Neuromelioidosis outbreak in Tamil Nadu, India: an investigation of transmission with genomic insights

Angel Miraclin Thirugnanakumar,[a,o] Prabu Rajkumar,[b,o] Karthik Gunasekaran,[c] Pavithra Mannam,[d] Manickam Ponnaiah,[b] Girish Kumar Chethrapilly Purushothaman,[b] Aravind Velmurugan,[e] Ayyan Raj Neeravi,[e] Jobin John Jacob,[e] Solomon D. Cruz,[e] Bhagteshwar Singh,[m] Prabhakar Appaswamy Thirumal,[a] Anitha Jasper,[d] Malu Mohan,[b] Devika Shanmugasundaram,[b] Ananth Abraham,[f] Senthil Thangarajan,[n] Tony Panicker,[f] Bijesh Nair,[f] Aravintharaj Selvakumar,[n] Ranjith K. Moorthy,[f] Baylis Vivek Joseph,[f] Krishna Prabhu,[f] Devarajulu Reddy Sirasanambati,[b] Shalini Nair,[i] Murali Umapathy,[b] Kishore Pitchamuthu,[i] Balusamy Murugesan,[b] Sowmya Sathyendra,[c] Samuel George Hansdak,[c] Hema Paul,[e] Rajesh Isaiah,[j] Kundavaram Paul P Abhilash,[h] Aditya V. Nair,[a] Vivek Mathew,[a] Ajith Sivadasan,[a] Maya Mary Thomas,[g] Sanjith Aaron,[a] John Antony Jude Prakash,[e] Geeta Chacko,[k] Priscilla Rupali,[l] Tom Solomon,[m] Somasundaram Anavarathan,[n] Selvavinayagam Thirumalaichiry Sivaprakasam,[n] Balaji Veeraraghavan,[e,**] and Manoj Murhekar[b,*]

[a]Department of Neurological Sciences (Division of Neurology), Christian Medical College, Vellore, Tamil Nadu, India
[b]Indian Council of Medical Research – National Institute of Epidemiology, Chennai, Tamil Nadu, India
[c]Department of Medicine, Christian Medical College, Vellore, Tamil Nadu, India
[d]Department of Radiodiagnosis, Christian Medical College, Vellore, Tamil Nadu, India
[e]Department of Clinical Microbiology, Christian Medical College, Vellore, Tamil Nadu, India
[f]Department of Neurological Sciences (Division of Neurosurgery), Christian Medical College, Vellore, Tamil Nadu, India
[g]Department of Neurological Sciences (Division of Pediatric Neurology), Christian Medical College, Vellore, Tamil Nadu, India
[h]Department of Emergency Medicine, Christian Medical College, Vellore, India
[i]Department of Critical Care Medicine, Christian Medical College, Vellore, Tamil Nadu, India
[j]Department of Radiation Oncology, Christian Medical College, Vellore, Tamil Nadu, India
[k]Department of General Pathology (Division of Neuropathology), Christian Medical College, Vellore, Tamil Nadu, India
[l]Department of Infectious Diseases, Christian Medical College, Vellore, Tamil Nadu, India
[m]Liverpool School of Tropical Medicine, United Kingdom
[n]Directorate of Public Health and Preventive Medicine, Tamil Nadu, India

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Summary

Background In May 2023, we investigated a cluster of neuromelioidosis notified from Tamil Nadu state in southern India to describe case characteristics and identify the infection source.

Methods We searched for probable cases presenting with fever and brainstem syndrome, supported by radiological findings suggestive of neuromelioidosis. Cases were confirmed by isolation of *Burkholderia pseudomallei* from tissue, blood, or cerebrospinal fluid (CSF), or by PCR. The cases were described by time (epidemic curve), place (spot map), and person (clinical characteristics). Infection sources and virulence markers were identified by genome sequencing of the clinical and environmental isolates. Whole genome sequencing data were analysed to investigate the expression of *Burkholderia mallei*-like $bimA_{Bm}$ gene, and a phylogenetic tree was constructed to study sequence similarity to the global isolates.

Findings We identified 21 probable cases between July 2022 and April 2023 (median age = 33 years; 11 females; five confirmed) across four districts in Northern Tamil Nadu. Seventeen cases were from a single district and 10 reported prior dental treatment at a clinic. Cases with dental exposure had higher fatality (8/10 vs. 1/11) and shorter time to death (median 17 days vs. 1 death at day 56) than sporadic cases. The $bimA_{Bm}$ gene, which is associated with neurotropism, was identified in all three clonal isolates (two from the cases and one from the environmental isolate from the in-use saline bottle). Whole genome sequencing identified the ST1553 strain as being associated with the current outbreak. Genetic analysis of 209 isolates available in the public database with metadata revealed that ST1553, the strain responsible for the outbreak, clustered with isolates from India and Australia that expressed the *B. mallei-like* $bimA_{Bm}$ *allele.*

Interpretation We confirmed a large cluster of neuromelioidosis from South India, likely representing sporadic cases from environmental sources and cases linked to an iatrogenic source at a dental clinic. Rapid and high case fatality among dental cases supports the direct trans-neural spread of *B. pseudomallei* to the brainstem following inoculation

*Corresponding author. ICMR-National Institute of Epidemiology, Chennai, Tamil Nadu 600077, India.
**Corresponding author. Department of Clinical Microbiology, Christian Medical College, Vellore 632004, India.
E-mail addresses: mmurekhar@nieicmr.org.in (M. Murhekar), vbalaji@cmcvellore.ac.in (B. Veeraraghavan).
[o]Both are joint first authors.

via contaminated saline. Expression of *B. mallei-like bimA$_{Bm}$ allele may have contributed to the increased neurological manifestations of melioidosis.*

Funding None.

Keywords: Neuromelioidosis; Neuro virulence; Outbreak; *Burkholderia pseudomallei*; Dental procedures; *Burkholderia mallei*–like *bimA$_{Bm}$* gene; South India

Research in context

Evidence before this study

We searched PubMed on December 1, 2024, using the terms ('melioidosis,' '*Burkholderia pseudomallei*,' and 'neurological') and identified 86 articles. One was a systematic review, the remainder were case reports or series. Majority reported secondary neurological involvement, such as parenchymal abscesses, parameningeal osteomyelitis, or myelitis. Reports of primary neuromelioidosis are increasing, with the typical radiological characteristic of progression along the corticospinal and white matter tracts referred to as 'tunnel sign'. Evidence from these reports point to the role of *Burkholderia mallei-like bimA$_{Bm}$* gene in increased neurovirulence and motility, potentially facilitating neurotropism through neuronal pathways. However, no studies have reported primary neuromelioidosis following iatrogenic exposure.

