Melioidosis in Mali: a retrospective observational study

Sabine Lichtenegger, Isabel Klugherz, Gabriel E Wagner, Justine Michel, Bastien Mollo, Adama Sanogo, Moussa K Diawara, Soumaila Traore, Hyacinthe G Kodo, Max-Yvon Mbangui, Marie-Hortense Koudika, Youssouf Diam Sidibé, Johanna Dabernig-Heinz, Christian Kohler, Karsten Becker, Karoline Assig, Direk Limmathurotsakul, Matthias Kohl, Carolina Jimenez, Rupa Kanapathipillai, Janet Ousley, Ivo Steinmetz

Summary

Background Melioidosis is a neglected tropical bacterial infection with a high mortality rate caused by the Gram-negative soil bacterium *Burkholderia pseudomallei*. Although the disease is increasingly recognised in Asian and Pacific regions, the situation in Africa is characterised by a scarcity of data and great uncertainty regarding the disease burden and distribution. Here, we aimed to report cases of melioidosis in children younger than 5 years in Mali, where no confirmed melioidosis had been reported previously.

Methods Médecins Sans Frontières maintains a paediatrics programme in Koutiala, Mali, for children younger than 5 years, including a microbiology laboratory. Between January 2018, and September 2021, biochemical characteristics of bacterial isolates suggested the presence of *B pseudomallei* in clinical samples from children admitted with severe signs of infection. Isolated strains were characterised by whole genome sequencing. Clinical data on the course and outcome of confirmed melioidosis cases were retrospectively analysed from the hospital records.

Findings 31 melioidosis cases of children younger than 5 years were confirmed. 15 (48%) cases were in infants aged 12 months or younger. *B pseudomallei*-positive samples included 28 blood cultures, two pleural fluids, and one pus sample. Of 19 patients with available outcome data, 12 (63%) died. Phylogenetic analysis of the *B pseudomallei* isolates revealed high genetic diversity suggesting long-standing persistence of the bacterium in this region. We estimated an annual melioidosis incidence of 8·8 per 100 000 (95% CI 5·7–11·9) in the paediatric population and the derived 15·5 per 100 000 (10·0–20·8) for the overall population.

Interpretation This is, to the best of our knowledge, the first case series reported in Mali and the largest cohort of melioidosis cases ever reported in Africa. Our annual incidence estimates suggest that melioidosis is a significant public health problem in this part of Africa. These findings clearly highlight the need for improved diagnostics and observational studies to learn more about the African melioidosis burden. They also support the inclusion of melioidosis in national health strategies to inform surveillance and empiric treatment protocols. As melioidosis is resistant to common empirical antibiotic regimens, these measures are essential to reduce the high mortality rate.

Funding None.

Copyright © 2025 The Author(s). Published by Elsevier Ltd. This is an Open Access article under the CC BY-NC-ND 4.0 license.

Lancet Glob Health 2025; 13: e1964–72

See **Comment** page e1794

For the French translation of the abstract see **Online** for appendix 1

Diagnostic and Research Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz, Graz, Austria (S Lichtenegger PhD, I Klugherz MD, G E Wagner PhD, J Dabernig-Heinz PhD, K Assig PhD, Prof I Steinmetz MD); **Médecins Sans Frontières, New York, NY, USA** (J Michel BSc, R Kanapathipillai MD); **Médecins Sans Frontières, Paris, France** (B Mollo MD, C Jimenez MD, J Ousley MPH); **Médecins Sans Frontières, Koutiala, Mali** (A Sanogo MSc, M K Diawara MD, S Traore MD, H G Kodo MD, M-Y Mbangui MD, M-H Koudika MD); **Ministry of Health, Bamako, Mali** (Y D Sidibé MD MSc); **Friedrich-Loeffler-Institute of Medical Microbiology, University Medicine, Greifswald, Germany** (C Kohler PhD, K Becker MD); **Mahidol–Oxford Tropical Medicine Research Unit and Department of Tropical Hygiene, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand** (D Limmathurotsakul MD); **Health, Medical and Life Sciences Faculty, Furtwangen University, Villingen-Schwenningen, Germany** (M Kohl PhD); **BioTechMed-Graz, Graz, Austria** (S Lichtenegger)

Correspondence to: Prof Ivo Steinmetz, Diagnostic and Research Institute of Hygiene, Microbiology and Environmental Medicine, Medical University of Graz, Graz 8010, Austria **ivo.steinmetz@medunigraz.at**

Introduction

Melioidosis is a frequently fatal infectious disease affecting humans and animals in subtropical and tropical regions. It is caused by the Gram-negative soil bacterium *Burkholderia pseudomallei*. Melioidosis is mostly acquired through environmental exposure; human-to-human transmission is exceptionally rare.[1] Rural populations, often exposed through agricultural activities, are at greatest risk of infection, especially during times of heavy rain. Infection can be acquired through inhalation of contaminated aerosols, percutaneous inoculation, or ingestion of contaminated water or food.[1]

There is increasing evidence that melioidosis is massively underdiagnosed in many low-income and middle-income countries. Clinical manifestations range from acute sepsis to chronic or acute localised forms with abscesses in almost any organ, with lower respiratory tract infections in about half of all cases. Bacteraemia is detected in at least 50% of adult melioidosis cases.[1] Melioidosis is generally considered a disease of older people with a median age of approximately 50 years. Its risk factors include co-morbidities such as chronic lung diseases, kidney disease, and diabetes, the major risk factor of acquiring melioidosis upon pathogen exposure. The clinical features of paediatric melioidosis are less well described. Paediatric melioidosis is generally believed to be a more localised disease with much less bacteraemic courses. In the two most thoroughly studied melioidosis endemic areas, northeast Thailand and northern Australia, 4% of all melioidosis cases are paediatric cases, and more than 80% of paediatric cases had no recognisable risk factors identified.[2,3]

Early diagnosis of melioidosis is essential, because *B pseudomallei* is resistant to many drugs that are commonly used as empiric treatment of severe infections in endemic areas. Once the disease is diagnosed, specific treatment can reduce mortality substantially.[1] The

Research in context

Evidence before this study

In 2016, a modelling study predicted that large parts of Africa, south of the Sahara, were environmentally suitable for *Burkholderia pseudomallei* and likely represent the world's second-largest melioidosis burden after the south Asia, east Asia, and Pacific region. These predictions sharply contrasted with the limited literature on melioidosis from various parts of Africa, which primarily consisted of rare, sporadic case reports, often from travellers diagnosed after returning to high-income countries, a few environmental studies, and isolated reports of animal infections, some published decades ago. To update the evidence, we searched PubMed for articles published between Jan 1, 2015, and March 31, 2025, using the keywords "melioidosis" and "Africa" with no language restrictions. We selected studies, including reviews, that addressed melioidosis acquired in African countries. Newly identified possible endemic regions included reports from Benin with a single autochthonous case, as well as from Ghana and Cameroon, both based on infections in travellers diagnosed after their return. In Kenya's Kilifi County, five autochthonous cases were reported, among them four retrospectively identified archived blood culture isolates. The continuing lack of data from Africa on the exposure risk, disease burden, and virulence potential of local *B pseudomallei* strains still leads to great uncertainty in assessing the public health significance of melioidosis in Africa.

