laying, 1 clearing drains, 1 commando crawling, 1 cleaning
Swimming in river or waterhole	7 (3%)	

SUPPLEMENTARY TABLE 2A ASSOCIATIONS WITH PRESENTATION WITH PNEUMONIA

	Patients		Prim Pneu		Bivariate		Multivariable				
	n	%	*n*	%	*p*[1]	OR	*p*	OR	OR 95% CI		
Age ≥50											
No	572	50%	274	48%	0.012		0.065				
Yes	576	50%	321	56%		1.4		1.3	0.98	-	1.7
Diabetes											
No	635	55%	286	45%	<0.0001		0.0004				
Yes	513	45%	309	60%		1.8		1.6	1.2	-	2.1
Hazardous alcohol use											
No	693	60%	338	49%	0.013		0.089				
Yes	455	40%	257	57%		1.4		1.3	0.96	-	1.6
Chronic lung disease											
No	836	73%	382	46%	<0.0001		<0.0001				
Yes	312	27%	213	68%		2.6		2.4	1.8	-	3.3
RHD/CCF											
No	1046	91%	524	50%	0.0003		0.023				
Yes	102	8.9%	71	70%		2.3		1.7	1.1	-	2.7
Season											
Apr-Nov	381	33%	146	38%	<0.0001		<0.0001				
Dec-Mar	767	67%	449	59%		2.3		2.2	1.7	-	2.8
Indigenous											
No	548	48%	244	45%	<0.0001		0.0001				
Yes	600	52%	351	59%		1.8		1.8	1.4	-	2.5
Sex											
Male	720	63%	340	47%	0.0001		0.011				
Female	428	37%	255	60%		1.6		1.4	1.1	-	1.9
Region											
Darwin Urban	785	68%	424	54%	0.026		0.0011				
Remote Top End	363	32%	171	47%		0.8		0.6	0.4	-	0.8
Kava											
No	1109	97%	584	53%	0.0043		0.038				
Yes	39	3.4%	11	28%		0.4		0.4	0.2	-	1.0

[1]P values adjusted for multiple testing using the False Discovery Rate (FDR) method.

SUPPLEMENTARY TABLE 2B ASSOCIATIONS WITH PRESENTATION WITH SKIN ABSCESS(ES)

	Patients		Primary Skin		Bivariate		Multivariable			
	n	%	*n*	%	*p*[1]	OR	*p*	OR	OR 95% CI	
Age ≥50										
No	572	50%	101	18%	<0.0001		0.0021			
Yes	576	50%	48	8.3%		0.4		0.5	0.3	0.8
Diabetes										
No	635	55%	127	20%	<0.0001		<0.0001			
Yes	513	45%	22	4.3%		0.2		0.2	0.1	0.4
Hazardous alcohol use										
No	693	60%	111	16%	0.0002		0.0004			
Yes	455	40%	38	8.4%		0.5		0.5	0.3	0.7
Chronic lung disease										
No	836	73%	140	17%	<0.0001		<0.0001			
Yes	312	27%	9	2.9%		0.1		0.2	0.1	0.4
RHD/CCF										
No	1046	91%	146	14%	0.0006		*p*≥0.100			
Yes	102	8.9%	3	2.9%		0.2				
Season										
Apr-Nov	381	33%	73	19%	<0.0001		<0.0001			
Dec-Mar	767	67%	76	10%		0.5		0.5	0.4	0.8
Indigenous										
No	548	48%	114	21%	<0.0001		<0.0001			
Yes	600	52%	35	5.8%		0.2		0.3	0.2	0.4
Immunosuppression										
No	1042	91%	147	14%	0.0001		0.0074			
Yes	106	9.2%	2	1.9%		0.1		0.1	0.03	0.6
Malignancy										
No	1037	90%	146	14%	0.0002		0.011			
Yes	111	10%	3	2.7%		0.2		0.2	0.1	0.7
Chronic kidney disease										
No	1008	88%	147	15%	<0.0001		0.018			
Yes	140	12%	2	1.4%		0.1		0.2	0.04	0.8
Kava consumption										
No	1109	97%	149	13%	0.0065	(skin *n*=0)				
Yes	39	3.5%	0	0%						

[1]P values adjusted for multiple testing using the False Discovery Rate (FDR) method.

SUPPLEMENTARY TABLE 2C ASSOCIATIONS WITH BACTERAEMIA ON PRESENTATION

	Patients		Bacteremic		Bivariate		Multivariable				
	n	%	*n*	%	*p*[1]	OR	*p*	OR	OR 95% CI		
Indigenous											
No	548	48%	246	45%	<0.0001		<0.0001				
Yes	600	52%	387	65%		2.2		2.3	1.8	-	3.1
Age ≥50											
No	572	50%	276	48%	<0.0001		<0.0001				
Yes	576	50%	357	62%		1.7		2.0	1.5	-	2.6
Diabetes											
No	635	55%	289	46%	<0.0001		<0.0001				
Yes	513	45%	344	67%		2.4		2.1	1.6	-	2.8
Hazardous alcohol use											
No	693	60%	358	52%	0.0041		0.0001				
Yes	455	40%	275	60%		1.4		1.7	1.3	-	2.2
Chronic kidney disease											
No	1008	88%	525	52%	<0.0001		0.0003				
Yes	140	12%	108	77%		3.1		2.2	1.4	-	3.5
Malignancy											
No	1037	90%	552	53%	0.0001		0.0027				
Yes	111	10%	81	73%		2.4		2.1	1.3	-	3.5
Immunosuppression											
No	1042	91%	547	53%	<0.0001		<0.0001				
Yes	106	9.2%	86	81%		3.9		4.4	2.6	-	7.6
RHD_CCF											
No	1046	91%	565	54%	0.016		*p*≥0.100				
Yes	102	8.9%	68	67%		1.7					
Season											
Apr-Nov	381	33%	175	46%	<0.0001		0.0004				
Dec-Mar	767	67%	458	60%		1.7		1.6	1.2	-	2.1

[1]P values adjusted for multiple testing using the False Discovery Rate (FDR) method.

