



Systematic Multi-Platform Laboratory Investigation of a Disease of Unknown Aetiology in Rubirizi District, Uganda, December 2025

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Summary

Background: Outbreaks of unknown aetiology present diagnostic and therapeutic challenges to public health systems. In the absence of a confirmed diagnosis, empirical antimicrobial treatment may be necessary but can increase unnecessary antimicrobial exposure and selection pressure for antimicrobial resistance (AMR). On November 26, 2025, the Ministry of Health received an alert of an illness of unknown aetiology in Kyabakara Subcounty, Rubirizi District. Thirteen cases, including four deaths, were identified, characterized by unilateral, fluid-filled blisters on the lower limbs that progressed to severe, non-healing ulcers. We conducted systematic, multi-platform laboratory investigations to identify possible causes, guide clinical management, and support public health decision-making.

Methods: Clinical samples including wound swabs, blood, and skin snips were collected and analyzed from eighteen cases. Environmental samples were collected from affected cases homes. Multi-platform diagnostic framework was utilized including microscopy, serology, histopathology, molecular diagnostics, heavy metal analysis, and genomic sequencing. Bacterial cultures and antimicrobial susceptibility testing (AST) were used to identify and establish the resistance profiles to guide treatment decisions.

Results: Of the eighteen (18) patients tested, 1 (6%) was positive for malaria, while 1 (6%) was positive for filarial worms. One (6%) tested positive for leishmaniasis, while another (6%) was positive for Rift Valley fever and 1 (6%) had Kaposi sarcoma. No acid-fast bacilli were detected for *Mycobacterium* species. Among the heavy metals analysed only Iron was detected in the soil samples. All eighteen patients (100%) were negative for anthrax and whole genome sequencing did not identify any pathogen that adequately explained the patients' clinical symptoms. Bacterial culture and antimicrobial susceptibility testing identified *Pseudomonas putida*, *Pseudomonas* spp, *Citrobacter freundii*, and *Klebsiella pneumoniae* and *Staphylococcus aureus*. One (6%) of the patients had extended-spectrum β -lactamase (ESBL) producing *K. pneumoniae*. *S. aureus* isolates from one patient (6%) demonstrated methicillin resistance (MRSA) and β -lactamase production.

Conclusion: Establishing a standardized national testing algorithm improves diagnostic efficiency, and supports public health decision-making during outbreaks of



unknown aetiology. The detection of ESBL-producing *K. pneumoniae* and methicillin-resistant and β -lactamase-producing *S. aureus* highlights the resistance to commonly used antibiotics. These results guided appropriate treatment of secondary bacterial wound infections and supported antimicrobial stewardship.

Background

On 26 November 2025, Uganda's Ministry of Health received an alert of community deaths from an unknown illness in Kyabakara Sub-county, Rubirizi District. Between March and December 2025, 13 cases were identified, including four deaths (31%). The illness was characterized by unilateral fluid-filled blisters on the lower limbs that progressed to severe, non-healing ulcers.

Chronic ulcers can have infectious and non-infectious causes, making diagnosis difficult when clinical features are non-specific. In outbreaks of unknown aetiology, delayed diagnosis may lead to prolonged empirical treatment and inappropriate antimicrobial use. Laboratory investigation is therefore essential to identify potential causes and guide clinical management and public health response (1).

We used a multi-platform laboratory testing approach to investigate the aetiology of the illness and inform treatment, antimicrobial stewardship, and outbreak-control decisions.

Methods

We conducted the investigation in Rubirizi District, western Uganda, a predominantly rural farming district that includes part of Queen Elizabeth National Park.

Clinical specimens, including wound swabs, blood, and skin snips, were collected from 18 case-patients, together with soil samples from affected households. Microscopy and serology were performed at Rugazi Health Centre IV, histopathology at Mulago National Referral Hospital, heavy-metal testing at the Directorate of Government Analytical Laboratory, pathogen discovery at the Uganda Virus Research Institute, bacterial culture and antimicrobial susceptibility testing at the National Microbiology Reference Laboratory, and genomic sequencing at the National Genomic Sequencing Laboratory.

The investigation was conducted as part of the Ministry of Health emergency public health response and was determined to be non-research by US CDC. Verbal informed consent was obtained from participants, and confidentiality was maintained through secure data storage.

