Fundamentals and Frontiers of Medicine:

An Academic Perspective

Edited by
Marileia Chaves Andrade

Afya FACULDADE DE MEDICINA DE ITAJUBÁ

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Preface

There are books that emerge from a single perspective, and there are books that emerge from many perspectives converging toward a shared commitment. This work belongs to the latter category. Brought together under the organization of Professor Mariléia, a name inseparable from the internationalization movement of the Faculty of Medicine of Itajubá and a researcher with a distinctive academic trajectory, whose scientific production transcends institutional and geographical boundaries with the same ease with which it crosses disciplinary fields, this collection is, above all, a portrait of the plurality that sustains knowledge production in health sciences.

Professor Mariléia was entrusted not only with the organizational task, but also with the construction of a space in which research of such diverse natures could engage in dialogue under the same roof. It is this gesture—that of embracing diversity without diluting it—that gives this e-book its most distinctive identity.

The chapters that comprise this work span a broad yet coherent spectrum of the health sciences. Laboratory investigations into the antimicrobial activity of natural compounds and probiotics, including buriti oil, pitaya peel, and commercial strains against hospital pathogens, engage in dialogue with reflections on the future of healthcare, such as the use of artificial intelligence to support the diagnosis of complex cases and the development of three-dimensional prototypes for teaching congenital malformations and endocrine disorders. Alongside these areas of investigation, public and collective health issues are also given prominence: the panorama of breastfeeding in Brazil over a fifteen-year period; the assessment of toxicity in cancer patients undergoing radiotherapy; the experience of infertility and the role of spirituality as a coping mechanism; the quality of routine prenatal care in Primary Health Care; and, finally, a topic of particular relevance to contemporary medical education: the development of inclusion policies through the Individualized Educational Plan.

What brings together the laboratory bench, classroom, clinical practice, and community in this collection is not merely the methodological rigor present in each chapter, but also a shared conviction: that medicine is taught and practiced at the intersection of science, technology, human care, and social responsibility. Each author represented here embraces this conviction in their own way, whether at the laboratory bench, through critical literature review, or through attentive listening to those experiencing illness or exclusion.

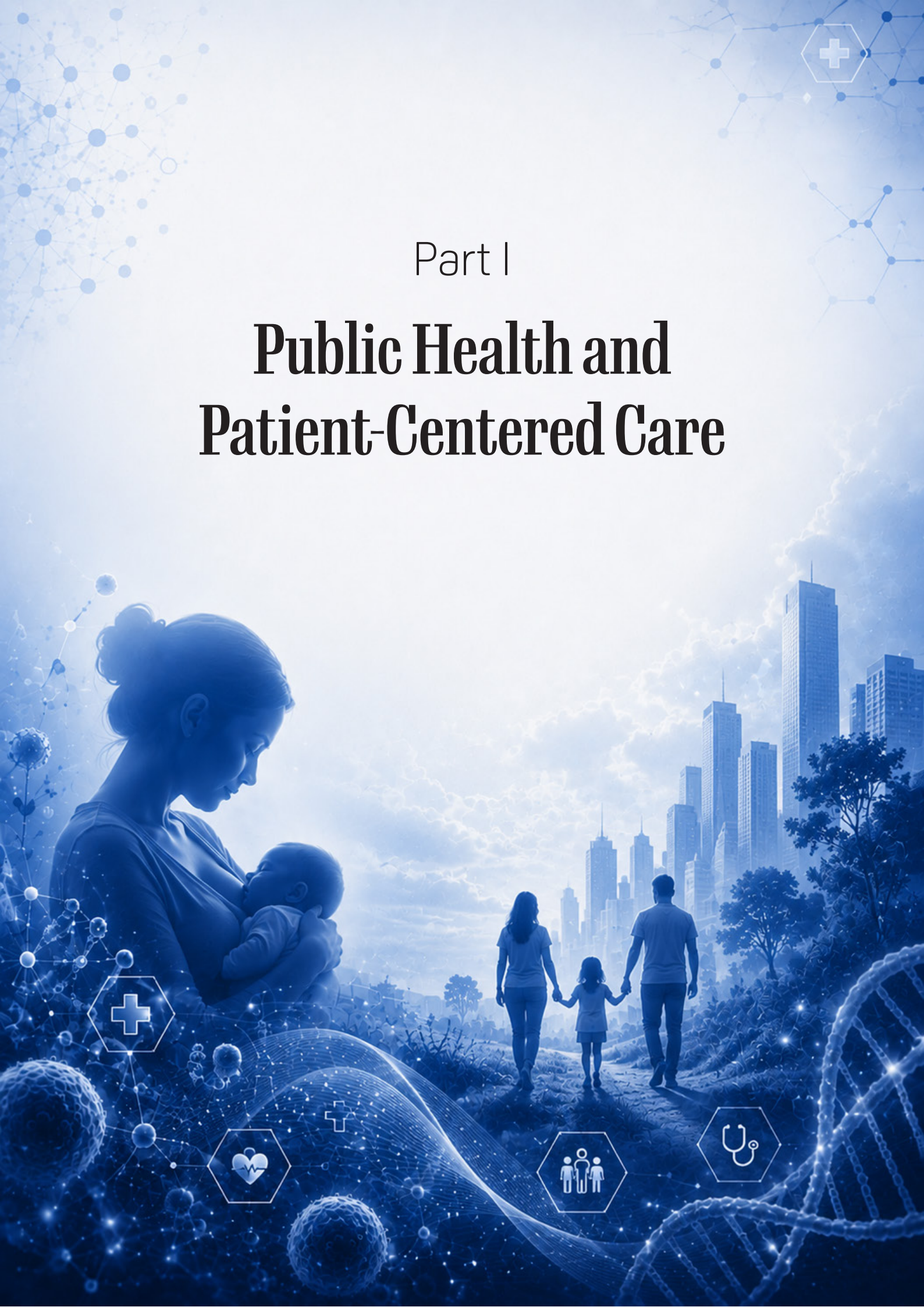
I invite the reader to move through these pages not as a collection of isolated studies, but as a collective testimony to what it means to educate and conduct research in health in contemporary Brazil: with scientific rigor, social sensitivity, and an enduring commitment to transforming knowledge into care.

Renata de Castro Matias

Coordinator of COPEXII (coordenação de pesquisa, extensão, internacionalização e inovação) - FMIT

Part I

Public Health and Patient-Centered Care



CHAPTER 01

Breastfeeding Trends in Brazil (2008–2023): Progress Toward Global Targets, Persistent Challenges, and Future Perspectives

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Introduction

Exclusive breastfeeding during the first six months of life is one of the most effective interventions for reducing infant mortality and promoting comprehensive child health. Consequently, it is strongly recommended by both national and international health organizations[1-12]. In addition to its well-established nutritional and immunological benefits, breastfeeding provides substantial economic advantages by reducing household expenditures and lowering public healthcare costs associated with the hospitalization and treatment of preventable diseases[2].

Globally, the United Nations Children's Fund (UNICEF) has established ambitious breastfeeding targets for 2030, including increasing the prevalence of exclusive breastfeeding during the first six months of life to 70%, achieving breastfeeding continuation rates of 80% at 12 months and 60% at 24 months[3]. Despite notable progress, Brazil remains below these targets. In 2023, the prevalence of exclusive breastfeeding up to six months reached only 35.5%, while breastfeeding continuation rates were 56% at 12 months and 32% at 24 months[4-5].

In this context, understanding national trends, identifying regional disparities, and evaluating the effectiveness of public health policies are essential for supporting evidence-based strategies aimed at promoting, protecting, and supporting breastfeeding. Breastfeeding remains a cornerstone of public health due to its direct contribution to reducing infant morbidity and mortality and promoting optimal child growth and development.

Within the state of Minas Gerais, characterized by marked socioeconomic and geographic inequalities, the assessment of breastfeeding indicators is particularly relevant for identifying

vulnerable populations and guiding targeted interventions that improve access to information and breastfeeding support services. For women, especially those facing challenges in balancing childcare responsibilities with professional and family demands, greater awareness of the benefits of breastfeeding may empower informed decision-making regarding infant health.

From a public health perspective, critical evaluation of national and state-level trends provides valuable evidence for strengthening intersectoral policies that promote equity and facilitate achievement of the global breastfeeding targets established for 2030. Furthermore, for medical students, this topic represents an opportunity to integrate scientific evidence with social realities, fostering the development of critical thinking, ethical responsibility, and humanistic competencies that are fundamental to healthcare professionals committed to improving population health.

Objectives

To describe the evolution of breastfeeding indicators in Brazil between 2008 and 2023.

To compare national breastfeeding indicators with the WHO/UNICEF global breastfeeding targets for 2030.

To identify regional disparities and knowledge gaps that should be addressed through public health policies.

Methods

This study is a narrative review with a quantitative approach based on the analysis of publicly available secondary data. The primary data sources included the Brazilian National Survey on Child Nutrition (ENANI-2019), the Food and Nutrition Surveillance System (SISVAN; 2008–2023)[5], and reports published by the World Health Organization (WHO) and the United Nations Children's Fund (UNICEF) [3-7].

The following breastfeeding indicators were analyzed: prevalence of exclusive breastfeeding (EBF) during the first six months of life, breastfeeding initiation within the first hour after birth, continued breastfeeding at 12 and 24 months, and early introduction of ultra-processed foods. Data were analyzed descriptively through the development of comparative tables and graphical representations to characterize temporal trends and compare national indicators with international breastfeeding targets.

Results and Discussion

The findings indicate that Brazil remains substantially below the international breastfeeding targets established by the WHO and UNICEF. In 2023, the prevalence of exclusive breastfeeding during the first six months of life reached 35.5%, corresponding to only half of the global target of 70%. Likewise, breastfeeding initiation within the first hour after birth was reported in 58.3% of live births, remaining below the recommended target of 80%. Continued breastfeeding rates reached 56% at 12 months and only 32% at 24 months (**Table 1**).

Table 1. Breastfeeding indicators in Brazil compared with global targets and international averages (2008–2023).

Indicator	Brazil (ENANI 2019)	Brazil (SISVAN 2023)	Global Goal (OMS/UNICEF)	Overall Average (UNICEF 2023)
Exclusive breastfeeding up to six months of age	45,8%	35,5%	70% by 2030	48%
Breastfeeding in the first hour of life	62,4%	58,3%	80% by 2030	44%
Breastfeeding up to 12 months	60%	56%	-	74%
Breastfeeding up to 24 months	35%	32%	-	52%
Early introduction of ultra-processed foods	20,5%	25%	-	-

Source: Prepared by the authors based on data obtained from SISVAN 2023[5], ENANI 2019[6], UNICEF[3], and WHO[7].

The data demonstrate that, although Brazil has achieved gradual improvements in breastfeeding practices over recent decades, national indicators remain considerably below the international targets established by the WHO and UNICEF. The prevalence of exclusive breastfeeding during the first six months of life (35.5%) compared with the global target of 70% indicates that a substantial proportion of Brazilian infants do not fully benefit from this highly protective practice during early life.

This scenario is likely influenced by multiple interacting factors, including mothers' early return to work[1-13-19], premature introduction of infant formula and ultra-processed foods[14], and limited access to qualified breastfeeding counseling and professional support during the postpartum period[15].

Breastfeeding initiation within the first hour after birth reached 58.3%, representing an important advance but remaining below the recommended target of 80%. This indicator deserves particular attention because early initiation of breastfeeding has consistently been associated with reductions in neonatal mortality[16] and improved mother–infant bonding[17]. The findings also suggest persistent challenges in the implementation of evidence-based maternity care practices, including full adherence to the Baby-Friendly Hospital Initiative (BFHI).

Regarding breastfeeding continuation, the prevalence of 56% at 12 months and 32% at 24 months highlights additional barriers to sustained breastfeeding. The marked decline after the first year of life reflects social and cultural factors, including misinformation regarding the nutritional value of breast milk after infancy, persistent misconceptions about prolonged breastfeeding, and insufficient workplace policies that support breastfeeding mothers, such as breastfeeding-friendly environments and childcare facilities[18].

Collectively, these findings indicate that although current public health initiatives have contributed to gradual improvements in breastfeeding indicators, they have not yet been sufficient to establish sustainable breastfeeding practices across the Brazilian population[8]. These results reinforce the need for comprehensive, intersectoral strategies that strengthen support for breastfeeding women from prenatal care through the child's first two years of life, in alignment with global child health goals.

The temporal trend in exclusive breastfeeding (EBF) prevalence in Brazil over the past decade demonstrates slow but consistent progress, although the observed increase remains insufficient to achieve the WHO/UNICEF targets for 2030. **Figure 2** illustrates the evolution of EBF prevalence among infants younger than six months between 2008 and 2023.

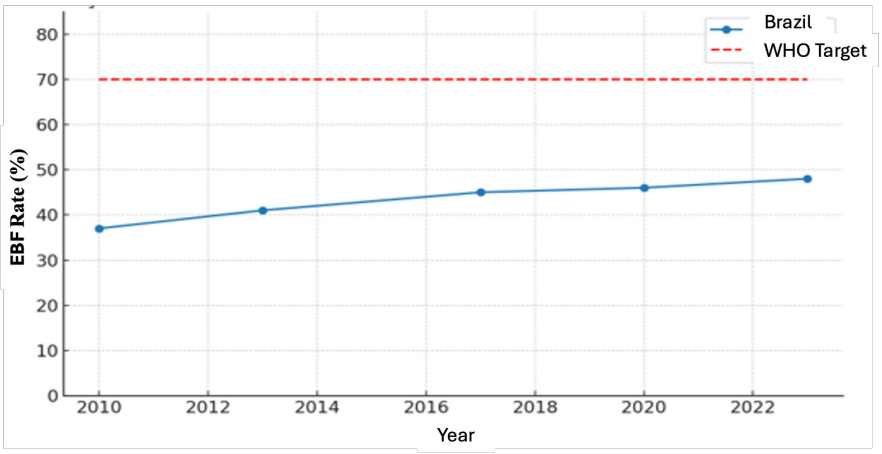

Figure 2. Temporal trends in exclusive breastfeeding prevalence among infants younger than six months in Brazil (2008–2023). Source: SISVAN[5] and WHO[7].

The prevalence of exclusive breastfeeding in Brazil increased gradually between 2008 and 2023, reflecting modest but sustained improvements[10]. Nevertheless, the current rate remains substantially below the international target established by the WHO and UNICEF, highlighting the need for more effective public health interventions.

Although the upward trend suggests positive effects of national breastfeeding promotion initiatives—including the Baby-Friendly Hospital Initiative (BFHI), the Breastfeeding and Complementary Feeding Strategy (*Estratégia Amamenta e Alimenta Brasil*), the Stork Network (*Rede Cegonha*)[9], and nationwide health promotion campaigns—these measures have not yet produced the structural changes required to substantially improve infant feeding practices.

Several factors likely contribute to this persistent stagnation. Social barriers, particularly mothers' early return to work[11] and insufficient maternity leave policies[20], remain major obstacles to sustained exclusive breastfeeding[21]. Cultural factors, including myths and misconceptions regarding breast milk adequacy, continue to negatively influence breastfeeding practices. In addition, structural inequities in access to healthcare services and qualified breastfeeding support[22] contribute to significant regional disparities throughout the country.

Furthermore, the COVID-19 pandemic likely exacerbated these challenges by disrupting maternal and child healthcare services, including breastfeeding counseling and support programs, thereby negatively affecting breastfeeding indicators nationwide[23].

Taken together, the findings presented in **Figure 2** indicate that, despite incremental progress, Brazil requires more comprehensive, equitable, and evidence-based public health policies capable of accelerating improvements in exclusive breastfeeding prevalence, reducing regional inequalities, and creating favorable conditions that enable mothers to maintain exclusive breastfeeding throughout the first six months of life.

Conclusion

Exclusive breastfeeding during the first six months of life in Brazil remains substantially below the international targets established by the WHO and UNICEF, despite gradual improvements observed between 2008 and 2023. Similarly, breastfeeding continuation rates at both 12 and 24 months remain unsatisfactory, underscoring the need for more effective and sustainable interventions.

Strengthening targeted public health policies, expanding health education initiatives, extending maternity leave, and promoting breastfeeding-friendly workplace environments are essential strategies for overcoming the social, cultural, and structural barriers that continue to hinder breastfeeding practices.

Investments in comprehensive breastfeeding promotion, protection, and support programs have the potential to substantially reduce infant morbidity and mortality while fostering healthy child growth and development. Achieving the global breastfeeding targets for 2030 will require coordinated intersectoral actions that ensure equitable access to qualified breastfeeding support across all regions of Brazil.

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CHAPTER 02

Routine Prenatal Care in Primary Health Care: A Literature Review

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Introduction

Prenatal care provided within Primary Health Care (PHC) represents one of the most important strategies for promoting maternal and neonatal health. It plays a fundamental role not only in reducing the immediate risks associated with pregnancy and childbirth but also as a public health intervention aimed at preventing future maternal and neonatal complications[1].

Prenatal care may be delivered across the three levels of healthcare—primary, secondary, and tertiary—according to the pregnant woman's obstetric risk stratification. Women identified as having conditions associated with an increased risk of maternal or fetal morbidity and mortality should be referred to specialized services with greater clinical complexity while maintaining continuous follow-up within Primary Health Care. In this context, obstetric risk assessment should be performed continuously and reassessed at every prenatal visit[2].

Obstetric risk assessment is a dynamic and comprehensive process that extends beyond the evaluation of the woman's current clinical condition[2]. It also encompasses sociodemographic characteristics, reproductive history, and pre-existing medical conditions. Such a comprehensive approach enables healthcare professionals to identify high-risk situations at an early stage, facilitating timely referral to specialized care whenever necessary[2].

Conditions requiring referral to high-risk prenatal services include cardiovascular diseases; severe respiratory disorders, including bronchial asthma; chronic kidney disease and kidney transplantation; endocrine disorders, particularly diabetes mellitus, hypothyroidism, and hyperthyroidism; hematological disorders such as sickle cell disease and thalassemia; chronic hypertension and women receiving antihypertensive therapy (blood pressure >160/110 mmHg before 20 weeks of gestation); neurological disorders including epilepsy; psychiatric disorders requiring specialized follow-up; autoimmune diseases; maternal genetic disorders; previous

venous thromboembolism or pulmonary embolism; gynecological disorders; infectious diseases, including viral hepatitis, toxoplasmosis, HIV infection, tertiary syphilis, other sexually transmitted infections, leprosy, and tuberculosis; substance use disorders; and any other clinical condition requiring specialized medical care[2].

Given the complexity of obstetric risk assessment, prenatal care should adopt a comprehensive and multidimensional approach. In addition to routine medical evaluations, prenatal care should include nutritional counseling and supplementation, psychosocial and mental health support, screening for infectious diseases, and preventive interventions such as immunization[1]. National recommendations establish a minimum of six prenatal consultations, alternating between physicians and nurses, with care initiated before the 12th week of gestation[3].

Data from the Brazilian Primary Health Care Information System (SISAB) (**Figure 1**) indicate a gradual increase in the number of pregnant women receiving their first prenatal consultation before the 12th gestational week between 2020 and 2024. The lowest rates were observed in 2020, likely reflecting the impact of the COVID-19 pandemic on access to healthcare services. Conversely, 2023 and 2024 recorded the highest levels of early prenatal care initiation, suggesting improved adherence to recommended prenatal follow-up. Nevertheless, the proportion of women initiating prenatal care within the recommended timeframe remains below the desired target, reflecting the influence of demographic, socioeconomic, and healthcare accessibility barriers[4].

