University of Venda

THE INFLUENCE OF WATER, SANITATION AND HYGIENE AND FEEDING PRACTICES ON GROWTH STATUS AND ANTIBIOTIC RESISTANCE IN INFANTS AGED 0-6 MONTHS IN DZIMAULI RURAL VILLAGES

By

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The dissertation submitted in fulfilment of the requirements for the degree of Master of Science in Nutrition in the Department of Nutrition, Faculty of Health Sciences, at the University of Venda.

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DECLARATION

I, **Ramalepe Audrey Kgomotso**, hereby declare that the dissertation titled **"The influence of water, sanitation and hygiene and feeding practices on growth status and antibiotic resistance in infants aged 0-6 months in Dzimauli rural villages"** for the Master of Science in Nutrition degree at the University of Venda hereby submitted by me has not been submitted previously for a degree at this or any other university, that it is my own work in design and execution, and that all reference material contained therein has been duly acknowledged.

______________ 23 February 2026

Signature **Date**

DEDICATION

- To my dear parents, this research study is for you. I hope you feel proud of what your daughter has achieved. Your love and guidance have shaped me, and I carry your hopes with me in everything I do.
- To my husband, thank you for always being there for me. Your constant support and encouragement have meant the world to me. This work reflects your belief in me, and I could not have done it without you.
- To my children, I want you to know that this journey has been one of hard work and perseverance. One day, when you look back, I hope you will see the dedication it took and feel inspired to pursue your own dreams with the same determination.
- To all my siblings, I share with you the words of a Xitsonga proverb: *“Dyondzo a yi lumi”*, meaning “education does not bite”. I hope this inspires you to continue learning, growing, and reaching for even greater success.

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ABSTRACT

Malnutrition and antibiotic resistance remain critical and interconnected public health challenges in low- and middle-income countries, particularly during early infancy. This study explored the influence of water, sanitation and hygiene practices and feeding practices on growth status and antibiotic resistance in infants aged 0-6 months in the Dzimauli rural villages, Limpopo Province, South Africa. A prospective cohort design was used, involving 156 mother-infant pairs who were followed monthly from birth to six months. Data were collected using structured questionnaires assessing socio-demographic characteristics, water, sanitation, and hygiene practices, and feeding practices, alongside monthly anthropometric measurements and stool sample analyses to determine antibiotic resistance patterns in *Escherichia coli*. Generalised estimating equations were used to examine associations between water, sanitation and hygiene practices and growth outcomes, while multivariable logistic regression identified factors associated with multidrug resistance. Ethical approval was obtained from relevant university committees and provincial authorities, and written informed consent was secured from all participants, with confidentiality maintained through coded data and secure storage. Results showed that exclusive breastfeeding practices declined sharply from 71.0% in month one to 2.0% by month six. Although most infants had normal growth indicators, progressive declines in length-for-age z-scores and weight-for-age z-scores were observed over time. After adjustment, the water, sanitation and hygiene practices were not significantly associated with growth outcomes, despite temporal growth faltering. High resistance to tetracycline and cefepime (70.9%) was observed, and multidrug resistance was detected in 23.6% of infants. No independent predictors of multidrug resistance were identified in adjusted models, partly due to limited sample size and sparse data. The study highlights the coexistence of growth faltering and early-life antibiotic resistance in rural settings and underscores the need for integrated interventions that strengthen water, sanitation and hygiene practices infrastructure, promote optimal infant feeding practices, and support antimicrobial stewardship to improve child health outcomes.

Keywords: Antibiotic resistance, growth status, hygiene, infant feeding practice, malnutrition, sanitation, and water.

ABBREVIATIONS

AMR	Antimicrobial Resistance
aOR	Adjusted Odd Ratio
AR	Antibiotic Resistance
ARG	Antibiotic-Resistant Genes
BMI	Body Mass Index
BMIZ	BMI-for-age z-score
BMS	Breast Milk Substitutes
CF	Complementary Feeding
CI	Confidence Interval
DoH	Department of Health
DWS	Department of Water and Sanitation
EBF	Exclusive Breastfeeding
EDD	Environmental Enteric Dysfunction
LAZ	Length-for-age z-score
MDR	Multi-Drug Resistance
NE	Not Estimable
NDoH	National Department of Health
SAM	Severe Acute Malnutrition
SAMRC	South African Medical Research Council
SDGs	Sustainable Development Goals
UNICEF	United Nations Children's Fund
WASH	Water, Sanitation and Hygiene
WAZ	Weight-for-age z-score
WHO	World Health Organisation
WLZ	Weight-for-length z-score