Added value of this study

This investigation of primary neuromelioidosis documented 'tunnel sign' as a potential early radiological marker and detected *B. mallei-like bimA$_{Bm}$* gene in the available isolates from cases. Additionally, the clustering of cases was epidemiologically linked to invasive dental procedures performed under suboptimal infection prevention and control conditions with the clonality of environmental isolate further supporting an iatrogenic source. Rapid clinical progression and higher fatality among the dental cases are suggestive of potential direct trans-neural route of central nervous system invasion by *B. pseudomallei*.

Implications of all the available evidence

These findings suggest a possible novel mechanism of neuromelioidosis pathogenesis and highlight the importance of evolving molecular epidemiology in the identification of the circulating neurovirulent strain of *B. pseudomallei* in Southeast Asia. Increased awareness of clinical manifestations and characteristic imaging findings and potential risk factors, including dental procedures, may facilitate timely diagnosis and management in endemic regions to enable faster recognition and management. These findings also underscore the importance of enforcing rigorous infection prevention and control practices across healthcare facilities, particularly those performing invasive procedures.

Introduction

Melioidosis is an infectious disease caused by *B. pseudomallei*, a primary soil saprophyte found in tropical and temperate regions.[1] Transmission typically occurs through skin inoculation, ingestion, or inhalation of *B. pseudomallei* with exposure to soil and consumption of contaminated food and water being the important environmental exposure pathways.[1] Unusual modes of transmission, including iatrogenic infections, have been reported from endemic and other regions.[2–8]

The clinical presentations of melioidosis are diverse, with high variability in case fatality (50–90%) influenced by multiple factors such as co-morbidities, bacterial load, mode of infection, strain virulence, and tropism.[1,9,10] Melioidosis in the head and neck region is often severe, with a shorter incubation period, and may develop following inhalation or aspiration of contaminated water, or ingestion of contaminated food.[1,11] Such cases are commonly reported from Southeast Asia and Northern Australia and can further progress to central nervous system (CNS) infection through trans-neural spread along free nerve endings or through contiguous anatomical structures.[12,13]

CNS manifestations of melioidosis are rare and typically secondary to haematogenous spread from a distant site of inoculation.[5,14] Evidence of primary neuromelioidosis (without evidence of disease else-where) is mostly limited to isolated case reports and lacks crucial epidemiological characterization to identify transmission routes.[14–18] Molecular characterization of virulence has identified *B. mallei*-like actin polymerization (*bimA$_{Bm}$*) gene, which is associated with higher virulence and serves as a predictor of neuromelioidosis.[19,20]

On May 9, 2023, the Christian Medical College and Hospital, Vellore (CMC), a tertiary care referral centre in the Southern Indian state of Tamil Nadu, notified the district and state public health authorities and the Indian Council of Medical Research – National Institute of Epidemiology of an increase in the number of neuromelioidosis cases. Some cases were linked to a particular

dental clinic at Vaniyambadi, a small town in the district of Tirupattur, Tamil Nadu. Additionally, local newspapers reported suspicious deaths among people who received treatment at dental clinic.[21] We investigated this cluster to describe the clinical and epidemiological characteristics and identify the infection source.

Methods

Ethics statement

This outbreak investigation was undertaken as part of an emergency public health response and approved by the Institutional Review Boards of the ICMR-National Institute of Epidemiology [NIE/IHEC/A/202306-01] and CMC [16,199]. Informed written consent has been obtained from the cases or caregivers for interviews and publication of MRI images.

Outbreak setting

Tamil Nadu is the tenth largest state in India, covering an area of 130,058 square kilometres (50,216 sq. mi). The state has distinct periods of rainfall: south-west monsoon (from June to September) with strong south-west winds, north-east monsoon (from October to December) with dominant north-east winds, and dry season (from January to May). The normal annual rainfall of the state is approximately 945 mm (37·2 in), of which 48% is through the north-east monsoon and 32% through the south-west monsoon. The outbreak occurred in Tamil Nadu, with most cases in the Tirupattur district. Additional cases were reported from the adjoining districts of Ranipet, Krishnagiri, and Tiruvannamalai (Fig. 1). These districts receive most of their rainfall during the south-west monsoon period. September and October are the wettest months, with approximately 400 mm of rain. Melioidosis has been previously reported in southern India, including Tamil Nadu, and the reporting has increased in the last five years due to improved recognition of clinical syndromes and laboratory diagnosis.[17,22] Most transmissions occur in the monsoon seasons, driven by the translocation of the bacteria to the upper layers of soil, where conditions support their multiplication.[9,11,22,23] During 2022–2023, the reporting hospital identified 83 cases of culture-proven melioidosis, the majority being septicaemic melioidosis followed by osteoarticular and pulmonary melioidosis.

The strategy for outbreak investigation

A married couple presented with acute-onset fever followed by brainstem syndrome, with imaging features indicative of necrotizing brainstem encephalitis consistent with neuromelioidosis to CMC. The relatives of the couple reported that both had undergone dental procedures at a specific clinic in the Tirupattur district, shortly before the onset of illness. Simultaneously, local newspapers reported multiple deaths among cases treated at the clinic.[21] This prompted a report to the District Public Health Authority in Vellore on May 9, 2023. Following a review of the couple's details and the media reports, a collaborative outbreak investigation was initiated, which included a comprehensive case search and environmental investigation to confirm the outbreak and detect the source of infection. A multidisciplinary team consisting of epidemiologists, clinicians, microbiologists, and public health officials coordinated the investigation following the 10-step WHO approach.

Case definitions

Brainstem syndrome

Cases presenting with one or multiple cranial nerve palsies in combination with pyramidal weakness, sensory disturbances, or altered mentation.

Radiological features consistent with neuromelioidosis

Magnetic Resonance Imaging (MRI) evidence of cytotoxic edema in the brainstem and/or spinal cord with radiological progression along the corticospinal/white matter tracts.