SUPPLEMENTARY TABLE 2D ASSOCIATIONS WITH MORTALITY

	Patients		Died		Bivariate		Multivariable				
	n	%	*n*	%	*p*[1]	OR	*p*	OR	OR 95% CI		
Age ≥50											
No	572	50%	48	8.4%	0.0034		0.0027				
Yes	576	50%	85	15%		1.9		1.8	1.2	-	2.7
Chronic lung disease											
No	836	73%	88	11%	0.0788		*p*≥0.100				
Yes	312	27%	45	14%		1.4					
RHD/CCF											
No	1046	91%	114	11%	0.0543		0.012				
Yes	102	9%	19	19%		1.9		2.0	1.2	-	3.6
Immunosuppression											
No	1042	91%	115	11%	0.0788		*p*≥0.100				
Yes	106	9.2%	18	17%		1.6					
Malignancy											
No	1037	90%	113	11%	0.0543		0.0198				
Yes	111	10%	20	18%		1.8		2.0	1.1	-	3.4
Chronic kidney disease											
No	1008	88%	109	11%	0.0543		0.058				
Yes	140	12%	24	17%		1.9		1.6	0.98	-	2.7
Season											
Apr-Nov	381	33%	31	8.1%	0.0285		0.033				
Dec-Mar	767	67%	102	13%		1.7		1.6	1.04	-	2.5
Decade											
1989-1998	252	22%	51	20%	0.0001						
1999-2008	286	25%	28	9.8%		0.43	0.0005	0.41	0.24	-	0.67
2009-2018	610	53%	54	8.9%		0.38	<0.0001	0.32	0.21	-	0.50

[1]P values adjusted for multiple testing using the False Discovery Rate (FDR) method.

Note: diabetes and hazardous alcohol use each had p>0.100 on bivariate analysis and therefore were not included in the model.

SUPPLEMENTARY TABLE 3 INTERNAL ORGAN ABSCESSES AND OTHER FOCI OF INFECTION FROM 1148 CASES OF MELIOIDOSIS

Site	Number (%)
Prostatic abscess(es)	143 (20%)[1]
Mediastinal lymphadenopathy/mass	99 (9%)
Splenic abscess(es)	72 (6%)
Liver abscess(es)	47 (3%)
Kidney abscess(es)	37 (3%)
Muscle abscess(es) [2]	37 (3%)
Lymphadenitis	24 (2%)
Pericarditis	11 (<1%)
Para-intestinal mass	10 (<1%)
Brain abscess	7 (<1%)
Subphrenic abscess	7 (<1%)
Mycotic (pseudo)aneurysm	6 (<1%)
Epididymo-orchitis	6 (<1%)
Adrenal abscess	5 (<1%)
Mastitis/breast abscess	5 (<1%)
Extradural abscess	3 (<1%)
Parotid abscess	2 (<1%)

[1] Calculated for males

[2] Psoas, calf, thigh most common

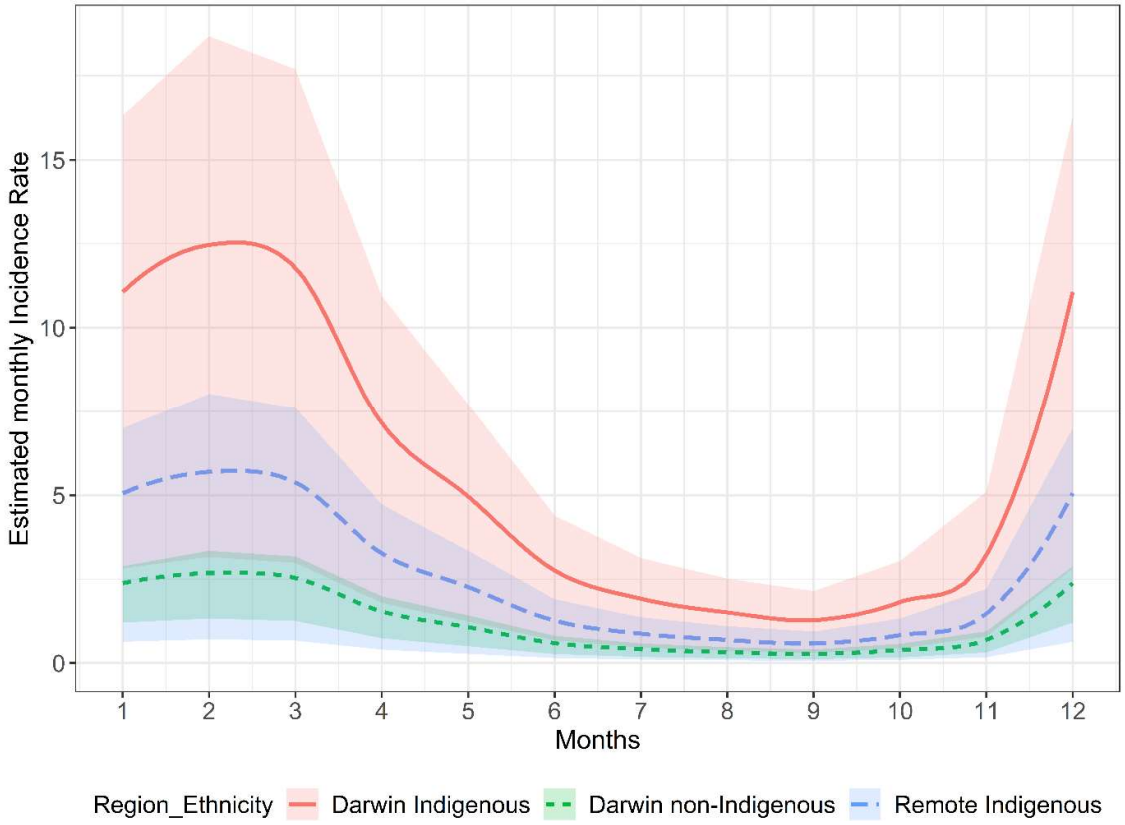
Note: Routine abdominal imaging on all cases commenced in 1995. Prior to 1995 abdominal imaging was performed at clinician discretion.

