

## Case Report

# Bacteremia Following *Alkalihalobacillus clausii* (Formerly *Bacillus clausii*) Administration in Immunosuppressed Adults: A Case Series

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**Abstract:** (1) Background: Given the widespread use of *Alkalihalobacillus clausii* (*A. clausii*) as a probiotic in recent decades and the detection of bacteremia cases in a group of patients, we sought to analyze cases of *A. clausii* bacteremia following oral probiotic use (2) Methods: A retrospective observational study was conducted at a private hospital in San Jose, Costa Rica. Cases of bacteremia caused by *A. clausii* confirmed by the microbiology laboratory were analyzed in patients who received oral treatment with this probiotic between January 2020 and January 2022. In addition, an isolate (HCB-AC2) was compared through whole genome sequencing to demonstrate the correlation of bacteremia and *A. clausii*. Possible vulnerability factors related to the development of this condition were determined. (3) Results: Four cases were identified in this hospital over 2 years. Genomic analysis of isolate HCB-AC2, using two different methods, showed identical results. This indicates that HCB-AC2 is genomically identical to ENTpro and the Enterogermina<sup>®</sup> reference genome. The median age was 71 years, and all patients had some degree of immunosuppression. All patients met at least three sepsis criteria at the time of bacterial identification. Most patients were treated with vancomycin and levofloxacin. Three of the identified patients died. (4) Conclusion: *A. clausii* can be used as a probiotic, but caution is advised when used in immunosuppressed and elderly patients. These findings align with those reported in similar case studies.

**Keywords:** *Alkalihalobacillus clausii*; bacteremia; probiotics; immunosuppressed



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## 1. Introduction

The Food and Agriculture Organization of the United Nations and the World Health Organization (WHO) defined probiotics as live microorganisms that, when administered in adequate amounts, improve host health [1,2]. In recent decades, there is widespread interest worldwide for commercially available probiotics, not only because of their robust safety profile but also because of the wide range of human health benefits [2]. Actual available products contain bacterial agents such as *Lactobacillus* spp. [3], *Bifidobacterium*

spp. [3], strains of *Saccharomyces cerevisiae*, *Aspergillus niger*, and *Bacillus* spp. that are common members of the endogenous gut microbiota [4,5].

There is strong evidence supporting the benefits of probiotics predominantly in the treatment of gastrointestinal conditions such as acute gastroenteritis, antibiotic-associated diarrhea, Crohn's disease, celiac disease, inflammatory bowel disease, and *Clostridiodes* and *Helicobacter pylori* infection [6–11]. Others under study include constipation, necrotizing enterocolitis and colorectal cancer [2,12–15].

Over the years, the precise mechanism by which probiotics generate beneficial gastrointestinal effects has been studied [16]. However, multiple pathways have been found by which they exert their effect, and it is suggested that they act through a multifaceted mechanism. Among the reported pathways are the colonization and reestablishment of the intestinal microbiota balance, the competitive exclusion of pathogens through the secretion of antimicrobials such as bacteriocins [17,18], competition for the environment and adherence to the intestinal wall [19,20], improvement of mucin production [19], immunomodulatory effects [21,22], and secretion of short-chain fatty acids [2,5,23].

Probiotics also exhibit nerve, endocrine, anticancer, and metabolic functions [6,21,24,25]. For example, there are reports of decreases in cholesterol levels that have been associated with metabolites of probiotics such as bacteriocins, biosurfactants, exopolysaccharides, and siderophores [4,5]. There is ample evidence to support the notion that probiotics can be considered valuable not only for treating diseases but also for their prevention.

*Bacillus* spp. strains are aerobic Gram-positive rod-shaped bacteria that form endospores [26], consisting of a core with a condensed and inactive chromosome, and additional layers, including a peptidoglycan-rich cortex [24]. The onset of sporulation occurs when the environment in which the bacterium is located presents a decrease in available nutrients [27]; then, the bacterium initiates the irreversible process to produce spores, which takes approximately 8 h [24,28].

The sporulation process preserves the life of the bacterium through the development of bacterial spores which survive in the long term under extreme environmental and physiological conditions [29–31], such as ultraviolet radiation, extreme heat, humidity, and the acidic environment of the stomach and gastrointestinal tract [27]. These properties allow them to be found in nature and in soil, air, foods undergoing fermentation, and the human intestine [24,28].

