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An outbreak of *Alkalihalobacillus clausii* bacteraemia linked to probiotic use in private-sector hospitals, Gauteng and North West provinces, South Africa, February–October 2024

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Summary

Alkalihalobacillus clausii (*A. clausii*) is widely used in probiotic formulations for the management of antibiotic-associated diarrhoea. Although generally regarded as safe, increasing reports of *A. clausii* bacteraemia have raised concerns about its safety in vulnerable populations. On 11 June 2024, the National Institute for Communicable Diseases was alerted to an unusual increase in *A. clausii* bacteraemia in several South African private-sector hospitals. An outbreak investigation was initiated, including retrospective clinical review of laboratory-confirmed cases (defined as *A. clausii* isolated from blood culture) and advanced isolate characterisation, including whole-genome sequencing (WGS) of clinical isolates and open probiotic sachets. Between February and October 2024, 11 cases (patients) with *A. clausii* bacteraemia were identified across eight hospitals in South Africa's Gauteng and North West provinces. The median age was seven years (interquartile range: 2–55 years), and 55% of patients were female. Five patients with available information had underlying conditions, and seven had consumed *A. clausii*-containing probiotics; a majority (5/8) had persistent bacteraemia. Three patients died (27% case fatality ratio). WGS demonstrated that isolates from four patients and two *A. clausii* containing probiotic samples were genetically almost identical (≤3 single nucleotide polymorphisms), confirming the probiotic as the most likely source of the outbreak. This outbreak demonstrates that *A. clausii* probiotics, while therapeutically beneficial in some settings, can cause severe invasive infections in vulnerable patients. These findings highlight the importance of rigorous safety assessments, quality control, and evidence-based clinical guidelines for probiotic use in hospitals. Strengthened case-based surveillance and clinician awareness are recommended to mitigate risks and protect patients.

Introduction

Alkalihalobacillus clausii (*A. clausii*), previously known as *Bacillus clausii*, is a non-pathogenic, antibiotic-resistant, spore-forming gram-positive bacillus that has demonstrated probiotic properties with significant therapeutic potential.[1] Research indicates that *A. clausii* spores can mitigate antibiotic-induced intestinal injury by maintaining colonic integrity, decreasing inflammation, and restoring the balance of gut microbiota in mice.[2] In a clinical trial, *A. clausii* was effective in treating antibiotic-associated diarrhoea in both paediatric and adult patients, resulting in reduced frequency and severity of diarrhoea without causing adverse effects.[3]

Despite its intended benefits, there have been documented cases and outbreaks of bacteraemia associated with the use of *A. clausii* probiotics among immunocompromised and immunocompetent adults and children alike.[4–7] One report detailed a four-month-old infant who developed septicaemia after receiving a probiotic containing *A. clausii* for management of acute diarrhoea.[8] Furthermore, a recent case series involving immunosuppressed adults provided genetic evidence linking *A. clausii* bacteraemia to probiotic therapy, marking the first confirmed association between *A. clausii* probiotic and invasive disease.[9] Beyond individual cases, large-scale healthcare-associated outbreaks related to *A. clausii* have also been linked to contaminated medical products, including hospital linen and calcium gluconate solutions.[10,11]

On 11 June 2024, the National Institute for Communicable Diseases (NICD), a division of the National Health Laboratory Service (NHLS), was notified of a suspected outbreak of *A. clausii* bacteraemia in patients admitted to various private-sector hospitals in Gauteng, South Africa. The suspected outbreak was notified by a clinical microbiologist from a private laboratory in the City of Tshwane Metropolitan. At the time of notification, the source of the infections was suspected to be related to the use of *A. clausii*-containing probiotic. The probiotic was reported to be widely used in both inpatient and outpatient settings in private-sector hospitals. According to the literature available at the time of the investigation, an outbreak associated with *A. clausii* use had never been reported in South Africa. An outbreak investigation was conducted to determine the extent of the outbreak, identify patients potentially at risk of infection, and confirm the source of the outbreak.

Methods

Following the outbreak notification, a retrospective review of laboratory-confirmed *A. clausii* bacteraemia cases was conducted. A line list of patients with *A. clausii* isolated from blood cultures between February and October 2024 was obtained from the reporting private sector laboratory. A case was defined as any patient with *A. clausii* isolated from a blood culture from February to October 2024. This period was chosen because the reporting clinical microbiologist indicated that no cases had been diagnosed in the laboratory during the previous year. Demographic and clinical characteristics were described using data collected with a standardised case report form. A timeline of cases was generated using Microsoft Excel.