Probable case

Occurrence of fever followed by brainstem syndrome and radiological features of neuromelioidosis among the residents of five districts of Northern Tamil Nadu (Tirupattur, Krishnagiri, Vellore, Ranipet, and Tiruvannamalai) between June 2022 and May 2023, and in the absence of other systemic infections such as tuberculosis, listeriosis, and other viral infections.

Confirmed case

B. pseudomallei isolation or PCR positivity in a clinical sample (blood/cerebrospinal fluid (CSF)/brain abscess pus/brain tissue) of probable cases or brainstem syndrome.

Primary neuromelioidosis

Index clinical presentation as a neurological syndrome (meningitis/encephalitis/myelitis).

Secondary neuromelioidosis

Index clinical presentation as a systemic syndrome (septicaemia, pulmonary, hepatic or renal involvement) and developing neurological manifestations during the course of illness.

Case search

We searched for probable cases by using multiple methods. On May 13, 2023, a meeting was held at the Tirupattur district health office with healthcare facilities, diagnostic imaging centres, and local professional medical and dental associations from Tirupattur and neighbouring districts of northern Tamil Nadu. The participants were sensitized to the case definition, clinical manifestations, laboratory diagnosis, and management

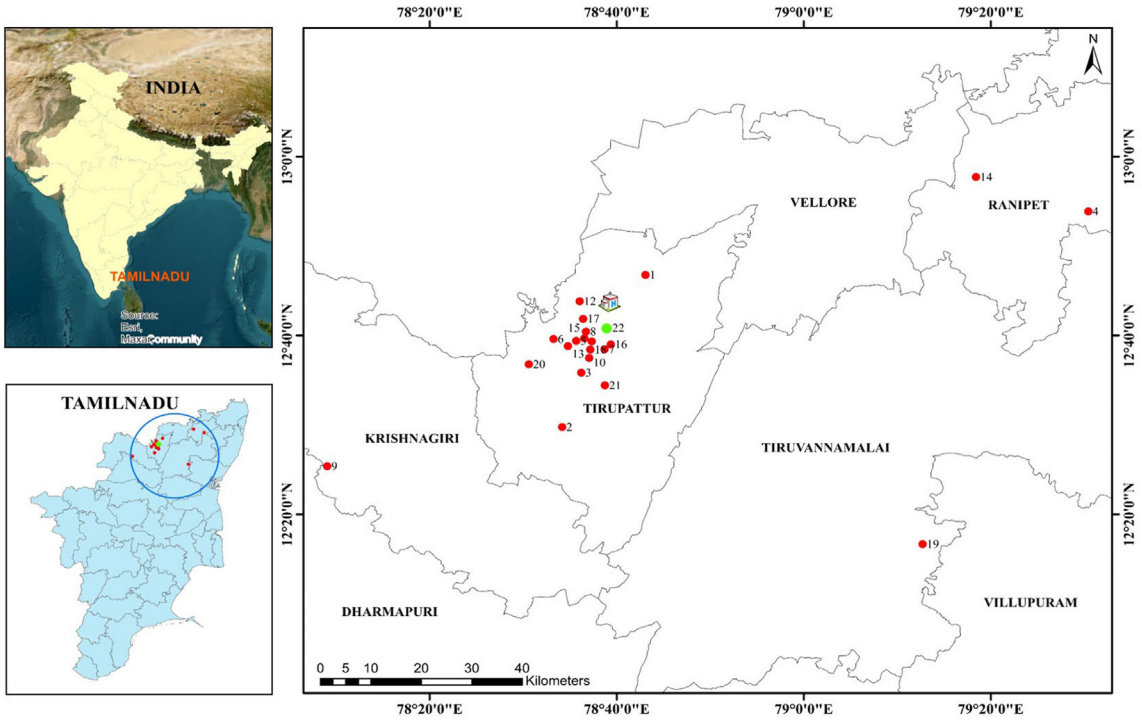

Fig. 1: Distribution of neuromelioidosis cases by residence in Tamil Nadu, India, 2022–2023. Indian subcontinent (top-left). Southern state of Tamil Nadu (Bottom-left). Five districts in Northern Tamil Nadu showing the distribution of neuromelioidosis cases (right). Dental clinic. Residence of cases. Residence of index case.

strategies. Through district health offices, this information was further communicated to various healthcare entities with requests to report such cases.

In addition, local newspapers were scanned to identify reports of similar events. We interviewed probable cases and/or relatives of severely disabled or deceased cases to collect information on sociodemographic details, exposure history, symptoms, illness progression, laboratory investigations, imaging findings, management, and outcomes from medical records. They were also asked to report any other known cases of similar manifestations (Supplementary Appendix 1). The cohort of survivors was followed up to record their functional status at six months.

Identification of potential iatrogenic source of outbreak

As initial clues pointed to the cases from Tirupattur district experiencing illness after undergoing invasive dental procedures at a local dental clinic, the dentist was interviewed to gather information on various dental procedures, associated complications, and infection prevention and control (IPC) practices. This was followed by an environmental investigation at the clinic to identify potential sources of infection. This involved swabbing dental equipment, common areas, water taps, sampling medications, and irrigation fluids.

At the time of the environmental sampling, the clinic was disinfected, water supply cut-off, and closed following a public outcry, making a comprehensive investigation impossible. As *B. Pseudomallei* was identified from an in-use saline bottle sample, additional samples were collected, including unopened saline bottles of the same and different batches, to check for batch contamination.

Laboratory methods

Blood and CSF samples were cultured using automated methods, and *B. pseudomallei* isolates were identified using standard biochemical testing, followed by validation with the VITEK MS-MALDI TOF (bioMerieux) system. Genomic DNA was extracted from 24 h bacterial colonies using the QIAamp DNA Mini kit (Qiagen), according to the manufacturer's instructions. The primer pair used were BpTT4176F (5'-CGTCTC TATACTGTCGAGCAATCG-3') and BpTT4290R (5'-CG TGCACACCGGTCAGTATC-3'), respectively, targeting the type III secretion system gene cluster of *B. pseudomallei*, generating a 115-bp product. The probe used was BpTT4208P (5'-CCGGAA TCTGGATCACCACCACTTT CC-3').[24] Species prediction was performed using BactInspector (v0.1.3) (https://gitlab.com/antunderwood/bactinspector). Multilocus Sequence Typing (MLST) was done using the *B. pseudomallei* scheme with MLST

(v2.23.0) (https://github.com/tseemann/mlst) to determine sequence types. Virulence genes were screened using Abricate (v1.0.0) (https://github.com/tseemann/abricate) in the VFDB (2022) database (http://www.mgc.ac.cn/VFs/). $BimA_{Bm}$ and $BimA_{Bp}$ were identified using BLASTn (v2.13.0) against MSHR668 (CP009546.1) and K96243 (CP009537.1), respectively. The detailed methodology is presented in Supplementary Appendix 2.