Currently, there are multiple brands of probiotics containing *Alkalihalobacillus clausii* (*A. clausii*), formerly *Bacillus clausii*, available worldwide [32]. This probiotic is an over-the-counter medicinal supplement and is manufactured in the form of an oral suspension, containing 2 billion or 4 billion spores of this microorganism. It is approved for the treatment and prophylaxis of alterations in intestinal bacterial flora due to endogenous avitaminosis, as well as for the recovery from dysbiosis caused by antibiotic or chemotherapy agents [1,2,11,24,26].

There is substantial proof to back up the therapeutic use of this probiotic around the world [2,11,33]. However, there have been reports questioning its efficacy and safety [33]. Post-marketing, the main potential side effects reported by the WHO include gastrointestinal disorders, general disorders, and conditions at the administration site, skin and subcutaneous tissue disorders, and infections [34].

Upon conducting a comprehensive analysis of various strains of *Bacillus* spp., including *A. clausii*, several disadvantages have come to light regarding their suitability as probiotics. These limitations primarily arise from their potential to transmit resistance genes, such as the *erm* gene responsible for macrolide resistance, and their ability to generate enterotoxins and biogenic amines [35]. Nevertheless, it is essential to acknowledge that different strains of *A. clausii*, which have also undergone studies, have demonstrated safety profiles and an inability to transfer such genes [5,35]. This underscores the significance of careful strain selection and rigorous testing to ensure the probiotic's safety and effectiveness.

This study involved a retrospective analysis of the cases of patients with some level of immunosuppression who developed bacteremia following administration of the probiotic *A. clausii* and were hospitalized in a private hospital in San Jose, Costa Rica. This research aims to comprehensively evaluate the safety of this probiotic and identify its potential associations.

## 2. Results

Four patients who developed bacteremia following the administration of *A. clausii* were identified. This microorganism was detected in the hospital's clinical laboratory using an automated blood culture system; then, it was isolated and identified through a proteomic methodology known as MALDI-TOF.

Table 1 shows the baseline characteristics of the identified patients. The median age was 71 years. The distribution of patients by gender was balanced. All patients had underlying medical conditions, including 75.0% ( $n = 3$ ) with hypertension (HTA), 50.0% ( $n = 2$ ) with diabetes mellitus (DM), 50.0% ( $n = 2$ ) with dyslipidemia, and 25.0% ( $n = 1$ ) with cancer. Importantly, every patient in this series exhibited some level of immunosuppression.

**Table 1.** Baseline characteristics of the 6 patients with detection of *A. clausii* in the bloodstream.

Case	Age/Gender	Comorbidities	Patient Profiles	Admission
HCB-AC1	73/M	HTA, DLP, DM	Bacterial pneumonia and SARS-CoV-2	COVID-19
HCB-AC2	39/F	Asthma	Cardiogenic shock, septic shock, and intrahepatic cholangiocarcinoma	Left intrahepatic cholangiocarcinoma
HCB-AC3	93/M	Basal cell carcinoma, sternal metastasis, HTA, SVT	Bacterial pneumonia, mechanical ventilation	Thoracoabdominal dissociation and pleural effusion
HCB-AC4	78/F	HTA, DLP, DM, CVD	Aspiration pneumonia	Pacemaker placement

Abbreviations: DLP: dyslipidemia, HTA: hypertension, DM: diabetes mellitus, Ca: cancer, BPH: benign prostate hyperplasia, UTI: urinary tract infection, Fa: atrial fibrillation, M: male, F: female, SVT: supraventricular tachycardia, CVD: cardiovascular disease.

All patients experienced an adverse drug reaction associated with antibiotic use, specifically a disruption in their bacterial flora, which prompted the prescription of this probiotic. The alteration of the microbiota led to diarrhea in most cases ( $n = 3$ ), as shown in Table 2. The most used dosage of *A. clausii* was 4 billion in 75.0% ( $n = 3$ ) of patients. The median duration of *A. clausii* treatment before the onset of bacteremia was 9 days (ranging from 4 to 15 days). Additionally, it was observed that three patients continued *A. clausii* treatment for an average of 1 day after a positive bacteremia culture was detected.

**Table 2.** *A. clausii* administration parameters and time to bacteremia development.

Case	<i>A. clausii</i> Administration			Time to the Onset of Bacteremia (Days)
	Disruptions in Bacterial Microbiota	Dosage (Billion IU)	Duration (Days)	
HCB-AC1	Diarrhea	4 every 12 h	7	6
HCB-AC2	Diarrhea, vomiting, and nausea	2 every 8 h	4	4
HCB-AC3	Diarrhea	4 every 24 h	9	8
HCB-AC4	Nausea	4 every 12 h	15	20

Abbreviations: IU: international units.