DEFINITION OF CONCEPTS

Antibiotic resistance (AR) is the ability of bacteria to survive and evade the effects of antibiotics through multiple mechanisms, making infections harder to treat (Habboush and Guzman, 2018). This study focused on identifying the prevalence of antibiotic resistance in infants aged 0-6 months**.**

Feeding practices refer to the behaviours and methods, including timing, type, frequency, and mode of feeding that caregivers use to nourish infants and young children (WHO, 2003). This study focused on the initiation of breastfeeding, exclusive breastfeeding, mixed feeding, and complementary feeding in infants aged 0-6 months**.**

Growth status refers to the measurement of an infant's physical development compared to internationally accepted standards established by the World Health Organization (WHO, 2006). In this study, WHO child growth indicators, namely, weight-for-age, length-for-age, and weight-for-length z-score, were used to assess the growth status in infants aged 0-6 months.

Hygiene practice is the practice of handwashing with soap after defecation and the disposal of child faeces, prior to preparing and handling food, before eating, and, in health care facilities, before and after examining patients and conducting medical procedures (WHO, 2025). In this study, hygiene practices refer to handwashing and food-handling practices among mothers with infants aged 0-6 months.

Sanitation refers to the provision and use of facilities and services that safely dispose of human urine and faeces, thereby preventing environmental contamination (UNICEF, 2016). In this study, sanitation focused on household toilet facilities for participating infants.

Stunting is characterised by a low length/height-for-age (LAZ) z-score of less than minus two standard deviations below the median of the WHO reference point for children of the same age (WHO, 2006). The study used the same growth indicator to assess stunting among in infants aged 0-6 months.

Underweight refers to low weight-for-age (WAZ) of less than minus two standard deviations of the median of the WHO reference point (WHO, 2006). The study used the same growth indicator to assess infant underweight in infants aged 0-6 months.

WASH practices refer to behaviours related to the use of safe water, improved sanitation, and proper hygiene, such as handwashing with soap, which help prevent infections and promote child health (WHO, 2019$_a$). The WASH components assessed in the study include household water sources, availability, and collection; sanitation, focusing on toilet facilities; and handwashing practices, including hand washing and food-handling practices.

Wasting is defined as a low weight-for-length (WLZ) of less than minus two standard deviations of the median of the WHO reference point (WHO, 2006). The study used the same growth indicator to assess wasting in infants aged 0-6 months.

Water is a clear, odourless liquid indispensable to life, supporting vital biological functions and everyday domestic, agricultural, and industrial activities; ensuring universal access to safe water is recognised as a basic human right (WHO, 2023). This study assessed water, focusing on availability, sources, and collection.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1: INTRODUCTION

1.1 Background information of the study

Malnutrition persists as a significant global health concern, affecting millions of children worldwide. It encompasses both undernutrition and overnutrition, posing a major public health concern among children under five. According to the United Nations Children's Fund (UNICEF), the World Health Organization (WHO), and the World Bank Joint Child Malnutrition Estimates (2024), approximately 150.2 million children under five were stunted, and 42.8 million were wasted. Concurrently, overnutrition is emerging as a growing challenge, with about 35.5 million children under five classified as overweight. These figures highlight the dual burden of malnutrition globally, particularly in low and middle-income countries (LMICs), where both insufficient and excessive nutrient intake contribute to poor growth outcomes and increased risk of morbidity and mortality. A study conducted in several LMICs by Kerac *et al.* (2025) on infants under six months indicates that approximately 10.3 million globally are underweight, 9.2 million are wasted, 11.8 million are stunted, and 8.9 million are overweight.

In Eastern and Southern Africa, UNICEF (2024) estimated that approximately 5.5 million infants under six months suffer from undernutrition, including approximately 1.6 million stunted, 0.6 million wasted, and 0.8 million underweight. The high rates of stunting, wasting, and underweight among infants indicate significant challenges in ensuring adequate nutrition during the crucial first six months of life. Nigeria faces significant challenges with child malnutrition, in which the national survey revealed that in Nigeria, approximately 32% of children under five years were malnourished, encompassing stunting, wasting, and underweight (UNICEF, 2025). These results make Nigeria one of the countries with the highest child malnutrition rates in Africa. The high prevalence of these conditions underscores the urgent need for targeted interventions to improve child nutrition in Nigeria.