Added value of this study

Through the microbiological diagnostics available at our study site, which are rarely available in much of Africa, we identified 31 melioidosis cases among children admitted to a clinic dedicated to children aged younger than 5 years in Koutiala, Mali run by Médecins Sans Frontières. To the best of our knowledge, these are the first cases of melioidosis ever described in this region. The cases in our study comprise approximately half of all melioidosis cases ever reported from Africa. Our estimations of incidence, together with the high mortality observed, indicate a regional disease burden comparable with highly endemic regions of the Asia Pacific region. Furthermore, our whole genome single nucleotide polymorphism-based phylogenetic analyses showed that clinical *B pseudomallei* strains in Mali are genetically diverse, suggesting long-standing persistence rather than more recent introduction.

Implications of all the available evidence

The history of melioidosis in highly endemic areas in Asia shows that this infection can go almost completely undetected when clinical awareness and laboratory capacity is missing. The previous reports on sporadic melioidosis cases across different parts of Africa, along with our identification of an endemic focus through local diagnostics, indicate a potentially grossly underestimated silent presence of this disease on the continent. Given the high mortality rate of melioidosis with untargeted therapy, our study shows the urgent need to raise awareness and introduce simple but effective laboratory algorithms to improve *B pseudomallei* identification. Clinical, environmental, and genomic studies are imperative to further characterise African melioidosis and risk factors. These will extend our study and help to map the true distribution of *B pseudomallei*, determine its routes of transmission, and develop targeted prevention strategies in Africa.

number of cases actually detected in many endemic countries is much lower than predicted. The observed discrepancy is mainly the result of a combination of a limited awareness of the disease, insufficient diagnostic facilities, and the wide clinical spectrum.

Modelling approaches predict that 165 000 human melioidosis cases occur annually worldwide, resulting in 89 000 deaths.[4] The global mortality and disease burden of melioidosis was estimated to be substantially higher than those of many WHO neglected tropical diseases.[5] African countries south of the Sahara likely account for the largest melioidosis burden after the South Asia, East Asia, and Pacific region with 24 000 predicted cases annually (credible interval 8000–72 000).[4] In contrast to these predictions, there are only sporadic reports of melioidosis cases from African countries south of the Sahara.[6]

Although *B pseudomallei* has been detected in the environment in various African countries,[7,8] the scarcity of clinical data leaves uncertainty regarding the risk of exposure and the virulence of local *B pseudomallei* strains. Also, there are only very few isolates available for genetic analysis preventing a more in-depth comparison of African isolates with those from other endemic areas.

Médecins Sans Frontières maintains a paediatrics programme in Koutiala, Mali, for children younger than 5 years, including a clinical microbiology laboratory that can perform routine diagnostics to a standard that is usually not accessible to patients in large parts of Africa, south of the Sahara. The region around Koutiala is characterised by a high under-5 mortality rate, with infections accounting for a large proportion of deaths.[9] We conducted a retrospective observational study to compile clinical data from 31 children in whom we could detect *B pseudomallei* in clinical samples between 2018 and 2021. Our study comprises the largest series of melioidosis cases from Africa ever reported and has enabled us to carry out, to the best of our knowledge, the first melioidosis incidence estimates for this region. Furthermore, also to the best of our knowledge, we conducted the first in-depth phylogenetic analysis of *B pseudomallei* isolates from human infections in Africa, offering an unprecedented insight into the genetic diversity in this region. By identifying cases in Mali and

estimating disease burden, we emphasise the importance of integrating *B pseudomallei* into diagnostic protocols and surveillance systems to support a more efficient use of limited health-care resources.

Methods

Study design and participants

We conducted a retrospective observational study of confirmed, hospitalised melioidosis cases admitted to the study clinic in Koutiala, Mali between January 2018, and September 2021. Médecins Sans Frontières maintains a paediatric programme in Koutiala, for children younger than 5 years, including a microbiology laboratory (appendix 2 p 1). Hospitalisation was limited to children with signs of severity. All clinical samples and data were collected during routine medical care of patients. This retrospective observational study was approved by the Comité National d' Ethique Pour la Santée Et Les Sciences De La Vie, Bamako, Mali (2024002) and the institutional Ethics Review Board at Médecins Sans Frontières. Due to the retrospective nature of the study and in accordance with the ethics approval, no informed consent was obtained.

Procedures

All available clinical data were compiled from the medical records of confirmed cases. Nutritional status was assessed by measuring the median upper arm circumference with an age-adjusted Z score bracelet. Haematology parameters were analysed and malaria was diagnosed using standard laboratory techniques (appendix 2 pp 1–2). Clinical data were recorded by medical doctors during patient care, supported by an Antimicrobial Stewardship activity within the project and subsequently compiled into a Microsoft Excel sheet by an infectious disease physician to maintain accuracy. However, the challenging humanitarian environment of the study site led to a limited ability to access some patient files. The primary outcome of this study was patient mortality. Secondary outcomes were clinical presentations, risk factors, laboratory parameter changes, seasonal association of melioidosis cases, annual incidence estimates, and a phylogenetic analysis of *B pseudomallei* isolates.

Clinical specimens were incubated at 35°C on standard microbiological culture media. Blood cultures were incubated for 5 days using the BACT/ALERT system (bioMérieux, Marcy l'Etoile, France; appendix 2 p 1) and subcultured on standard media. Identification of bacterial cultures included Gram staining, oxidase testing, and biochemical API 20NE or 20E strips (bioMérieux). Isolates were considered suspicious, when the reaction profile obtained matched possible *B pseudomallei* profiles or profiles of related bacteria such as *Burkholderia cepacia* in the API 20 NE database. The presence of *B pseudomallei* isolate was confirmed by TTSS1-PCR and whole genome sequencing data (appendix 2 p 1). Antimicrobial susceptibility testing was performed using disc diffusion testing according to the European Committee on Antimicrobial Susceptibility Testing and gradient concentration strips (bioMérieux; appendix 2 p 1).