To determine the clustering and sequence similarities, we performed whole-genome sequencing (WGS) of the clinical and environmental isolates from the present outbreak and 10 contextual isolates *of B. pseudomallei* from cases with septicemic melioidosis admitted during the study period in two hospitals in the outbreak districts (detailed methodology is provided in Supplementary Appendix 2). In addition, genomes of 50 *B. pseudomallei* isolates from an ongoing hospital-based surveillance from 2009 to 2023 and those from other geographical regions were retrieved from the NCBI database (n = 3195) (https://www.ncbi.nlm.nih.gov). Genomes lacking metadata were excluded from analysis. The remaining assemblies (n = 209) were mapped to the reference genome of *B. pseudomallei* (GCA 000959305.1) by using Snippy (v4.6.0) (https://github.com/tseemann/snippy). Recombination regions were removed from the core genome alignment using Gubbins (v2.3.1) to generate recombination-free core genome alignment. Hierarchical Bayesian clustering was performed using fastBAPS (v1.0.8) (https://github.com/gtonkinhill/fastbaps). A phylogenetic tree was constructed using IQTree2 with the GTR + F + ASC + R4 model and 1000 bootstrap replicates. The resulting phylogenetic tree was visualized and annotated using Interactive Tree of Life software (iTOL v.3). Strains were considered clonal if there were no differences in the SNPs. The metadata of the sequences used are provided in Supplementary metadata.

Data analysis

We constructed an epidemic curve by month of onset and calculated the time to key events. We plotted cases by residence on a spot map. The functional outcome of the cases at six months was scored using the modified Rankin Scale (mRS); a score of ≤2 was defined as a favourable outcome. We defined the date of symptom onset as day 0 and used Kaplan–Meier survival analysis to estimate survival time in the sporadic and dental exposure groups. The log-rank test was used to compare survival differences. All statistical analyses were performed using STATA V.17.0.

Role of funding source

No specific funding was obtained to conduct the outbreak investigation.

Results

Descriptive epidemiology

We identified 21 cases of neuromelioidosis reported in four districts in Northern Tamil Nadu between July 6, 2022, and April 13 2023 (Figs. 1, 2a and 2b).

Sporadic cases

Of these cases, 11 reported in the initial period were sporadic in nature, without any particular intervention performed preceding the onset of illness. The median age of these sporadic cases was 26 years (interquartile range [IQR] 16–45), and 6 (55%) were males. More than half of the cases reported regular contact between soil and surface water. The median time from symptom onset to hospitalization was 7 days (IQR 5, 14) (Table 1, Fig. 2b). One case presented with suppurative parotitis (Supplementary Appendix 3), and four cases had cervical lymphadenopathy with buccal mucosal inflammation. Nine cases exhibited brainstem encephalitis with five cases showing radiologically confirmed myelitis. Contrast-enhanced MRI of the brain revealed patchy hyperintensities in the brainstem with varying degrees of diffusion restriction, including abscess-related restrictions in all cases. Brainstem abscesses located in the brachium-pontis and middle cerebellar peduncles were observed in six cases (Supplementary Appendix 3). A distinctive 'tunnel sign' in the supratentorial white matter was observed in all the 11 sporadic cases (Fig. 3).

Cases with exposure to dental procedures

The remaining ten cases reported from December 2022 to April 2023 were clustered around Vaniyambadi town in the Tirupattur district, and all of them reported undergoing invasive procedures that required injection of local anaesthetic and repeated irrigation with saline at a dental clinic in the same locality, thus constituting a dental case series (Figs. 1 and 2). The median age of these dental cases was 44 years (IQR 34–49), with six cases being female (Table 1). Over half of the cases reported frequent contact with soil and surface water. Three cases had diabetes with poor glycaemic control. The median time between the dental procedure and symptom onset (incubation period) was 8 days (IQR 5, 29), while the median time from symptom onset to hospitalization was 9 days (IQR, 5, 13). All the cases initially presented with facial pain, multiple cranial nerve palsies involving the trigeminal and facial nerves, and limb weakness. Inflammation at the dental procedure site ranges from facial cellulitis to maxillary or submandibular abscesses. The radiological features were similar to those of sporadic cases, although local abscess formation was more pronounced in dental cases.

Laboratory investigations

Biochemical and haematological parameters were not different between sporadic cases and those linked to