Results

Of the eighteen patients' samples tested, one (6%) was positive for malaria, while one (6%) was positive for filarial worms by microscopy and antigen testing. One (6%) tested positive for leishmaniasis, while another (6%) was positive for Rift Valley fever and 1 (6%) was positive for Kaposi sarcoma. No (0%) acid-fast bacilli were detected by microscopy for *Mycobacterium* species, Random blood glucose, complete blood count, and renal function tests results were within normal limits for all patients tested.



Among the heavy metals analysed only Iron was detected in the soil samples, Aluminium, Iron, Arsenic, Cadmium, Mercury and lead were not detected. All eighteen patients (100%) were negative for anthrax and whole genome sequencing did not identify any pathogen that adequately explained the patients' clinical symptoms. Pathogen discovery identified *Mycobacteroides abscessus* and *Paraburkholderia fungorum* in two cases (11%). Bacterial culture and antimicrobial susceptibility testing identified several bacterial species, including Gram-negative organisms such as *Pseudomonas putida*, *Pseudomonas* spp, *Citrobacter freundii*, and *Klebsiella pneumoniae*, as well as the Gram-positive bacterium including *Staphylococcus aureus*. Out of the eighteen (18) patients whose samples were tested One (6%) patients had extended-spectrum β -lactamase (ESBL) producing *K. pneumoniae*. *S. aureus* isolates from one patient (6%) demonstrated methicillin resistance (MRSA) and β -lactamase production.

Discussion

The detection of ESBL-producing *K. pneumoniae* and methicillin-resistant and β -lactamase-producing *S. aureus* highlights the presence of clinically important antimicrobial resistance and the potential limitations of empirical antibiotic treatment applied before the investigation (2). These results underscored the importance of antimicrobial susceptibility testing to guide appropriate treatment and support antimicrobial stewardship (3).

The detection of iron in soil samples was also noteworthy but cannot, by itself, establish an environmental cause of illness. Its relevance requires assessment of concentration, chemical form, exposure pathways, and evidence of exposure among affected individuals. The detection of leishmaniasis and Kaposi sarcoma in individual patients, suggests that some cases may have had distinct underlying conditions rather than a common disease process.

The identification of *Mycobacteroides abscessus* and *Paraburkholderia fungorum* in the two cases could explain the secondary bacterial infection observed in the cases. *M. abscessus* is usually found in soil and water, and has intrinsic and acquired antimicrobial resistance. This bacteria could easily colonise the wounds and lead to the severe non healing ulcers observed in this investigation (4).

The negative findings for anthrax and acid-fast bacilli reduced the likelihood of several suspected infectious causes, while the metagenomic analysis did not identify a pathogen that adequately explained the clinical presentation. However, this negative finding does not exclude infection as the sample type or timing of collection may have affected the results. Overall, the investigation did not identify a single infectious etiology that could explain the clinical manifestations among the eighteen cases.

Study limitation: The recovery of several bacterial species, including *Pseudomonas*, *Citrobacter freundii*, *Klebsiella pneumoniae*, and *Staphylococcus aureus* may represent colonization or environmental contamination rather than the causes of disease. To mitigate this limitation, findings were assessed alongside clinical presentation and antimicrobial susceptibility results, with emphasis on significant resistance patterns such as ESBL production and methicillin resistance.



Conclusion: The systematic multi-platform testing approach excluded high-consequence pathogens. Although no primary etiological agent was identified, AST a guided appropriate treatment of secondary bacterial wound infections and supported antimicrobial stewardship. Establishing a standardized national testing algorithm improves diagnostic efficiency, and supports public health decision-making during outbreaks of unknown aetiology. The detection of ESBL- and selection producing *K. pneumoniae* and methicillin-resistant and β -lactamase-producing *S. aureus* highlights that empirical antimicrobial treatment may increase unnecessary antimicrobial exposure pressure for antimicrobial resistance (AMR) to commonly used antibiotics. We recommend establishment of a standardized national testing algorithm to improve diagnosis, and support public health decision-making during future outbreaks of unknown aetiology.

Conflict of Interest: The authors declare no conflict of interest.

Author contribution: CM led the study conceptualization, data collection, analysis, and article drafting, AA, MN, IM, WS, VN, WO, ST, DK, ADA, SK, DA, MM, SN, AN, SNN, contributed to data collection and writing. SG, GE provided supervision, and article review. All authors read and approved the final manuscript.

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