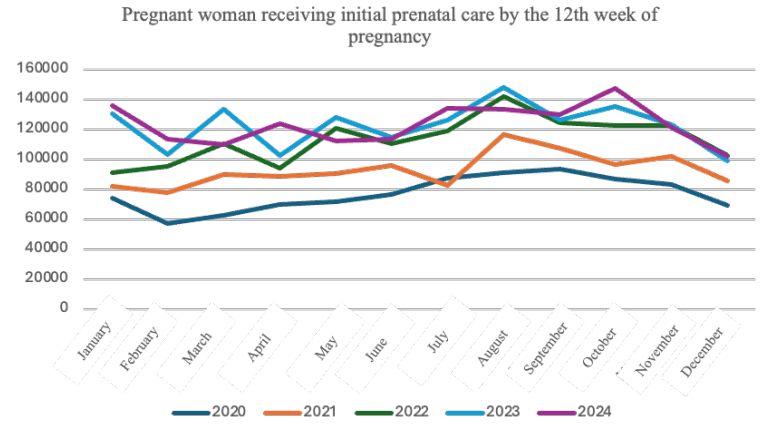

Figure 1. Number of pregnant women seen by the 12th week of gestation between 2020 and 2024, according to SISAB data.

The recommended frequency of prenatal consultations varies according to gestational age: monthly until the 28th week, every two weeks between the 28th and 36th weeks, and weekly from the 36th to the 41st week of pregnancy. During these visits, healthcare professionals should perform comprehensive clinical assessments, including nutritional evaluation, blood pressure measurement, abdominal palpation, assessment of fetal movements, uterine fundal height

measurement, and fetal heart rate auscultation. Pregnancies extending beyond 41 weeks require referral for fetal well-being assessment, including evaluation of the amniotic fluid index and fetal heart rate monitoring[3].

Laboratory and imaging examinations constitute essential components of prenatal care because they enable the early detection of clinical abnormalities and obstetric risk factors. During the first trimester, recommended tests include a complete blood count, blood typing and Rh factor determination, indirect Coombs test (for Rh-negative women), fasting plasma glucose, serological testing for syphilis, HIV, hepatitis B and C, toxoplasmosis (IgM and IgG), thyroid-stimulating hormone (TSH), urinalysis, urine culture, and obstetric ultrasonography. During the second trimester, laboratory tests should be repeated when clinically indicated, and additional investigations—including the oral glucose tolerance test (OGTT) and second-trimester morphological ultrasound—should be performed. During the third trimester, clinical evaluation focuses on identifying late pregnancy complications, including anemia, urinary tract infections, glycemic abnormalities, and fetal growth and presentation. All findings should be appropriately documented in the Pregnant Woman's Health Record, which serves as a key instrument for ensuring continuity and integration of prenatal care across different healthcare providers and levels of care[3].

Prenatal care should also include oral health assessment, with referral for dental evaluation early in pregnancy. Maternal oral health is particularly important because periodontal disease and other dental conditions have been associated with adverse pregnancy outcomes, including preterm birth, low birth weight, and other obstetric complications[3].

Preventive supplementation and pharmacological prophylaxis are fundamental components of routine prenatal care. Folic acid should be administered at a dose of 0.4 mg/day until the 12th week of gestation to prevent neural tube defects. Women at increased risk, including those with a previous pregnancy affected by neural tube defects, anticonvulsant use, or a history of bariatric surgery, should receive 4–5 mg/day. Iron supplementation, consisting of 40 mg of elemental iron daily, should begin as soon as pregnancy is confirmed and continue until the third month postpartum to prevent iron-deficiency anemia[3].

Maternal immunization is another cornerstone of prenatal care, providing direct protection to pregnant women while conferring passive immunity to newborns through transplacental antibody transfer. Recommended vaccines include the Tdap vaccine, administered after the 20th week of each pregnancy to prevent neonatal pertussis; the influenza vaccine, recommended during any trimester of pregnancy; the hepatitis B vaccine for susceptible women; and, more recently, the COVID-19 vaccine, which has demonstrated safety and effectiveness in reducing severe maternal outcomes associated with SARS-CoV-2 infection[3].

Objective

This chapter aims to discuss the importance of adequate routine prenatal care and to describe the principal clinical practices recommended for its implementation within Primary Health Care.

Methods

This study consists of a narrative literature review aimed at analyzing the importance of adequate routine prenatal care and the clinical practices adopted within the context of Primary Health Care (PHC). The literature search was conducted using the SciELO and PubMed databases with the following search terms: prenatal care, primary health care, and Brazilian Unified Health System (Sistema Único de Saúde – SUS).

Results and Discussion

The findings reinforce the importance of prenatal care as an essential public health strategy for promoting maternal and neonatal health. By ensuring systematic obstetric risk assessment and regular prenatal consultations, Primary Health Care (PHC) serves as the principal gateway to comprehensive care for pregnant women.

The literature consistently demonstrates that continuous obstetric risk assessment is indispensable because maternal health conditions may change throughout pregnancy. Early identification of high-risk conditions—including hypertension, diabetes mellitus, and infectious diseases—enables timely clinical interventions that substantially reduce maternal and neonatal morbidity and mortality, corroborating the evidence synthesized in this review.

Similarly, adherence to the laboratory and imaging investigations recommended in national prenatal care guidelines plays a decisive role in monitoring pregnancy progression. Previous studies have shown that the early detection of laboratory abnormalities and ultrasonographic findings supports more effective clinical decision-making, preventing pregnancy-related complications and reducing the need for emergency obstetric interventions.

Taken together, the available evidence highlights that the effectiveness of prenatal care depends not only on ensuring access to consultations but also on implementing comprehensive, evidence-based clinical practices encompassing risk stratification, preventive interventions, nutritional support, immunization, laboratory screening, oral healthcare, and multidisciplinary follow-up. These integrated actions contribute substantially to improving maternal and neonatal outcomes and reinforce the central role of Primary Health Care in coordinating the continuum of maternal healthcare.

Conclusion

In summary, routine prenatal care delivered within Primary Health Care plays a fundamental role in promoting maternal and neonatal health by integrating continuous obstetric risk assessment, multidimensional care, and systematic clinical follow-up. Early identification of maternal and fetal risk factors, combined with regular prenatal consultations, complementary diagnostic testing, and preventive interventions, enables timely clinical management and contributes significantly to reducing maternal and fetal morbidity and mortality.

The findings further demonstrate that the effectiveness of prenatal care depends not only on the availability of consultations and diagnostic examinations but also on the implementation of systematic, high-quality, and integrated healthcare practices capable of ensuring continuous, safe, and timely maternal care. Strengthening Primary Health Care and enhancing coordination with specialized healthcare services therefore remain essential strategies for improving maternal and fetal health outcomes throughout pregnancy.

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CHAPTER 03

Quality of Life in Infertile Women in Brazil: Religious Spirituality as a Coping Mechanism

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Introduction

Infertility is clinically defined as the inability to achieve pregnancy after at least 12 months of regular, unprotected sexual intercourse. Although this technical definition is widely adopted in medical practice, it fails to capture the profound emotional, psychological, and social burden experienced by couples undergoing infertility. For many individuals, infertility is characterized by prolonged uncertainty, repeated medical consultations, unsuccessful treatment attempts, and the persistent frustration associated with unfulfilled reproductive expectations. In recognition of its broad health implications, the World Health Organization (WHO) considers infertility a major global public health issue. In Brazil, it is estimated that 15–20% of couples experience infertility, with prevalence continuing to increase.

The psychosocial consequences of infertility disproportionately affect women, even when infertility is attributable to male factors. Deeply rooted cultural and societal expectations surrounding motherhood frequently led women to perceive childlessness as a personal failure. Consequently, many infertile women report feelings of frustration, shame, guilt, and social isolation. Scientific evidence consistently demonstrates elevated rates of anxiety, psychological distress, and depressive symptoms among women undergoing infertility treatment, highlighting that infertility extends far beyond a biomedical condition and substantially compromises multiple dimensions of quality of life.

Within this context, spirituality has emerged as an important coping resource. Religious practices—including prayer, participation in faith-based activities, and the search for meaning through spiritual beliefs—may provide emotional comfort, hope, and resilience while women navigate the uncertainties associated with infertility. Rather than representing solely a religious ritual, spirituality may function as a psychological resource that facilitates emotional adaptation and promotes resilience during infertility treatment.

Understanding the influence of religiosity and spirituality on women's experiences of infertility is therefore essential for achieving a more comprehensive understanding of the coping strategies adopted by these patients. Such knowledge may also contribute to the development of more holistic and patient-centered healthcare approaches that recognize not only the physical but also the emotional, social, and spiritual dimensions of infertility care.

Objectives

The present study aimed to evaluate whether religiosity and spirituality, expressed through different forms of religious belief and practice, function as coping mechanisms for women experiencing infertility and to investigate their potential impact on emotional well-being, social functioning, and fertility-related quality of life.

Methods

This cross-sectional study included 104 women diagnosed with infertility, aged between 18 and 50 years, who were receiving treatment at an assisted reproduction clinic in the city of São Paulo, Brazil. Data collection was conducted in 2020 using an electronic questionnaire distributed to eligible participants. All participants voluntarily agreed to participate in the study and provided written informed consent prior to enrollment.

The study protocol was approved by the Research Ethics Committee of the Faculty of Medicine of Itajubá (FMIT) (Approval No. 3,846,249) and was conducted in accordance with Brazilian National Health Council Resolution No. 196/96, which governed ethical standards for research involving human participants at the time the study was approved.

Two instruments were used for data collection:

FertiQoL (Fertility Quality of Life): a translated and validated questionnaire specifically designed to assess fertility-related quality of life among individuals undergoing infertility treatment.

Religiosity Questionnaire: a 12-item instrument developed by the investigators to evaluate participants' religiosity and spirituality, including religious affiliation, frequency of religious practices, participation in religious services, and perceptions regarding the role of spirituality in coping with infertility.

Statistical analyses were performed using SPSS Statistics version 18.0® (IBM Corp., Armonk, NY, USA). Descriptive statistics were calculated to characterize the study population, and associations between variables were assessed using the chi-square test. Statistical significance was established at $p < 0.05$.

Results and Discussion

The findings revealed that spirituality played a significant role in helping women cope with infertility. Approximately 90% of participants reported that spirituality was an important source of support throughout infertility treatment. Likewise, 93% indicated that they regularly engaged in prayer or the reading of religious texts, describing these practices as sources of calm, hope, inner peace, and emotional comfort. These results demonstrate that religiosity was deeply integrated into the participants' lives and represented an important coping resource during treatment.

Despite this spiritual support, participants also reported considerable psychological distress. Approximately 79% described experiencing a persistent alternation between hope and despair throughout the treatment process, while 45% reported frequent episodes of sadness or depressive symptoms. In addition, 67% experienced difficulty concentrating because of recurrent thoughts related to infertility, highlighting the substantial psychological burden associated with this condition.

Infertility also had a marked impact on social functioning. Half of the participants reported that infertility interfered with their daily activities, whereas 58% experienced discomfort during social gatherings, often because of comparisons with relatives or friends who had already become mothers. Nevertheless, 76% expressed satisfaction with their marital relationships, although 68% acknowledged that infertility had generated tension and negatively affected their relationship with their partner. Women who reported more frequent religious practices also demonstrated greater resilience in coping with infertility, suggesting that spirituality may function as a protective factor against the emotional and relational burden associated with infertility.

These findings are consistent with both national and international studies identifying faith and spirituality as important coping resources for individuals experiencing infertility. Previous research has also shown that infertile patients are more likely to seek support from religious

leaders than from psychologists or formal support groups. In recognition of the psychological burden associated with infertility, the European Society of Human Reproduction and Embryology (ESHRE) recommends integrating psychosocial care into assisted reproduction services, emphasizing that infertility management should address not only medical aspects but also the emotional, psychological, and social needs of patients.

However, the role of religiosity is not universally beneficial. The participants' experiences suggest that spirituality may have different meanings depending on individual beliefs and religious interpretations. For some women, faith represented an essential source of hope, resilience, and emotional support. For others, particularly when associated with more rigid religious beliefs, spirituality could intensify feelings of guilt, self-blame, or hopelessness related to their inability to conceive. These findings reinforce the importance of adopting a patient-centered approach in infertility care, recognizing that although religiosity often promotes psychological well-being, it may also generate emotional conflicts in certain circumstances. Healthcare professionals should therefore be prepared to acknowledge and respect the diversity of patients' spiritual experiences when providing comprehensive infertility care.

Conclusion

The present study demonstrated that religiosity and spirituality play a meaningful role in helping women cope with infertility by fostering resilience throughout the challenges associated with infertility treatment. Religious practices—including prayer, reading religious texts, and participation in spiritual activities—were associated with greater emotional well-being, stronger perceptions of social support, and improved relationship satisfaction.

Although the cross-sectional design does not allow causal inferences, the findings suggest that spirituality may serve as a complementary resource capable of mitigating the psychological and social burden of infertility. Nevertheless, the experience of religiosity is not uniform. While faith represents a source of comfort, hope, and emotional strength for many women, it may also generate feelings of guilt, self-blame, or distress when accompanied by rigid religious beliefs or moral interpretations regarding infertility.

These findings underscore the importance of adopting a holistic, patient-centered approach to infertility care. Healthcare professionals should recognize that infertility management extends beyond medical treatment and laboratory procedures, encompassing emotional, social, and spiritual dimensions that influence patients' overall well-being. Respecting each woman's individual beliefs and spiritual experiences may contribute to more compassionate, comprehensive, and person-centered care.

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CHAPTER 04

Long-Term Patient-Reported Toxicity Following Conventional Fractionated Radiotherapy for Localized Prostate Cancer

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Introduction

Prostate cancer remains the most frequently diagnosed malignancy among men worldwide and represents a major public health challenge. According to the 2023 cancer statistics, an estimated 288,300 new cases were diagnosed, accounting for approximately 29% of all newly diagnosed cancers in men, while approximately 34,700 deaths were attributed to the disease, corresponding to nearly 11% of all male cancer-related deaths. Furthermore, the incidence of prostate cancer increased by approximately 3% annually between 2014 and 2019, highlighting the growing burden of this malignancy[1].

Early diagnosis plays a fundamental role in improving clinical outcomes. Current screening strategies recommended for men aged 50 years or older include measurement of prostate-specific antigen (PSA) levels and digital rectal examination, both of which contribute to earlier detection and facilitate individualized therapeutic decision-making[2].

Treatment selection depends on multiple factors, including patient age, tumor stage, life expectancy, comorbidities, and overall clinical condition. Therapeutic options include radical prostatectomy, radiotherapy, androgen deprivation therapy, chemotherapy, or combinations of these modalities according to disease stage and individual patient characteristics[3].

Radiotherapy constitutes one of the principal treatment modalities for localized prostate cancer and may be delivered with either curative or palliative intent. Conventional fractionated radiotherapy and hypo fractionated radiotherapy are the two most employed treatment schedules. Despite its well-established efficacy, ionizing radiation may induce both acute and

late adverse effects that negatively affect patients' quality of life. The most frequently reported toxicities involve the urinary tract, including increased urinary frequency, urgency, and dysuria, as well as gastrointestinal symptoms such as bowel urgency, rectal discomfort, and altered bowel habits[3-6].

Given the increasing number of long-term prostate cancer survivors, understanding the late toxicity associated with radiotherapy has become increasingly important. Assessment of treatment-related morbidity provides valuable information for shared clinical decision-making and contributes to improving long-term survivorship care.

Objective

This study aimed to evaluate the long-term toxicity associated with conventional fractionated radiotherapy in patients treated for prostate cancer.

Materials and Methods

Study Design and Ethical Approval

This cross-sectional observational study was approved by the Research Ethics Committee of the Faculty of Medicine of Itajuba under protocol No. 4,660,960, following institutional approval granted by the participating oncology clinic.

Eligible patients were contacted primarily through telephone calls and electronic questionnaires distributed via WhatsApp®. During the initial contact, participants received detailed information regarding the objectives of the study, the importance of their participation, and the procedures involved. Verbal informed consent by inform consent form (TCLE) was obtained prior to questionnaire administration, and participants were given the opportunity to clarify any questions concerning the study.

Assessment of Treatment-Related Toxicity

Treatment-related toxicity was evaluated using the Expanded Prostate Cancer Index Composite (EPIC), a validated patient-reported outcome instrument designed to assess health-related quality of life following prostate cancer treatment[7,8]. The questionnaire evaluates functional outcomes and symptom burden across four domains: urinary, bowel, sexual, and hormonal function. Because the present study focused specifically on urinary, bowel, and sexual outcomes, the hormonal domain was not included in the analysis.

The EPIC questionnaire comprises 32 items, each referring to symptoms experienced during the previous four weeks[7-9]. Responses are recorded using Likert-type scales and subsequently converted into standardized scores ranging from 0 to 100, with higher scores indicating better health-related quality of life, as shown in **Table 1**[7,8,10].

Table 1. Instructions for matching Likert scale values with the EPIC questionnaire (translated scale).

Item Number	Value of the Item Answered	Standardized Value
23, 24, 25, 42, 43, 48, 56, 57, 58, 60, 61, 62, 63, 64, 69, 70, 71, 72	1	0
	2	25
	3	50
	4	75
	5	100
26, 59	1	0
	2	33
	3	67
	4	100
27	0	100
	1	67
	2	33
	3	0
28, 29, 30, 31, 32, 33, 49, 50, 51, 52, 53, 54, 65, 66, 67, 74, 75, 76, 77, 78, 79	0	100
	1	75
	2	50
	3	25
	4	0
34,44,45,46,55,68	1	100
	2	75
	3	50

A digital version of the validated Brazilian Portuguese EPIC questionnaire was created using the Google Forms® platform to facilitate participant recruitment and maximize response rates. During data processing, minor discrepancies between the Brazilian validated version and the original University of Michigan EPIC questionnaire were identified. Specifically, one response option from Question 6 was absent from the Brazilian version and was therefore excluded from the analysis. In addition, the scoring system of Question 17 differed between the two versions;

consequently, responses were converted to ensure consistency with the original scoring system prior to statistical analysis. Thus, the 0-to-4-point scale was converted to a 1-to-5-point scale for data analysis[8,10].

Clinical Data Collection

Clinical and oncological data were retrieved from the Hospital Cancer Registry maintained by the Oncominas Oncology Clinic, Itajuba, Minas Gerais, Brazil. Information regarding radiotherapy treatment protocols was obtained from patients' medical records.

Descriptive statistical analyses were performed using Microsoft Excel® 2010 following double data entry and validation procedures. Quantitative variables were summarized using measures of central tendency, including means and medians. Correlation analyses were planned to investigate associations between treatment characteristics and toxicity outcomes.

Results and Discussion

This observational cross-sectional study initially identified 294 patients diagnosed with prostate cancer who had undergone conventional fractionated radiotherapy and were considered eligible for participation. However, only 24 patients completed the EPIC questionnaire, resulting in an overall response rate of 8.19% (**Figure 1**). Among these participants, 10 questionnaires were incomplete, primarily because of patient discomfort in answering questions related to sexual and bowel function. Additional losses resulted from patient deaths (n = 31) and unsuccessful attempts to establish contact.

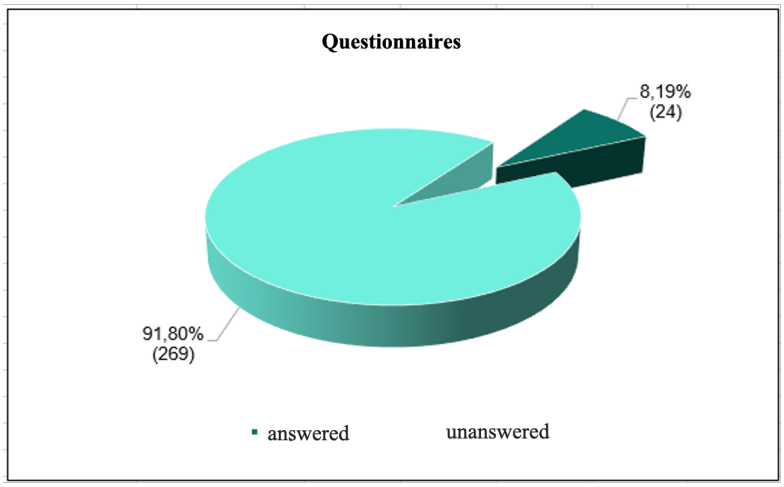

Figura 1. Graph showing the number of completed and uncompleted questionnaires.
Source: Authors' elaboration.