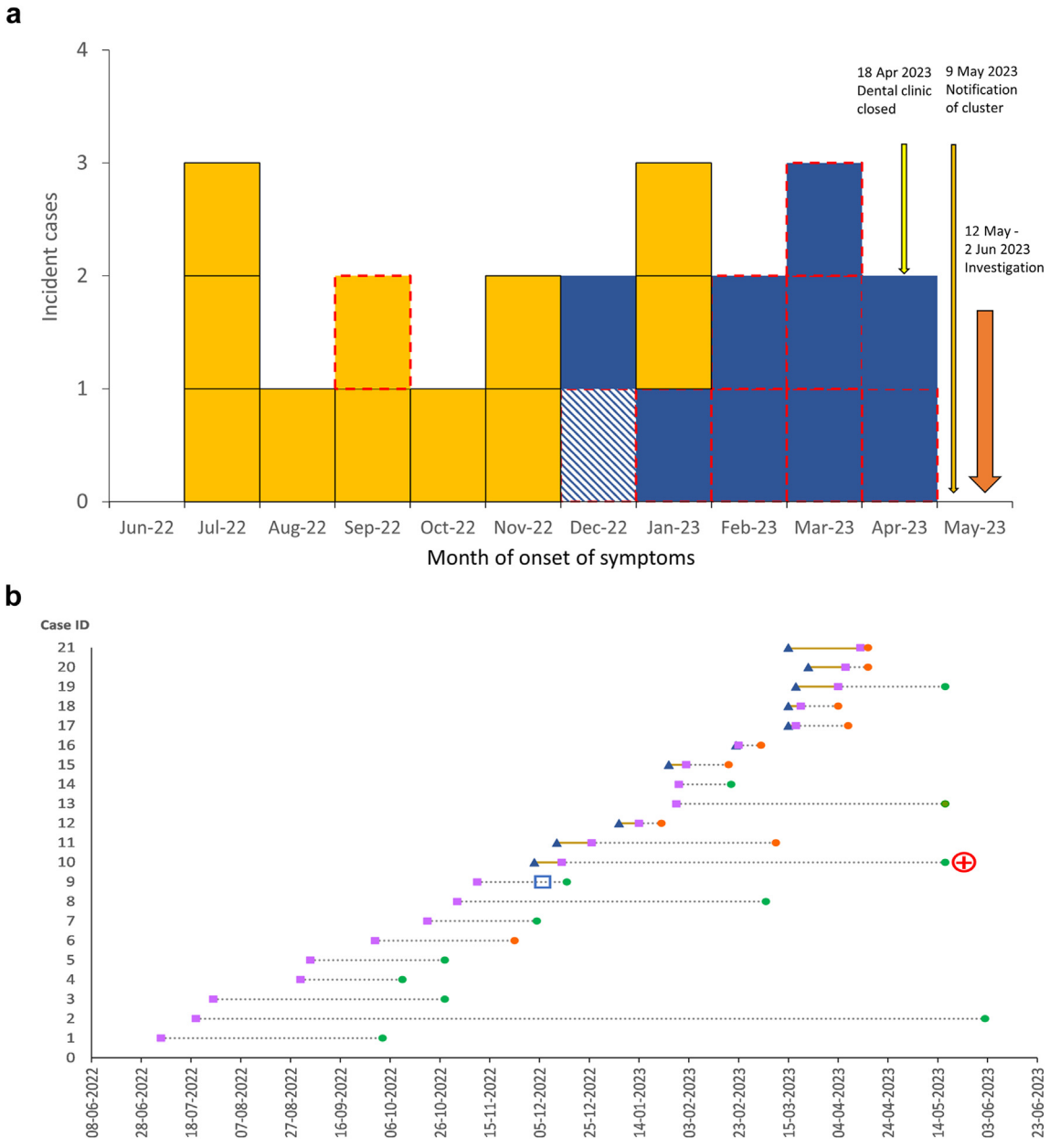

Fig. 2: **a**—Distribution of neuromelioidosis cases by month of symptom onset and type of exposure in Tamil Nadu, India, June 2022–April 2023. Sporadic. Dental. --- Died. Index case. **b**—Time intervals between exposure, symptom onset and outcome of neuromelioidosis cases by type of exposure in Tamil Nadu, India, June 2022–June 2023. Date of exposure to dental procedures. Date of symptom onset. Date of outcome (died). Date of outcome (alive). — Interval between exposure and symptom onset. ···· Interval between symptom onset and outcome. *B. pseudomallei* isolated from a brain sample. *B. pseudomallei* isolated from an environmental sample in the dental clinic.

dental exposure (Table 1). *B. pseudomallei* was isolated from two of 11 sporadic cases (one from surgical aspirate of parotid abscess and the other from the blood culture) and three of ten dental cases (two from the brain tissue and one from the facial abscess). Culture (n = 21) and PCR (n = 10) analyses of the CSF samples were negative (Supplementary Appendix 4).

Management

Fourteen cases (sporadic = 10, dental = 4) were treated at CMC Hospital, while the rest received treatment at other hospitals. The median hospital stay was 43 days (IQR 2, 97) and 23 days (IQR 9, 69) in the intensive care unit. Sixteen cases (76%) were critically ill at presentation, all required invasive ventilation, and 13 cases (47%)

	Sporadic cases (n = 11)	Dental cases (n = 10)	p value
Characteristics and exposure details			
Median age, year (IQR)[a]	26 (16,45)	44 (33,49)	0·09
Male sex	6	4	0·67
Regular contact with soil	7	7	0·56
Regular contact with surface water	7	0	0·004
Time to key events, day (IQR)[a]			
Exposure to symptom onset	NA	8 (5,29)	NA
Symptom onset to first hospitalization	7 (5, 14)	9 (5, 13)	NA
Symptom onset to any known outcome[b]	56 (41, 108)	19 (9, 46)	NA
Symptom onset to death	56	16 (9, 34)	NA
Hospitalization to death	38	9 (2,23)	NA
Clinical presentation			
Fever	11	10	
Headache	4	7	0·198
Seizures	3	1	0·586
Facial cellulitis	3	8	0·030
GCS, Mean (±SD)[c]	12	11	0·706
Facial nerve palsy	9	8	0·669
Bulbar palsy	9	6	0·361
Hemiparesis	5 (54)	2 (20)	0·361
Paraparesis	2 (18)	0 (0)	0·476
Quadriparesis	5 (45)	5 (50)	0·590
Brain stem encephalitis	9 (82)	9 (90)	0·479
Myelitis	5 (46)	0	0·030
Laboratory features, mean (±SD)[c] [reference range]			
Haemoglobin [11–15 g/dl]	11·6 (1·6)	12·9 (1·8)	0·142
Total White blood cell counts (4000–12,000/cu mm)	11,663·6 (5816·4)	17,571·4 (8495)	0·030
CSF[d] Total cell count (n/dl)	259	107	0·478
CSF[d] Neutrophil count (per dl)	190	59	0·404
CSF[d] Glucose (40–70 mg/dl)	65	107	0·946
CSF[d] Protein (15–45 mg/dl)	126	84	0·347
Laboratory confirmation (%)	2 (20)	3 (27)	
Blood culture positive (n = 21, %)	1 (4·7)	0	
CSF culture positive (n = 21, %)	0	0	
CSF PCR positive (n = 10, %)	0	0	
Brain tissue culture positive (n = 2, %)	0	2 (100)	
Lymph node culture positive (n = 7, %)	0	0	
Parotid/facial abscess culture positive (n = 2, %)	1 (50)	1 (50)	
Radiological characteristics (%)			
Isolated cranial nerve (5,7) enhancement	0 (0)	2 (20)	0·214
Cranial nerve enhancement with involvement of pons, middle cerebellar peduncles and cerebellum	1 (9)	3 (30)	0·311
Extension to subcortical white matter through CST[e]/white matter tracts	11 (100)	7 (70)	0·09
Brain stem abscesses	6 (54)	4 (40)	0·670
Cervical lymphadenopathy	4 (36)	3 (20)	0·562
Buccal mucosal thickening and inflammation	4 (36)	7 (70)	0·072
Management (%)			
Inotropic support	5 (72)	8 (90)	0·183
Invasive ventilation	6 (54)	9 (90)	0·243
Intravenous Meropenem	10 (91)	3 (30)	0·004
Outcome (%)			
Death	1 (9)	8 (80)	0·008
Follow-up status at six months (%)	**10**	**2**	
Favourable outcome (mRS[f] ≤ 2)	3 (30)	1 (50)	0·586
Unfavourable outcome (mRS[f] 4–5)	7 (70)	1 (50)	NA

[a]Interquartile range. [b]Any known outcome includes death, recovery or referral for further management. [c]Standard deviation. [d]Cerebrospinal fluid. [e]Cortico-spinal tract. [f]Modified Rankin's scale. NA Not applicable.