The clinical characteristics of the identified patients are presented in Table 3. Following the use of the SIRS criteria as a method to standardize the potential severe disease associated with *A. clausii* bacteremia, it was found that all patients had at least three criteria of this scale. The most common signs and symptoms were temperature greater than 38 °C, followed by heart rate greater than 90 beats per minute, and respiratory rate greater than 20 breaths per minute. Among the patient group, renal dysfunction was detected in one of them, along with one case of respiratory dysfunction and another case of hypotension.

**Table 3.** Clinical characteristics, treatment, and outcome of patients with *A. clausii* bacteremia.

Case	SIRS Criteria	Organic Dysfunction	Evolution	Antibiotic Treatment	Other Culture Findings	In-Hospital Fatality and Other Remarks
HCB-AC1	4/4	Resp	Pulmonary necrosis, ICU, MV, neurological disorders, upper gastrointestinal bleeding, septic shock.	Cefotaxime, Levofloxacin	Blood culture: <i>Pseudomonas aeruginosa</i> , <i>Staphylococcus epidermidis</i> .	Died
HCB-AC2	3/4	HT	Peritonitis, cardiac arrhythmias, bacteremia, hemodynamic instability.	Vancomycin	Peritoneal fluid: <i>Candida tropicalis</i> , <i>Pseudomonas aeruginosa</i> , <i>Enterococcus faecalis</i> , <i>Ochrobactrum anthropic</i> .	Died
HCB-AC3	3/4	-	Hemodynamic instability, acute renal failure, pneumonia due to superinfection.	Ceftazidime-Avibactam + Vancomycin	Bronchial secretion: <i>Burkholderia cepacian</i> group.	Transfer to another hospital
HCB-AC4	3/4	Ren	Hemodynamic instability, cardiac arrhythmias, pneumonia due to superinfection, neurological disorders.	Ampicillin+ Levofloxacin + TMP-SMX	Bronchial secretion: <i>Stenotrophomonas maltophilia</i> , <i>Escherichia coli</i> , <i>C. albicans</i> .	Died

Abbreviations: Res: respiratory dysfunction; HT: hypotension; Ren: renal dysfunction. The comma (,) means a change of antibiotic. The plus sign (+) means multiple antibiotic therapy.

The antibiotic therapy used in patients upon the presentation of *A. clausii* bacteremia was in line with what is found in the literature in 75.0% ( $n = 3$ ) of the patients. After the detection of bacteremia, three patients passed away, and one patient was discharged, making it impossible to ascertain their outcome. On average 17 days after the detection of *A. clausii* in the blood culture, the patients died.

Mapping of HCB-AC2 reads to a reference ENTPro genome revealed 21 G or C insertions, with nearly half of them seen in homopolymer G or C stretches, and a 1 bp deletion in the intergenic region upstream of a cat gene encoding a chloramphenicol acetyltransferase (Table 4). Moreover, the HCB-AC2 assembly was identical to a draft genome that we generated in parallel for a bacterial isolate that we cultivated from an Enterogermina® vial (data not shown). The aforementioned variations align with sequencing by synthesis errors. Hence, it is plausible that the ENTPro genome and our Enterogermina®/draft genome are the same.