Clinical isolates and two opened *A. clausii*-containing probiotic packets used by affected patients were sent to the Centre for Healthcare Associated Infections, Antimicrobial Resistance and Mycoses (CHARM) at the NICD for testing. Bacterial isolates were obtained from culture on blood agar, and identification was conducted using Matrix-Assisted Laser Desorption/Ionisation Time-of-Flight Mass Spectrometry (MALDI-TOF MS) (Microflex, Bruker Daltonics, USA). Genomic DNA was extracted with the QIAamp Mini kit (Qiagen, Germany), and the DNA concentration was quantified on the Qubit-4 fluorometer (Thermo Fisher Scientific, Waltham, MA, United States).

Library preparation was done with the Nextera DNA Flex library prep kit (Illumina, USA). Whole-genome sequencing was done using the NextSeq system (Illumina, USA) at a 2x300 bp read length at a 100x coverage. Bioinformatic analysis was conducted using the Jekesa pipeline (https://github.com/stanikae/jekesa). Briefly, Trim Galore! (v0.6.2; https://github.com/FelixKrueger/-TrimGalore) was used to filter the paired-end reads (Q>30 and length >50 bp). De novo assembly was performed using SPAdes v3.13, and the assembled contigs were polished using Shovill (v1.1.0; https://github.com/tseemann/shovill).[12,13]

The multi-locus sequence typing (MLST) profiles were determined using the MLST tool (v2.16.4; https://github.com/tseemann/mlst). Assembly metrics were calculated using QUAST (v5.0.2; http://quast.sourceforge.net/quast). Whole-genome single nucleotide polymorphism (SNP) differences

were determined using a reference-free approach using the SKA toolkit. The Resistance Gene Identifier (RGI) tool (v5.2.0) is hosted at the web portal of the Comprehensive Antibiotic Resistance Database (CARD) (https://card.mcmaster.ca/), and ResFinder (https://cge.cbs.dtu.dk/services/ResFinder/) was used to describe the resistome of *A. clausii* from the assembled genome sequences.[14]

Results

From February 2024 to October 2024, 11 cases (patients) were reported across eight private-sector hospitals (Figure 1) in the Gauteng (n=10) and North West (n=1) provinces. Seven patients had more than one positive culture during their hospital stay. The median age was seven years (IQR: 2–55 years), and 55% (6/11) were female. Available clinical information for all patients is provided in Table 1.