Table 1: **Characteristics, exposure details, time course of disease, clinical profile, management and outcome of 21 neuromelioidosis cases.**

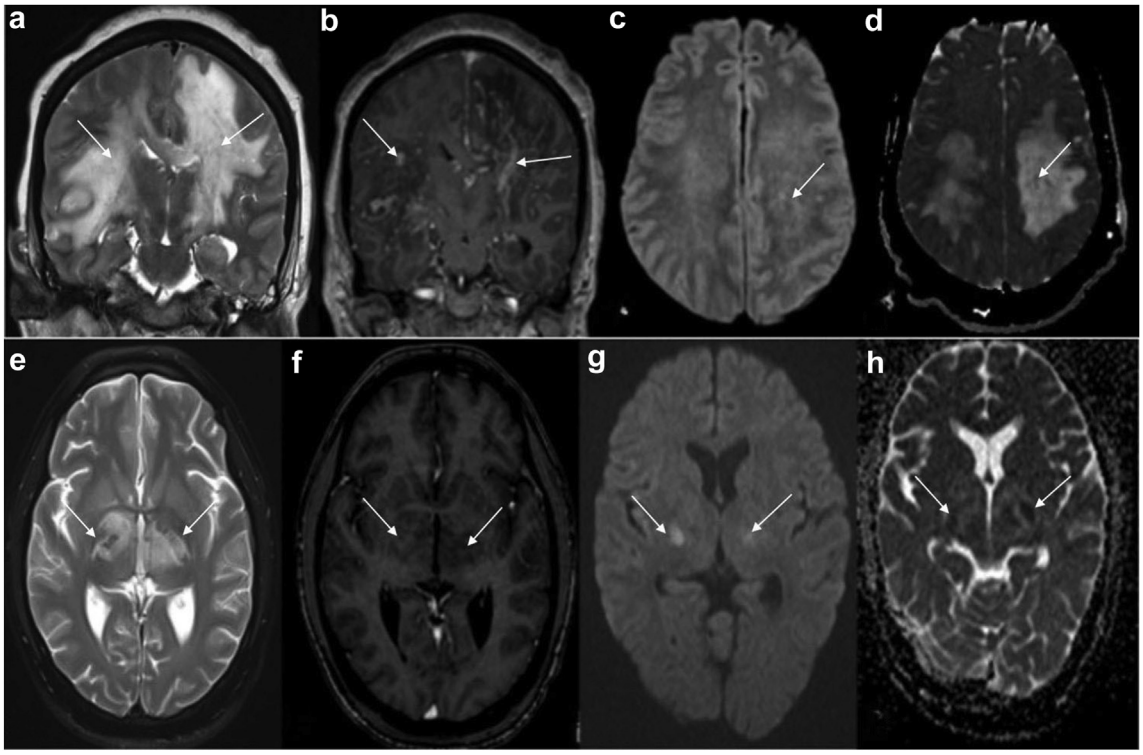

Fig. 3: **(a–d):** MRI T2 weighted coronal imaging showing 'tunnel-sign', involvement of white matter tracts causing cerebritis, **(e–h):** MRI showing typical progression along the corticospinal tracts, with involvement of posterior limb of internal capsule and ascent along the fibres, causing cerebritis.

required haemodynamic support. Thirteen cases received meningitic doses of intravenous meropenem and five cases received a combination of parenteral doxycycline and trimethoprim-sulfamethoxazole in addition to meropenem.[25] Adjunctive corticosteroids were administered to seven cases.

Clinical outcomes

The median time from symptom onset to any outcome (death, recovery, or referral for further management) was 19 days (IQR 9–46) in the dental exposure group and 56 days (IQR 41–108) in the sporadic group (Table 1). The overall case-fatality was 43% (9/21), which was higher in the dental exposure group than in the sporadic group [80% (8/10) vs. 9% (1/11)]. The survival distributions differed between the sporadic and dental exposure groups (p = 0.0008) (Supplementary Appendix 3). The median time from symptom onset to death in the dental exposure group was 16 days (IQR, 9, 34). One case in the sporadic group died on day 56. Of the deaths, two occurred in cases treated with meropenem, while seven occurred in those who did not. Six-month follow-up was completed for all 12 survivors. Among them, eight (67%) (seven in sporadic and one in dental groups) showed unfavourable outcomes in the form of severe neurological sequelae and disability (Table 1).

Confirmation of source of infection

Based on the interview with the dentist, it was evident that the dental clinic offered a wide range of services, including fixation of partial dentures, full-mouth prophylaxis, root canal treatments, and tooth extraction. The clinic was staffed with a dentist, nursing staff, and receptionist, and none of them had any formal training in hospital infection control. Normal saline supplied in sterile 500 mL plastic bottles was used for wound irrigation during surgical procedures as well as for dilution of the local anaesthetic used for infiltration. These bottles were opened using a non-sterile periosteal elevator, loosely resealed, and used over subsequent days until they were empty. An increase in failed dental procedures and complications was reported during the period when neuromelioidosis cases started to occur in the district. Of the 28 environmental samples initially collected from the clinic, *B. pseudomallei* was isolated from an in-use saline bottle. An additional 30 samples from unopened bottles (from the same and other batches), swabs from various surfaces of the dental clinic, and drug storage area tested negative. The WGS of *B. pseudomallei* isolated from the brain tissue of the first dental exposure case showed clonality with the isolate from the in-use saline bottle (Fig. 4).