**Table 4.** Mapping of Illumina reads obtained for HCB-AC2 to a long-read based reference genome (*A. clausii* ENTPro).

Position	Mutation	Annotation	Gene	Description
246,999	+G	coding (1215/1416 nt)	DB29_00287 ←	Membrane protein, putative
505,267	(C) <sub>5→6</sub>	coding (137/204 nt)	DB29_00564 ←	hypothetical protein
670,059	(C) <sub>8→9</sub>	intergenic (+16/+23)	DB29_00722 →/← DB29_00723	Putative glycoside hydrolase/Stage V sporulation protein B
912,77	(C) <sub>6→7</sub>	coding (406/459 nt)	DB29_00956 ←	Phosphoribosylformylglycinamide cyclo-ligase
1,607,340	(C) <sub>6→7</sub>	intergenic (−783/+1)	DB29_01660 ←/← DB29_01661	putative rhamnogalacturonan lyase in rhamnose utilization cluster/hypothetical protein
1,738,248	+G	coding (269/330 nt)	DB29_01784 →	Integral membrane protein
1,767,258	+C	coding (754/2934 nt)	DB29_01815 ←	hypothetical protein
1,810,315	+C	coding (338/483 nt)	DB29_01851 ←	KinB signaling pathway activation protein
2,359,157	(G) <sub>5→6</sub>	coding (557/609 nt)	DB29_02403 →	Transposase
2,826,475	+C	coding (574/618 nt)	DB29_02867 ←	TetR family transcriptional regulator
3,061,533	+G	intergenic (+94/+14)	DB29_03110 →/← DB29_03111	Teichuronic acid biosynthesis glycosyl transferase TuaC/hypothetical protein
3,265,972	+C	intergenic (−12/+157)	DB29_03326 ←/← DB29_03327	Isocitrate lyase/hypothetical protein
3,481,536	(G) <sub>5→6</sub>	coding (743/828 nt)	DB29_03557 →	Hypothetical protein
3,528,009	+C	intergenic (−11/+264)	DB29_03604 ←/← DB29_03605	Cytochrome c-type biogenesis protein DsbD, protein-disulfide reductase/hypothetical protein
3,552,355	+G	coding (745/750 nt)	DB29_03624 →	Uroporphyrinogen-III synthase
3,648,617	(G) <sub>6→7</sub>	coding (392/408 nt)	DB29_03715 ←	Hypothetical protein
3,888,513	+G	coding (3166/3186 nt)	DB29_03977 →	Chromosome partition protein smc
3,998,002	(G) <sub>6→7</sub>	coding (380/468 nt)	DB29_04092 →	DNA mismatch repair protein MutL
4,192,551	(C) <sub>5→6</sub>	coding (2671/2835 nt)	DB29_04308 ←	Multimodular transpeptidase-transglycosylase
4,259,750	(A) <sub>7→6</sub>	coding (532/540 nt)	DB29_04378 ←	Flagellar motor rotation protein MotB
4,263,161	+G	intergenic (−198/−156)	DB29_04381 ←/→ DB29_04382	Hypothetical protein/hypothetical protein
4,264,808	Δ1 bp	intergenic (+35/−)	DB29_04384 →/−	Chloramphenicol acetyltransferase/−

### 3. Discussion

In this study, we included data from four new cases of patients who developed bacteremia after the administration of *A. clausii* and were admitted to a single private hospital in the country. Currently, there are only 10 clinical cases of bacteremia attributed to *A. clausii* (Table 5), which represents a small number of patients. None of these documented cases have demonstrated the correlation between the development of bacteremia and the administration of *A. clausii* through genetic testing; it has only been reported from microbiological cultures.

This is the first case report to present genetic evidence of the correlation of the development of bacteremia after the initiation of *A. clausii* therapy in one of the patients. Proof of this is that the HCB-AC2 isolate is genomically indistinguishable from ENTpro and our own Enterogermina<sup>®</sup> reference genome, except for a few minor changes that we believe to be sequencing artifacts based on previous observations [36].

For the other cases, it was not possible to perform such genomic analysis. However, there is a high probability that this probiotic may translocate from the intestinal tract into the bloodstream, particularly in individuals with specific predisposing factors [34,37–42]. This underscores the need for further microbiological and genetic studies in future reports for the detection of risk factors associated with such translocation and their possible clinical implications.

While it is true that the evidence for bacteremia caused by *A. clausii* is limited, there are at least eight reports in which the association between the development of bacteremia and the use of probiotics such as *Lactobacilli*, including *Lactobacillus acidophilus*, *Lactobacillus casei*, and *Lactobacillus GG*, has been demonstrated. In addition, there are more than nine cases of sepsis due to *Saccharomyces boulardii*, *Lactobacillus GG* [43–47], *Bacillus subtilis* [48,49], *Bifidobacterium breve* [50], or a combination of probiotics. Particularly these cases develop in patients with chronic anatomical conditions, patients with some degree of immunosuppression, and patients with intestinal conditions [43–50].

As established by the evidence and in our study, the use of oral probiotics is mainly indicated for the treatment of diarrhea, nausea, and vomiting associated with the use of antibiotics. However, a common problem lies in the use of these in populations considered at high risk due to their level of immunosuppression, whether due to age, high doses of steroids, chronic diseases, cancer treatments, or severe acute illnesses [38,39,51]. In fact, all patients in our study met the criteria for SIRS, and three of them experienced organ dysfunction.