SUPPLEMENTARY TABLE 4 EPIDEMIOLOGY, CLINICAL RISK FACTORS, CLINICAL PRESENTATIONS AND OUTCOMES BY DECADE

		1989/1990-1998/1999 *n*=252		1999/2000-2008/2009 *n*=286		2009/2010-2018/2019 *n*=610		*p* (trend)
		n	%	*n*	%	*n*	%	
Epidemiology	Male	190	75%	180	63%	351	58%	<0.0001
	Indigenous Australian	130	52%	148	52%	322	53%	-
	Age <15y	9	3.6%	15	5.2%	24	3.9%	-
	Age 15-49y	129	51%	141	49%	254	42%	
	Age 50+	114	45%	130	46%	332	54%	0.0051
	Urban Darwin and surrounds	152	60%	179	63%	455	75%	<0.0001
Clinical Risk Factors	Diabetes	93	37%	117	41%	303	50%	0.0002
	Hazardous alcohol use	95	38%	115	40%	245	40%	-
	Chronic lung disease	64	25%	77	27%	171	28%	-
	Chronic renal disease	24	10%	40	14%	76	13%	-
	Malignancy	11	4.4%	21	7.3%	79	13%	<0.0001
	Immunosuppressive therapy and other immunosuppression	14	5.6%	17	5.9%	75	12%	0.0004
	Rheumatic heart disease and/or congestive cardiac failure	17	6.7%	23	8.0%	62	10%	0.091
	Kava use	20	7.9%	7	2.4%	12	2.0%	<0.0001
	No clinical risk factors	54	21%	52	18%	80	13%	0.0015
Clinical Presentations and Outcomes	Pneumonia	127	50%	149	52%	319	52%	-
	Genitourinary	37	15%	38	13%	65	11%	0.081
	Bacteraemia no evident focus	23	9.1%	37	13%	70	12%	-
	Skin infection	31	12%	38	13%	80	13%	-
	Soft tissue abscess(es)	13	5.2%	6	2.1%	27	4.4%	-
	Neurological	9	3.6%	5	1.7%	5	0.8%	0.0045
	Osteomyelitis	3	1.2%	4	1.4%	8	1.3%	-
	Septic arthritis	6	2.4%	7	2.4%	16	2.6%	-
	Other diagnosis	3	1.2%	2	0.7%	20	3.3%	-
	Bacteraemic	118	47%	180	63%	335	55%	0.15
	Septic shock	49	19%	72	25%	119	20%	-
	ICU admission	39	16%	92	32%	147	24%	0.069
	Mechanical ventilation	38	15%	66	23%	76	12%	-
	Died from melioidosis	51	20%	28	10%	54	9%	<0.0001

SUPPLEMENTARY FIGURE 1

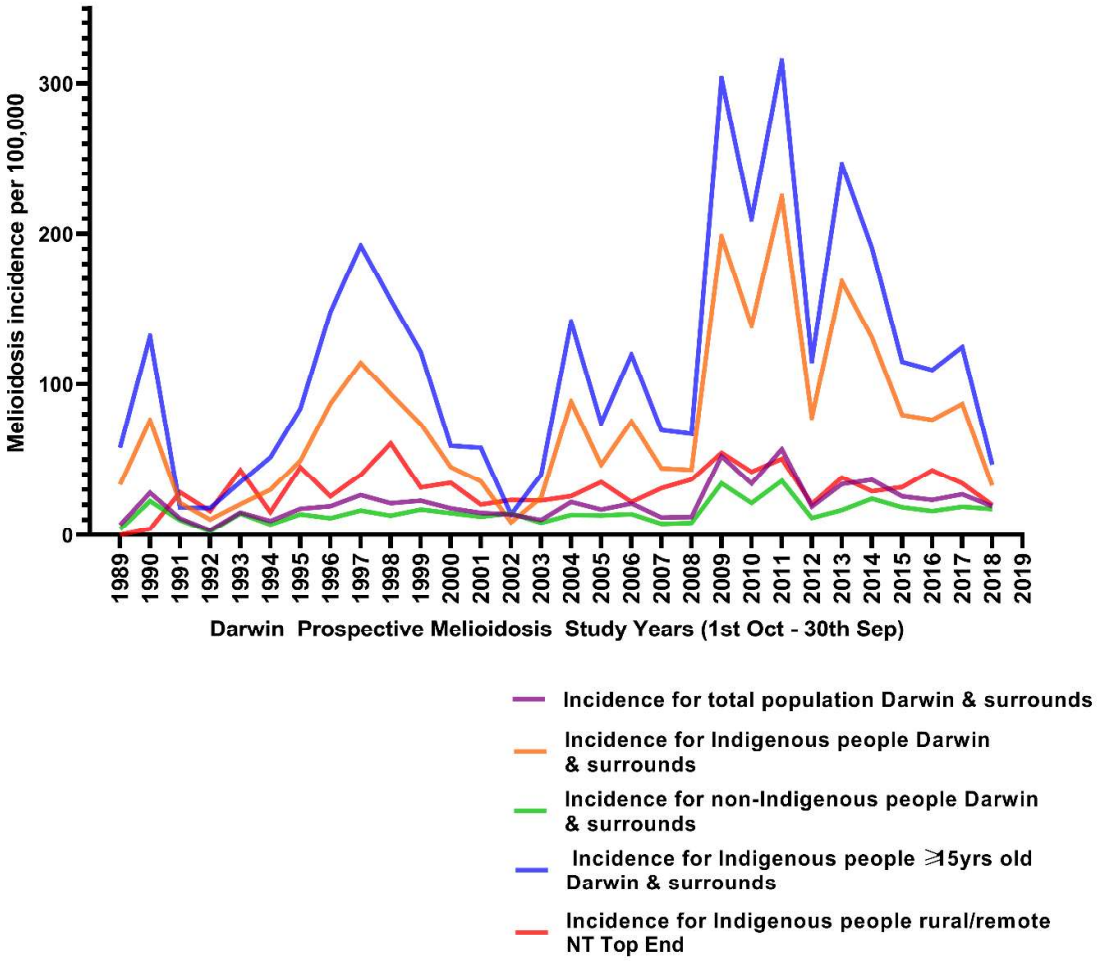
ESTIMATED INCIDENCE RATES BY MONTH, ETHNICITY AND REGION FOR THE 30 YEARS



See Supplementary Document 1 for generalized additive model (GAM) methods. Shaded colour represents 95% CI for the estimated monthly incidence rates for each of the three groups.