DNA was isolated using the NucleoSpin microbial DNA kit (Macherey-Nagel, Düren, Germany) according to Weigl and colleagues.[10] DNA concentration was determined using the Qubit-BR assay kit on a Qubit four fluorometer (Thermo Fisher Scientific, Vienna, Austria). Next-generation sequencing was performed on an Illumina MiSeq DX instrument (Illumina, San Diego, CA, USA) using Illumina DNA Prep Kit for a 2×300-bp paired-end sequencing run. Raw data were assembled with SKESA (default setting, version 2.3.0) implemented in SeqSphere (version 9.0.7; Ridom GmbH).

See **Online** for appendix 2

Core genome multi-locus sequence typing analyses were performed using SeqSphere (version 9.0.7).[11] A contamination check was performed using Mash screen implemented in SeqSphere. Distance matrices were generated in SeqSphere by cross-comparison of individual alleles of found target genes. Minimum spanning trees were created in SeqSphere (default parameters, option "pairwise ignore missing values").

Single-nucleotide polymorphisms were identified from whole genome sequencing data using SPANDX (version 4.0.2) and *K96243* as the reference genome.[12] The phylogenetic tree was visualised using iTOL.

For **SPANDX** see https://github.com/dsarov/SPANDx

For **iTOL** see https://itol.embl.de

Monthly rainfall data were obtained from the gridded Climatic Research Unit Time-Series data (version 4.09; University of East Anglia, Norwich, UK) for the grid cell covering the region around Koutiala (12·5° N, 5·5° W).[13] Average precipitation was calculated for each month over the study period.

Statistical analysis

All children with confirmed *B pseudomallei* infection, between 2018 and 2021, were included in the study (n=31). Detailed clinical records were available for 12 children (the subgroup); however, parameter documentation was incomplete. Sample sizes therefore vary by analysis because missing observations were excluded. We assessed the association between monthly case numbers and precipitation using Spearman's rank correlation in R (version 4.4.0). The melioidosis incidence per 100 000 population in children aged 0–4 years in Koutiala was calculated assuming a diagnostic test sensitivity of 60%, then compared with the corresponding incidence in Northeast Thailand (appendix 2 p 3). A normalisation factor was derived from this comparison and applied across all age groups in Mali (appendix 2 p 4). The mean number of cases was estimated considering the population structure in Mali and the assumption that the risk to acquire melioidosis is the same throughout the environmentally suitable zones for *B pseudomallei*,[4] where approximately 91% of the Malian population live. A

For the **API 20 NE database** see https://apiweb.biomerieux.com

	Sex	Age, months	Admission	*Burkholderia pseudomallei* isolated from	Outcome
1	Male	9	January, 2018	Blood	Deceased
3	Female	36	March, 2018	Blood	NA
4	Male	<1	March, 2018	Blood	NA
6	Male	12	April, 2018	Blood	Deceased
7	Male	36	August, 2018	Blood	NA
8	Male	12	August, 2018	Blood	NA
12	Female	<1	September, 2018	Blood	Deceased
13	Male	24	September, 2018	Blood	Deceased
14	Male	24	September, 2018	Blood	NA
16	Male	36	September, 2018	Blood	NA
17	Male	48	October, 2018	Blood	NA
19	Male	<1	May, 2019	Blood	NA
20	Male	12	May, 2019	Blood	Deceased
24	Male	13	July, 2019	Blood	Deceased
26	Male	24	August, 2019	Blood	Deceased
29	Male	36	September, 2019	Blood	NA
30	Female	36	September, 2019	Blood	Deceased
32	Male	24	October, 2019	Blood	Cured
33	Female	48	October, 2019	Blood	Deceased
34	Male	12	October, 2019	Blood	Deceased
36	Male	12	March, 2020	Blood	Deceased
37	Male	<1	February, 2020	Blood	Cured
40	Female	9	September, 2020	Blood	Referral (for surgery)
41	Male	7	August, 2020	Pleural fluid	NA
43	Female	36	September, 2020	Pus	Cured
50	Female	6	May, 2021	Pleural fluid	Cured
51	Male	<1	June, 2021	Blood	NA
52	Male	7	July, 2021	Blood	Cured
54	Female	36	July, 2021	Blood	Cured
55	Male	24	July, 2021	Blood	Deceased
58	Male	58	September, 2021	Blood	NA

NA=not available.

Table 1: **Demographic and basic clinical data of all 31 melioidosis cases admitted from 2018 to 2021 by patient identification number**

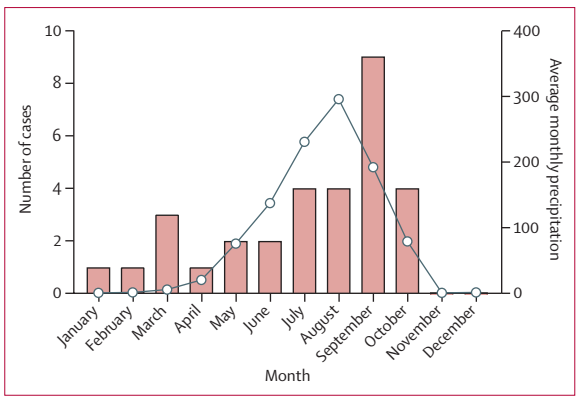

Figure 1: **Monthly distribution of melioidosis cases and average precipitation in Koutiala from 2018 to 2021**
Number of cases is shown as light brown bars, the monthly precipitation in mm as connected circles.

step-by-step calculation is provided in appendix 2 (pp 3–4). We used the negative binomial distribution to estimate the average number of cases and the associated 95% CI using the MASS package in R (version 4.4.0; appendix 2 p 4). Plots were created using R package ggplot2.

Role of the funding source

There was no funding source for this study.

Results

In 31 children admitted with severe signs of infection between January, 2018 and September, 2021 biochemically suspicious isolates were confirmed as *B pseudomallei* through a positive TTSS1-PCR and whole genome sequencing data. A retrospective analysis of clinical data from confirmed melioidosis cases revealed that of 19 patients with available outcome data 12 (63%) patients died (male to female ratio 9:3; table 1). Although specific causes of death were not recorded, all deceased children had positive blood cultures (table 1). Blood cultures were taken based on clinical criteria for sepsis suggesting that the likely cause of death was sepsis.