These findings highlight the importance of conducting pharmacovigilance studies of the use of *A. clausii* as a probiotic. As of now, official safety information regarding this probiotic has not been updated, and there are no established recommendations concerning its utilization in high-risk populations. Furthermore, it is crucial to investigate the potential correlation between *A. clausii* overdose and the risk of microbial infection, especially considering that one case in this study was prescribed doses exceeding the recommended levels. No existing evidence was found regarding the analysis of dosage effects [5,24,51]. This underscores the need for further research and vigilant oversight to establish safe and effective guidelines for the use of *A. clausii* as a probiotic, particularly in vulnerable patient populations.

The recommended antibiotics for the treatment of infections caused by *A. clausii*, which are susceptible to various species of the *Bacillus* genus, are vancomycin (as the first choice) or clindamycin, with fluoroquinolones or imipenem as alternatives. If the above antibiotics are not available, Linezolid can be used [52,53]. Four of the cases detected in this center received optimal antibiotic treatment. It should be considered that in all patients other microorganisms were detected, so they required more than one antibiotic (Table 3). One case received non-optimal treatment with trimethoprim/sulfamethoxazole (TMP-SMX). The resistance of this antibiotic to *Bacillus* has been reported in several studies, so its use is not recommended for the management of *A. clausii* infections [52–54].

Due to the small number of bacteremia cases in our study, we cannot calculate mortality rates accurately. However, when reviewing the literature, we found that 5 of the 10 previously reported cases resulted in the death of patients, which seems to indicate a higher mortality than is considered typical for this infection [37,38,51].

**Table 5.** Profile of patients documented with bacteremia due to *A. clausii* administration [37,38,40,41,50,53,54].

Age (Years)	Patient Profile	Treatment	Antibiotic Susceptibility	Outcome	Authors	Year of Publication	Country
69	Ventilator-associated pneumonia, BSA, progresses to acute diarrhea.	Vancomycin + Levofloxacin + Rifampicin	ND	Recovery	Nacinovich, F. et al.	2017 [54]	Argentina
71	MI, catheter-associated infection, in treatment with BSA evolution for acute diarrhea.	Vancomycin + Levofloxacin	ND	Recovery	Nacinovich, F. et al.	2017 [54]	Argentina
ND	Pneumonia and Stage IV lung cancer.	ND	Resistance to clindamycin, penicillin, and tetracycline.	Discharge	Gargar, J.D. et al.	2019 [37]	ND
ND	Pneumonia and Stage IV lung cancer.	ND	ND	Recovery	Gargar, J.D. et al.	2019 [37]	ND
ND	Septic shock from ischemic colitis.	ND	ND	Recovery	Gargar, J.D. et al.	2019 [37]	ND
5 months	Congenital heart disease, recurrent respiratory tract infections, repeated hospital/ICU admissions, BSA.	Vancomycin	Susceptibility to vancomycin and penicillin.	Discharge	Joshi, S. et al.	2019 [41]	ND
ND	DM 2, with decompressive craniotomy and BSA.	Teicoplanin	Susceptibility to ciprofloxacin and vancomycin. Penicillin resistant.	Discharge	Princess et al.	2020 [38]	India
87	HT, EPOC, cholecystectomy, hypertension, urinary tract infection caused by <i>Klebsiella pneumoniae</i> .	Vancomycin + Cefepime	ND	Discharge	García, J. et al.	2021 [50]	Colombia
1 & 5 month	None.	Ceftriaxone, Ampicillin, Levofloxacin, Vancomycin, Gentamicin	Susceptibility to ceftriaxone, levofloxacin, and vancomycin. Penicillin, rifampin, and clindamycin resistant.	Recovery	Khatri, A. et al.	2021 [40]	United States
37	Postoperative bariatric surgery type sleeve, presented thoracoabdominal fistula, treated with BSA evolves to acute diarrhea.	Vancomycin	ND	Discharge	Schierling, N. et al.	2022 [53]	Brazil

Abbreviations: BSA: broad-spectrum antibiotics; ND: no documentation; HT: hypertension; MI: myocardial infarction; ICU: intensive care unit.

Limitations of this analysis should be considered, including the lack of sufficient microbiological analysis to guarantee that the *A. clausii* isolated from three of the patients is the same as the medication supplied and the absence of antimicrobial susceptibility testing on *A. clausii*. The results should be interpreted cautiously, and further research with a larger sample size would be necessary to confidently identify and characterize risk factors and clinical complications.