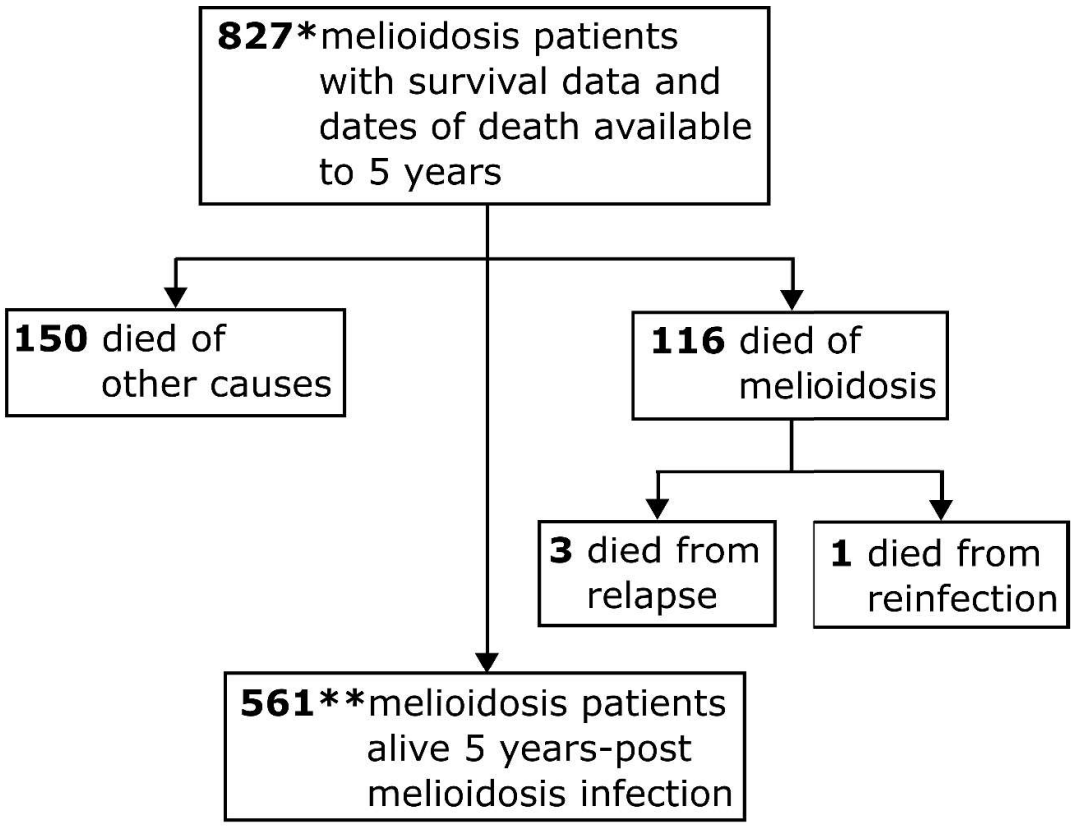
SUPPLEMENTARY FIGURE 2

INCIDENCE RATES BY REGION AND ETHNICITY OVER 30 YEARS



SUPPLEMENTARY FIGURE 3A

KAPLAN-MEIER SURVIVAL ANALYSIS DATA

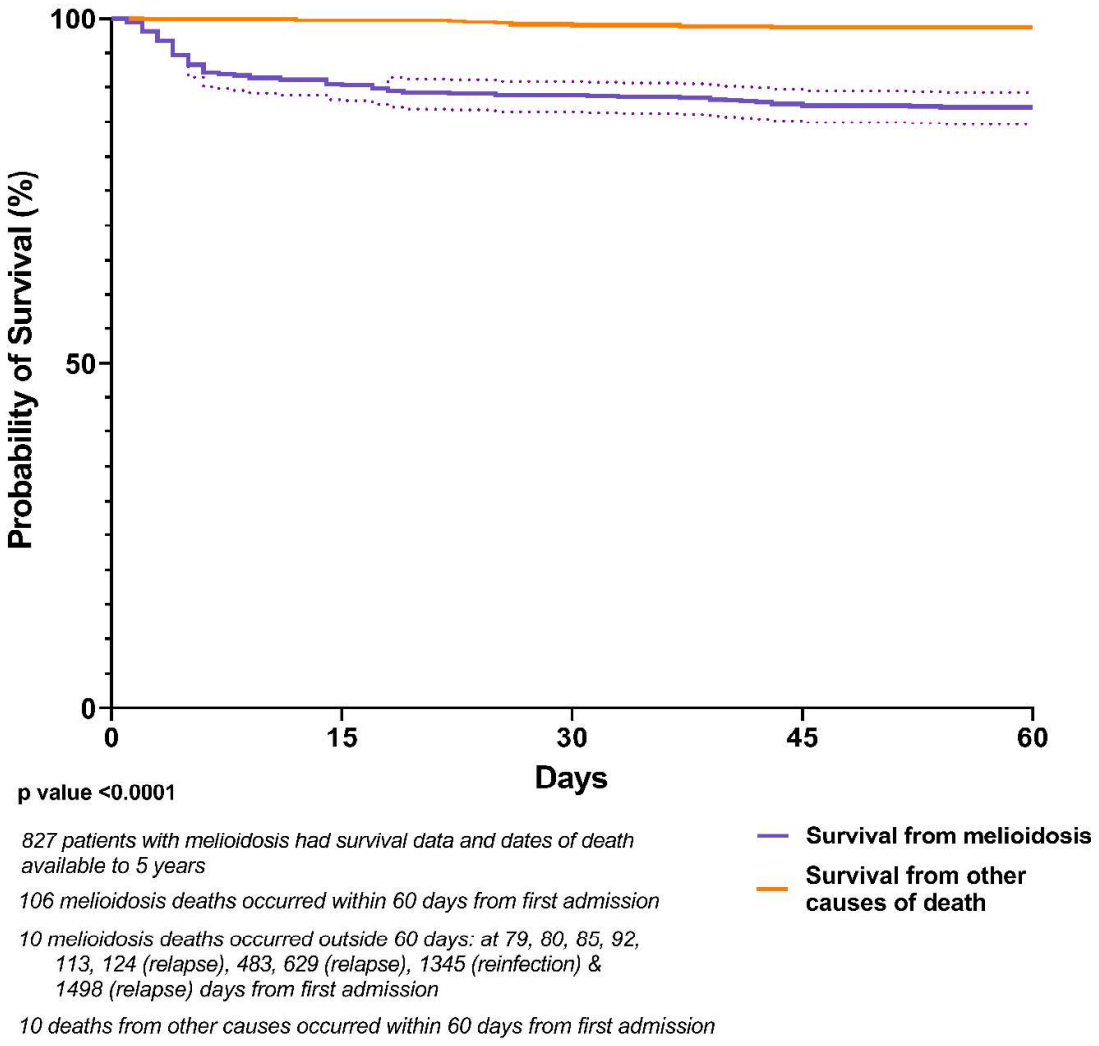


** 321 DPMS patients were not included in the Kaplan-Meier survival analysis because either their admission date was after September 30th 2014 or they were