Tables 2 and **3** show, respectively, the reasons why the questionnaires were not answered and the circumstances in which the completed questionnaires were found.

Table 2. Reasons why the questionnaires were not answered.

Patient's condition	N	%
Dead	31	11,52
They didn't want to participate	13	4,8
Other reasons (absence or incorrect contact details)	225	83,6

Source: Prepared by the authors

Table 3. Circumstances in which the completed questionnaires are found.

Questionnaire status	N	%
Complete	14	58,3
Incomplete	10	41,6
Urinary Function	4	16,6
Bowel Function	8	33,3
Sexual Function	12	50

Source: Prepared by the authors

Most participants were recruited through electronic questionnaires distributed via WhatsApp® (83%), whereas the remaining 17% were contacted by telephone. Participants ranged in age from 43 to 70 years, with a median age of 68 years.

Sociodemographic and Clinical Characteristics

Analysis of the participants' demographic and clinical characteristics revealed that 70.8% reported a positive family history of cancer, with 54.2% having at least one affected first-degree relative. Regarding lifestyle characteristics, 62.5% reported alcohol consumption, whereas 16.6% were former smokers. Most tumors (83.3%) were classified as Stage I, and the most frequent Gleason score was 7 (3 + 4), observed in 41.6% of patients. Detailed clinical and demographic characteristics are presented in **Table 4**.

Table 4. Population, oncological, and sociodemographic data analyzed as frequency percentages in the sample.

Population / Oncological / Sociodemographic Data	N	%
Positive family history	17	70,83
First-degree relatives	13	54,16
Second-degree or more distant relatives	4	16,6
Lifestyle habits		
Alcohol consumer	15	62,5
Recovering alcoholic	1	4,1
Smoker	4	16,6
Former smoker	4	16,6
Stages		
1	20	83,3
3	1	4,1
4	3	12,5
Gleason		
6 (3+3)	7	29,16
7 (3+4)	10	41,6
7 (4+3)	4	16,6
8 (4+4)	3	12,5

Source: Prepared by the authors

EPIC Questionnaire Outcomes

Finally, the results obtained from the EPIC questionnaires—total and the sexual, bowel, and urinary subscales—are presented in **Table 5** and **Figure 2**.

Overall health-related quality-of-life scores obtained using the EPIC questionnaire demonstrated distinct patterns among the evaluated functional domains. Urinary and bowel function presented relatively preserved scores, whereas sexual function exhibited substantially lower values, indicating greater impairment following treatment.

Median scores (Q2) were 80.1 for urinary function, 77.1 for bowel function, and 45.7 for sexual function. The overall EPIC median score was 67.6, indicating moderate long-term treatment-related toxicity among the evaluated patients.

Table 5. Analysis of the domains evaluated.

	Total	FU	FI	FS
Minimum	41,2162162	40,09	25	17,3
Q3	80,15789475	92,5	92,85	57,69
Q2	67,63759842	80,14916667	77,055	45,71875
Q1	58,49342108	69,7	65,6525	29,7375
Maximum	86,8421053	100	100	71,15

Source: Prepared by the authors.

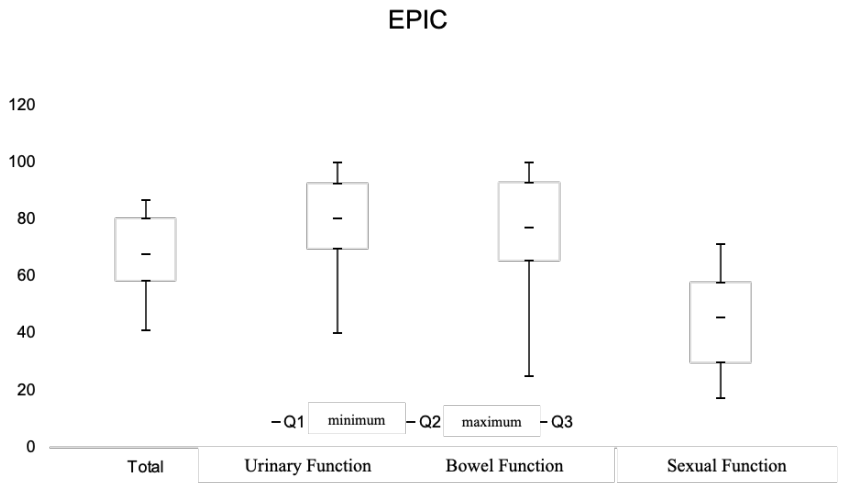

Figure 2. Illustration of variables derived from EPIC scores of patients recruited for the study. Source: Authors' elaboration.

The present study evaluated long-term patient-reported toxicity following conventional fractionated radiotherapy for prostate cancer using the validated EPIC questionnaire. The findings demonstrated that urinary and bowel functions remained relatively preserved over time, whereas sexual function showed the greatest decline, suggesting that sexual dysfunction represents the most significant long-term adverse effect experienced by these patients.

These results are consistent with previous studies reporting that urinary symptoms—including urinary frequency, nocturia, urgency, and reduced urinary stream—are frequently present even before treatment because they are directly associated with prostate enlargement and tumor progression. Likewise, erectile dysfunction has been documented in a proportion of patients prior to radiotherapy, indicating that treatment is not the sole determinant of impaired sexual function[11,12].

Long-term toxicity remains one of the principal concerns associated with radiotherapy for localized prostate cancer because of its direct impact on patients' health-related quality of life[13]. Previous studies have reported increased risks of secondary malignancies, including bladder and colorectal cancers, following pelvic irradiation. Conversely, comparisons between treatment modalities have demonstrated that patients undergoing radical prostatectomy often experience worse urinary and sexual outcomes than those treated with radiotherapy, whereas bowel function tends to be more adversely affected after radiation therapy. These observations reinforce the importance of individualized treatment selection based on patient preferences and expected toxicity profiles[13].

Urinary toxicity has been extensively investigated because it represents one of the most common adverse effects of prostate cancer treatment. Tamihardja et al. reported acute urinary toxicity rates of 30.1% and late toxicity rates of 26.3%, with a median patient age of 73 years[14]. Other longitudinal studies have shown that although nearly 90% of patients experience urinary symptoms after radiotherapy, symptom severity generally decreases over time, with acute manifestations predominating during the first six weeks after treatment and late toxicities occurring up to five years thereafter[11,14,15].

Furthermore, according to Bhatnagar, post-radiation urinary symptoms may be related to the dose received, given that toxicity was higher in patients treated with brachytherapy compared to radiotherapy[16]. The most reported urinary symptoms include nocturia, urinary urgency, increased voiding frequency, and reduced urinary stream[16,17].

With respect to gastrointestinal toxicity, previous studies have demonstrated that bowel symptoms generally peak approximately 60 months after radiotherapy before gradually improving. Acute gastrointestinal toxicity has been reported in approximately 13% of patients, whereas late toxicity at five years reaches approximately 12.1%[14]. Comparative studies have also shown poorer bowel function scores among patients receiving radiotherapy than among those managed with active surveillance or radical prostatectomy[13,18].

In the present study, bowel function was the second most frequently omitted domain in incomplete questionnaires (33.3%), possibly reflecting patient discomfort when discussing bowel symptoms[16]. The most reported manifestations described in the literature include rectal bleeding and fecal incontinence[14].

Among all evaluated domains, sexual function was the most profoundly affected. Sexual dysfunction has substantial physical, psychological, and social consequences and remains one of the principal determinants of quality of life after prostate cancer treatment[11,13].

Previous studies have shown that 69% of patients treated with external beam radiotherapy report erectile dysfunction one year after treatment, increasing to approximately 74% after six years of follow-up[17]. Likewise, the overall risk of erectile

dysfunction after conventional radiotherapy has been estimated at 52%, whereas loss of libido has been reported in approximately 14.7% of treated patients[11,16]. Interestingly, brachytherapy has been associated with better long-term sexual outcomes than conventional external beam radiotherapy[17]. It is worth noting that this domain showed the lowest level of adherence in the current study (50%), that erectile dysfunction was already present prior to radiotherapy in some studies, and that advanced age is a major risk factor for this sexual disorder[11,13].

It is of great importance to address the significance of the patient's active and autonomous decision regarding the treatment method (radical prostatectomy, active surveillance, brachytherapy, or conventional fractionation or hypofractionation radiotherapy) and its negative impacts on the domains examined in this study and on their quality of life[13,19]. Given the foregoing, the physician is responsible for establishing a physician-patient relationship that fosters clear and precise communication and understanding, while assessing the patient's need and willingness to undergo radiation[13].

Study Limitations

This study has several limitations that should be acknowledged. First, the relatively small sample size, primarily resulting from the low response rate to the EPIC questionnaire, limits the generalizability of the findings. Nevertheless, studies evaluating long-term toxicity following conventional fractionated radiotherapy remain scarce in the state of Minas Gerais, highlighting the relevance of the present investigation.

A second limitation is the limited availability of studies assessing late toxicity beyond six years after conventional radiotherapy. Although one long-term follow-up study evaluated patients between 13 and 21 years after treatment, approximately 92% of the original cohort had died[20], substantially restricting the availability of long-term patient-reported outcomes.

An additional methodological consideration concerns the use of the Brazilian validated version of the EPIC questionnaire. Minor differences in item scoring between the Brazilian adaptation and the original University of Michigan version required score conversion during data analysis. Although this approach may have introduced a small degree of measurement variability, the use of standardized mean values and graphical data representation minimized its potential impact on the interpretation of results.

Conclusion

The present study evaluated the long-term toxicity associated with conventional fractionated radiotherapy in patients treated for prostate cancer using the validated EPIC questionnaire. Overall, urinary and bowel functions remained relatively preserved during long-term follow-up, whereas sexual function demonstrated substantially greater impairment, confirming that sexual dysfunction remains one of the most significant long-term adverse effects associated with conventional radiotherapy.

The study also highlighted important methodological challenges, particularly the low adherence of patients to remotely administered questionnaires. Sensitive topics, especially those related to sexual function, were frequently left unanswered, emphasizing the need for alternative strategies to improve patient engagement in quality-of-life research.

Despite these limitations, the findings are consistent with previous studies reporting comparable urinary and bowel outcomes after conventional radiotherapy. The greater deterioration observed in sexual function reinforces the importance of discussing potential long-term treatment-related adverse effects during therapeutic decision-making and survivorship care. Future studies including larger cohorts and prospective longitudinal follow-up are warranted to better characterize the long-term impact of radiotherapy on patient-reported quality of life.

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Part II

Translational Research and Novel Therapeutic Strategies

CHAPTER 05

Modulatory Activity of *Caryocar brasiliense* Against Bacteria Isolated from Patients with Healthcare-Associated Infections

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Introduction

Over the past decades, bacterial resistance has emerged as one of the most significant threats to global public health. Both common and opportunistic pathogens have developed resistance to antimicrobial agents, making bacterial infections increasingly difficult to treat and substantially reducing the effectiveness of currently available therapeutic options (World Health Organization, 2024). This phenomenon has been observed across multiple bacterial species and is associated with prolonged hospital stays, increased healthcare costs, and higher morbidity and mortality rates. Consequently, the discovery of novel compounds with antimicrobial activity has become a priority for preserving the effectiveness of antibiotic therapy and safeguarding public health (Santos, 2004).

The Brazilian Cerrado, a tropical savanna biome recognized as one of the world's biodiversity hotspots, harbors a wide variety of plant species with considerable pharmacological potential (Pereira et al., 2011). Among these, *Caryocar brasiliense*, popularly known as pequi, is one of the most economically, nutritionally, and culturally important native fruits. In addition to its widespread culinary use, pequi has attracted growing scientific interest because of its high concentration of bioactive compounds, including carotenoids, polyphenols, fatty acids, and triterpenes, which have been associated with antioxidant, anti-inflammatory, antimicrobial, and antifungal activities (Miranda-Vilela et al., 2009; Silva et al., 2023). These characteristics suggest that *C. brasiliense* may represent a promising natural source of biologically active compounds with therapeutic applications.

The antimicrobial activity of pequi has been attributed to several of its bioactive constituents (Melo et al., 2024). Unsaturated fatty acids, particularly oleic acid and linoleic acid, have demonstrated inhibitory activity against a broad spectrum of bacteria and fungi by disrupting microbial cell membrane integrity (Rocha et al., 2019). Likewise, carotenoids—especially β-carotene—have shown antimicrobial effects against clinically relevant bacterial strains. In addition, phenolic compounds, including flavonoids, contribute to microbial growth inhibition through multiple mechanisms, reinforcing the therapeutic potential of this fruit (Toscan, 2010).

The pulp of *Caryocar brasiliense* is particularly rich in carotenoids, flavonoids, essential fatty acids, and phenolic compounds, all of which are widely recognized for their antioxidant properties and potential antimicrobial activity (Lima et al., 2007; Delgado-Vargas et al., 2000). Carotenoids have also been reported to modulate immune responses and intercellular communication. Furthermore, previous studies have demonstrated the antimicrobial activity of carotenoid-rich extracts against multidrug-resistant bacterial strains, including methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Escherichia coli*. Fatty acids present in pequi pulp further enhance its antimicrobial potential by destabilizing bacterial lipid bilayers, ultimately inhibiting microbial growth and reducing bacterial viability (Kusmita et al., 2023).

Bacterial resistance poses a particularly serious challenge in healthcare settings (Loureiro et al., 2016), where patients are frequently exposed to prolonged antibiotic therapy, invasive procedures, immunosuppression, and multiple comorbidities. Under these conditions, multidrug-resistant microorganisms employ diverse resistance mechanisms—including enzymatic antibiotic inactivation, modification of antimicrobial targets, and activation of efflux pumps—that substantially compromise the efficacy of conventional antimicrobial therapies. Consequently, healthcare-associated infections caused by resistant pathogens are associated with increased morbidity and mortality, prolonged hospitalization, and elevated healthcare expenditures. This scenario highlights the urgent need to identify and develop novel antimicrobial agents capable of overcoming existing resistance mechanisms and expanding available therapeutic options (Damiani et al., 2013; Ambler et al., 2018).

Despite increasing interest in the biological properties of *Caryocar brasiliense*, the available scientific literature regarding the antimicrobial activity of pequi pulp against clinically relevant bacterial isolates remains limited. Therefore, the present study aimed to evaluate in vitro the antimicrobial activity of *Caryocar brasiliense* pulp against pathogenic bacterial strains isolated from patients with healthcare-associated infections. It is expected that the findings will contribute to expanding current knowledge regarding the antimicrobial properties of pequi pulp and provide scientific evidence supporting its potential application as a natural antimicrobial agent for the management of bacterial infections.

Materials and Methods

This study was designed as a descriptive, quantitative, non-probabilistic experimental investigation employing the broth microdilution assay to evaluate the antibacterial activity of pequi (*Caryocar brasiliense*) pulp. The study protocol was approved by the Research Ethics Committee of the Faculdade de Medicina de Itajubá (FMIT), Brazil (Protocol No. 7,376,571/2025).

A total of 22 bacterial isolates obtained from the Academic Microorganism Collection (MPA) of the Microbiology Laboratory at FMIT were included in the study. The collection comprised 10 clinical isolates of *Proteus spp*. and 12 clinical isolates of *Staphylococcus aureus* recovered from hospitalized patients between 2001 and 2008. The isolates originated from patients admitted to the institution's teaching hospital and were recovered from multiple clinical specimens, including urine, skin, bloodstream, surgical sites, and other anatomical locations, representing patients admitted to different hospital units, including Pediatrics, Surgical Ward, Internal Medicine, Private Ward, and the Intensive Care Unit (ICU).

Among the *Staphylococcus aureus* isolates, 40% were obtained from female patients and 60% from male patients, ranging in age from 46 to 88 years. Regarding the *Proteus spp.* isolates, 58.33% originated from female patients and 41.67% from male patients, with ages ranging from 53 to 86 years. All laboratory procedures were performed between February and April 2025 at the Microbiology Laboratory of the Faculdade de Medicina de Itajubá.

Before antimicrobial testing, frozen bacterial stocks were thawed at room temperature and subjected to a reactivation procedure. Under sterile conditions using a Bunsen burner, approximately 10 µL of each bacterial stock was collected with a sterile inoculating loop and streaked onto sterile nutrient agar plates. Each plate was labeled, divided into four quadrants, and inoculated with the respective bacterial isolates. Following inoculation, the cultures were incubated at 37°C for 48 hours. Only isolates exhibiting satisfactory bacterial growth were selected for antimicrobial evaluation.

All *Staphylococcus aureus* isolates demonstrated adequate viability and were included in the study. Among the twelve *Proteus spp.* isolates, ten remained viable after reactivation and were therefore included in the experimental analyses.

Subsequently, bacterial biomass from each viable culture was collected using a sterile inoculating loop and aseptically transferred into microcentrifuge tubes containing 5 mL of sterile 0.85% saline solution. The suspensions were homogenized using a vortex mixer, and inoculum density was standardized by turbidity comparison using a Wickerham turbidity card until reaching the 3+ grade, corresponding to the disappearance of the reference lines. This turbidity is equivalent to

the 0.5 McFarland standard, representing approximately 1 × 10^8 colony-forming units (CFU)/mL (McFarland, 1907).

Commercially preserved 130 g pequi pulp, containing 90 kcal, 5.3 g carbohydrates, 0.9 g protein, 7.2 g total fat, 7.6 g dietary fiber, and 623 mg sodium, was dehydrated in a conventional laboratory oven at 60°C for 120 minutes and subsequently ground using a domestic blender. From the resulting material, 10 g of dried pulp was suspended in sterile distilled water to obtain a stock solution of 0.1 g/mL, followed by filtration through 14-µm paper filters.

Serial two-fold dilutions of the stock solution were subsequently prepared, ranging from 1:2 to 1:256 (1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, and 1:256).

Antimicrobial activity was evaluated using the broth microdilution method in 96-well microplates. Two microplates were used to evaluate 20 bacterial isolates (10 *Proteus spp.* and 10 *Staphylococcus aureus*) (**Figure 1**). Each plate consisted of eight rows (A–H) and twelve columns.

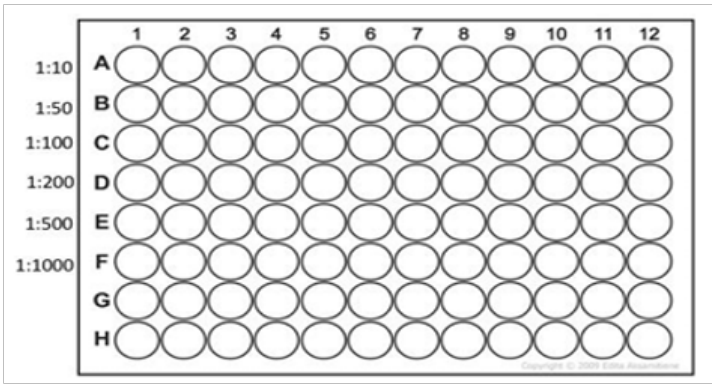

Figure 1. Schematic representation of the 96-well microplate used for the broth microdilution assay. Source: Prepared by the authors.