The median age of melioidosis patients was 13 months (IQR 8–36) and 23 (74%) participants were male and eight (26%) were female (table 1). Ten (32%) patients were younger than 12 months. The age distribution of melioidosis cases reflects the general age distribution of patients from whom blood cultures were collected in Koutiala during the study period (4989 [30%] of 16711 children aged <12 months). 28 (90%) of 31 children had *B pseudomallei*-positive blood cultures from a total of 2095 children with positive blood cultures during the study period. Two children had positive pleural fluids, and one had a positive pus sample. Nine (29%) of 31 patients were diagnosed in September, during the rainy and the malaria season (figure 1). The cases per month strongly correlated with both the average monthly precipitation of the same month (Spearman's r=0·844) and the previous month (0·713).

In a subgroup of 12 patients with detailed clinical data available (table 2), eight of 11 with documented temperature values presented with fever on admission. The most prevalent clinical symptom was respiratory distress (n=10). In seven children, gastrointestinal symptoms such as vomiting, diarrhoea, or a refusal to breastfeed were reported. Two children showed abscesses in the cranial area, and one child additionally had an elbow abscess. The clinical parameters on admission predominantly showed elevated heart rates (six of 12) and hypotonia (eight of 11 with documented values), indicating the systemic effects of infection. Three children presented with critically low peripheral oxygen saturation that called for oxygen therapy. The median time from symptom start to hospital admission was 4·5 days (data

available for the 12 children). The median length of stay for the children who were cured was 24 days (data available for six children). The median time to death from admission (available for four children) was 8 days. In four (44%) of nine children with an available differential blood count, leucocytosis was observed, driven by elevated numbers of granulocytes. In three of the deceased children lymphopenia was observed. All children presented with moderate to severe anaemic haemoglobin levels (11 of 11 children with documented values). Five children were also diagnosed with malaria. Of the seven children with severe anaemia (ie, haemoglobin <7·0 g/dl) four had malaria potentially contributing to the anaemia. Apart from one child with described trisomic facies, no underlying medical conditions were noted. Eight of 10 children with known nutritional status showed signs of undernutrition, with median upper arm circumference Z scores at least one SD below the median value for age and sex.

Seven (58%) of 12 children received antibiotic treatment with ceftazidime or a carbapenem as recommended for the intensive phase of melioidosis treatment.[1] Antimicrobial susceptibility testing of isolates showed no resistance to ceftazidime, carbapenems, amoxicillin–clavulanic acid, and trimethoprim–sulfamethoxazole drugs recommended for the treatment of melioidosis.

Based on the 31 confirmed paediatric melioidosis cases in Koutiala, we estimated an incidence rate of 5·3 per 100 000 (95% CI 3·4–7·2) for the paediatric population and 9·3 per 100 000 (6·0–12·6) for the total population per year (appendix 2 pp 3–4). However, if we consider that blood cultures for melioidosis only have a sensitivity of about 60%,[1,14] then the estimated incidence rate increased to 8·8 per 100 000 (5·7–11·9) for the paediatric population and 15·5 per 100 000 (10·0–20·8) for the total population. From the two incidence rates of the total population one can extrapolate an average of either 1907 (95% CI 1230–2584) or 3178 (2050–4264) annual melioidosis cases in Mali. However, it is reasonable to assume that the sensitivity of paediatric blood cultures is well below 60%. If the sensitivity is assumed to be 40% the estimated incidence rate increases to 13·2 per 100 000 (8·5–17·9) for the paediatric population and 23·1 per 100 000 (14·9–31·3) for the total population (appendix 2 p 5), extrapolating to an average of 4746 (3054–6434) annual melioidosis cases.

All 31 available clinical isolates were subjected to whole genome sequencing and were found to represent 27 distinct sequence types. 17 sequence types were novel (figure 2). Three of the sequence types were previously found in isolates from the environment in Ghana and Nigeria (sequence type 12 and 930) and a traveller returning from western Africa (sequence type 349) to Spain.[7,8,15] Seven known sequence types with deposited origin were also found in the Americas and the neighbouring island of Martinique. Comparative analysis of the 31 *B pseudomallei* genomes with a global set of

	Baseline characteristics and clinical presentation									Signs on admission				Haematological parameters				
	Sex	Age, months	Outcome	Time from symptom start to hospital admission, days	RD*	GIS*	Abscesses*	AC*	PMT*	Maximum temp (°C)	Temp (°C)	SPO_2 (%)	Systolic BP	HR_2 per min	MUAC Z score	WBC (cells per µL)	Neutrophils (cells per µL)	Lymphocytes (cells per µL)	Haemoglobin (g/dL)
12	Female	<1	Deceased	4	1	0	0	0	0	NA	38·2†	100	68	163	Median	NA	NA	NA	NA
24	Male	13	Deceased	10	1	1	0	1	1	39·8†	39·8†	91‡	72	195†	−2 to −3	6200	3200	2500‡	6·6‡
32	Male	24	Cured	4	1	0	0	1	0	39·8†	34·6‡	81‡	NA	72‡	≤3	NA	NA	NA	7·6‡
33	Female	48	Deceased	2	1	1	0	0	1	41·8†	40·7†	100	48‡	176†	Median	3600‡	2700	800‡	9·2‡
34	Male	12	Deceased	5	1	1	0	0	1	NA	40·3†	NA	54‡	182†	≤3	12 500	8000	3700‡	5·6‡
36	Male	12	Deceased	1	1	0	0	0	0	NA	40·5†	100	56‡	199†	≤3	18 600†	13 400†	4500	6·9‡
37	Male	<1	Cured	4	0	1	0	1	0	39·4†	36·4	100	56‡	139	NA	41 000†	34 100†	4700	5·5‡
40	Female	9	Referred	8	1	1	0	0	0	NA	40·1†	100	58‡	177†	−2 to −3	74 500†	67 800†	3500‡	9·5‡
43	Female	36	Cured	7	1	0	1	0	1	40·0†	38·1†	100	48‡	129	NA	11 600	5800	5000	5·1‡
50	Female	6	Cured	7	1	1	0	0	0	39·6†	37·8	100	70	195†	−1 to −2	24 100†	16 200†	6400	8·1‡
52	Male	7	Cured	3	1	1	0	1	1	39·5†	NA	55‡	52‡	114	≤3	9300	2600	5800	4·6‡
54	Female	36	Cured	5	0	0	1	0	0	40·7†	38·8†	100	48‡	135	−1 to −2	NA	NA	NA	6·1‡

Gastrointestinal symptoms include vomiting, diarrhoea, and refusal to breastfeed. AC=altered consciousness. BP=blood pressure. GIS=gastrointestinal symptoms. HR=heart rate. MUAC Z score=medium upper arm circumference Z score. NA=not available. PMT=positive malaria test. RD=respiratory distress. SPO_2=peripheral oxygen saturation. Temp=temperature. WBC=white blood cell count. *Values 0 and 1 represent the presence or absence of each clinical presentation ie, 0=absence and 1=presence. †Values are greater than the age-adjusted reference value (appendix 2 p 2). ‡Values are less than the age-adjusted reference value (appendix 2 p 2).