lost to follow-up or their date of death was not recorded*

*** An additional 142 deaths occurred from other causes amongst these 561 patients over subsequent years but there have been no further deaths from melioidosis*

SUPPLEMENTARY FIGURE 3B

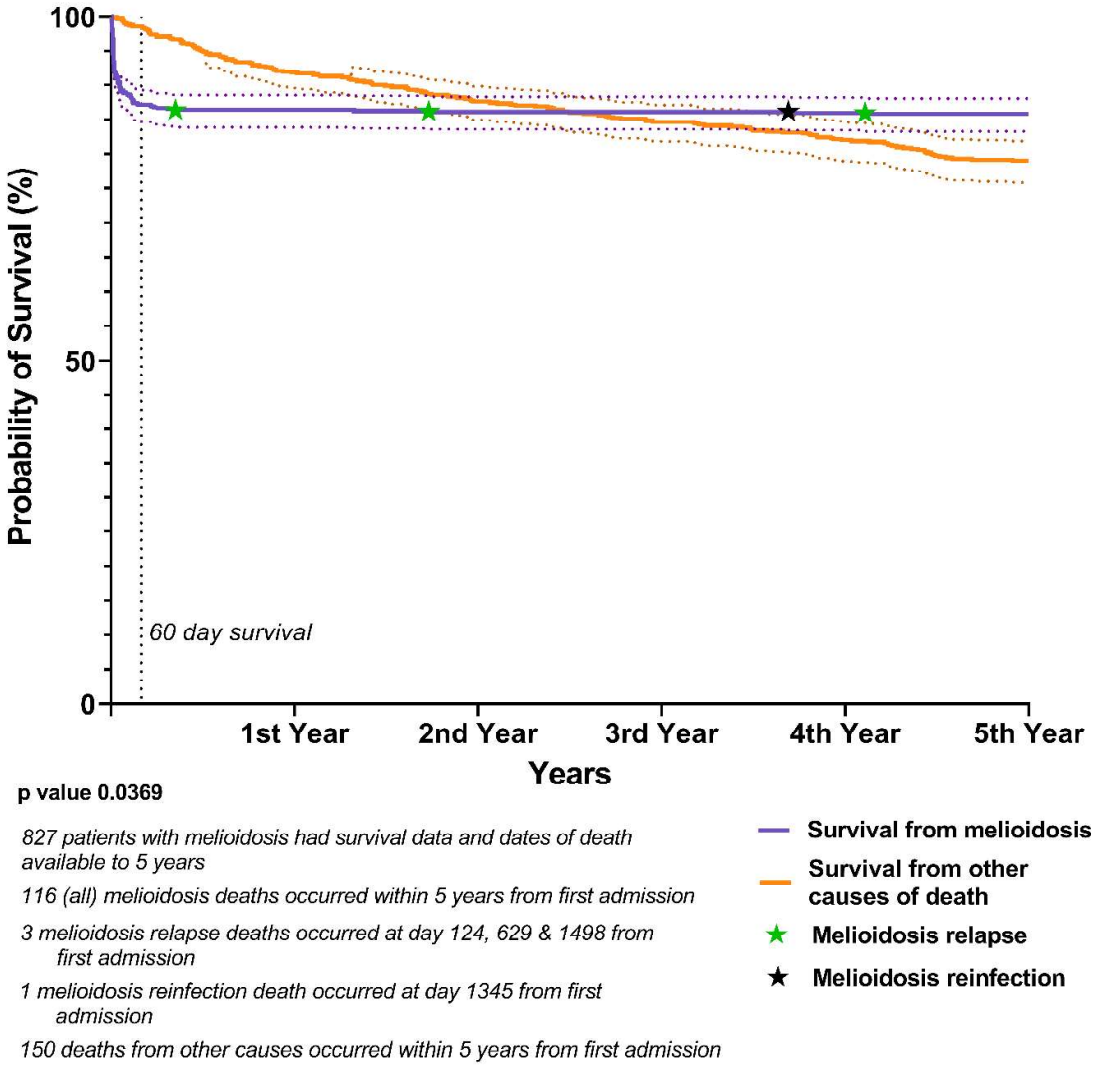
KAPLAN-MEIER 60 DAY SURVIVAL ANALYSIS



95% confidence intervals were used for error bars.