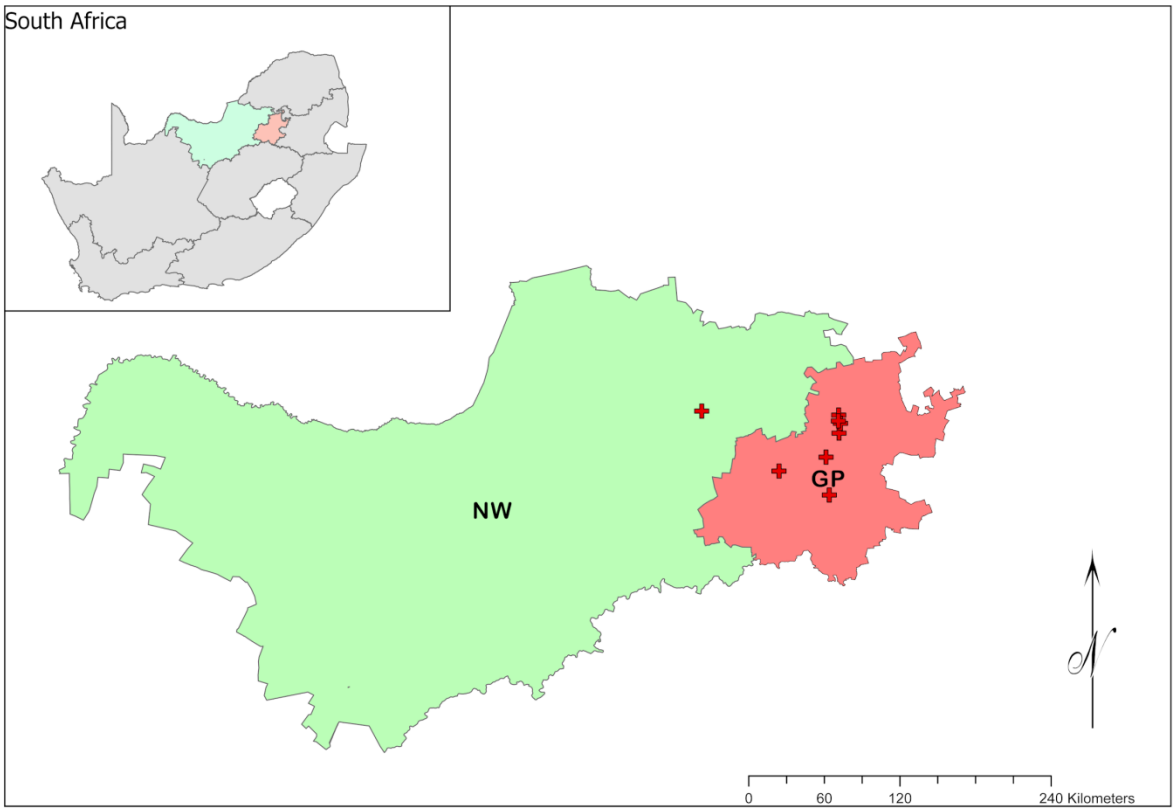

Figure 1. Hospitals reporting *Alkalihalobacillus clausii* patients in the Gauteng (GP) and North West (NW) provinces, South Africa, February 2024 to October 2024. Map created using ArcGIS Pro (V3.2).

The first case (Patient A) was a 24-year-old male admitted on 27 February 2024 with *A. clausii*-positive blood culture collected on 03 and 26 March 2024. This patient reported using the *A. clausii*-containing probiotic over an unknown period and frequency since November 2023 (Figure 2). Patient D, an 82-year-old female, was admitted on 20 May 2024, and blood specimens were collected from her on 30 May 2024 and 01 June 2024. The patient was administered the *A. clausii*-containing probiotic in hospital from 25 May 2024 to 01 June 2024. She had a perforated peptic ulcer and demised on 02 June 2024. Patient

E, a 55-year-old male, reported purchasing the probiotic on 05 June 2024. He was admitted on 06 June 2024, and his blood specimens were collected on 06 and 08 June 2024. The patient was discharged on an unknown date. An open package of the probiotic used by him, along with isolates obtained from his blood cultures, was sent to the NICD. Patients B, C, H, and I (age range 1–12 years of age) were all admitted to the same hospital, with three in the paediatric intensive care unit and one in the general paediatric ward. Three of these patients were administered *A. clausii*-containing probiotics in hospital prior to blood culture collection. The *A. clausii*-containing probiotic packet used by Patient J was also sent to the NICD. The last reported case (Patient K) was a two-year-old female whose blood culture specimen was collected on 18 October 2024, with probiotic exposure status and outcome unknown (Table 1).

Treatment information for bacteraemia was available for three patients; Patient A was administered a combination of vancomycin, ciprofloxacin, and doripenem. Patient E was administered vancomycin, ciprofloxacin, and imipenem, while Patient D received vancomycin alone. Of the nine patients with known outcome information, six were discharged and three died (27% case fatality ratio) (Table 1).

Clinical isolates from Patients E, H, I, and J, as well as isolates cultured from the probiotic samples, were identified as *A. clausii*. An SNP analysis showed a mean SNP difference of 1.4, with differences ranging 0–3 between six isolates (Table 2). The Sequence Type (ST) for the isolates was undefined. All six isolates, both clinical and probiotic, carried the same set of acquired and intrinsic antibiotic resistance genes (Table 3). These included genes conferring resistance to aminoglycosides (*ANT(4')-Ib*, *aadD2*), beta-lactams (*blaBCL-1*), macrolides (*Erm(34)*), phenicols (*catA*), tetracyclines (*tetB(P)*), oxazolidinones (*clbB/C*), and glycopeptides. Glycopeptide resistance was associated with genes from the *vanG* cluster (*vanG*, *vanT*, and *vanY*) as well as *vanW* from the *vanI* cluster.