Initially, 40 µL of Brain Heart Infusion (BHI) broth was dispensed into every well. Subsequently, 40 µL of each pequi pulp dilution was added horizontally across the rows, with row A receiving the 1:2 dilution, row B the 1:4 dilution, and so forth through the remaining serial dilutions. Thereafter, 20 µL of standardized bacterial suspension (1 × 10^8 CFU/mL) from each isolate was inoculated into the wells of columns 1 through 10. For the growth control, column 11 received bacterial inoculum plus 40 µL of BHI broth without pequi extract. The microplates were incubated at 37°C for 24 hours. Following incubation, 30 µL of 2.5% 2,3,5-triphenyl tetrazolium chloride (TTC) solution was added to every well to assess bacterial metabolic activity using a colorimetric assay (Tanaka, 2001). The plates were incubated for an additional 24 hours at 37°C. An additional control plate was prepared containing 20 µL of bacterial suspension, 40 µL of BHI broth, and 20 µL of ciprofloxacin (25 mg/mL) for each isolate. Bacterial growth was interpreted according to the colorimetric reaction produced by TTC. Wells displaying red or pink coloration were considered indicative of bacterial

growth, whereas colorless or weakly stained wells indicated inhibition of bacterial growth and, consequently, potential antimicrobial activity of the pequi pulp extract (Figure 2).

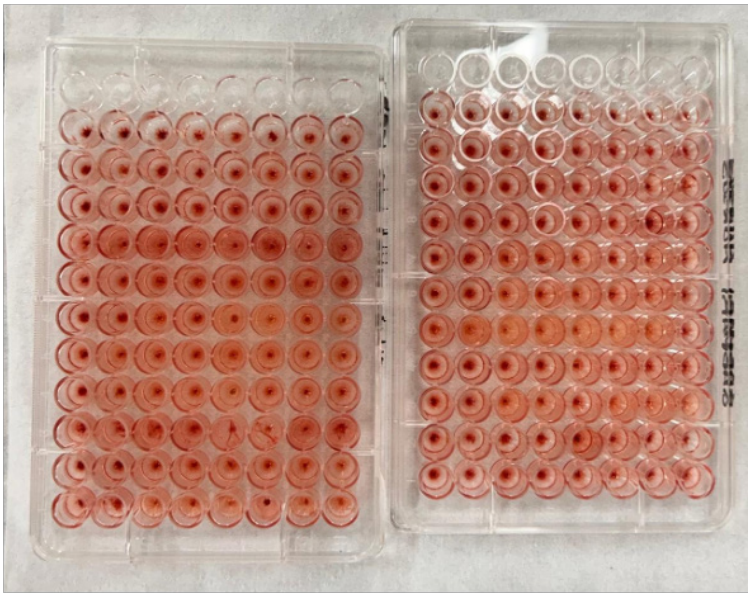

Figure 2. Representative colorimetric results of the broth microdilution assay using 2,3,5-triphenyl tetrazolium chloride (TTC) as the metabolic indicator. The left microplate illustrates the analysis of Proteus spp. isolates, whereas the right microplate corresponds to Staphylococcus aureus isolates. Wells showing inhibitory activity exhibited no color development or only faint staining, whereas wells without inhibition displayed intense red coloration. Source: Prepared by the authors.

Throughout the handling of biological specimens and preparation of *Caryocar brasiliense* fruit pulp extracts, appropriate biosafety measures were adopted. Laboratory personnel wore disposable latex gloves, laboratory coats, protective face masks, and safety goggles to minimize contamination risks and occupational exposure. In addition, all microbiological procedures were performed within a Class II biological safety cabinet to ensure aseptic conditions and operator safety.

Contaminated materials were disposed of in designated biological waste containers following the recommendations of Brazilian Health Regulatory Agency (ANVISA) Resolution RDC No. 222/2018.

Research data management complied with the recommendations of the Brazilian National Research Ethics Commission (CONEP). All data generated during the study were securely stored with restricted access available only to the research team. At the completion of the study, physical records, including laboratory notebooks and experimental documentation, were archived in a locked cabinet under the responsibility of the principal investigator. Digital records were stored using secure cloud-based platforms to ensure data integrity, confidentiality, and protection against information loss.

Results

The findings demonstrated a selective modulatory effect of the crude *Caryocar brasiliense* (pequi) pulp extract on bacterial growth. Among the bacterial species evaluated, the extract exhibited greater modulatory activity against *Proteus spp.* (**Figure 3**), particularly at dilutions of 1:32, 1:64, 1:128, and 1:256, with modulatory activities of 60%, 60%, 70%, and 70%, respectively.

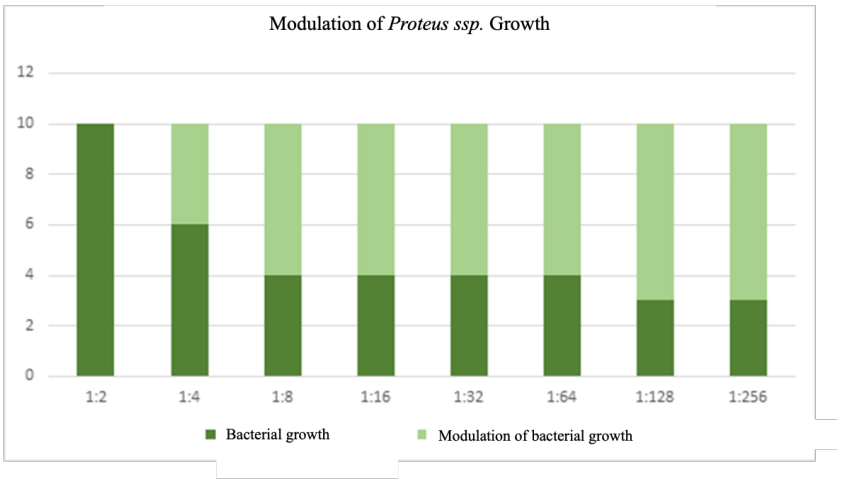

Figure 3. Analysis of the activity of the crude extract of *Caryocar brasiliense* (pequi) on the growth of *Proteus ssp.* bacteria isolated from patients with hospital-acquired infections. Source: Author's own work (research data).

In contrast, the extract showed a less pronounced modulatory effect against *Staphylococcus aureus* (**Figure 4**), reaching 40% modulatory activity at dilutions of 1:32, 1:64, and 1:128. These findings indicate that the biological activity of the crude pequi extract varies according to the bacterial species tested, suggesting differential susceptibility among clinically relevant pathogens.

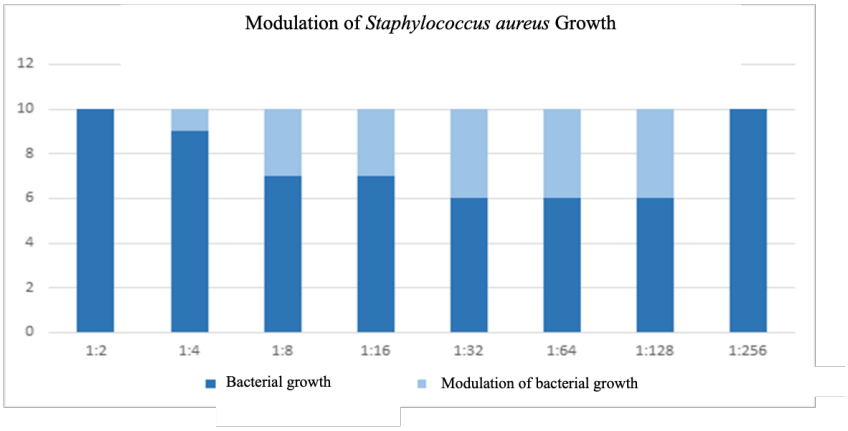

Figure 4. Analysis of the activity of the crude extract of *Caryocar brasiliense* (pequi) on the growth of *S. aureus* bacteria isolated from patients with hospital-acquired infections. Source: Author's own (research data).

Discussion

The present findings indicate that *Caryocar brasiliense* pulp extract possesses a potential modulatory effect on bacterial growth, corroborating previous reports in the literature (Sousa, 2021). This biological activity is likely associated with the presence of bioactive constituents, including fatty acids, flavonoids, and phenolic compounds, which are recognized for their antimicrobial properties. Acting synergistically, these compounds may interfere with essential bacterial metabolic pathways and alter cell membrane permeability, ultimately reducing bacterial viability (Zhang et al., 2022).

Despite this modulatory activity, the extract did not exhibit a significant direct inhibitory effect against the bacterial strains evaluated. This finding suggests that the antimicrobial efficacy of the crude extract may be limited under the experimental conditions employed or that higher extract concentrations may be required to achieve bacteriostatic or bactericidal activity.

Differences in bacterial cell wall architecture may partially explain the distinct responses observed between the tested microorganisms. *Staphylococcus aureus*, a Gram-positive bacterium, possesses a thick peptidoglycan layer but lacks an outer membrane, facilitating the penetration of bioactive compounds (Mueller & Levin, 2020). This structural characteristic may account for the greater modulatory activity observed, as evidenced by the reduction in color intensity within the tested wells. In contrast, *Proteus spp.*, a Gram-negative bacterium, contains an outer membrane rich in lipopolysaccharides (LPS), which constitutes an additional permeability barrier against antimicrobial agents (Fivenson et al., 2023). Nevertheless, the pequi extract also demonstrated modulatory activity against *Proteus spp.*, suggesting that its bioactive compounds—including flavonoids, fatty acids, and phenolic compounds—may interfere with the integrity of the outer membrane or affect bacterial resistance mechanisms.

To further validate these findings, future investigations should include a larger number of bacterial isolates, particularly Gram-positive strains, together with extended experimental periods that allow greater assay reproducibility and methodological refinement. In addition, complementary antimicrobial susceptibility assays, such as the determination of the minimum inhibitory concentration (MIC) and the inclusion of standardized positive controls, would provide more robust evidence regarding the antimicrobial potential of *Caryocar brasiliense* pulp extract.

Conclusion

The findings of the present study indicate that *Caryocar brasiliense* pulp extract exhibits a significant modulatory effect on bacterial growth, with more pronounced activity against Proteus spp. than against *Staphylococcus aureus*.

These results suggest that pequi may represent a promising natural source of bioactive compounds for combating bacterial resistance, particularly against Gram-negative pathogens, which are responsible for a substantial proportion of healthcare-associated infections and frequently exhibit multidrug resistance. Conversely, the modulatory effect observed against the Gram-positive strain *S. aureus* was comparatively less pronounced. Given the worldwide increase in antimicrobial resistance, identifying novel natural products with antimicrobial activity remains an important research priority.

Nevertheless, additional studies employing broader methodological approaches are required to validate and expand the therapeutic applicability of *Caryocar brasiliense*. Future investigations should evaluate different bacterial species, experimental conditions, and biological models while further elucidating the bioactive compounds, mechanisms of action, pharmacological safety, and clinical relevance of pequi-derived products. Such studies will be essential for determining the potential role of *Caryocar brasiliense* as an alternative strategy for managing antimicrobial-resistant infections.

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CHAPTER 06

Antimicrobial Modulatory Effects of *Hylocereus megalanthus Peel* Extract Against *Staphylococcus aureus*

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Introduction

The fruit commonly known as yellow dragon fruit (*Hylocereus megalanthus*), a member of the Cactaceae family, is native to the tropical and subtropical regions of the Americas, particularly Mexico. In recent years, it has attracted increasing scientific interest because of its nutritional value and pharmacological properties (Erazo-Lara et al., 2024; Morillo et al., 2023). Historically, this fruit has been used not only as a food source but also in traditional medicine, particularly by Central American communities, owing to its recognized therapeutic potential.

The scientific relevance of yellow dragon fruit is primarily associated with its high content of natural pigments, particularly betalains, which are responsible for its characteristic coloration and exhibit potent antioxidant and antimicrobial activities (Yuan et al., 2023). In addition, the peel contains high concentrations of polyphenols and flavonoids, bioactive compounds that have been associated with the modulation of inflammatory processes, the reduction of oxidative stress, and inhibitory activity against pathogenic microorganisms (Silva et al., 2021; Li et al., 2020).

The increasing prevalence of antimicrobial resistance (AMR) has become a major global public health concern, largely driven by the excessive and inappropriate use of antimicrobial agents, which promotes the selection of multidrug-resistant microorganisms. Among the pathogens of greatest clinical importance, Staphylococcus aureus deserves particular attention because of its involvement in severe infections, including pneumonia, infective endocarditis, and septicemia. The emergence of resistant strains, especially methicillin-resistant *Staphylococcus aureus* (MRSA), has further complicated this scenario and highlights the urgent need for the development of novel and effective therapeutic alternatives (CDC, 2022; WHO, 2022).

Within this context, the search for natural compounds with antimicrobial properties has stimulated growing interest in fruits with therapeutic potential, including yellow dragon fruit. Different anatomical parts of the fruit have demonstrated beneficial biological activities, such as the regulation of lipid metabolism, reduction of hyperglycemia, and anti-inflammatory effects. The peel has attracted considerable attention because of its high concentration of phenolic compounds and its reported ability to inhibit the growth of pathogenic microorganisms (Nguyen & Vo, 2022; Yuan et al., 2023).

Despite these promising findings, the antimicrobial properties of *Hylocereus megalanthus* remain insufficiently characterized, particularly under Brazilian experimental conditions. Consequently, further investigations are needed to identify and characterize the bioactive compounds responsible for its biological activity (Figueroa-Hernández et al., 2023). Moreover, elucidating the mechanisms by which these compounds exert antimicrobial effects may contribute significantly to the development of more effective therapeutic strategies against resistant bacteria, promoting safe, natural, and sustainable alternatives to address the global antimicrobial resistance crisis (CDC, 2022; WHO, 2022).

Therefore, the present study aimed to evaluate the in vitro antimicrobial potential of yellow dragon fruit (*Hylocereus megalanthus*) peel extract against clinical isolates of *Staphylococcus aureus*. It is anticipated that the findings will provide scientific evidence supporting future investigations and contribute to the development of natural antimicrobial agents for combating bacterial resistance.

Materials and Methods

Bacterial Isolates

This study was approved by the Research Ethics Committee (REC) under approval number 7,300,864/2025. A total of 10 clinical isolates of *Staphylococcus aureus* were selected from the Academic Microorganism Collection of the Microbiology Laboratory at a medical school located in southern Minas Gerais, Brazil. The isolates had been recovered from hospitalized patients with confirmed bacterial infections admitted to the institution's affiliated teaching hospital between 2006 and 2008.

Five isolates were preserved in microcentrifuge (Eppendorf) tubes (identification numbers: 21, 87, 13, 16, and 40), whereas the remaining five were stored in culture tubes (identification numbers: 6543, 6568, 444, 6330, and 250).

The original laboratory records were reviewed to identify the anatomical sites from which the isolates had been obtained. This information was available only for the isolates stored in culture tubes. These isolates originated from the tibia (osteomyelitis), bloodstream (thrombophlebitis), hallux (osteomyelitis), surgical wound following right lower-limb amputation, and abdominal secretion.

Among the isolates with available clinical information, 80% originated from male patients

and 20% from female patients. Although the isolates preserved in Eppendorf tubes were identified as Staphylococcus aureus recovered from infected patients treated at the same hospital, the original anatomical sites of isolation were unavailable.

This stage of the investigation was conducted in February 2025, and all ten selected isolates remained viable after bacterial reactivation and were subsequently included in the study.

Bacterial Reactivation

The frozen bacterial isolates were removed from storage and allowed to thaw at room temperature. Under aseptic conditions, sterile inoculating loops were flamed using a Bunsen burner and allowed to cool before transferring approximately 10 µL of each bacterial suspension onto nutrient agar plates. Four Petri dishes were divided into four quadrants, each receiving one *Staphylococcus aureus* isolate using the streak plate technique. Six isolates were cultured in duplicate to ensure adequate bacterial recovery. Following inoculation, the plates were incubated at 37°C for 24 hours. All isolates exhibited satisfactory bacterial growth and were therefore included in the subsequent antimicrobial assays. After incubation, the cultures were stored at 4°C to maintain bacterial viability while minimizing environmental contamination.

Preparation of the Bacterial Inoculum

The ten viable bacterial isolates were removed from refrigerated storage, and representative colonies were aseptically transferred, using sterile inoculating loops, into test tubes containing 5 mL of sterile 0.85% saline solution.

The bacterial suspensions were standardized according to the McFarland turbidity method. Turbidity was adjusted using a Wickerham turbidity card until the 3+ endpoint (disappearance of the reference lines), corresponding to the 0.5 McFarland standard, equivalent to approximately 1×10^8 colony-forming units (CFU)/mL (Clinical and Laboratory Standards Institute, 2020). Following inoculum standardization, the bacterial suspensions were maintained at 4°C until antimicrobial susceptibility testing.

Preparation of the *Hylocereus megalanthus* Peel Extract

Two fresh fruits of *Hylocereus megalanthus* (yellow dragon fruit) were purchased from a local market in southern Minas Gerais, Brazil. The fruits were washed under running water and dried with paper towels.

The pulp was manually separated and discarded, while the peels were dried in a conventional oven at 180°C for 2 hours. After drying, the peels were allowed to cool to room temperature and transported to the Microbiology Laboratory.

The dried peels were manually ground using a laboratory mortar and pestle until a fine powder was obtained. Subsequently, 5 g of powdered peel was accurately weighed using an ultra-microanalytical balance and suspended in 50 mL of distilled water, producing a stock solution with a final concentration of 100 mg/mL. The

suspension was filtered through filter paper to remove solid residues, yielding the final aqueous peel extract used throughout the study.

To evaluate antimicrobial activity at different concentrations, serial two-fold dilutions of the stock solution were prepared. Eight sterile test tubes were each filled with 2 mL of sterile 0.85% saline solution. Two milliliters of the stock solution were added to the first tube, and sequential two-fold dilutions were performed by transferring 2 mL from one tube to the next until the eighth tube. Finally, 2 mL were discarded from the last tube so that all tubes contained equal final volumes (2 mL).

This procedure generated the following concentrations: 1:2, 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, and 1:256. After preparation, all tubes were wrapped in aluminum foil to protect the extract from light exposure and stored under refrigeration until antimicrobial testing.

Antimicrobial Activity Assay

The antimicrobial activity of the *Hylocereus megalanthus* peel extract against *Staphylococcus aureus* was evaluated using the broth microdilution method in a sterile 96-well microplate. Each well received: 40 µL of Brain Heart Infusion (BHI) broth; 40 µL of the peel extract at one of the eight serial dilutions (1:2–1:256); 20 µL of standardized bacterial suspension (approximately 1×10^8 CFU/mL).

Rows A to H corresponded to the eight extract concentrations, whereas columns 1–10 were assigned to the ten bacterial isolates, with each isolate tested independently. Columns 11 and 12 were reserved for experimental controls. The positive growth control (column 11) consisted of 40 µL of BHI broth plus 20 µL of bacterial suspension, confirming bacterial viability under the experimental conditions. The negative control (column 12) consisted of 40 µL of BHI broth, 40 µL of trimethoprim-sulfamethoxazole (prepared by dissolving one-half tablet containing 800 mg sulfamethoxazole and 160 mg trimethoprim in 20 mL of sterile saline, resulting in a final concentration of 25 mg/mL), and 20 µL of bacterial suspension. Following inoculation, the microplates were incubated at 37°C for 24 hours. After incubation, 30 µL of 2.5% 2,3,5-triphenyl tetrazolium chloride (TTC) was added to each well as a metabolic indicator, and the plates were reincubated at 37°C for an additional 3 hours. Bacterial growth was evaluated according to the colorimetric reaction produced by TTC. Wells exhibiting red or pink coloration were considered indicative of active bacterial growth, whereas colorless or faintly stained wells indicated inhibition of bacterial growth, demonstrating the antimicrobial activity of the tested extract.