Table 2: **Clinical information and laboratory values for the subgroup of 12 children by identification number**

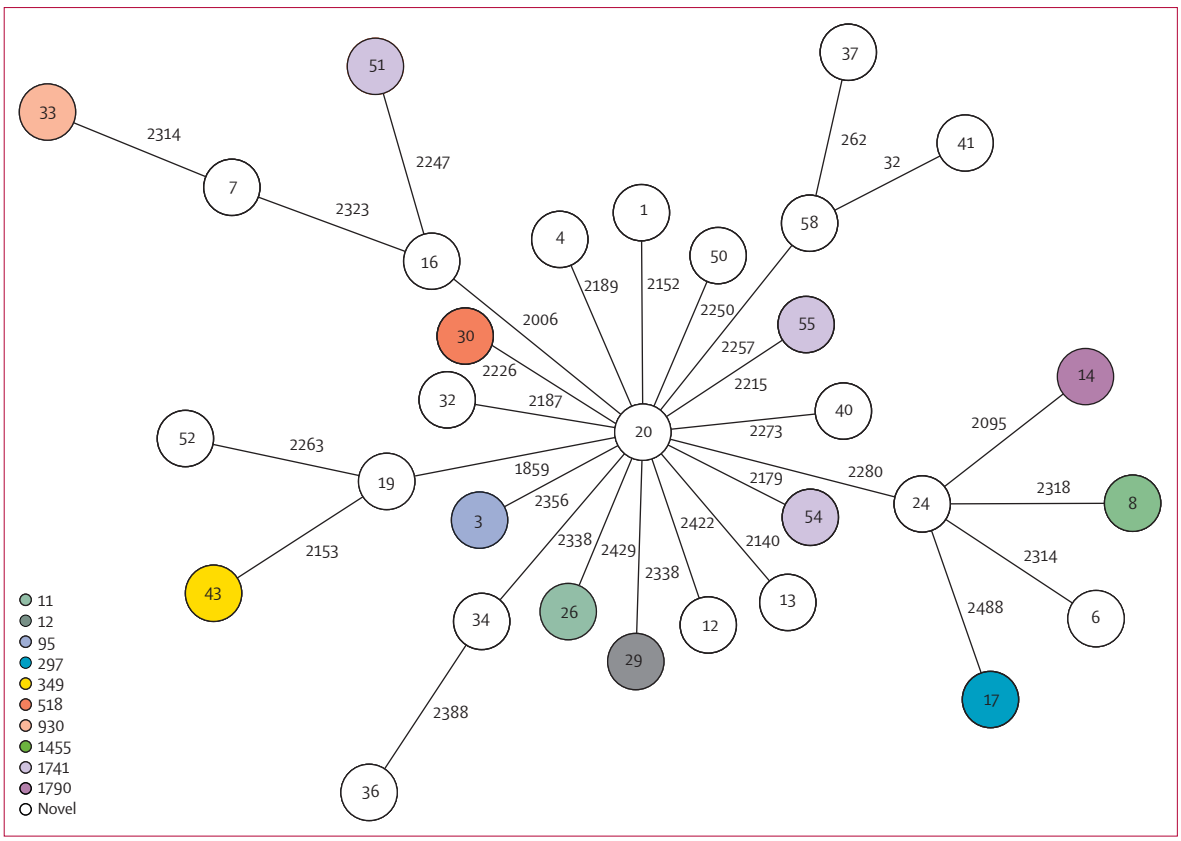

Figure 2: **Core genome multi-locus sequence typing minimum-spanning tree of paediatric isolates from Koutiala**
Each circle represents an allelic profile based on a sequence analysis of 4221 core genome multi-locus sequence typing target genes. The numbers on the connecting lines refer to the number of allele differences. Circles are coloured according to sequence type. White circles represent novel sequence types.

218 genomes placed our isolates in a clade with *B pseudomallei* strains from other African countries and the Americas (figure 3). Within the African clade, isolates from Mali share a distinct clade with isolates from Gabon.

The 31 isolates from Mali are genetically diverse (figure 2) since most of them differ in more than 2000 alleles in the analysis of the 4221 core genome multi-locus sequence typing targets.

Discussion

Of the 19 children with known outcome data, 12 (63%) died, a mortality similar to what is reported from other countries such as Viet Nam.[17] In line with known endemic areas, most of our 31 paediatric melioidosis cases occurred during periods of heavy rainfall. Based on our cases, we estimated an annual incidence of 15·5 per 100 000 for the total population per year in Mali considering a blood culture sensitivity of 60%.[14] From the incidence rate one can extrapolate an average of 3178 (95% CI 2050–4264) annual melioidosis cases in Mali, clearly extending beyond the 580 (190–1912) melioidosis cases predicted for 2015 by a modelling study[4] (adjusted to 668 cases for 2018–21 considering population growth). These numbers are comparable to highly endemic regions such as northeast Thailand and Australia's Top End.[2,18] Assuming that the sensitivity of blood cultures in our paediatric setting is likely well below 60%, our estimates of incidence would be considerably higher (appendix 2 p 5). Furthermore, additional factors suggest that the identified cases substantially under-represent the actual incidence of melioidosis in the study region. Since only severe cases were admitted to the clinic, localised less severe disease, which might have occurred in outpatients, is not represented in our study. Additionally, long distances from the clinic likely hinder health-care access. Despite the limitations of our study, such as the retrospective design, small sample size, geographical restriction, and paediatric focus, this is the largest series of melioidosis cases ever reported from Africa, and to the best of our knowledge, the first case series ever reported from Mali.