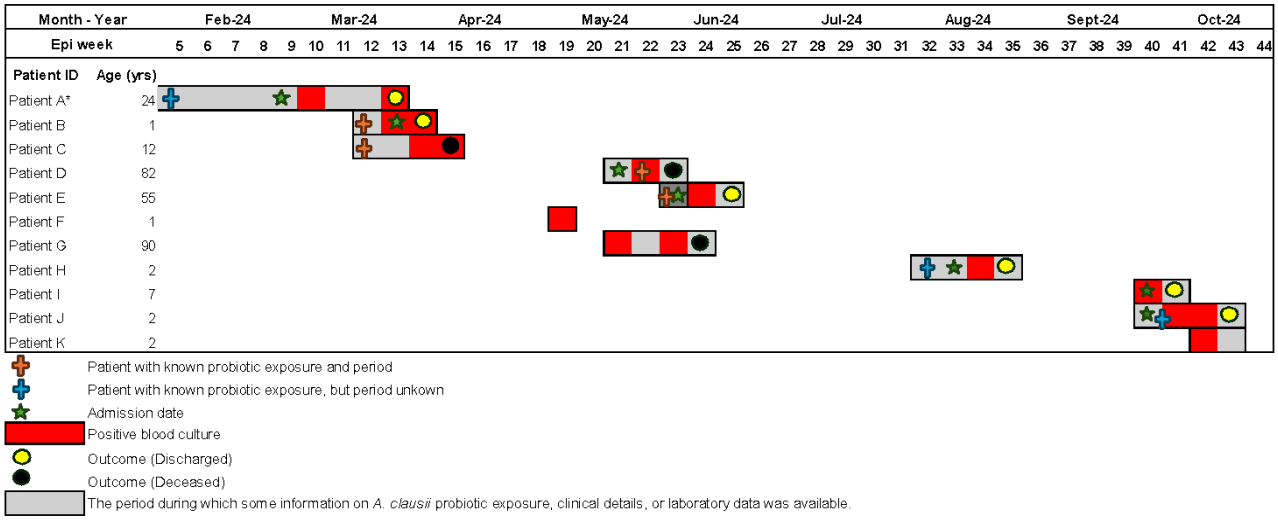

Figure 2. *Alkalihalobacillus clausii* outbreak timeline, clinical information, and age of cases in the Gauteng and North West provinces, South Africa, February 2024–October 2024.
*Patient A has been exposed to the probiotic since November 2023.

Table 1. Characteristics of patients of *Alkalihalobacillus clausii* bacteraemia in private-sector hospitals in the Gauteng and North West provinces, South Africa, February 2024–October 2024.

Patient ID	Age in years	Sex	Ward	Underlying condition	Antibiotics used	Probiotic use	*A.clausii* probiotic start date (duration)	Outcome
Patient A	24	Male	Orthopaedic ward	Solid tumour, developed infective endocarditis of aortic valve	Vancomycin, doripenem, ciprofloxacin	Yes	November 2023	Discharged
Patient B	1	Male	Paediatric ICU	Unknown	Unknown	Yes	26 March 2024 (8 days)	Discharged
Patient C	12	Female	Paediatric ICU	Ecthyma gangronosum	Unknown	Yes	19 March 2024 (9 days)	Died
Patient D	82	Female	Intensive care unit	Perforated peptic ulcer	Vancomycin	Yes	25 May 2024 (7 days)	Died
Patient E	55	Male	Medical ward	Pneumoconiosis, chronic obstructive pulmonary disease	Vancomycin, imipenem, ciprofloxacin	Yes	5 June 2024	Discharged
Patient F	1	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown
Patient G	90	Unknown	Unknown	Unknown	Unknown	Unknown	Unknown	Died
Patient H	2	Female	Paediatric ICU	Unknown	Unknown	Yes	Unknown	Discharged
Patient I	7	Female	Paediatric ward	Unknown	Unknown	Unknown	Unknown	Discharged
Patient J	2	Female	Paediatric ICU	Down syndrome, colitis, gastroenteritis, multiple organ failure	Unknown	Yes	Unknown	Discharged
Patient K	2	Female	Neonatal ward	Unknown	Unknown	Unknown	Unknown	Unknown

Table 2. Single nucleotide polymorphisms (SNP) of *Alkalihalobacillus clausii* isolated from clinical specimens and probiotic sachets used by the patients in the Gauteng and North West provinces, South Africa, February 2024–October 2024, n=6.

SNP differences	ML6922 (Patient E)	ML6923 (Probiotic)	ML6975 (Patient H)	ML7005 (Patient J)	ML7006 (Patient I)	ML7007 (Probiotic)
ML6922 (Patient E)		2	0	0	1	0
ML6923 (Probiotic)	2		2	3	3	2
ML6975 (Patient H)	0	2		0	2	1
ML7005 (Patient J)	0	3	0		2	0
ML7006 (Patient I)	1	3	2	2		3
ML7007 (Probiotic)	0	2	1	0	3	

Table 3. Acquired and intrinsic antibiotic resistance genes associated with *Alkalihalobacillus clausii* isolated from clinical specimens and probiotic sachets used by the patients in the Gauteng and North West provinces, South Africa, February 2024–October 2024.