Results

The yellow dragon fruit (*Hylocereus megalanthus*) peel extract demonstrated the ability to modulate the growth of *Staphylococcus aureus*, with inhibitory activity observed in 25% of the tested wells (orange-red coloration) at the 1:2 and 1:4 dilutions (**Figure 1**). The minimum inhibitory concentration (MIC) of the test solution was therefore established at the 1:4 dilution, indicating that bacterial growth modulation occurred only at the highest extract concentrations evaluated. At lower concentrations, corresponding to more diluted preparations, 75% of the wells exhibited dark red coloration, indicating the absence of growth modulation.

The positive control showed 100% bacterial growth, confirming the validity and reliability of the experimental assay. In contrast, the negative control, containing trimethoprim-sulfamethoxazole, demonstrated that 62.5% of the clinical isolates were resistant to the reference antibiotic (dark red coloration), whereas bacterial growth was inhibited in only 37.5% of the isolates (yellow-orange coloration) (**Figure 1**).

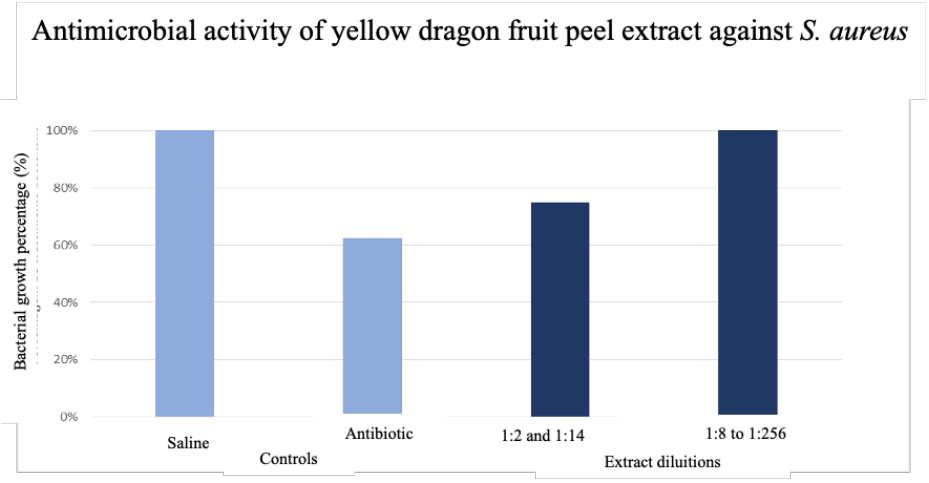

Figure 1. Antimicrobial activity of *Hylocereus megalanthus* peel extract against clinical isolates of *Staphylococcus aureus* recovered from patients with healthcare-associated infections. Source: Research data.

These findings reinforce the urgent need for alternative therapeutic approaches, as the increasing dissemination of antimicrobial-resistant bacteria continues to compromise the effectiveness of conventional antimicrobial therapy. Like the reference antibiotic, the yellow dragon fruit peel extract was able to modulate the growth of selected *S. aureus* isolates, including strains exhibiting antimicrobial resistance, thereby supporting its potential antimicrobial properties. Consequently, further investigations are warranted to better characterize the biological activity of *Hylocereus megalanthus* and to explore its potential application as a complementary strategy for combating the growing challenge of bacterial resistance.

Discussion

The evaluation of the antimicrobial activity of the aqueous peel extract of Hylocereus megalanthus against clinical isolates of *Staphylococcus aureus* demonstrated an inhibitory effect on bacterial growth, as determined by the broth microdilution assay performed according to Clinical and Laboratory Standards Institute (CLSI) guidelines (Clinical and Laboratory Standards Institute, 2020). These findings are consistent with recent studies highlighting the bioactive potential of yellow dragon fruit peel, which is rich in phenolic compounds, flavonoids, and betalains with recognized antimicrobial properties (Silva et al., 2021; Li et al., 2020). Furthermore, previous investigations have demonstrated that different anatomical parts of *Hylocereus megalanthus* exhibit significant antioxidant and antimicrobial activities, reinforcing the pharmacological value of this species (Yuan et al., 2023).

In the present study, the inhibitory activity observed against resistant *S. aureus* isolates suggests that these bioactive compounds may interfere with conventional bacterial resistance mechanisms. This finding is particularly relevant considering the prominent role of *S. aureus* in both healthcare-associated and community-acquired infections and the increasing prevalence of multidrug-resistant strains worldwide (World Health Organization, 2022; Centers for Disease Control and Prevention, 2022).

Nevertheless, several methodological limitations should be acknowledged. First, the exclusive use of an aqueous extract may have limited the extraction of lipophilic bioactive compounds naturally present in the fruit peel, potentially underestimating the overall antimicrobial activity of the plant material (Nguyen & Vo, 2022). Future investigations should therefore evaluate alternative extraction solvents and innovative approaches, including green extraction technologies and nanoparticle synthesis, to optimize compound recovery and enhance biological activity.

Another important limitation is that the present investigation was conducted exclusively in vitro. Although in vitro antimicrobial assays are essential for the preliminary screening of bioactive compounds, they do not fully reproduce the complexity of biological systems, in which factors such as bioavailability, metabolism, pharmacokinetics, and host immune responses may substantially influence therapeutic efficacy (Figueroa-Hernández et al., 2023).

In addition, although the study included ten distinct clinical isolates of *Staphylococcus aureus*, including potentially resistant strains, expanding the diversity and number of bacterial isolates would improve the representativeness of resistance profiles encountered in clinical practice and strengthen the external validity of the findings (Centers for Disease Control and Prevention, 2022).

From a translational perspective, the utilization of agricultural by-products, such as yellow dragon fruit peel, represents an attractive strategy for adding economic value to agro-industrial waste while promoting the development of natural antimicrobial agents that may exhibit lower toxicity and reduced environmental impact compared with conventional synthetic drugs (Erazo-Lara et al., 2024; Morillo et al., 2023). Such an approach aligns with current global efforts to develop sustainable and innovative strategies for addressing antimicrobial resistance, one of the greatest public health challenges of the twenty-first century (Valero et al., 2025).

Overall, the present study reinforces the potential of Hylocereus megalanthus peel as a promising source of antimicrobial bioactive compounds against *Staphylococcus aureus*. These findings support the continuation of research aimed at the detailed chemical characterization of its active constituents, evaluation in more complex biological models, and development of pharmaceutical formulations suitable for clinical application.

Conclusion

The findings of the present study demonstrate that the peel extract of *Hylocereus megalanthus* (yellow dragon fruit) exhibits growth-modulatory activity against *Staphylococcus aureus*, particularly at the higher concentrations evaluated (1:2 and 1:4 dilutions).

The presence of bioactive constituents in the fruit peel, particularly flavonoids and betalains, reinforces its potential as a complementary therapeutic agent in the context of the rapidly increasing global resistance to conventional antibiotics. Furthermore, the use of agricultural by-products, such as fruit peels, represents a sustainable and economically viable strategy for valorizing organic residues while simultaneously generating products with potential applications in public health and environmental sustainability.

Future studies should focus on the isolation and chemical characterization of the active compounds responsible for the observed antimicrobial activity, as well as on in vivo investigations to establish their efficacy, safety, and clinical applicability. Integrating expertise from microbiology, pharmacognosy, natural products chemistry, and public health will be essential for translating the promising antimicrobial potential of *Hylocereus megalanthus* into safe and effective therapeutic applications.

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CHAPTER 07

In Vitro Antimicrobial Activity of *Mauritia flexuosa* Fruit Oil Against Clinical *Staphylococcus aureus* Isolates from Hospital-Acquired Infections

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Introduction

Mauritia flexuosa L.f., commonly known as buriti, is a native palm species widely distributed throughout the wetlands of the Brazilian Midwest, North, and Northeast regions. It is also referred to by several regional names, including carandá-guaçu, palmeira-dos-brejos, and carnadaí-guaçu (Almeida et al., 1998). Its fruit, characterized by a yellow-orange pulp, is recognized for its exceptionally high carotenoid content (Rodriguez-Amaya, 2001) and for containing essential amino acids such as threonine, leucine, and tryptophan, the metabolic precursor of serotonin (Morais et al., 2022). These bioactive compounds confer remarkable antioxidant properties to buriti oil (Oliveira et al., 2020) and may contribute to the modulation of mood and anxiety-related disorders (Morais et al., 2022). Traditionally, the fruit pulp is widely consumed by local communities in the preparation of juices, jams, preserves, and fermented beverages (Morais et al., 2022).

Beyond its nutritional value, increasing evidence suggests that buriti possesses important antimicrobial properties, supporting its potential application in the prevention and treatment of infectious diseases (Mesquita et al., 2020). Buriti oil exhibits not only high nutritional quality but also promising pharmacological activity, with documented beneficial effects on human health (Cruz et al., 2020). Given the alarming global rise of antimicrobial resistance (O'Neill, 2016), the search for naturally derived compounds capable of combating multidrug-resistant microorganisms has become a major research priority. Despite these promising characteristics, however, the commercialization of buriti products remains largely restricted to their regions of origin, limiting

their broader availability throughout Brazil (Morais et al., 2022). Expanding the economic value of buriti may therefore not only strengthen local economies but also contribute to the conservation of native palm populations. Consequently, investigating plant-derived products with antimicrobial potential represents an important strategy for the development of novel therapeutic approaches against clinically relevant pathogens.

Previous studies have demonstrated that buriti oil exhibits antimicrobial activity against clinically important microorganisms, including *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus* (Mesquita et al., 2020). Moreover, when combined with conventional antibiotics such as norfloxacin, buriti oil has been shown to reduce the minimum inhibitory concentration (MIC) by up to 50% (Cruz et al., 2020). Human studies have also reported enhanced antimicrobial activity for non-encapsulated buriti oil, with inhibitory effects of 59% against *P. aeruginosa*, 62% against *K. pneumoniae*, and 43% against *S. aureus* compared with untreated controls (Mesquita et al., 2020). These biological effects have been attributed to the physicochemical characteristics of the oil droplets and the presence of bioactive phytochemicals, including quercetin, eugenol, and vanillic acid (Morais et al., 2022). In addition, Leão et al. (2019) demonstrated that buriti oil nano emulsions display pronounced antimicrobial activity against Gram-negative bacteria.

Similarly, Freire et al. (2016) reported that methanolic extracts obtained from different parts of *M. flexuosa* exhibit antimicrobial activity against pathogenic bacteria, an effect largely attributed to phenolic compounds such as chlorogenic acid. Nonato et al. (2018) further identified several flavonoids in buriti fruit pulp that not only possess antioxidant and antimicrobial properties but also enhance the activity of conventional antibiotics. More recently, Oliveira et al. (2022) isolated naturally occurring phenolic derivatives from buriti stems that demonstrated antibacterial activity against methicillin-resistant *Staphylococcus aureus* (MRSA), further supporting the therapeutic potential of this species against multidrug-resistant pathogens.

Despite these encouraging findings, studies specifically investigating the antimicrobial activity of buriti oil against *Staphylococcus aureus* strains isolated from hospital-acquired infections remain scarce (Morais, 2021). Although research on the biological properties of *M. flexuosa* has expanded considerably in recent years, relatively few studies have evaluated its activity against clinically relevant bacterial pathogens (Castro et al., 2020). Since *S. aureus* is one of the leading causes of both healthcare-associated and community-acquired infections and is increasingly associated with antimicrobial resistance (Tong et al., 2015), further investigation of natural products with potential antibacterial activity is warranted. Such studies may contribute to the development of alternative therapeutic strategies for the management of infections caused by resistant bacterial strains.

Objective

Considering the scientific relevance of this topic and its potential implications for both basic research and public health, this study aimed to evaluate the *in vitro* antimicrobial activity of buriti (*Mauritia flexuosa*) fruit oil against *Staphylococcus aureus* strains isolated from patients with hospital-acquired infections.

Materials and Methods

Study Design

This descriptive, quantitative in vitro study evaluated the antimicrobial activity of buriti (*Mauritia flexuosa*) fruit oil using the disk diffusion assay, following the methodology recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 2003). The study protocol was approved by the Research Ethics Committee of the Faculty of Medicine of Itajubá (approval no. 7,300,866/2025).

Bacterial Isolates

Initially, ten *Staphylococcus aureus* isolates obtained from hospitalized patients between 2001 and 2008 were selected from the Academic Microorganism Collection maintained by the Microbiology Laboratory of the Faculty of Medicine of Itajubá.

Revival and Culture of Bacterial Isolates

Frozen bacterial isolates were thawed at room temperature. Under aseptic conditions in a laminar flow cabinet, approximately 10 µL of each bacterial suspension was transferred using a sterile inoculating loop onto nutrient agar plates previously divided into four quadrants, allowing four isolates to be cultured per plate. The inoculum was streaked using the quadrant streak method, and the plates were incubated at 37°C for 48 h. After incubation, seven isolates exhibited satisfactory bacterial growth characterized by well-defined colonies and were therefore selected for subsequent analyses, following the procedures described by Sanders (2012).

Preparation of Bacterial Inocula

The viable bacterial isolates were collected from nutrient agar plates using a sterile inoculating loop and suspended in sterile 0.85% saline solution. The suspensions were thoroughly homogenized using a vortex mixer, and bacterial density was standardized by visual comparison with the Wickeman turbidity card until a turbidity of 3+ (complete disappearance of the reference lines) was achieved, corresponding to the 0.5 McFarland standard (approximately 1.5×10^8 colony-forming units [CFU]/mL), as previously described by Pelissari et al. (2010).

Preparation of Buriti Oil Dilutions

Buriti (*Mauritia flexuosa*) fruit oil was diluted in virgin coconut oil to obtain the following concentrations (v/v): 1:5, 1:10, 1:20, 1:40, 1:80, 1:160, 1:320, 1:640, and 1:1280. After preparation, all dilutions were stored in amber glass bottles at room temperature and protected from light until use.

Evaluation of In Vitro Antimicrobial Activity

The antimicrobial activity of buriti oil against *Staphylococcus aureus* was initially assessed using the broth microdilution method, following the protocol described by Telles and Mosca (2000). This assay employs 2,3,5-triphenyltetrazolium chloride (TTC) as a colorimetric indicator of bacterial growth.

However, no visible color development was observed following TTC addition (**Figure 1**), preventing reliable interpretation of bacterial growth. Consequently, the broth microdilution assay was considered unsuitable under the experimental conditions adopted in this study, and antimicrobial activity was subsequently evaluated using the agar disk diffusion method.

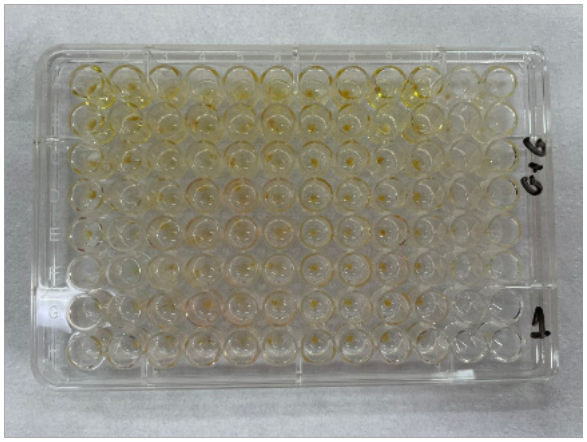

Figure 1. Microdilution test plate showing the absence of color after the use of the TTC developer. Source: research data.

For the disk diffusion assay, Mueller–Hinton agar plates were inoculated with 100 µL of each standardized bacterial suspension (0.5 McFarland standard). The inoculum was evenly distributed across the agar surface using a sterile Drigalski spatula to obtain a uniform bacterial lawn.

Sterile filter-paper disks were then impregnated with 10 µL of buriti oil at four selected dilutions (1:5, 1:40, 1:640, and 1:1280) and placed onto the inoculated agar plates.

As a positive control, sulfamethoxazole (800 mg), prepared at a final concentration of 25 mg/mL in sterile saline, was applied to filter-paper disks. Sterile 0.85% saline solution served as the negative control.

The number of disks placed on each plate followed the recommendations of the Brazilian Committee on Antimicrobial Susceptibility Testing (BrCAST) to prevent overlap of inhibition zones and minimize potential interference among test substances. Plates were incubated at 37°C, and inhibition halos were measured after 24 h and 48 h of incubation.

Data Analysis

The results were analyzed using a qualitative categorical approach, in which antimicrobial activity was classified simply as present (+) or absent (–) according to the test method, control substance, and buriti oil concentration evaluated.

Results

The antimicrobial activity of buriti oil was initially investigated using the broth microdilution assay at four different concentrations. Under the experimental conditions employed, none of the tested dilutions inhibited the growth of *Staphylococcus aureus*. Because the methodology did not allow reliable visualization of bacterial growth, definitive interpretation of antimicrobial activity using this approach was not possible.

Subsequently, bacterial growth inhibition was evaluated using the agar disk diffusion assay (antimicrobial susceptibility test). Consistent with the microdilution findings (**Table 1**), buriti oil failed to produce measurable inhibition zones at any of the concentrations tested, indicating the absence of significant antimicrobial activity against the evaluated *S. aureus* isolates.

Table 1. Evaluation of results obtained by plate microdilution (1) and nutrient agar disk diffusion (2), an antibiogram technique.

S. aureus Strains	Microdiluition	Antibiotic (Growth control)	Diluition 1:5	Diluition1:40	Diluition 1:640	Diluition 1:1280
250	-	-	-	-	-	-
6330	-	+	-	-	-	-
444	-	+	-	-	-	-
21	-	+	-	-	-	-
13	-	-	-	-	-	-
40	-	+	-	-	-	-
6959	-	-	-	-	-	-

Interestingly, 43% of the bacterial isolates exhibited resistance to the antibiotic used as the positive control, suggesting that a substantial proportion of the clinical isolates presented an expanded antimicrobial resistance profile (**Figure 2**).

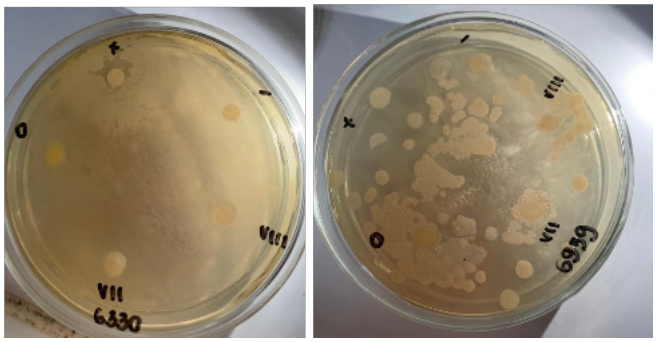

Figure 2. Petri dishes showing bacterial growth at all dilutions. On the left, an inhibition halo is observed in the positive control with antibiotic. On the right, resistance to the drug is demonstrated.

Discussion

The present study investigated the in vitro antimicrobial activity of buriti (*Mauritia flexuosa*) fruit oil against *Staphylococcus aureus* strains isolated from patients with hospital-acquired infections. Contrary to expectations based on previous reports describing the antimicrobial properties of buriti-derived products, the oil did not exhibit significant inhibitory activity against any of the clinical isolates evaluated under the experimental conditions employed.

These findings differ from previous studies demonstrating antimicrobial activity of buriti oil against clinically relevant pathogens. For example, buriti oil nano emulsions have been reported to inhibit the growth of Gram-negative bacteria, including *Escherichia coli* and *Pseudomonas aeruginosa*, as well as *Staphylococcus aureus*, particularly when administered in combination with conventional antibiotics such as norfloxacin, resulting in reductions of up to 50% in the minimum inhibitory concentration (MIC) (Morais et al., 2021). Such discrepancies may be explained by differences in oil extraction procedures, formulation strategies, physicochemical characteristics, and experimental methodologies adopted across studies.