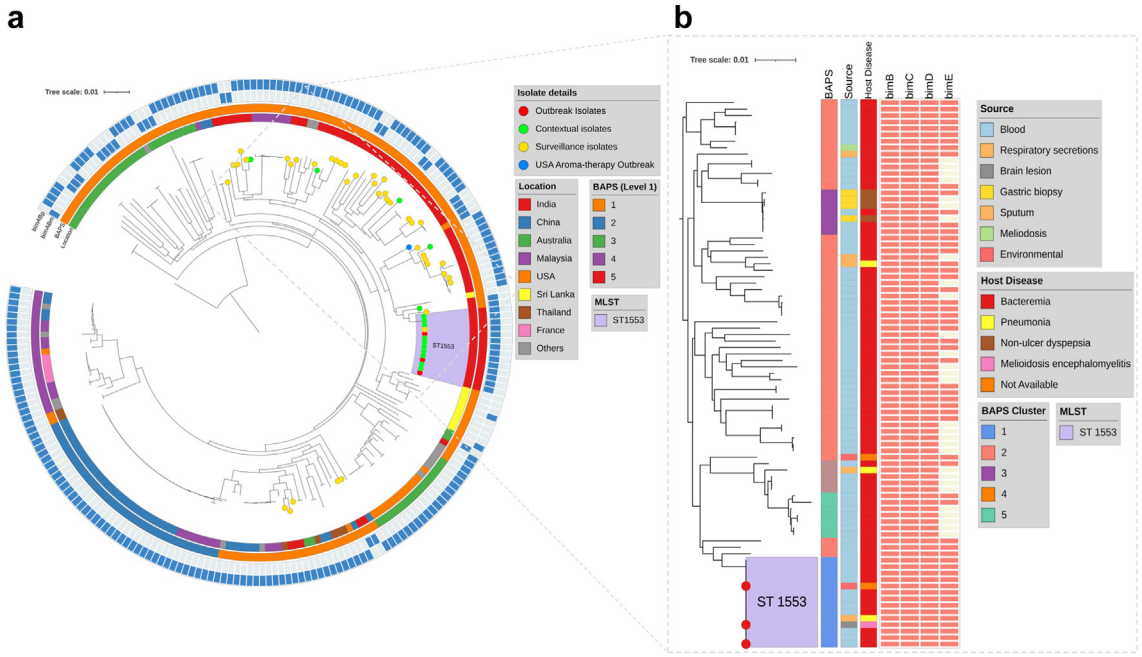

Fig. 4: Phylogenetic analysis of *Burkholderia pseudomallei* genomes, highlighting outbreak isolates within a global context. **(a)** A core SNP-based maximum likelihood phylogeny of *B. pseudomallei* genomes (n = 227), rooted on the MSHR5619 isolate. **(b)** A detailed view of the Indian *B. pseudomallei* genome cluster, depicting virulence profiles and host factors. Isolates belonging to ST1553 are shaded in lavender. Key metadata, including location, Bayesian Analysis of Population Structure (BAPS-Level 1), source of isolate, and host disease, are represented as coloured strips. The presence of $BimA_{Bm}$ is shown in a heatmap. Colour codes for variables are provided in the inset legend. Branches are marked with symbols: red for outbreak isolates, green for contextual isolates, and yellow for surveillance isolates from the study site. The scale bar indicates substitutions per site. The phylogenetic tree was visualized and annotated using iTOL (https://itol.embl.de/).

Molecular epidemiology

WGS was obtained from only two out of five isolates (one from the sporadic group – an isolate from blood and the other from the index case in the dental group – brain tissue); the other isolates could not be retrieved for WGS. All three isolates in this study (one each from sporadic and dental cases and one from the in-use saline bottle) were ST1553 and 100% clonal (Fig. 4), whereas the surveillance isolates from India showed diverse MLST profiles (Supplementary metadata). Notably, two of these surveillance isolates (one each from 2009 to 2020) were ST1553, indicating that this particular strain has been circulating in the northern districts of Tamil Nadu since 2009. The strain identified in the outbreak of melioidosis in Texas, USA, due to imported aromatherapy spray from India, was ST1941 and clustered with the Indian surveillance isolates.

All isolates belonging to the outbreak strain expressed the *B. mallei*–like $bimA_{Bm}$ gene. The majority of Indian and Australian isolates expressed these polymorphisms in the *bimA* gene, which is not exclusively associated with neurological diseases.

Discussion

We investigated a large cluster of neuromelioidosis cases from South India characterized by two distinct patterns: sporadic, epidemiologically unlinked cases, and a cluster of cases linked to a common iatrogenic source at a dental clinic. The iatrogenic source was confirmed by clonality in the whole-genome sequences of *B. pseudomallei* isolates from a case and an in-use saline bottle at the dental clinic. Head and neck involvement, such as inflammation of the parotid gland, buccal mucosa, and lymphadenopathy, was common in sporadic cases, while those with dental exposure showed rapid progression, presenting with facial cellulitis and soft tissue abscess. These clinical features suggest pathogenesis through oral mucosal ingestion or inoculation with contaminated water, leading to infection.[16,22]

However, reports on neurological involvement are rare and mostly documented as secondary to haematogenous dissemination from distant sites.[16,26] Prior to this report, only 18 cases of neuromelioidosis were recorded over an 11-year period in the reporting hospital and seven cases over a six-year period from another hospital in South India.[16,17] While the source of sporadic cases remains undetermined, most cases reported frequent contact with surface water potentially exposing them to increased aerosolization of bacteria or increased bacterial load in ground water during post-monsoon months (July to January).[27] Additionally, the presence of myelitis in the sporadic group could support the

hypothesis of bacterial inoculation in the limbs and spread through nerves or plexus.[28]

The isolation of *B. pseudomallei* from a saline bottle at the implicated dental clinic in May 2023 and its clonality to the isolate from the brain tissue of the first dental case in December 2022 strongly suggests an iatrogenic common source exposure. Saline bottles were often opened using available tools and washed in tap water. Inadequate IPC practices at the clinic, as evidenced by the observations, might have facilitated contamination of dental instruments from the dental unit water system, potentially exacerbated by prevailing post-monsoon conditions. Although previous reports of iatrogenic infections following exposure to contaminated irrigation fluid, handwash liquid, and invasive dental procedures exist, there are no reported CNS manifestations.[3–7,23]