Drug class	Antimicrobial resistance genes detected	ML6922 (Patient E)	ML6923 (Probiotic)	ML6975 (Patient H)	ML7005 (Patient J)	ML7006 (Patient I)	ML7007 (Probiotic)
Aminoglycoside	*ANT(4)*-Ib	Present	Present	Present	Present	Present	Present
	*aad*D2	Present	Present	Present	Present	Present	Present
Macrolide	*Erm*(34)	Present	Present	Present	Present	Present	Present
beta-lactam	*bla*BCL-1	Present	Present	Present	Present	Present	Present
Glycopeptide	*van*W gene in *van*I cluster	Present	Present	Present	Present	Present	Present
	*van*T gene in *van*G cluster	Present	Present	Present	Present	Present	Present
	*van*Y gene in *van*G cluster	Present	Present	Present	Present	Present	Present
	vanG	Present	Present	Present	Present	Present	Present
Oxazolidinone	*clbB/C*	Present	Present	Present	Present	Present	Present
Phenicol	*catA*	Present	Present	Present	Present	Present	Present
Tetracycline	*tet*B(P)	Present	Present	Present	Present	Present	Present

Discussion

In June 2024, an outbreak of *A. clausii* bacteraemia was reported in private hospitals across the Gauteng and North West provinces, affecting 11 patients over the nine months. No similar infections were identified from the reporting laboratory in the previous year, indicating a marked increase above baseline and confirming an outbreak. The affected patients varied in age and underlying conditions and were admitted to different hospitals, indicating that the outbreak was not confined to a specific demographic or clinical setting. However, the cluster of four patients within one facility's paediatric ward suggested potential healthcare-associated transmission, although limited clinical and epidemiological data prevented full assessment. Seven of the 11 patients affected had taken the probiotic prior to infection onset, and whole-genome sequencing demonstrated a close genetic match between isolates from four patients and two probiotic sachets, strongly implicating the probiotic as the likely source.

Alkalihalobacillus clausii is widely marketed as a probiotic supplement intended to restore intestinal flora and manage antibiotic-associated diarrhoea. However, commercially available *A. clausii* probiotic-related bacteraemia has been reported.[4,5,7–9,15] These document invasive *A. clausii* infections, particularly in vulnerable populations, and this is the first reported outbreak in South Africa.[5,9,16] In the present outbreak, isolates from four patients and two probiotic sachets were genetically similar, differing by fewer than three SNPs, strongly implicating the commercially available probiotic.[9] Although not clear, the

outbreak may have been triggered by changes in patient demographics, increased probiotic use in hospitals, or factors that heightened patient susceptibility.

Historical outbreaks of healthcare-associated infections linked to contaminated probiotics or other biological products, including *Bacillus cereus* in neonatal ICUs and *Saccharomyces boulardii* in critically ill adult patients, demonstrate the potential for such products to act as vectors in hospital settings.[17,18] In this outbreak, multiple cases occurred within a single paediatric ward. Genetic data indicated the probiotic as the probable source, but lack of data prevented a full assessment to rule out horizontal transmission. These observations highlight the need for surveillance in facilities where probiotics are administered to high-risk patients.

All patients with available clinical information had underlying medical conditions, and several were at the extremes of age. This highlights the elevated risk of probiotic-associated bacteraemia in these vulnerable populations. One patient with ulcerative colitis developed *A. clausii* bacteraemia following probiotic administration. While probiotics can be beneficial in some conditions like ulcerative colitis by enhancing mucosal barrier function, reducing inflammation, and inhibiting pathogenic bacterial growth, this outbreak underscores the need for careful patient selection, particularly in critically ill individuals.[19]

Although general risk categories for probiotic-related infections are known, the precise biological mechanisms of how these probiotics translocate into the bloodstream remain unclear, but potential factors include damage to the intestinal mucosa, dysbiosis of gut microbiota, altered intestinal permeability, and immune dysfunction.[20] In this outbreak, several patients required prolonged courses of multiple antibiotics, some experienced persistent bacteraemia, and a 27% case fatality ratio was observed. These complications were likely due to treatment difficulties associated with *A. clausii*, a multi-drug-resistant organism harbouring genes conferring resistance to last-line glycopeptides (*vanG* and *vanI*).[21]