In Australia cutaneous melioidosis is the most common presentation of paediatric melioidosis, whereas suppurative parotitis is common in Thailand and Cambodia.[19–21] In East Malaysia children commonly present with cervical node swelling and if disseminated disease is present, with pneumonia.[22] The detection of 28 *B pseudomallei*-positive blood cultures out of 31 children in our study is notably high, especially since

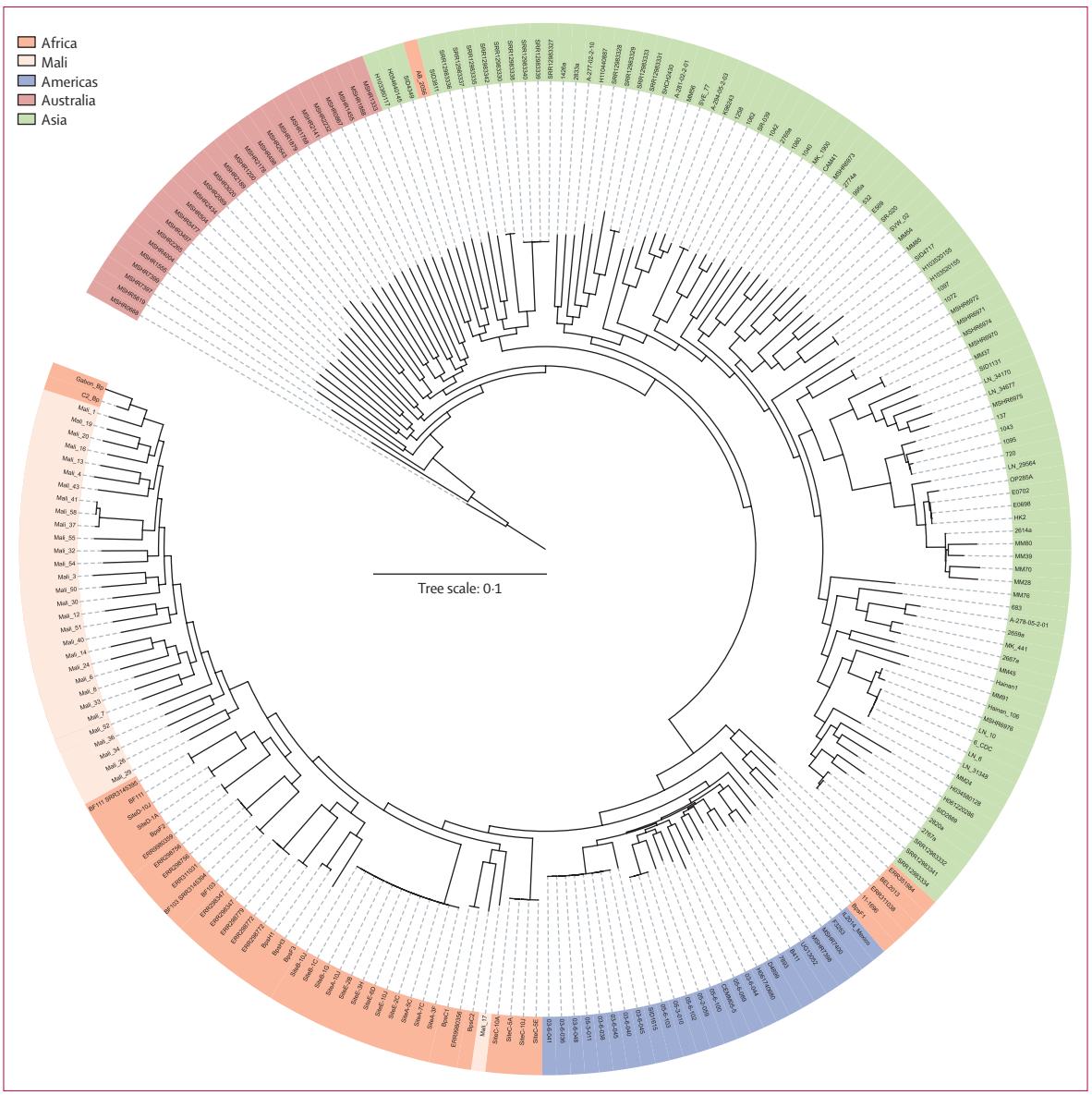

Figure 3: **Core single nucleotide polymorphism-based maximum likelihood phylogeny of 218 global *Burkholderia pseudomallei* isolates**
The tree was rooted on MSHR0668, the most ancestral *Burkholderia pseudomallei* strain, as identified by Price and colleagues.[16]

bacteraemic melioidosis is generally considered to occur at lower rates in children than in adults.[19,21] This observation could be the result of a diagnostic bias since only severely ill children were admitted to our study clinic. However, this finding could also suggest a high susceptibility to severe forms in our specific study population, increased virulence of the *B pseudomallei* isolates, or high environmental exposure to contaminated water or soil. The high proportion of children with respiratory and gastrointestinal symptoms suggests that inhalation and ingestion were the main routes of infection. In our study population, respiratory distress was a prominent sign of melioidosis, which was also described in other studies investigating paediatric melioidosis (eg, Viet Nam and Malaysia).[17,23] Gastrointestinal symptoms have also been documented in paediatric melioidosis and are often seen in conjunction with other systemic manifestations.[17] We have no data on whether or not haemoglobinopathies such as sickle cell disease or thalassemia were present in our study population, the latter being a known risk factor for paediatric melioidosis. The reason for the observed male to female imbalance with a majority being male patients is unclear and needs further investigations. Eight of ten melioidosis cases with known nutritional status showed signs of undernutrition, a known risk factor for severe infections in children younger than 5 years in Africa and possibly paediatric melioidosis.[24,25]

However, our clinic's malnutrition focus could have introduced bias and therefore further observational studies are needed to clarify associated risk factors and the clinical presentation of melioidosis in Mali and other African countries. Due to the wide clinical spectrum, the diagnosis of melioidosis is restricted to microbiological laboratories, posing an enormous hurdle for most low-income and middle-income countries. Apart from the modelling study that predicted melioidosis in Mali[4] there was only a single clinical report of cutaneous melioidosis in the region of Kita, although no information was provided on the bacterial identification method.[26]

In our Koutiala laboratory *B pseudomallei*-suspicious isolates could be detected, because a blood culture system was used routinely and costly biochemical identification systems were available to test non-fermenting Gram-negative rod bacteria. Even though such identification methods might not be available soon in many resource-limited laboratories in Africa, there is a solution for this bottleneck. Gram-negative, cytochrome c oxidase-positive rod bacteria can be tested for colistin and gentamicin resistance and amoxicillin-clavulanic acid susceptibility, an antibiotic resistance pattern characteristic for *B pseudomallei*. This unique antibiotic resistance profile can be identified using disc diffusion antibiotic susceptibility tests. These are accessible in resource-limited microbiology laboratories and were shown to be highly specific when used on bacterial cultures from clinical samples.[27] The applicability of this algorithm for Malian strains is proven by the fact that all our isolates show the respective resistance pattern, which was retrospectively analysed.