The absence of *B. pseudomallei* in unopened saline bottles reduces the likelihood of manufacturing contamination, and the temporal nature of the cases in the dental group suggests a localized common source of contamination within the clinic. Further investigation to determine the full extent of contamination within the dental clinic, including testing of dental unit water systems, was limited as the clinic was disinfected and closed following a public outcry. Notwithstanding the above proposition, we cannot rule out any contamination other than the above-mentioned environmental sources. Direct contamination of instruments and the clinical environment from infected body fluids cannot be ruled out, as environmental contamination with *B. pseudomallei* from infected body fluids has been reported previously.[29]

Most cases presented with brainstem syndrome, characterized by prominent facial pain and cranial nerve palsies, likely indicating neuronal spread along free trigeminal or facial nerve endings following inhalation or oral inoculation. The use of contaminated saline during invasive dental procedures could have introduced *B. pseudomallei* into the brainstem via trigeminal free nerve endings, thereby causing necrotizing encephalitis. Rapid disease progression and higher fatal outcomes following direct inoculation among dental clusters offer additional supportive evidence for the pathogenesis. Sporadic cases showed similar radiological findings, such as trigeminal and facial nerve enhancement, along with evidence of orofacial involvement, including suppurative parotitis, indicating common pathogenesis in both groups. Evidence from a study in Australia shows the development of classical neuromelioidosis after direct invasion by *B. pseudomallei*.[14] Studies on mouse models suggest that intranasal inoculation can lead to neurological involvement via the olfactory nerve as a portal of entry for *B. pseudomallei* into the brain.[30] Disability was much higher than that seen in other studies,[15] which is influenced by the mode of transmission, strain differences, and inadequate management due to lack of suspicion.

The diagnosis of primary neuromelioidosis is challenging as microbiological confirmation relies on tissue samples, with bacteremic developing later in disease course.[31] Typical imaging findings including cranial nerve enhancement, pattern of diffusion restriction and supratentorial spread along the white matter tracts also called 'tunnel sign,' recognition of which helped in timely initiation of empirical antibiotics that resulted in averting mortality in some cases. Radiological 'tunnel sign' is not specific for neuromelioidosis, as cerebral sparganosis and listeriosis often have the reported radiological finding. However, sparganosis has a typical exposure history to the consumption of raw or undercooked frogs or snakes, or using the flesh of an infected host as a poultice for open wounds, peripheral and/or CSF eosinophilia, evidence of calcifications, and retrieval of 'live worms' in surgical procedures. None of these features were observed in our series. Blood cultures are highly sensitive tests for listeriosis, which is an important differential diagnosis for necrotizing brainstem encephalitis, and none of the cases in our series had a positive blood or CSF culture for *Listeria monocytogenes*. As reported earlier,[32] cases responded well to meropenem. The higher case fatality in dental cases likely resulted from a lack of suspicion of neuromelioidosis in other centres where they were treated, which led to inappropriate antibiotic therapy in other hospitals.[22]

The presence of $bimA_{Bm}$ gene confers the advantage of neurotropism to *B. pseudomallei* and hence explains the higher proportion of neurological involvement and severe outcomes in this cluster.[10] This virulence factor offers a motility advantage to the bacteria, thereby helping in rapid progression along the nerves. This pathogenesis is clearly visualized in imaging as disease progression along the white matter tracts, indicating strong neurotropism. To date, only a few cases of $bimA_{Bm}$ variants have been reported in Australia, Sri Lanka, and India.[10,20] However, the contextual isolates also expressed the $BimA_{Bm}$ allele, and none had neurological involvement. Although evidence from an Australian study[10] suggests that the presence of this allele was five times more likely to lead to neurological involvement, the numbers are too small to make such a conclusion from the current investigation. Further research is warranted to determine markers of neurovirulence.[5,19]

Our study has certain limitations. Microbiological confirmation was only possible in a subset of cases because of limited consent for brain biopsy. Among the 5 isolates, only 2 were available for WGS. Despite this, the clinical, laboratory, and imaging similarities favour that the outbreak is likely to be due to the dominant strain in circulation with expression of the $bimA_{Bm}$ allele. The lack of availability of dental clinic registers limited us from determining the number of individuals exposed to contaminated saline. However, given the

severe and rapidly fatal outcomes, it is unlikely that the extent of the outbreak was underestimated.

Conclusions

We identified a cluster of primary neuromelioidosis cases in South India, likely arising from both sporadic environmental exposures and a potential iatrogenic source linked with a dental clinic. The temporal association between dental procedures and rapid symptom onset and the clonality of *B. pseudomallei* isolates from clinical and environmental samples suggests a plausible link to contaminated saline. Rapid clinical progression and high fatality among individuals exposed through dental procedures highlight the possibility of direct trans-neural spread to the brainstem. The presence of $bimA_{Bm}$ in the available isolates indicates the circulation of a potentially virulent strain in the region. The lack of clinical suspicion and diagnostic delays might have contributed to the higher fatality. These findings emphasize the importance of strict infection control and prevention practices in healthcare settings, including dental facilities, especially in melioidosis-endemic areas. Detailed clinical, radiological, and management data from these cases may help early recognition and appropriate management of neuromelioidosis.

Contributors

Conceptualisation: A.M.T, P.R, K.G, P.M, M.P, G.K.C.P, M.M and B.V; Formal analysis: A.M.T, P.R, K.G, P.M, M.P, G.K.C.P, M.M and B.V; Funding acquisition: NIL; Investigation: all authors; Methodology: A.M.T, P.R, K.G, P.M, M.P, G.K.C.P, A.V, A.R.N, J.J.J, M.M and B.V.; Project administration: A.M.T, P.R, K.G, P.M, M.P, G.K.C.P, M.M and B.V; Supervision: M.M, and B.V.; Validation: All authors; Visualisation: A.M.T, P.R, K.G, M.P, G.K.C.P.; Writing – original draft: A.M.T, P.R, K.G, P.M, M.P, G.K.C.P, M.M and B.V; and Writing – review & editing: all authors.

All authors had full access to all data in the study and had final responsibility for the decision to submit for publication. A.M.T, P.R, K.G, P.M, M.P, G.K.C.P, M.M, and B.V directly accessed and verified the underlying data reported in the manuscript.

Data sharing statement

De-identified participant data can be made available upon reasonable request and in accordance with the General Data Protection Regulation (GDPR) two years after the publication of the primary results. Proposals should be directed toward mmurekhar@nieicmr.org.in.

Editor note

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Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.lansea.2025.100602.

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