In the present investigation, neither the broth microdilution assay nor the agar disk diffusion method demonstrated significant antibacterial activity of buriti oil against the tested *S. aureus* isolates. One plausible explanation for this outcome is the high level of antimicrobial resistance exhibited by the bacterial strains included in the study. Notably, 43% of the isolates were resistant to the sulfamethoxazole/trimethoprim positive control, suggesting that these clinical isolates displayed an extensive resistance profile. Consequently, any intrinsic antibacterial activity of buriti oil may have been insufficient to inhibit bacterial growth under these challenging conditions. These findings further emphasize the growing global concern regarding antimicrobial resistance and reinforce the need to identify alternative therapeutic strategies capable of overcoming multidrug resistance.

Several methodological factors should also be considered when interpreting these results. First, the relatively small number of bacterial isolates limits the generalizability of the

findings. Second, only commercially available buriti oil was evaluated, whereas previous studies have reported that extraction procedures markedly influence the concentration of bioactive phytochemicals. Furthermore, advanced drug-delivery systems, such as nanoencapsulation, which have previously been shown to enhance the biological activity of buriti oil, were not investigated in the present study.

In addition, factors including oil concentration, extraction method, colorimetric indicators used for bacterial growth detection, and the mode of application may all influence antimicrobial performance. Previous investigations reporting favorable results commonly employed nano emulsion-based formulations, which increase the stability, dispersion, and bioavailability of hydrophobic bioactive compounds (Leão et al., 2019). By contrast, in the present study, buriti oil was diluted in virgin coconut oil, a formulation that may have altered compound diffusion through the agar matrix and consequently reduced its apparent antimicrobial activity.

Overall, the findings indicate that, under the experimental conditions adopted, buriti oil did not exhibit significant antibacterial activity against clinical isolates of *Staphylococcus aureus*. Future investigations should evaluate alternative extraction techniques, optimized formulations such as nano emulsions or nanoencapsulation systems, broader concentration ranges, and larger collections of bacterial isolates exhibiting distinct antimicrobial susceptibility profiles. Moreover, the potential synergistic effects between buriti oil and conventional antibiotics deserve further investigation, as they may represent a promising strategy for combating multidrug-resistant bacterial infections.

Conclusion

Several technical challenges were encountered during the development of this study. The principal limitation was the high frequency of resistance (43%) among the *Staphylococcus aureus* isolates to sulfamethoxazole/trimethoprim, the antibiotic used as the positive control in the antimicrobial susceptibility assay. This resistance profile hindered direct comparisons and raises the possibility that the antimicrobial potential of buriti oil may be more readily detected against less resistant bacterial strains.

These findings highlight the need for further research investigating the antimicrobial properties of buriti oil using different bacterial species, extraction techniques, formulation strategies, and concentration ranges, as well as comparisons with additional broad-spectrum antimicrobial agents. Such studies are essential to clarify the therapeutic potential of buriti oil as a natural complementary approach for the management of antimicrobial-resistant pathogens, particularly clinically relevant species such as *Staphylococcus aureus*.

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CHAPTER 08

Antimicrobial Activity of a Commercial Probiotic Against *Escherichia coli*: An In Vitro Study

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Introduction

The emergence and rapid dissemination of antimicrobial resistance (AMR) represent one of the greatest challenges facing global public health. The indiscriminate and inappropriate use of antibiotics in both human and veterinary medicine has accelerated the selection of multidrug-resistant microorganisms, substantially reducing the effectiveness of conventional antimicrobial therapies. As a result, infections caused by resistant bacteria are associated with increased morbidity and mortality, prolonged hospital stays, and escalating healthcare costs (Han et al., 2023; Kaper et al., 2004; Moura et al., 2012; Souza et al., 2016).

Among the pathogens of greatest clinical relevance, *Escherichia coli* occupies a prominent position because of its high prevalence in both community-acquired and healthcare-associated infections. Although *E. coli* is a natural constituent of the intestinal microbiota, pathogenic strains are responsible for a broad spectrum of diseases, including urinary tract infections, bloodstream infections, neonatal meningitis, gastroenteritis, and sepsis (Hernandez-Pastor et al., 2023; Kaper et al., 2004). The increasing frequency of multidrug-resistant isolates, particularly those producing extended-spectrum β-lactamases (ESBLs) and carbapenemases, has considerably restricted available therapeutic options, emphasizing the need for novel antimicrobial strategies (da Silva & Mendonça, 2012; Kaper et al., 2004; Rasko et al., 2008). In 1945, enteropathogenic *E. coli* (EPEC) was the first pathotype to be described following major outbreaks of infantile diarrhea in the United Kingdom, and over time, other pathogenic strains were discovered, such as enterohemorrhagic *E. coli* (EHEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), and enteroinvasive *E. coli* (EIEC) (Kaper et al., 2004).

Furthermore, as a highly adaptable microorganism, this pathogen poses a clear threat to global public health and is frequently associated with outpatient infections or prolonged hospital stays (da Silva & Mendonça, 2012; Leoncio et al., 2019; Pozzato & Parisi, 2018). Hospital-acquired infections, in turn, extend patient hospital stays, increase costs for hospitals and the public health system, and raise patient morbidity and mortality—with the latter reaching 15–20% within 30 days in cases involving *E. coli*—while also contributing to the emergence of bacterial resistance (Gopaul et al., 2023; Padoveze & Fortaleza, 2014; Pozzato & Parisi, 2018). There is a growing search for substances that act either complementarily to or independently of antibiotics.

In this context, probiotics have emerged as promising biological alternatives for the prevention and control of bacterial infections. According to the World Health Organization (WHO) and the Food and Agriculture Organization (FAO), probiotics are defined as live microorganisms that, when administered in adequate amounts, confer a health benefit on the host. Their beneficial effects extend beyond modulation of the intestinal microbiota and include enhancement of epithelial barrier function, regulation of immune responses, and inhibition of pathogenic microorganisms through multiple complementary mechanisms (Kim et al., 2019).

The antimicrobial activity of probiotics is primarily attributed to the production of organic acids, hydrogen peroxide, bacteriocins, and other bioactive metabolites capable of inhibiting pathogen growth. In addition, probiotic microorganisms compete with pathogens for adhesion sites and available nutrients, thereby reducing intestinal colonization and limiting the establishment of infectious processes (Saad, 2006; Vidhate et al., 2024). These mechanisms have stimulated growing scientific interest in the therapeutic use of probiotics as complementary or adjunctive agents to conventional antimicrobial therapy.

It is worth noting that the strongest evidence for the efficacy of probiotics lies in the treatment of acute diarrhea—particularly that caused by rotavirus—but there is also evidence of their effectiveness against antibiotic-associated diarrhea, hepatic encephalopathy, ulcerative colitis, and irritable bowel syndrome, for example (Williams, 2010; Wilkins & Sequoia, 2017).

Commercial probiotic formulations generally contain strains of *Lactobacillus*, *Bifidobacterium*, and other beneficial bacteria whose biological properties have been extensively investigated *(*Williams, 2010). They are used to improve the homeostasis of the internal microbiota and, consequently, to maintain human intestinal health (Bhutada et al., 2025; Kim et al., 2019). These organisms balance the gut microbiota by creating an acidic environment that reduces the survival of harmful bacteria and promotes the growth of beneficial bacteria (Kim et al., 2019).

The gut microbiota, acquired shortly after birth, consists of a diverse array of bacteria that perform various functions for the host, such as nutrient absorption, protection against pathogens, and immune system modulation

(Moraes et al., 2014). Consequently, the host relies on the gut microbiota for a range of vital functions, and it thus contributes positively to health (Carding et al., 2015). Given that probiotics support a healthy gastrointestinal system—which is essential for the proper functioning of the human body—they play a significant role in promoting individual health (Carding et al., 2015; Williams, 2010).

Several studies have demonstrated their ability to inhibit clinically relevant pathogens, including *Escherichia coli*, *Staphylococcus aureus*, *Salmonella spp*., and *Clostridioides difficile*. Nevertheless, the antimicrobial activity of commercial probiotic products may vary considerably depending on their microbial composition, bacterial viability, and concentration of active microorganisms, highlighting the importance of experimentally evaluating individual formulations.

Despite increasing evidence supporting the beneficial effects of probiotics, relatively few studies have investigated the antimicrobial activity of commercially available probiotic formulations against clinical isolates of *Escherichia coli*. Expanding knowledge in this field may contribute to the development of complementary therapeutic approaches capable of mitigating the impact of antimicrobial resistance while reducing reliance on conventional antibiotics.

Objective

The present study aimed to evaluate the in vitro antimicrobial activity of a commercial probiotic against clinical isolates of *Escherichia coli* obtained from patients with healthcare-associated infections, thereby contributing to the understanding of the probiotic's potential as an adjunctive strategy for controlling antimicrobial-resistant bacteria.

Materials and Methods

Selection of Bacterial Isolates

The *Escherichia coli* (*E. coli*) isolates used in this study were obtained from the Academic Microorganism Collection (MPA) maintained by the Microbiology Laboratory of the Faculdade de Medicina de Itajubá (FMIT), Brazil. These clinical isolates had been recovered from hospitalized patients between 2006 and 2008 from a variety of clinical specimens, including urine, cerebrospinal fluid (CSF), peritoneal fluid, purulent skin secretions, catheter tips, fecal samples, and contaminated biological materials.

All experimental procedures were conducted between February and April 2025 at the FMIT Microbiology Laboratory. The study protocol was approved by the Research Ethics Committee (REC) of the Faculdade de Medicina de Itajubá (Approval No. 7,300,899/2025).

Thirteen *E. coli* isolates were selected from the collection. Following bacterial reactivation, all isolates remained viable and were therefore included in the study.

Bacterial Reactivation

Frozen bacterial stocks were removed from storage and allowed to thaw at room temperature. Under aseptic conditions, sterile inoculating loops, previously sterilized by flaming over a Bunsen burner and allowed to cool, were used to transfer approximately 10 µL of each bacterial suspension onto sterile nutrient agar plates.

Three Petri dishes were previously divided into four quadrants, allowing the inoculation of four bacterial isolates per plate, totaling 12 isolates. A fourth Petri dish was used to culture the remaining isolate individually.

Following inoculation, all plates were incubated at 37°C for 48 hours. After incubation, every isolate exhibited abundant colony growth, demonstrating satisfactory viability for inclusion in the antimicrobial assays. Consequently, all thirteen previously selected isolates met the inclusion criteria for the study.

Preparation of the Bacterial Inoculum

Representative colonies from each of the thirteen isolates were aseptically collected from the nutrient agar plates using sterile inoculating loops and transferred into microcentrifuge tubes containing 5 mL of sterile 0.85% saline solution.

The bacterial suspensions were standardized by turbidity using a Wickerham turbidity card until reaching the 3+ endpoint, corresponding to the disappearance of the reference lines. This turbidity is equivalent to the 0.5 McFarland standard, representing approximately 1×10^8 colony-forming units (CFU)/mL.

Following standardization, the bacterial suspensions were stored at 4°C until antimicrobial susceptibility testing.

Probiotic Preparation and Serial Dilution

The commercial probiotic Repoflor® (*Saccharomyces boulardii*) was purchased from a local pharmacy within its expiration date. A single 250-mg capsule was aseptically opened, and its

contents were homogenized using a sterile spoon before being transferred to a microcentrifuge tube containing 5 mL of sterile saline solution, yielding a stock suspension of 50 mg/mL. Serial two-fold dilutions were subsequently prepared by transferring 1 mL of each suspension into a new microcentrifuge tube containing 5 mL of sterile saline, repeating the procedure sequentially until the eighth dilution.

This protocol generated the following probiotic concentrations: 1:2 (25.0 mg/mL), 1:4 (12.5 mg/mL), 1:8 (6.25 mg/mL), 1:16 (3.125 mg/mL), 1:32 (1.562 mg/mL), 1:64 (0.781 mg/mL), 1:128 (0.390 mg/mL), 1:256 (0.195 mg/mL).

Evaluation of Antimicrobial Activity

Antimicrobial activity was assessed using the broth microdilution method, with minor modifications to previously standardized protocols.

Sterile 96-well microplates were used to evaluate all thirteen *E. coli* isolates (Figure 1). Each microplate consisted of eight horizontal rows (A–H) and twelve vertical columns. Initially, 40 µL of Mueller–Hinton broth was dispensed into every well of Microplate 1. Subsequently, 40 µL of each probiotic dilution (1:2 to 1:256) was added to the wells so that each horizontal row corresponded to one probiotic concentration, with row A receiving the 1:2 dilution, row B the 1:4 dilution, and so forth through row H (1:256). Next, 20 µL of each standardized E. coli suspension was inoculated into columns 1–12, with each column containing a different bacterial isolate. To complete the evaluation of the thirteenth isolate, a second microplate was prepared following the same experimental design. Only column 1 was used, receiving 40 µL of Mueller–Hinton broth, 40 µL of each probiotic dilution, and 20 µL of the remaining bacterial suspension.

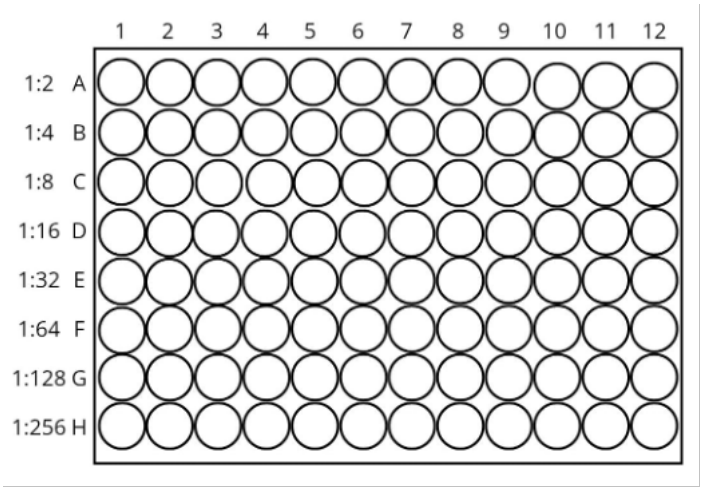

Figure 1. Schematic representation of the broth microdilution assay. Each bacterial isolate occupied one vertical column, whereas probiotic concentrations were distributed across the horizontal rows. Twelve isolates were analyzed in Microplate 1, and the remaining isolate was evaluated in Microplate 2. Source: Authors (2025).

Thus, each vertical column contained a single bacterial isolate exposed to all probiotic concentrations. For example, wells A1 through H1 contained the same bacterial isolate at eight different probiotic concentrations, whereas wells A2 through H2 contained a different isolate under identical concentration gradients. Growth controls were included in Microplate 2. Columns B and C contained bacterial suspensions (20 µL) together with 40 µL of Mueller–Hinton broth, serving as positive growth controls. Columns D and E contained bacterial suspensions together with 40 µL of Mueller–Hinton broth and 10 µL of ciprofloxacin, to which the isolates were previously known to be susceptible (Artero et al., 2023), serving as the negative (inhibition) control. Following inoculation, the microplates were incubated at 37°C for 24 hours. After incubation, 20 µL of 2.5% 2,3,5-triphenyl tetrazolium chloride (TTC) solution was added to every well as a metabolic indicator. The plates were then incubated at 35°C for an additional 3 hours (**Figure 2**).

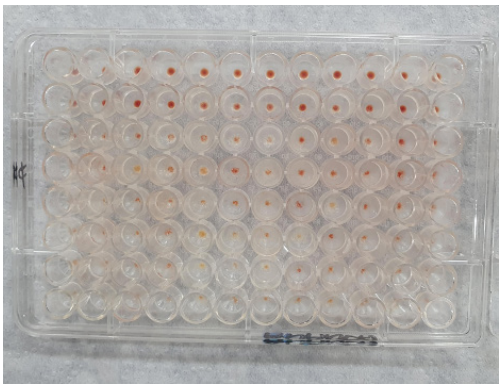

Figure 2. Plates showing colorimetric aspects of the microdilution test using the TTC indicator. Wells showing a modulatory effect on *E. coli* exhibit no coloration, while wells with no modulatory effect show reddish coloration. Plate 1 on the right and Plate 2 on the left. Source: Authors (2025).

Bacterial growth was assessed according to the colorimetric reaction produced by TTC. Wells exhibiting red or pink coloration were considered indicative of active bacterial growth, whereas colorless wells indicated inhibition of bacterial growth and were interpreted as evidence of antimicrobial activity by the probiotic preparation.

Biosafety Procedures

To minimize occupational risks, all researchers received laboratory training before initiating the study under the supervision of the project advisor. The training emphasized safe handling of bacterial cultures and compliance with microbiological laboratory procedures.

Throughout the study, all experimental procedures were conducted in accordance with established biosafety guidelines. Laboratory personnel wore appropriate personal protective equipment (PPE), including laboratory coats, gloves, face masks, and protective eyewear. Additional safety measures included the use of biological safety cabinets, appropriate antiseptic procedures, controlled laboratory conditions, and the proper disposal of biological and chemical waste.

Confidentiality and Data Protection

The identity of all bacterial isolates remained strictly confidential throughout the study. No patient-identifying information was accessible during data collection or analysis. All research records were securely stored and made accessible only to the principal investigator for a minimum period of five years, or until publication of the study results, in accordance with the Brazilian General Data Protection Law (Lei Geral de Proteção de Dados—LGPD, Law No. 13,709/2018).

Results and Discussion

The probiotic *Saccharomyces boulardii* exhibited a variable growth-modulatory effect against clinical isolates of *Escherichia coli* recovered from patients with healthcare-associated infections, as illustrated in **Figure 3**. Although all *E. coli* isolates demonstrated some degree of susceptibility to the probiotic, the highest dilution tested (1:256) produced the greatest inhibitory effect, resulting in reduced bacterial growth in nearly all isolates. Conversely, the highest probiotic concentration (1:2) exerted the least inhibitory activity, suggesting that bacterial growth modulation increased as the probiotic concentration decreased.

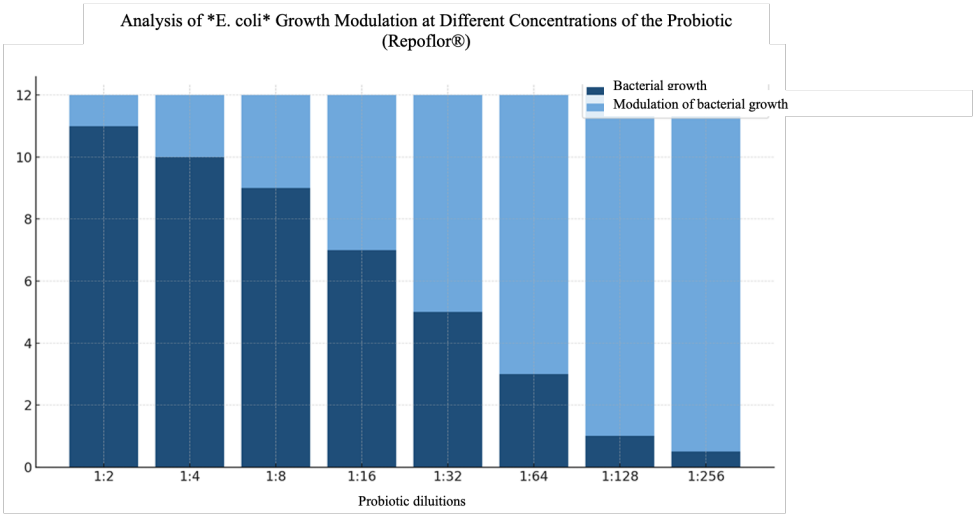

Figure 3. Growth-modulatory activity of the probiotic Repoflor® (*Saccharomyces boulardii*) against clinical *Escherichia coli* isolates. Dark blue bars represent bacterial growth, whereas light blue bars indicate growth modulation across the different probiotic dilutions. Greater bacterial growth inhibition was observed at the higher dilutions of the probiotic. Source: Authors (2025).