Apart from *B pseudomallei* identification challenges, the limited availability of blood cultures is a major issue in low-resource settings. This limitation is highly relevant for melioidosis diagnostics, since there is a high prevalence of bacteraemic melioidosis in affected adults. However, the availability of blood culture systems alone does not guarantee the diagnosis of melioidosis, because non-fermenting Gram-negative, oxidase-positive bacterial blood culture isolates might not be further differentiated and labelled as *Pseudomonas* spp.[28] It cannot be ruled out that *B pseudomallei* isolates can be found among such strains and that the introduction of the mentioned simple laboratory algorithm could substantially improve identification.

Once suspicious cases are detected, definitive confirmation of *B pseudomallei* can be obtained at reference centres. Most importantly, a positive triple-disc test can prompt the initiation of appropriate melioidosis treatment.[27] Treatment comprises an intensive therapy for a minimum of 10–14 days with either ceftazidime or a carbapenem (meropenem or imipenem) with or without trimethoprim–sulfamethoxazole followed by an eradication therapy with trimethoprim–sulfamethoxazole for 3–6 months.[1]

Our case series enabled us to address the local population structure of African clinical *B pseudomallei* strains in detail. Phylogenetic analysis places our novel isolates within the African clade and revealed high genetic diversity aligning with diversity patterns observed in environmental strains from Nigeria and Ghana (appendix 2 pp 6–7). Among the 31 strains 17 unknown sequence types were identified. Three of the known sequence types with documented origins were previously detected in Nigeria and/or Ghana. Previous studies indicate that *B pseudomallei* originates from Australia and spread to southeast Asia and further to south Asia and east Asia and then arrived in Africa approximately 2000 years ago.[29,30] These studies suggested that the subsequent migration from Africa to the Americas took place during the slave trade of 1650–1850.[29,30] The introduction of *B pseudomallei* from Africa to the Americas fits our multi-locus sequence typing data, as seven of the ten known sequence types found in our study population are also present in the Americas.

Although our findings are specific to Mali, they have broader public health implications for other countries in African regions, since many of these countries share comparable environmental and socio-economic conditions and health system structures. As practical next steps, we suggest a stringent application of the simple and cost-effective laboratory algorithm for identifying *B pseudomallei* cultures in patients with a fever of unknown origin to improve the timely diagnosis of melioidosis. Additionally, the reanalysis of archived cultures, sero-surveillance, and environmental studies might provide indications for the presence of melioidosis in other parts of Africa.

Our findings reveal a significant burden of paediatric melioidosis in Koutiala highlighting the urgent need for increased clinical awareness and improved diagnostic capacity, as a basis for appropriate treatment to reduce mortality. This need is further strengthened by the sharp rise of diabetes in Africa, a major risk factor for melioidosis. New serological methods for cost-efficient and less invasive melioidosis diagnostics are encouraging, including point-of-care assays, but need to be validated in prospective studies.[31] To inform the implementation of context-specific diagnostic protocols, we emphasise the need for expanded research efforts to assess the burden and clinical characteristics of melioidosis in Africa.

Contributors

SL: conceptualisation, data analysis and curation, investigation, development of the method, supervision, writing of the original manuscript, and editing subsequent drafts. IK: clinical data analysis and curation, investigation, writing of the original manuscript, and editing subsequent drafts. GEW: genomics data curation and interpretation and development of the method. JM, BM, and JO: conceptualisation, data extraction and interpretation, field coordination, and technical review. AS, MKD, ST, HGK, and M-YM: data extraction and interpretation, field coordination, and technical review. M-HK: field coordination and technical review. YDS: data interpretation and technical review. JD-H: genomics data analysis and curation. CK: development of the method and data validation. KB and KA: development of the method. DL: epidemiological data curation and analysis, writing of the original manuscript, and editing subsequent

drafts. MK: development of the method and data validation. CJ: supervision and conceptualisation. RK: conceptualisation, data extraction and interpretation, field coordination, technical review. IS: conceptualisation, data analysis and curation, supervision, writing of the original manuscript, and editing subsequent drafts. BM, ST, HGK, JM, SL, IK, GEW, RK, JO, and IS directly assessed and verified the underlying data reported in this study. All authors had access to all the data and accept responsibility for the decision to submit for publication. SL and IS were responsible for the final submission.

Equitable partnership declaration

The authors of this paper have submitted an equitable partnership declaration (appendix 3). This statement allows researchers to describe how their work engages with researchers, communities, and environments in the countries of study. This statement is part of *The Lancet Global Health's* broader goal to decolonise global health.

Declaration of interests

We declare no competing interests.

Data sharing

Sequencing data were deposited (BioProject PRJNA1279036) in the National Center for Biotechnology Information Sequence Read Archive repository and will be released upon publication.

Acknowledgments

We thank Michaela Lipp and Fabian Granitz for their contribution to the laboratory work and data visualisation. We thank the Médecins Sans Frontières team on the ground in Koutiala, Mali and supporting work from Bamako, Mali, as well as Médecins Sans Frontières's valuable partners at the Ministry of Health (Bamako, Mali), for their coordination and collaboration on this work. During the preparation of this work, the authors used DeepL and ChatGPT in order to check grammar, spellings, and improve readability. After using these tools, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