SUPPLEMENTARY FIGURE 3C

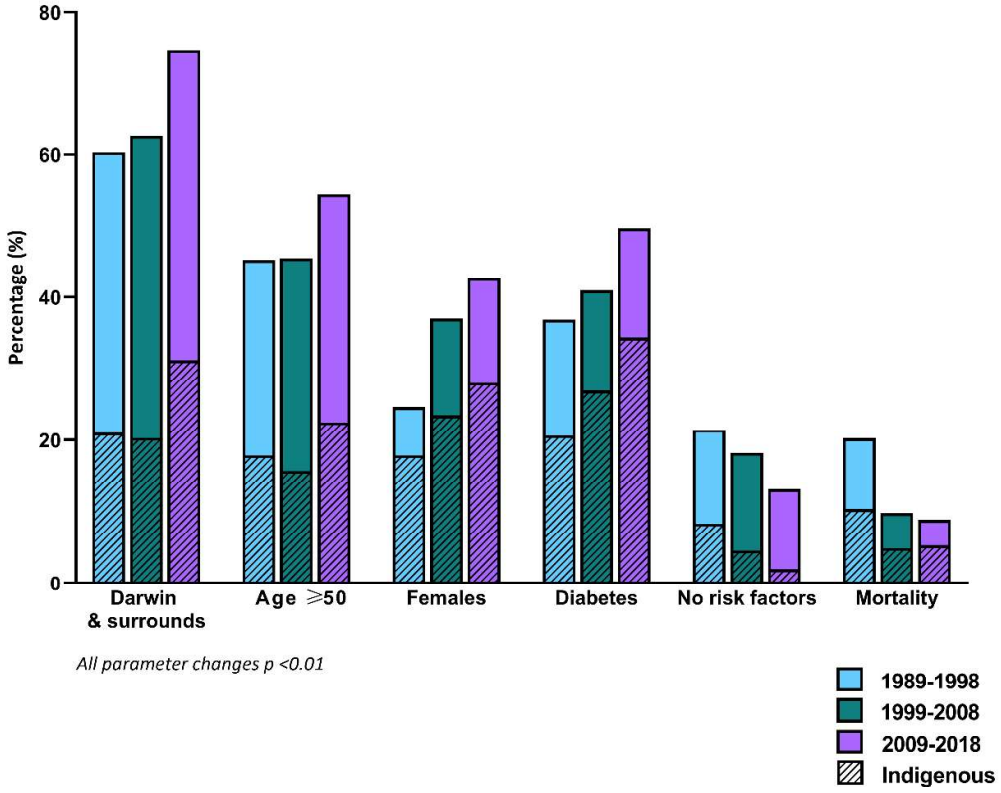
KAPLAN-MEIER 5 YEAR SURVIVAL ANALYSIS



95% confidence intervals were used for error bars.

SUPPLEMENTARY FIGURE 4

SIGNIFICANT PARAMETER CHANGES OVER THE 30 YEARS OF THE DARWIN PROSPECTIVE MELIOIDOSIS STUDY BY DECADE



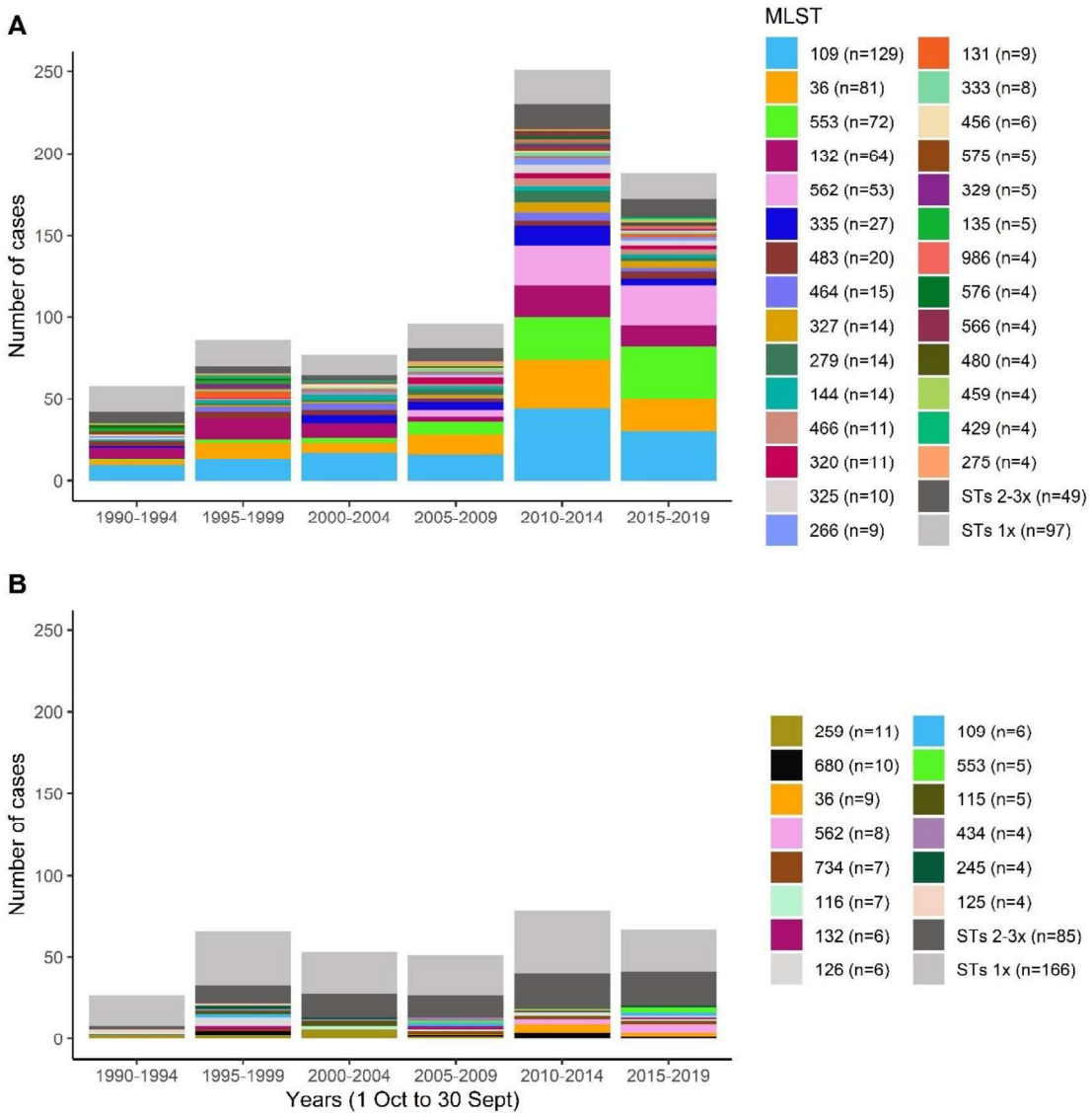
See Supplementary Table 4 for data on trends by decade.