The probiotic effect did not follow a strictly linear dose–response relationship across the tested concentrations. Instead, fluctuations in growth modulation were observed among the different dilutions. This variability may reflect the complex interactions between *S. boulardii* and the bacterial isolates, as well as the influence of factors such as bacterial genetic diversity, culture conditions, and possible alterations in the stability or activity of probiotic-derived metabolites

at different concentrations. These findings highlight the need for further studies to optimize probiotic dosage and administration protocols to achieve more consistent antimicrobial activity.

Several mechanisms have been proposed to explain the antimicrobial activity of *Saccharomyces boulardii*. In addition to secreting antimicrobial compounds, this probiotic modifies the intestinal microenvironment, thereby impairing pathogen adhesion, colonization, and proliferation (Bhutada et al., 2025). The findings of the present study are consistent with these protective mechanisms, demonstrating that *S. boulardii* could modulate the growth of clinical *E. coli* isolates, particularly at higher dilutions. This observation may have important clinical implications, especially considering the increasing prevalence of antimicrobial-resistant *E. coli* strains associated with healthcare-associated infections.

Antimicrobial resistance has emerged as one of the most significant global public health challenges, compromising the effectiveness of conventional antibiotic therapy and emphasizing the need for safe and effective alternative strategies (da Silva & Mendonça, 2012). Within this context, probiotics such as *Saccharomyces boulardii* represent a promising complementary therapeutic approach. Beyond their potential antimicrobial activity, probiotics contribute to the restoration of intestinal microbial homeostasis, reduce the adverse effects commonly associated with antibiotic therapy, and may help decrease the selective pressure that drives the emergence of multidrug-resistant bacterial strains (Bhutada et al., 2025; Kim et al., 2019; Saad, 2006).

Nevertheless, several limitations should be acknowledged. The relatively small number of bacterial isolates analyzed, together with the variability in antimicrobial responses among the isolates, warrants cautious interpretation of the findings. Future investigations should include larger collections of clinical isolates, evaluate additional probiotic concentrations, and further elucidate the molecular mechanisms underlying the observed antimicrobial activity.

Recent advances in metabolic engineering and microbial biotechnology may enable the development of genetically optimized *Saccharomyces boulardii* strains with enhanced production of antimicrobial metabolites, thereby improving their therapeutic efficacy and expanding their clinical applicability (Gopaul et al., 2023).

Overall, the present findings indicate that *Saccharomyces boulardii* possesses considerable potential as an adjunctive strategy for the management of Escherichia coli infections in healthcare settings. Although additional experimental and clinical studies are required, this probiotic may contribute to future antimicrobial stewardship strategies aimed at reducing dependence on conventional antibiotics while supporting the control of bacterial infections.

Final remarks

The study demonstrated that the commercial probiotic Repoflor® (*Saccharomyces boulardii*) exhibited a probable modulatory effect on the growth of *Escherichia coli* strains isolated from hospital-acquired infections, particularly at higher dilutions. These findings may reinforce the potential of probiotics as complementary therapeutic tools for controlling this bacterium, thereby contributing to a reduction in the exclusive reliance on antibiotics.

Ultimately, it is speculated that the use of the probiotic Repoflor® (*Saccharomyces boulardii*) could be a promising strategy for modulating bacterial growth, though further research is required to validate its therapeutic application.

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Part III

Innovation in Medical Education

CHAPTER 09

Individualized Education Plan (IEP): An Inclusion Policy for Medical Students

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Introduction

Inclusive education is a process aimed at ensuring that all students, regardless of their individual differences or disabilities, have access to high-quality education within mainstream educational settings. It is grounded in the principle that diversity is an enriching value and that educational institutions should adapt to meet the specific needs of each learner. Beyond promoting equal educational opportunities, inclusive education fosters a sense of belonging, encourages social participation, and contributes to the development of a more equitable and inclusive society (UNESCO, 2020).

In Brazilian higher education, the inclusion of students with disabilities is supported by a comprehensive legal framework designed to guarantee equitable access, academic retention, and successful educational outcomes. The Brazilian Law for the Inclusion of Persons with Disabilities (Law No. 13,146/2015) establishes that higher education institutions must provide physical, communicational, and pedagogical accessibility, including curricular adaptations and accessible educational resources. In addition, Decree No. 9,034/2017 regulates admission policies for students with disabilities in public universities and federal educational institutions, while the National Policy on Special Education from the Perspective of Inclusive Education (2008) promotes inclusive pedagogical practices that ensure quality education for all learners. Complementary legislation, including the Quota Law (Law No. 12,711/2012) and National Education Council Resolution No. 3/2016, further reinforces institutional responsibilities regarding accessibility, assistive technologies, and educational accommodations. More recently, Provisional Measure No. 1,025/2020 established the Individualized Education Plan (IEP) as an educational instrument within Brazil's inclusive education system, ensuring that teaching strategies are tailored to each student's specific needs while promoting meaningful participation in academic life. Together, these policies provide the legal and institutional foundation for a more accessible and inclusive university environment.

Recent data from the 2024 Brazilian Higher Education Census demonstrate substantial progress in the participation of students with disabilities in higher education. A total of 79,262 students with disabilities, developmental disorders, or high abilities/giftedness were enrolled in higher education institutions, representing an increase of approximately 171% compared with 2017. Physical disabilities (34.8%), low vision (26.1%), and hearing impairment (10.3%) accounted for more than 70% of reported cases, followed by intellectual disability (6.8%) and autism spectrum disorder (ASD) (5.3%). This remarkable increase reflects the positive impact of inclusive educational policies implemented during basic education, which have expanded opportunities for students with disabilities to progress into higher education. At the same time, these data underscore the growing responsibility of higher education institutions to strengthen accessibility services and pedagogical support, ensuring not only admission but also academic success and retention.

Educational inclusion in higher education presents specific challenges, requiring higher education institutions to develop support and monitoring policies that go beyond merely offering places. Initiatives such as the creation of Inclusion and Accessibility Centers have proven to be effective strategies in this regard. As reported by Tomelin et al. (2018), these centers carry out welcoming and support activities—including tutoring, remedial courses, faculty guidance, physical adaptations, and the use of assistive technologies—thereby fostering more effective inclusion.

Within this framework, the Individualized Education Plan (IEP) has emerged as a strategic pedagogical tool. The IEP is a collaborative document developed by a multidisciplinary team—including faculty members, specialists, educational psychologists, and the student—that establishes individualized educational goals, teaching strategies, and accommodations based on each learner's specific needs (BARBOSA; CARVALHO, 2019). Its primary purpose is to ensure equitable access to high-quality education through personalized curricular and pedagogical adaptations, thereby promoting full participation in academic life.

However, studies such as those by Anache and Cavalcante (2018) reveal the numerous obstacles faced by students with disabilities during their university journey, including architectural barriers, a lack of appropriate curricular adaptations, gaps in teacher training, and attitudinal prejudices. Such barriers compromise students' academic performance and motivation, contributing to high dropout rates. Given this scenario, the implementation of institutional accessibility policies is essential. The Individualized Education Plan (PEI) stands out as a valuable tool, enabling the personalization of the educational process according to each student's needs and fostering more inclusive pedagogical practices (SILVEIRA et al., 2023).

This chapter aims to present the experience of a higher education institution in implementing an institutional inclusion policy centered on the Individualized Education Plan (IEP). Furthermore, it discusses the role of the IEP in promoting educational equity and ensuring

high-quality learning opportunities for all students, thereby contributing to the development of a truly inclusive academic environment.

Development

The implementation of the Individualized Education Plan (IEP) at a higher education institution located in a medium-sized city in the state of Minas Gerais, Brazil, is part of a broader institutional policy aimed at promoting inclusion and accessibility in higher education. This policy recognizes that ensuring both access to and retention of students with disabilities, developmental disorders, or high abilities/giftedness requires coordinated actions among multiple institutional sectors, as well as the development of pedagogical practices that are responsive to the unique needs and circumstances of each student.

The institutional workflow for implementing the IEP begins with the identification of the student's specific educational needs. These needs may be identified through different pathways, including the admissions process (via self-disclosure and supporting documentation), enrollment procedures, or at any point during the student's academic journey, either by the student's own request or through referrals made by faculty members or program coordinators. Regardless of the referral pathway, all cases are directed to the Student Experience Center (Núcleo de Experiência Discente – NED), a department staffed by psychologists responsible for coordinating student support services, psychosocial follow-up, and institutional actions aimed at promoting academic success and retention.

Once a case has been identified, the NED conducts an individualized assessment to better understand the student's educational needs, barriers encountered in daily academic activities, and existing support resources. Whenever possible, this assessment incorporates active listening to the student's perspective and, when appropriate, input from family members or external healthcare professionals already involved in the student's care. Based on this assessment, the NED prepares a comprehensive report that serves as the foundation for subsequent decision-making.

The case is subsequently submitted to the Inclusion and Accessibility Committee (Comissão de Inclusão e Acessibilidade – CIA), a multidisciplinary committee composed of representatives from the NED, faculty members, administrative staff, and students. The CIA is responsible for evaluating each case comprehensively, determining the accessibility resources and accommodations to be provided, discussing improvements to the institution's inclusion policy, and promoting awareness initiatives within the academic community. This collaborative governance structure is essential for ensuring that decisions are made collectively, thereby avoiding the concentration of responsibility within a single department and fostering an institution-wide commitment to inclusive education.

The Individualized Education Plan is developed through a collaborative and interdisciplinary process. Following the committee's recommendations, the NED coordinates the preparation of the document in partnership with course coordinators, faculty members responsible for the student's courses, and other relevant institutional sectors. The IEP includes: (a) general information about the student and academic background; (b) identification of educational barriers and support needs; (c) educational objectives and developmental goals; (d) individualized teaching strategies and curricular accommodations; (e) accessibility resources and support services; and (f) clearly defined responsibilities for each institutional sector involved in implementing the proposed measures. Students are actively encouraged to participate in this process by reviewing the document, validating its content, and contributing their own perspectives, thereby reinforcing principles of shared decision-making and student empowerment.

Following formal approval by the CIA, the IEP is officially communicated to all faculty members responsible for the student's courses, program coordinators, and relevant administrative departments. The NED subsequently acts as the central coordinating body, monitoring implementation of the planned accommodations while serving as a communication bridge between students, faculty, and institutional leadership.

Implementation of the IEP is continuously monitored throughout each academic semester through regular student appointments, meetings with faculty members, and periodic revisions of the document whenever necessary. This ongoing monitoring and adjustment process enables educational strategies to be modified according to students' academic progress, changes in health status, or newly identified educational needs. The CIA also meets regularly to review ongoing cases, evaluate implemented actions, and propose improvements to institutional inclusion practices.

Regarding accessibility resources, the institution places particular emphasis on accommodations related to assessment conditions. The most frequently implemented measures include extended time for examinations and other assessments, as well as access to separate testing rooms, which provide quieter and more controlled environments that minimize distractions and facilitate concentration. These accommodations are commonly offered to students with learning disorders, attention-deficit/hyperactivity disorder (ADHD), and autism spectrum disorder (ASD). In addition, the institution provides reader assistance for students who require support in reading examination materials, thereby ensuring equitable access to assessment content.

Beyond complying with national legislation, the institution has established its own internal regulations governing the request, approval, and implementation of accessibility accommodations. These institutional guidelines specify the types of available accommodations, eligibility criteria, required documentation, and the responsibilities of

each administrative sector, ensuring consistency, transparency, and standardization across institutional procedures. This internal regulatory framework has become an important mechanism for consolidating and sustaining the institution's inclusion policy.

Despite these advances, implementation of the IEP continues to face significant challenges. One of the most important is the ongoing need to raise awareness among faculty members, students, and administrative staff regarding the principles and legal foundations of inclusive education. Misconceptions remain common, particularly concerning the meaning of pedagogical accommodations, which are frequently misinterpreted as reducing academic standards or lowering educational expectations. Such misunderstandings may generate resistance and compromise the effectiveness of inclusion initiatives. It is therefore essential to reinforce that curricular accommodations are intended to promote educational equity by recognizing diverse learning needs without diminishing academic rigor or altering curricular objectives.

Another key aspect of this institutional model is its commitment to continuous organizational learning. Each student case is addressed individually through ongoing dialogue with the student, allowing the NED to develop personalized solutions based on active listening and collaborative problem-solving. Consequently, every new IEP not only supports an individual student but also contributes to institutional learning by encouraging the university to refine its educational practices, improve internal procedures, and strengthen interdisciplinary collaboration. Inclusive education thus becomes a dynamic and continuously evolving process rather than a static set of administrative regulations.

The close collaboration between the Student Experience Center (NED) and the Inclusion and Accessibility Committee (CIA) has proven fundamental to the effectiveness of this institutional policy. While the NED maintains direct engagement with students and coordinates day-to-day support activities, the CIA provides institutional oversight through an interdisciplinary approach that promotes shared responsibility across different sectors. This organizational structure ensures that the development and implementation of IEPs are collective institutional processes rather than isolated responsibilities assigned to individual professionals.

Finally, the institutional model emphasizes continuous dialogue with faculty members. The institution recognizes that meaningful inclusion extends beyond providing assistive technologies or material accommodations and fundamentally requires the transformation of teaching practices. Accordingly, the NED regularly meets with faculty members to present each student's IEP, clarify questions, and collaboratively develop teaching strategies that address students' specific needs while preserving curricular learning objectives. These interactions have contributed to increasing awareness within the academic community and strengthening a sustainable culture of inclusion across the institution.

Final remarks

The implementation of the Individualized Education Plan (IEP) represents an important institutional strategy for strengthening inclusive education in higher education, particularly within medical programs where academic demands are substantial, and students present diverse educational needs. By adopting individualized pedagogical planning, higher education institutions can promote equitable learning opportunities while respecting the unique characteristics, abilities, and challenges of each student.

The institutional experience presented in this chapter demonstrates that the successful implementation of the IEP depends on coordinated, multidisciplinary collaboration involving students, faculty members, academic leadership, and specialized support services. The partnership between the Student Experience Center (NED) and the Inclusion and Accessibility Committee (CIA) has proven essential for identifying students' needs, planning individualized accommodations, monitoring academic progress, and continuously refining institutional inclusion practices.

The findings also highlight that effective inclusion extends beyond compliance with legal and regulatory requirements. Building an inclusive educational environment requires continuous faculty development, institutional commitment, interdisciplinary collaboration, and the adoption of pedagogical practices that recognize diversity as a fundamental component of educational excellence. In this context, the IEP should be understood not merely as an administrative document but as a dynamic educational tool that supports individualized learning and promotes meaningful participation in academic life.

Although important advances have been achieved, ongoing challenges remain, particularly regarding the dissemination of inclusive educational principles, the reduction of attitudinal barriers, and the consolidation of an institutional culture that embraces diversity as an essential value. Future initiatives should prioritize the continuous evaluation of IEP outcomes, faculty training in inclusive pedagogical practices, and the development of evidence-based institutional policies capable of improving both academic performance and student well-being.

Ultimately, the experience described in this chapter suggests that the Individualized Education Plan constitutes a valuable instrument for promoting accessibility, educational equity, and student success in higher education. Its implementation has the potential to transform institutional culture by fostering more inclusive learning environments in which all students are provided with equitable opportunities to achieve their full academic potential.

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CHAPTER 10

Three-Dimensional Prototypes for Teaching Congenital Malformations (Exencephaly and Acrania) and Endocrine Disorders (Acromegaly)

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Introduction

Neural tube defects (NTDs) are among the most prevalent congenital malformations worldwide and represent a major public health concern due to their substantial clinical, social, and economic impact. Among the most severe forms are exencephaly, characterized by the partial or complete absence of the brain and cranial vault, with most of the embryonic brain tissue exposed or protruding outside the skull, and acrania, defined by the absence of the calvaria while preserving partially developed but exposed brain tissue[1-3]. Both conditions are incompatible with extrauterine life and constitute a significant challenge for healthcare systems, particularly in socially vulnerable regions.

Although acromegaly is not classified as a neural tube defect, it was included in this project because of its considerable educational value in integrating morphophysiology, endocrinology, and clinical medicine. Acromegaly is an endocrine disorder resulting from excessive secretion of growth hormone (GH), most commonly caused by a pituitary adenoma, leading to progressive skeletal overgrowth, enlargement of the extremities, and thickening of soft tissues[4]. Including this condition broadens the range of educational content addressed while fostering interdisciplinary learning and the development of integrated clinical reasoning.

Teaching these topics in undergraduate health sciences programs presents unique challenges because it requires students to understand dynamic developmental processes and complex three-dimensional anatomical structures that are often inadequately represented in conventional

textbooks or lecture-based instruction. In this context, the use of three-dimensional (3D) printed anatomical prototypes has emerged as an innovative, accessible, and cost-effective educational strategy capable of enhancing anatomical visualization, promoting active learning, and supporting student-centered teaching methodologies[5-7].

This chapter describes the development of three-dimensional anatomical models representing exencephaly, acrania, and acromegaly as educational resources designed to support the teaching–learning process in undergraduate medical education.

Objectives

To develop and characterize three-dimensional anatomical prototypes representing exencephaly, acrania, and acromegaly for use as educational tools in undergraduate medical education.

Materials and Methods

This study was designed as an applied, qualitative, and descriptive investigation aimed at developing and evaluating three-dimensional educational resources for undergraduate medical education. An extensive literature review was conducted using the PubMed, SciELO, and LILACS databases, complemented by consultations of standard textbooks in embryology, anatomy, and physiology to ensure the anatomical and pathophysiological accuracy of the prototypes[1-6].

Three-dimensional digital modeling was performed using 3D Meshy.IA®, an artificial intelligence–based software platform for anatomical model generation (**Figure 1**). The modeling workflow consisted of the following sequential steps: Definition of anatomical descriptors based on specialized scientific literature; Generation of preliminary three-dimensional digital models using the software; manual refinement of anatomical proportions, morphological details, and structural features according to reference anatomical atlases; Export of the finalized models in STL (Standard Tessellation Language) format, compatible with additive manufacturing systems.

Figure 1. Development of the prototypes' three-dimensional structure in the 3D Meshy.IA® program. **Source:** Authors' own elaboration.

The anatomical models were subsequently fabricated using fused deposition modeling (FDM) 3D printing with polylactic acid (PLA) filament. After printing, each prototype underwent manual finishing and painting with non-toxic acrylic paints to improve anatomical realism and facilitate visualization of clinically relevant structures.

This production method was selected because it combines anatomical fidelity, structural durability, low manufacturing cost, and high reproducibility, making it an accessible alternative to more expensive educational technologies such as virtual reality (VR) and augmented reality (AR). Furthermore, all stages of prototype development were photographically documented to support future academic dissemination and the preparation of a reproducible manufacturing guide.

Results and Discussion

Three distinct three-dimensional prototypes were created (**Figure 2**): the exencephaly model (Figure 2A), characterized by the absence of cerebral hemispheres and the cranial vault, resulting in an abrupt termination of the skull; the acrania prototype (**Figure 2B**), featuring the absence of the cranial vault but the presence of exposed brain tissue; and the head model exhibiting acromegaly (**Figure 2C**), with characteristic facial and skeletal alterations, including a prominent mandible, enlargement of the extremities, and soft tissue thickening.