## 4. Materials and Methods

### 4.1. Setting and Study Design

A retrospective observational study was carried out at a private hospital located in San Jose, Costa Rica, from January 2020 to January 2022. It analyzed cases of bacteremia caused by *A. clausii* in adult patients who had undergone treatment with Enterogermina<sup>®</sup> and Enterogermina<sup>®</sup> Plus. Patients with incomplete medical records were excluded from the analysis, ensuring the integrity and reliability of the data.

### 4.2. Data Collection and Outcome Measures

The data were obtained from electronic clinical records of the hospital. This included patient information like age, gender, comorbidities, clinical history, admission details, and admission dates. Additionally, we gathered data on the use of *A. clausii*, including the reason for use, dosage, and length of treatment. Other collected information included the date of *A. clausii* detection, the patient's progress following bacteremia, the outcome, laboratory test results, culture findings, and the antibiotics used to treat the infection.

The systemic inflammatory response syndrome (SIRS) criteria were determined as described previously considering heart rate, respiratory rate, temperature, and leukocyte count and/or presence of bands [55], and organ dysfunction caused by the infection was classified according to the Swedish Society of Infectious Diseases guidelines as described by Senneby et al. [56]. Hypotension was defined as systolic blood pressure <90 mmHg or mean arterial pressure <70 mmHg; respiratory dysfunction was defined as peripheral oxygen saturation <90%; renal dysfunction was defined as an increase in serum creatinine levels of >45 µM, initiation of dialysis or urinary output <0.5 mL/kg/h for >2 h [55,57].

### 4.3. Bacterial Isolates

Blood was cultured using aerobic and anaerobic media bottles from the automated blood culture system Bactec FX40 (Becton-Dickinson<sup>®</sup>, Sparks, MD, USA). Positive bottles for the presence of Gram-positive rods were inoculated on Liofilchem<sup>®</sup> Columbia Agar (Roseto degli Abruzzi, Teramo, Italy) with 5% sheep blood. Plates were incubated for 24 h at 37 °C in an aerobic atmosphere. Finally, isolates were identified using MALDI-TOF mass spectrometry in a Microflex LT/SH (Bruker Daltonics<sup>®</sup>, Bremen, Germany) in association with the MALDI Biotyper<sup>®</sup> Version 3.1 software.

### 4.4. Statistical Analysis

Given the limited sample size in this study, it is not feasible to employ a statistically representative model or conduct inferential analysis to discern specific trends.

### 4.5. Bioinformatic Analysis

For this analysis, only the HCB-AC2 isolate was available from the processed sample of patient HCB-2. To determine whether HCB-AC2 indeed correspond to the strain included in the probiotic formulation Enterogermina<sup>®</sup> (Sanofi-Aventis, Paris, France), we mapped Illumina reads that were trimmed with fastp v.023.4 to the chromosomal sequence of *B. clausii* ENTPro (Genbank CP012475), which were obtained through PacBio sequencing with the P6C4 chemistry [58]. This mapping was performed with the Breseq pipeline for finding mutations in haploid microbial genomes [59], and snippy for core SNP detection [60]. We also generated a whole-genome assembly with skesa for an isolate that we cultivated from a vial of Enterogermina<sup>®</sup> purchased locally (lot 0I065).

### 4.6. Ethics Approval and Consent to Participate

Ethical approval to conduct this study was obtained from the Scientific Ethical Committee of the University of Medical Sciences (CEC-UCIMED), approval date of 2 June 2021, reference number CEC-632-10-2022, and approval date of 29 November 2022. Written consent was not necessary for this study according to this committee.

#### 4.7. Declaration of Generative AI and AI-Assisted Technologies

During the development of this project, the authors utilized ChatGPT to improve the clarity of translated sentences. Subsequently, after using this tool/service, the authors conducted a thorough review and made any necessary refinements to the content, fully taking responsibility for this publication's material.

### 5. Conclusions

*A. clausii* can indeed function as a probiotic agent; however, it is essential to exercise caution when contemplating its administration to immunosuppressed and elderly individuals. These research findings emphasize the crucial need for personalized medical assessments and customized therapeutic strategies when considering probiotic supplementation for such patients. Healthcare professionals should meticulously balance the potential advantages with potential risks, maintaining vigilant monitoring for any adverse reactions or indications of infection when incorporating *A. clausii* into patient care plans.

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