SUPPLEMENTARY FIGURE 5

BURKHOLDERIA PSEUDOMALLEI MULTILOCUS SEQUENCE TYPES DIVERSITY DYNAMICS OVER 30 YEARS

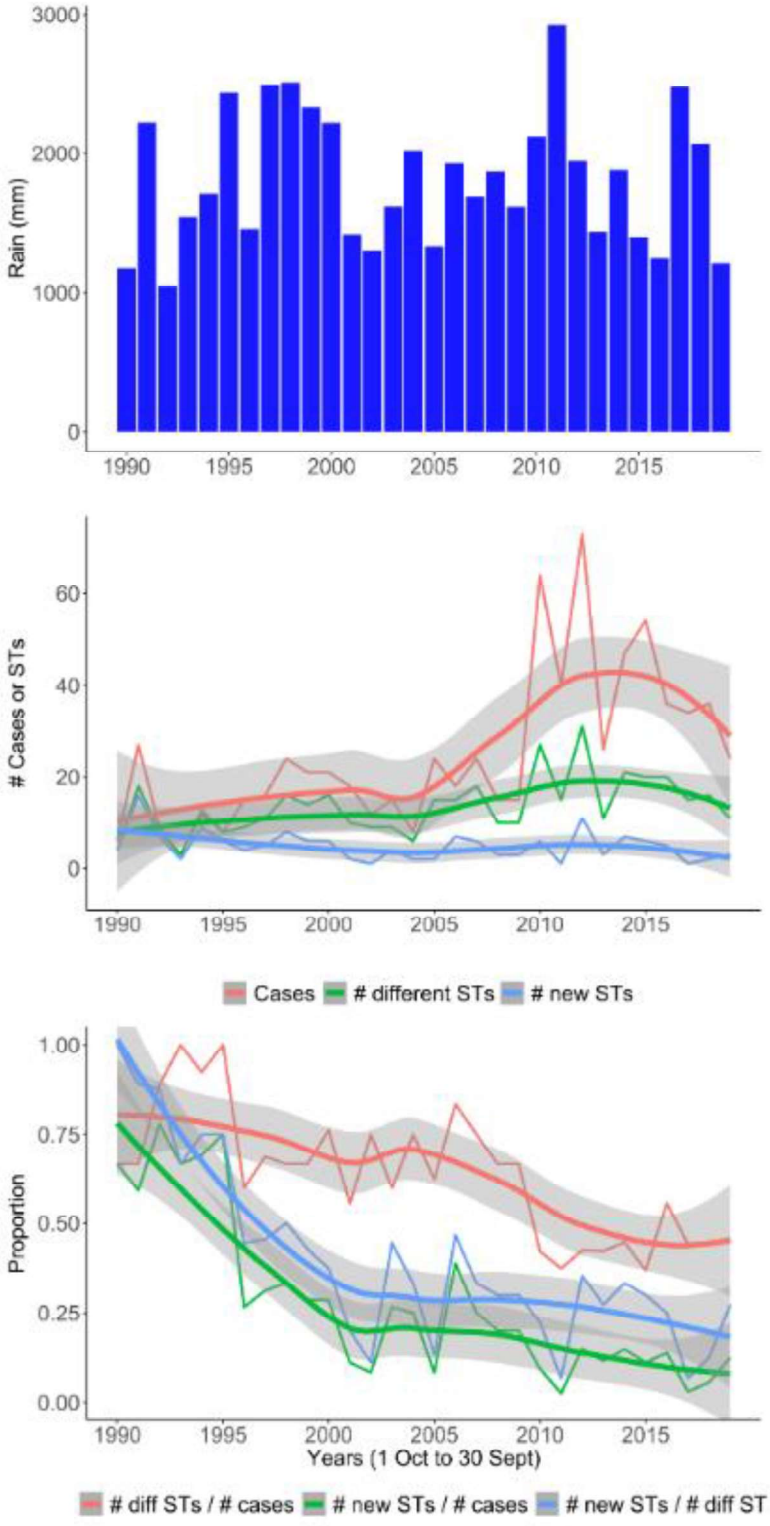
A. DARWIN AND SURROUNDS

B. RURAL AND REMOTE TOP END



SUPPLEMENTARY FIGURE 6

***BURKHOLDERIA PSEUDOMALLEI* MULTILOCUS SEQUENCE TYPES DIVERSITY DYNAMICS IN DARWIN AND SURROUNDS OVER 30 YEARS**



Supplementary Document 1

Supplementary Methods

Setting

The Top End of the Northern Territory encompasses around 500,000 km2 (Fig. 1). Darwin (12°S latitude) with surrounding rural hamlets has a population around 150,000. The remaining population of around 50,000 live in the rural towns of Katherine and Nhulunbuy and numerous remote Indigenous communities with populations of 50-3000 people.

Definitions for demographical, clinical risk factors and clinical illness parameters

Patient location was based on residence or a known likely location when infection occurred. Patients were each assigned to one of urban Darwin, Darwin rural hamlets, rural towns and remote Indigenous Top End communities or from outside the Top End (Fig. 1).