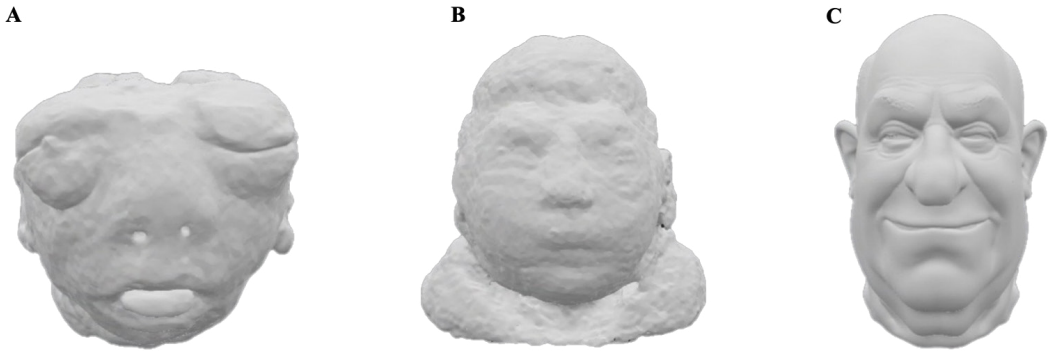

Figure 2. Developed prototypes. Source: Prepared by the author.

Table 1. summarizes the main differences between exencephaly and acrania, aiding in differential diagnosis and the understanding of the associated embryological abnormalities.

Features	Exencephaly	Acrania
Skullcap	Absent	Absent
Brain	Absent or vestigial	Present, yet exposed
Postnatal viability	Incompatible	Incompatible
Prenatal diagnosis	Morphological ultrasound (high sensitivity)	Morphological ultrasound (high sensitivity)
Main visual aspect	The brain does not develop	Brain present, without bone

The construction of these models demonstrated that 3D prototyping can faithfully and accessibly represent key morphological changes, enabling a better understanding of formation processes and neural tube closure defects[8]. From a pedagogical standpoint, the prototypes proved more effective than two-dimensional resources (atlases and slides), as they facilitate spatial perception[9], student manipulation[10], and active learning[11].

Furthermore, when compared to more advanced technologies such as virtuais and augmented reality, 3D physical models offer lower costs and greater accessibility—characteristics that make them viable in various educational settings.

Another important aspect is the potential to use these prototypes within active learning methodologies, such as Problem-Based Learning (PBL) and Team-Based Learning (TBL), thereby enriching clinical-pathological discussions regarding congenital malformations and endocrine conditions.

The results indicate that creating prototypes not only contributes to anatomical and embryological understanding[12] but also fosters basic-clinical integration, preparing students to recognize and correlate structural abnormalities with clinical manifestations[13].

In this initial phase, the models were fabricated and characterized, pending pedagogical validation in the classroom. The next stage will involve implementing the resource with undergraduate medical students—using satisfaction surveys and pre- and post-learning tests—to assess the material's effectiveness as a teaching tool.

Conclusion

The present study demonstrated the feasibility of developing anatomically accurate three-dimensional educational prototypes representing exencephaly, acrania, and acromegaly through the integration of artificial intelligence–assisted digital modeling and three-dimensional printing technology.

The resulting models constitute valuable educational resources capable of enhancing the teaching and learning of complex anatomical and pathological conditions by promoting active learning, improving spatial understanding, and facilitating the integration of basic anatomical sciences with clinical practice.

Furthermore, the proposed methodology offers an affordable, reproducible, and scalable alternative for producing high-quality anatomical teaching materials, making it particularly suitable for institutions with limited financial resources.

Future studies should focus on validating the educational effectiveness of these prototypes through controlled studies involving undergraduate medical students and faculty members, as well as expanding this methodology to other anatomical systems and pathological conditions. Such investigations will contribute to establishing evidence-based recommendations for the incorporation of three-dimensional printing technologies into contemporary medical education.

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Part IV

Artificial Intelligence and the Future of Healthcare

CHAPTER 11

Artificial Intelligence as a Decision Support Tool for Medical Diagnosis in Highly Complex Clinical Cases: A Literature Review

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Abstract

Artificial intelligence (AI) has emerged as a promising decision support tool for medical diagnosis, particularly in highly complex clinical scenarios that require the rapid and accurate integration of large volumes of clinical information. This study aimed to review recent evidence on the application of AI as a diagnostic support tool in complex clinical settings, highlighting its potential benefits, current limitations, and implementation challenges. A narrative literature review was conducted based on scientific articles published between 2004 and 2025 and retrieved from the PubMed, Scopus, and Web of Science databases. Studies evaluating AI models applied to complex clinical cases were included, with emphasis on diagnostic accuracy, response time, interpretability, and clinical validation. The available evidence indicates that AI systems may achieve diagnostic performance comparable to—or, in some circumstances, superior to—that of experienced physicians, while providing shorter response times and high diagnostic sensitivity. Large language models, particularly GPT-4, demonstrated substantial clinical diagnostic accuracy and outperformed groups of medical specialists in controlled diagnostic scenarios. Despite these promising findings, several barriers continue to limit the widespread implementation of AI in clinical practice, including the lack of external validation, algorithmic bias, limited interpretability, and the occurrence of AI hallucinations. These limitations underscore the importance of cautious implementation and rigorous clinical oversight. In conclusion, although artificial intelligence has demonstrated considerable potential to enhance diagnostic decision-making in highly complex clinical settings, its safe incorporation into healthcare practice requires multicenter validation studies, robust regulatory frameworks, and continuous physician supervision to ensure safe, ethical, and reliable clinical use.

Keywords: Artificial intelligence; Clinical diagnosis; Clinical decision support systems.

Introduction

Artificial intelligence (AI) is broadly defined as a field of computer science dedicated to the development of systems capable of performing tasks that typically require human intelligence, including learning, reasoning, pattern recognition, and decision-making. Over recent decades, AI has been increasingly incorporated into a wide range of professional fields, particularly medicine, where its clinical applications have expanded considerably. One of the earliest reported medical applications of AI dates back to 1976, when computer-assisted systems were used to support the diagnosis of acute abdominal pain. Since then, advances in computational methods, data availability, and machine learning algorithms have substantially broadened the scope of AI in healthcare[1].

Medical diagnosis requires the integration of heterogeneous sources of information, including patient history, physical examination findings, laboratory results, imaging studies, and extensive clinical knowledge. The growing complexity of healthcare, coupled with the exponential increase in available medical data, has made the acquisition, interpretation, and application of clinical information increasingly challenging. In this context, AI has emerged as a promising tool to assist healthcare professionals in managing complex clinical problems, particularly in patients with multiple comorbidities or atypical clinical presentations[1].

Recent technological advances have led to the development of increasingly sophisticated AI models capable of supporting clinical reasoning across multiple medical specialties. Among these, the Generative Pre-trained Transformer 4 (GPT-4) has attracted considerable attention. In a recent diagnostic challenge involving complex clinical cases, GPT-4 correctly identified the differential diagnosis in 64% of cases and provided the correct primary diagnosis in 39%. Nevertheless, the study also highlighted important limitations, including the omission of relevant clinical information resulting from standardized case presentation protocols, which may have underestimated the model's true diagnostic capabilities[2].

Diagnostic decision-making remains one of the fundamental components of medical practice. Accurate diagnosis frequently relies on complementary examinations, including ultrasonography, radiography, computed tomography, and magnetic resonance imaging, requiring physicians to interpret increasingly complex imaging data. In this context, Convolutional Neural Networks (CNNs)—a class of deep learning algorithms specifically designed for image analysis—have demonstrated remarkable performance across several medical applications, including automated interpretation of chest radiographs during the COVID-19 pandemic.

Despite these advances, significant challenges remain regarding algorithm interpretability, data quality, and ethical and regulatory considerations[3,4]. Simultaneously, diagnostic imaging

has undergone substantial technological evolution, with remarkable improvements in image acquisition, processing, and analytical precision. The rapid increase in the volume of imaging examinations has required radiologists to continuously expand their expertise to interpret increasingly sophisticated diagnostic information.

These developments have been particularly evident in oncology, where imaging findings now provide information extending far beyond the traditional distinction between benign and malignant lesions. Modern imaging techniques contribute to tumor staging, identification of molecular alterations, prediction of therapeutic response, assessment of recurrence risk, and estimation of patient survival. As AI technologies continue to evolve, there is growing expectation that these systems will facilitate increasingly personalized approaches to patient care by incorporating individual clinical characteristics into diagnostic decision-making[5].

AI-based technologies have become valuable complementary tools for medical diagnosis, particularly considering the enormous global burden of disease. Every day, billions of clinical events related to physical and mental health conditions are evaluated worldwide, generating vast amounts of healthcare data. Automated image analysis has demonstrated diagnostic performance comparable to that of medical specialists in several fields, including cancer detection, pulmonary disease assessment, and ophthalmology, particularly in diabetic retinopathy screening. Effective implementation of these technologies, however, depends on clinicians' ability to critically interpret AI-generated outputs within the broader clinical context. Furthermore, recently developed AI models have demonstrated the ability to identify mental disorders with diagnostic accuracies approaching 89% using only 28 structured questions, without requiring direct human intervention, thereby supporting clinical decision-making[6,7].

Beyond improving diagnostic accuracy, AI has the potential to reduce diagnostic turnaround times, lower healthcare costs, and expand access to specialized medical expertise, particularly in underserved regions with limited availability of healthcare professionals. However, the successful implementation and widespread adoption of AI in healthcare require substantial organizational changes, including the establishment of clear professional responsibilities, modernization of health information systems, and development of secure infrastructures capable of storing, protecting, and analyzing large-scale clinical datasets[8].

In the field of infectious diseases, where timely diagnosis and appropriate therapeutic interventions are essential, AI has emerged as a promising strategy for improving healthcare preparedness, diagnostic performance, and clinical outcomes[9,10]. Recent literature also highlights the successful application of AI to rare disease diagnosis, interpretation of complex medical images, and prediction of clinical deterioration. At the same time, persistent limitations—including algorithmic bias, inadequate representativeness of training datasets, poor generalizability across

healthcare centers, and unresolved ethical and regulatory issues—continue to hinder widespread clinical implementation, reinforcing the importance of maintaining a collaborative human–AI partnership in medical decision-making[11-14].

Accordingly, the present literature review aims to summarize current applications of artificial intelligence as a diagnostic support tool in highly complex clinical settings, evaluate the available evidence regarding diagnostic accuracy and clinical impact, identify the principal technical, ethical, and regulatory barriers to implementation, and discuss strategies for the effective integration of AI systems into physicians' clinical decision-making processes.

Methods

Eligibility Criteria

This research was conducted in 2025 and although this study was designed as a narrative review, the study identification and selection process was structured according to selected PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses recommendations to improve transparency. Studies published between 2004 and 2025 that evaluated artificial intelligence as a diagnostic support tool for highly complex clinical cases were considered eligible.

The inclusion criteria comprised original research articles, systematic reviews, and narrative reviews investigating AI applications in complex diagnostic scenarios. Eligible studies evaluated the performance of AI systems in supporting medical diagnosis, including diagnostic accuracy, response time, interpretability, and clinical applicability.

Studies were excluded if they were duplicates, did not address the proposed topic, or consisted of opinion articles, editorials, commentaries, conference abstracts, book chapters, dissertations, or theses.

Information Sources and Study Selection

A comprehensive literature search was conducted using the PubMed, SciELO, and Virtual Health Library (VHL) databases.

The search strategy employed both individual and combined controlled vocabulary terms using the Boolean operators AND and OR, in Portuguese and English. Search terms were selected from the Health Sciences Descriptors (DeCS) and the Medical Subject Headings (MeSH) databases and included:Artificial Intelligence, Clinical Diagnosis, Clinical Medicine, Clinical Decision Support Systems, Medical Informatics, Biomedical Technology.

After the initial screening, potentially relevant studies were evaluated according to the predefined eligibility criteria. Studies that did not investigate artificial intelligence as a diagnostic support tool for highly complex clinical cases were excluded.

The final review included original studies, systematic reviews, and clinical guidelines published between 2004 and 2025 that investigated AI-based diagnostic systems in complex clinical settings, including intensive care units, rare diseases, and atypical multisystem disorders**. Figure 1** shows the flowchart of the scientific article selection process.

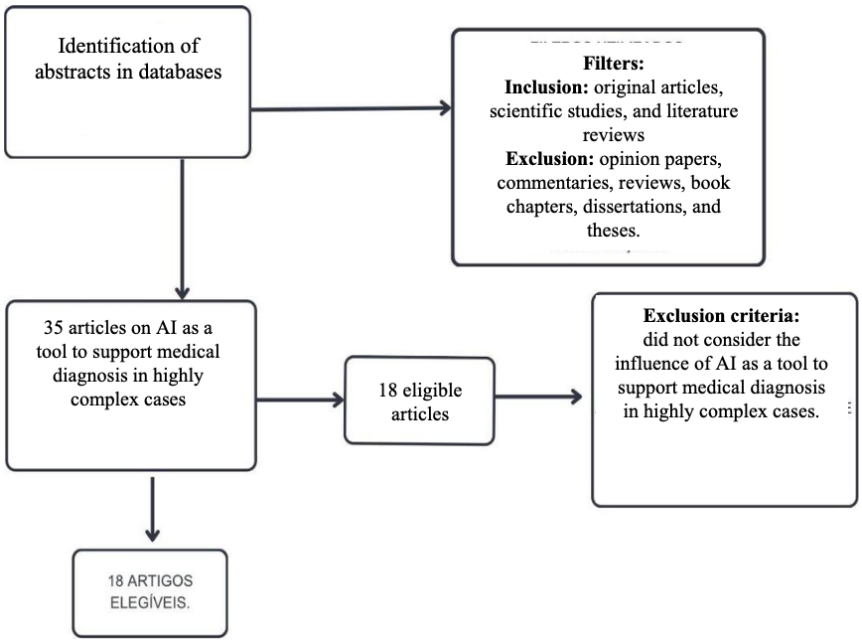

Figure 1. PRISMA flow diagram illustrating the study selection process. Source: Prepared by the authors.

Results and Discussion

The use of artificial intelligence (AI) as a diagnostic support tool has expanded rapidly in recent years, particularly due to its ability to integrate, process, and interpret large volumes of complex clinical data. The literature consistently demonstrates that AI has the potential to improve diagnostic performance and clinical decision-making; however, its implementation in routine medical practice should be approached with caution, given the methodological and operational limitations still associated with these technologies.

Advances in machine learning have enabled the incorporation of AI into multiple areas of clinical practice. Current evidence indicates that AI-assisted diagnostic systems have progressively improved diagnostic accuracy while facilitating the analysis of large and complex datasets that would otherwise be difficult to interpret using conventional approaches[5]. Furthermore, studies

have shown that ensemble learning classifiers can further enhance diagnostic accuracy and demonstrate considerable potential for the early diagnosis of infectious diseases[10,11].

In oncology, AI-driven digital image analysis has become an important tool for prognosis prediction and disease monitoring. Similarly, studies in ophthalmology have demonstrated that AI systems can be successfully deployed in real-world clinical settings while maintaining high diagnostic performance. Nevertheless, implementation in primary healthcare remains challenging because of heterogeneous clinical data, limited digital infrastructure, and variability in healthcare resources across institutions[9,12,13].

Among the recently developed AI systems, GPT-4 has demonstrated particularly promising diagnostic capabilities. In a study conducted by Kanjee et al., GPT-4 achieved superior performance compared with physicians and simulated clinical readers when evaluating highly complex diagnostic cases, correctly identifying 57% of the diagnoses compared with 36% among human participants[2].

Likewise, a subsequent investigation by Sarvari et al. reported a diagnostic sensitivity of approximately 94% when GPT-4 was applied to electronic health records from the MIMIC-IV database, outperforming PaLM2, another state-of-the-art large language model developed for medical applications[15]. These findings suggest that large language models may substantially improve diagnostic reasoning when integrated into clinical workflows.

Similar results have been reported in neurology. A machine learning model specifically trained to solve highly complex neurological cases achieved an overall diagnostic accuracy of 86.2%, significantly outperforming a panel of 13 neurologists, whose mean diagnostic accuracy was 55.1%. In addition to greater accuracy, the AI system generated diagnostic responses within approximately 20 seconds, whereas human specialists required an average of 9–10 minutes to reach their conclusions[16].

Despite these impressive results, caution is warranted when interpreting such findings. Most validation studies have been conducted using standardized or simulated clinical scenarios that do not fully reflect the complexity and heterogeneity of real-world medical practice. Consequently, current evidence cannot yet be directly extrapolated to routine clinical care.

Another noteworthy development is the Microsoft AI Diagnostic Orchestrator (MAI-DxO), which evaluated complex clinical cases published in the New England Journal of Medicine. The system achieved an overall diagnostic accuracy of 85.5%, approximately four times higher than the average performance of 21 participating physicians[14]. However, these studies were also performed under controlled experimental conditions and did not account for epidemiological diversity, incomplete medical records, or the operational challenges routinely encountered in healthcare settings.

Although the rapid evolution of AI has generated considerable enthusiasm, several important limitations continue to restrict its widespread clinical implementation. Algorithmic bias,

unrealistic expectations regarding AI capabilities, and methodological weaknesses in validation studies remain major concerns. Many published investigations are based on relatively small datasets and employ heterogeneous evaluation protocols, making comparisons across studies difficult. Furthermore, integrating AI into clinical decision support systems requires careful adaptation to existing clinical workflows to ensure usability and physician acceptance[3,4,6,8].

Another critical challenge involves the interpretability of AI-generated recommendations. The "black-box" nature of many deep learning models reduces clinicians' confidence in algorithm-assisted decision-making, particularly in high-risk clinical situations. In addition, limitations in training datasets may lead to diagnostic errors, including inaccurate identification of anatomical structures or inappropriate clinical recommendations. These findings reinforce that human supervision remains indispensable, particularly in situations requiring clinical judgment, ethical reasoning, contextual interpretation, and empathy.

Therefore, current evidence supports the view that artificial intelligence should be considered a clinical decision support tool rather than a replacement for physician expertise. The integration of AI into healthcare should aim to augment clinical reasoning, improve diagnostic efficiency, and enhance patient care while preserving the central role of physicians in medical decision-making[17,18].

Conclusion

Artificial intelligence has demonstrated considerable potential as a clinical decision support tool for the diagnosis of highly complex medical conditions, offering opportunities to improve diagnostic accuracy, reduce time to clinical decision-making, and enhance the interpretation of large and complex healthcare datasets.

However, despite the substantial advances achieved in recent years, the safe implementation of AI in clinical practice remains dependent on rigorous external validation, robust regulatory frameworks, transparent algorithm development, and continuous physician oversight. Current evidence indicates that AI should be incorporated as an adjunct to, rather than a replacement for, clinical reasoning and professional judgment.

Furthermore, several challenges—including algorithmic bias, limited interpretability, variability in training datasets, and difficulties integrating AI systems into routine clinical workflows—must be addressed before these technologies can be widely adopted in healthcare.

Future research should prioritize multicenter validation studies conducted under real-world clinical conditions, the development of explainable and trustworthy AI models, and the establishment of ethical and regulatory standards that ensure patient safety, transparency, and accountability.

Overall, artificial intelligence represents a transformative technology with the potential to significantly strengthen medical diagnosis in highly complex clinical scenarios. Nevertheless, its greatest value lies in fostering collaborative decision-making between clinicians and intelligent systems, thereby enhancing healthcare quality while preserving the indispensable role of physicians in patient care.

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A organizadora do e-book declara que ferramentas de Inteligência Artificial generativa foram utilizadas como apoio técnico ao processo de tradução de textos para o inglês. A IA foi empregada como recurso auxiliar para tradução, revisão linguística e adequação terminológica, sem substituir o contexto elaborado pelos autores.