Currently, South Africa lacks formal surveillance systems or adverse event reporting mechanisms for probiotic-associated infections. Probiotics are classified as dietary supplements rather than therapeutic agents, meaning they bypass the rigorous safety evaluations and post-market surveillance required for medicines.[22] This regulatory gap complicates outbreak response, making it difficult to track adverse events. To address this, the establishment of formal surveillance systems or making probiotic-related infections notifiable is crucial. There is a need for updated regulatory standards that include robust manufacturing quality control and post-market monitoring. The use of multidrug-resistant bacterial strains in probiotics raises additional ethical and medico-legal concerns, particularly regarding their potential to disseminate resistance genes in hospital and community settings.

This outbreak also revealed a need for greater clinician awareness. Many healthcare professionals may not be fully aware of the risks associated with probiotic use in immunocompromised patients.[23] Best practices include assessing patient risk and selecting products with robust supporting evidence in specific patient groups.[23] At the time of the investigation and writing of this report, no specific clinical guidelines

were available. Education efforts targeting professionals at the levels of tertiary education institutions and as part of continuing professional development (CPD) activities are essential to ensure appropriate and safe prescribing. A probiotic stewardship framework, modelled on antimicrobial stewardship principles (i.e., the right probiotic is given to the right patient, for the right indication, at the right dose and for the right duration), could provide a structured approach for safe clinical use. This would include defining clinical indications, identifying high-risk populations, guiding product selection and dosing, and monitoring adverse events. Additionally, formal guidance documents and targeted educational efforts for both healthcare professionals and the public are essential for promoting informed decision-making and ensuring patient safety.

Public health actions

The NICD reported the outbreak to the National Department of Health and engaged the South African Health Products Regulatory Authority (SAHPRA) to review product safety. Public communications, including a media statement and a frequently-asked-questions list, informed healthcare providers and the public about potential risks. Alerts were also disseminated to clinical laboratories to enhance early case detection. As of August 2025, no additional cases of *A. clausii* bacteraemia linked to the probiotic were reported. Pharmacists at affected hospitals were informed, and they may have communicated with the doctors as well. As the NICD, we did not implement direct measures; therefore, any actions taken by clinicians or the general public are unknown.

Conclusion

This investigation identified a commercially available *A. clausii*-containing probiotic as the most likely source of the bacteraemia in South Africa. The event highlights that while probiotics may offer therapeutic benefits, their unregulated use in vulnerable populations can pose serious risks. The outbreak exposed critical gaps in surveillance, regulation, and clinician awareness. This outbreak serves as a sentinel event, urging the need for public health action. We recommend establishing national guidance on probiotic use, including clear contraindications and criteria for patient selection. Furthermore, the notification of severe probiotic-related infections is essential to enable early detection and response to future outbreaks. Probiotic stewardship should be integrated into clinical practice to ensure a balanced approach that weighs potential benefits against infection risks. Finally, continued research into host–microbe interactions and evidence-based guidelines is critical to inform safe clinical decision-making and prevent similar incidents from occurring in the future.

Recommendations

- Pharmacists and microbiologists should report all probiotic-linked cases of *A. clausii* bacteraemia to SAPHRA for further investigations.

- Public health authorities should inform the broader healthcare community and patients about the outbreak and provide education on the safe use of probiotics, emphasising adherence to indications and recommended dosages and protocols, especially for high-risk populations.

- Microbiology laboratories should continuously monitor laboratory-confirmed cases of *A. clausii* bacteraemia and promptly report probiotic-linked cases to the NICD and submit isolates for further characterisation. Submission of these isolates should ideally be accompanied by clinical information to inform appropriate public health action.

- Clinicians should advise patients with co-morbidities or at the extremes of age to monitor symptoms and seek medical advice before and during probiotic use.

- A national guideline, developed by relevant clinical societies, with endorsement from the DoH, is needed to ensure appropriate clinical practice and to prioritise patient safety.

Ethical considerations

This investigation was conducted in accordance with the National Institute for Communicable Diseases' institutional ethics approval for surveillance and outbreak investigations granted by the University of the Witwatersrand Human Research Ethics Committee (HREC) (Medical) (protocol reference M1809107).

Conflict of interest

None to declare.

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