Clinical risk factors and clinical illness parameters used constant definitions over the 30 years. Variables recorded were age, sex, ethnicity (Indigenous Australian Aboriginal or other), and the previously identified clinical risk factors of diabetes, hazardous alcohol use, chronic renal disease and chronic lung disease. Hazardous alcohol use was defined as greater than an average daily consumption of six standard drinks (60 g alcohol total) for males and four (40g alcohol total) for females. Chronic renal disease was defined as a creatinine of > 150 umol/L (N. R. <90 umol/L) before the admission with melioidosis, or after completion of therapy if not previously documented. Chronic lung disease was defined as a documented diagnosis of chronic obstructive airways disease or bronchiectasis. Recent or current malignancy, immunosuppressive illness or immunosuppressive therapy, confirmed rheumatic heart disease or congestive cardiac failure and a history of recent kava ingestion were also

documented. “No clinical risk factors” referred to any patient with none of the above presumptive clinical risk factors.

Each patient was assigned to a single primary clinical diagnosis on presentation, representing the dominant organ involvement on clinical assessment by the Infectious Diseases team; pneumonia, skin infection without systemic symptoms, genitourinary melioidosis, bacteraemia with no evident focus, soft tissue abscess(es) either subcutaneous and/or lymph node, septic arthritis, osteomyelitis, neurological melioidosis and other. Presence or absence of bacteraemia was recorded. Septic shock was defined as the presence of hypotension not responsive to fluid replacement, together with hypoperfusion abnormalities manifest as end organ dysfunction (American College of Chest Physicians/Society of Critical Care Medicine Consensus Conference: definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. Crit Care Med 1992;20: 864–874). This historical definition of septic shock was used throughout the 30 years and for this analysis contemporary pneumonia and sepsis severity scores such as the pneumonia severity index (PSI) and the APACHE score were not used, having not been created when this prospective study commenced.

Statistical and graphical methods

Patient demographic, epidemiological, clinical and laboratory details were stored in MariaDB v10.2.31 (Oracle, California) and analysed using Stata v15.1 (Stata, Texas). Relative risks were calculated to assess the risks of clinical risk factors among melioidosis patients on binary outcomes such as mortality and 2-sided Fisher’s exact test and 95% confidence intervals were computed. P values of bivariate analyses were adjusted for multiple testing using the False Discovery Rate (FDR) method. A multivariable logistic regression model was constructed to identify demographic, clinical risk factor, seasonal and regional associations with various clinical presentations (pneumonia, skin abscess), bacteraemia and a fatal

outcome from melioidosis. P values of bivariate analyses to select parameters for inclusion in the models were adjusted for multiple testing using the False Discovery Rate (FDR) method. All variables with $p<0.100$ in bivariate analyses were included in the initial models. Variable selection for the final multivariable models was through backward stepwise elimination ($p<0.100$ threshold). Of note, for the outcome of mortality, diabetes and hazardous alcohol use each had $p>0.100$ on bivariate analysis and therefore were not included in the model.

Incidence rate (IR) trends over time were estimated with the package "mgcv" in R (version 4.0.2, R Development Core Team 2018) using a generalized additive model (GAM) with outcome being monthly melioidosis cases and parameters being region (Darwin vs remote), ethnicity (Indigenous vs non-Indigenous), season (dry vs wet season) and smoothing terms months (cyclic cubic regression spline) and years (1989-2019). The latter was estimated by region and ethnicity using thin-plate regression splines. In order to get monthly IR per 100,000 population, an offset (log) population and a negative binomial family (log link) were applied. To account for temporal autocorrelation, a first-order autoregressive (AR1) error model with estimated rho 0.08 was used. Nested models were compared using the Akaike's information criterion (AIC) and model residuals were checked for no temporal autocorrelation and meeting distributional assumptions.

The association between IR trends over time and rainfall (monthly rainfall, Australian Bureau of Meteorology Darwin Airport weather station) were estimated in a separate model for the Darwin region and wet season (Nov to April) only. The same GAM model structure was used (estimated rho 0.23) with outcome being monthly cases in Darwin in the wet season, offset (log) population, parameters being ethnicity, rainfall and smoothing term years by ethnicity.

Multilocus sequence typing

The multilocus sequence typing (MLST) scheme for *B. pseudomallei* was first developed in 2003 and targets the genetic sequence of seven housekeeping loci: ace (acetoacetyl coenzyme A reductase), gltB (glutamate synthase), gmhD (ADP-L-glycero-D-manno-heptose 6-epimerase), lepA (GTPbinding elongation factor), lipA (lipoic acid synthetase), narK (nitrite extrusion protein) and ndh (NADH dehydrogenase). For each housekeeping locus, the different sequences obtained from the *B. pseudomallei* isolates are assigned as distinct alleles. Each isolate is then defined by a string of seven integers (the allelic profile), which correspond to the allele numbers at the seven loci. Each unique allelic profile is assigned a sequence type (ST), which can then be assigned to any *B. pseudomallei* strain having that specific allelic profile. New allelic profiles not on the database are assigned the next available ST number by JRW, the curator of the global *B. pseudomallei* MLST database (previously housed at http://bpseudomallei.mlst.net; now at http://pubmlst.org/bpseudomallei/). In addition to conventional MLST with sequencing of each of the seven alleles, *in silico* MLST is now possible using allelic sequence data derived from whole genome sequencing to determine the ST.(16)

Supplementary Results

Melioidosis and severe weather events

The flooding and evacuation of the regional town of Katherine in January 1998, which followed extensive inland movement of tropical cyclone Les was associated with 6 cases of melioidosis (Fig. 1). The Category 5 severe tropical cyclone Thelma in December 1998 (Fig. 1), resulted in 6 cases from the remote Tiwi Islands north of Darwin and 6 mainland cases from the Darwin region (Fig. 1)(17). Several severe weather events with high rainfall particularly in the Darwin region were associated with the two highest 12-monthly total case

numbers in the Top End – 91 cases in the 2009-2010 12 months and 96 cases in 2011-2012, with tropical cyclone Grant causing widespread flooding in December 2011 (Fig. 1, Fig. 3A).