References

1 Meumann EM, Limmathurotsakul D, Dunachie SJ, Wiersinga WJ, Currie BJ. *Burkholderia pseudomallei* and melioidosis. *Nat Rev Microbiol* 2024; **22:** 155–69.
2 Currie BJ, Mayo M, Ward LM, et al. The Darwin Prospective Melioidosis Study: a 30-year prospective, observational investigation. *Lancet Infect Dis* 2021; **21:** 1737–46.
3 Hantrakun V, Kongyu S, Klaytong P, et al. Clinical epidemiology of 7126 melioidosis patients in Thailand and the implications for a national notifiable diseases surveillance system. *Open Forum Infect Dis* 2019; **6:** ofz498.
4 Limmathurotsakul D, Golding N, Dance DA, et al. Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. *Nat Microbiol* 2016; **1:** 15008.
5 Birnie E, Virk HS, Savelkoel J, et al. Global burden of melioidosis in 2015: a systematic review and data synthesis. *Lancet Infect Dis* 2019; **19:** 892–902.
6 Steinmetz I, Wagner GE, Kanyala E, et al. Melioidosis in Africa: time to uncover the true disease load. *Trop Med Infect Dis* 2018; **3:** 62.
7 Savelkoel J, Oladele RO, Ojide CK, et al. Presence of *Burkholderia pseudomallei* in Soil, Nigeria, 2019. *Emerg Infect Dis* 2023; **29:** 1073–75.
8 Schully KL, Voegtly LJ, Rice GK, et al. Phylogenetic and phenotypic characterization of *Burkholderia pseudomallei* isolates from Ghana reveals a novel sequence type and common phenotypes. *Front Microbiol* 2024; **15:** 1401259.
9 Yakum MN. The challenges of reducing child mortality at the district level: the case of Koutiala, Mali. Epicentre Scientific Day Paris 2023; June 8, 2023.
10 Weigl S, Dabernig-Heinz J, Granitz F, et al. Improving Nanopore sequencing-based core genome MLST for global infection control: a strategy for GC-rich pathogens like *Burkholderia pseudomallei*. *J Clin Microbiol* 2025; **63:** e0156924.
11 Lichtenegger S, Trinh TT, Assig K, et al. Development and validation of a *Burkholderia pseudomallei* core genome multilocus sequence typing scheme to facilitate molecular surveillance. *J Clin Microbiol* 2021; **59:** e0009321.
12 Sarovich DS, Price EP. SPANDx: a genomics pipeline for comparative analysis of large haploid whole genome re-sequencing datasets. *BMC Res Notes* 2014; **7:** 618.
13 Harris I, Osborn TJ, Jones P, Lister D. Version 4 of the CRU TS monthly high-resolution gridded multivariate climate dataset. *Sci Data* 2020; **7:** 109.
14 Limmathurotsakul D, Jamsen K, Arayawichanont A, et al. Defining the true sensitivity of culture for the diagnosis of melioidosis using Bayesian latent class models. *PLoS One* 2010; **5:** e12485.
15 Cuadros J, Gil H, De Miguel J, et al. Case report: melioidosis imported from West Africa to Europe. *Am J Trop Med Hyg* 2011; **85:** 282–84.
16 Price EP, Currie BJ, Sarovich DS. Genomic insights into the melioidosis pathogen, *Burkholderia pseudomallei*. *Curr Trop Med Rep* 2017; **4:** 95–102.
17 Pham TS, König E, Bui TT, et al. Newly detected paediatric melioidosis cases in a single referral children's hospital in Ho Chi Minh City indicate the probable underrecognition of melioidosis in South Vietnam. *Trans R Soc Trop Med Hyg* 2024; **118:** 190–98.
18 Limmathurotsakul D, Wongratanacheewin S, Teerawattanasook N, et al. Increasing incidence of human melioidosis in Northeast Thailand. *Am J Trop Med Hyg* 2010; **82:** 1113–17.
19 McLeod C, Morris PS, Bauert PA, et al. Clinical presentation and medical management of melioidosis in children: a 24-year prospective study in the Northern Territory of Australia and review of the literature. *Clin Infect Dis* 2015; **60:** 21–26.
20 Lumbiganon P, Chotechuangnirun N, Kosalaraksa P, Teeratakulpisarn J. Localized melioidosis in children in Thailand: treatment and long-term outcome. *J Trop Pediatr* 2011; **57:** 185–91.
21 Turner P, Kloprogge S, Miliya T, et al. A retrospective analysis of melioidosis in Cambodian children, 2009–2013. *BMC Infect Dis* 2016; **16:** 688.
22 Mohan A, Podin Y, Tai N, et al. Pediatric melioidosis in Sarawak, Malaysia: epidemiological, clinical and microbiological characteristics. *PLoS Negl Trop Dis* 2017; **11:** e0005650.
23 Mohan A, Paranchothy M, Segaran S, et al. Fatal pediatric melioidosis and the role of hyperferritinemic sepsis-induced multiple-organ dysfunction syndrome. *Am J Trop Med Hyg* 2022; **107:** 393–96.
24 Madewell ZJ, Keita AM, Das PM, et al. Contribution of malnutrition to infant and child deaths in Sub-Saharan Africa and South Asia. *BMJ Glob Health* 2024; **9:** e017262.
25 Chandna A, Bonhoeffer M, Miliya T, Suy K, Sao S, Turner P. Improving treatment and outcomes for melioidosis in children, Northern Cambodia, 2009–2018. *Emerg Infect Dis* 2021; **27:** 1169–72.
26 Dicko A. Mélioïdose cutanée: a propos d'un cas au Mali. *Rev Mali Infect Microbiol* 2022; **17:** 97–99.
27 Trinh TT, Hoang TS, Tran DA, et al. A simple laboratory algorithm for diagnosis of melioidosis in resource-constrained areas: a study from north-central Vietnam. *Clin Microbiol Infect* 2018; **24:** 84.e1–e4.
28 Rahden P, Barrow E, Bah H, Bittaye SO, Nygren D, Badjan A. Bloodstream infections at a tertiary hospital in the Gambia—a one-year retrospective study. *BMC Infect Dis* 2025; **25:** 170.
29 Chewapreecha C, Holden MT, Vehkala M, et al. Global and regional dissemination and evolution of *Burkholderia pseudomallei*. *Nat Microbiol* 2017; **2:** 16263.
30 Sarovich DS, Garin B, De Smet B, et al. Phylogenomic analysis reveals an Asian origin for African *Burkholderia pseudomallei* and further supports melioidosis endemicity in Africa. *mSphere* 2016; **1:** e00089–15.
31 Wagner GE, Föderl-Höbenreich E, Assig K, et al. Melioidosis DS rapid test: a standardized serological dipstick assay with increased sensitivity and reliability due to multiplex detection. *PLoS Negl Trop Dis* 2020; **14:** e0008452.

See **Online** for appendix 3

For the **National Center for Biotechnology Information Sequence Read Archive repository** see https://www.ncbi.nlm.nih.gov/bioproject/PRJNA1279036