In South Africa, malnutrition among children under five years also remains a major public health concern, encompassing both undernutrition and overnutrition. According to the 2016 South Africa Demographic and Health Survey (SADHS), approximately 27% of children under five were stunted, indicating chronic undernutrition, which was more prevalent in rural areas (29%) than in urban areas (26%), highlighting disparities in access to food, healthcare services, and socioeconomic resources between rural and urban populations National Department of Health (NDoH), Statistics South Africa (Stats SA), South African Medical Research Council (SAMRC) and Inner City Fund (ICF), 2019). The prevalence of wasting was estimated at 2.5%-3.4% among children under five (NDoH, Stats SA, SAMRC, and ICF, 2019). Although lower than stunting, wasting also remains a key contributor to child morbidity and mortality. Meanwhile, underweight affected about 5.9% of children under five, suggesting moderate national vulnerability to chronic and acute nutritional deprivation. The survey further reported the prevalence of overweight at 13%, while the prevalence of obesity was 4% in children under five years. Given the prevalence of malnutrition, South Africa faces the simultaneous challenge of undernutrition and overnutrition among young children, complicating the country's nutrition landscape.

Malnutrition in Limpopo Province remains a major public health concern. Modjadji and Mashishi (2020) found that 45.3% of children under five were stunted, and 29% were underweight, with boys more affected than girls. The latter study also indicated that approximately 9% of children under five were overweight, while 3% were obese. The 2023 Limpopo Provincial Food and Nutrition Security Survey further reported an underweight prevalence of 11.5%, including 6.3% severely underweight and 5.2% moderately underweight children, with the Mopani District recording the highest rate (16.6%) (Mutanga *et al*., 2023). In a study conducted by Mafhungo *et al.* (2025) in Limpopo, Thabazimibi sub-district, among 409 children (median age: 18 months), 38% were stunted, 13% were underweight, 5% were wasted, and 17% were overweight. Limpopo continues to experience higher undernutrition levels than the national rate, particularly for stunting, alongside rising overweight rates. Although underweight appears to have declined, persistently high stunting indicates slow improvement and continued challenges.

Risk factors for malnutrition in infants include inadequate water, sanitation and hygiene (WASH) conditions, inappropriate infant feeding practices, and antibiotic resistance (AR). Access to clean and safe drinking WASH can reduce the prevalence of childhood malnutrition by preventing diarrhoea, parasitic diseases, and environmental enteropathy, which negatively affect the body's ability to absorb nutrients and impede normal growth and cognitive development (UNICEF and WHO, 2023). Improving access to safe WASH interventions could prevent at least 1.4 million deaths annually (WHO, 2023_a). In infants under six months of age, diarrhoea can cause permanent damage to intestinal development, reducing a child's ability to absorb nutrients and putting their future health and development at risk. According to Bhutta *et al*. (2017), infants living in poor sanitary conditions develop an idiopathic chronic inflammatory enteropathy of the intestine, termed Environmental Enteric Dysfunction (EDD). EDD contributes to a cascade of adverse effects, including muscle wasting, gastrointestinal malabsorption, and infectious disease, which result in severe acute malnutrition (SAM) (Lauer, 2023). Lauer (2023) confirms that inadequate infant growth and development can also be attributed to EED.

As identified in the Global Plan of Action to combat antimicrobial resistance (AMR) (WHO, 2020), improving WASH and wastewater management in all sectors is critical to prevent infections and reduce the spread of AMR. However, poor infrastructure and access to WASH increase the prevalence of AR (Collignon *et al*., 2018). The effectiveness of hygiene in reducing infection rates, reducing antimicrobial use, and preventing infections, was demonstrated in a study conducted in Cape Town, South Africa. The study assessed 685 households with children under five years of age in two regions and found that hygiene education was associated with handwashing, regular washing, and cleaning floors at critical times (Essack *et al*., 2021). Furthermore, a lack of access to water, sanitation and hygiene (WASH) leads to the spread of infectious diseases, thereby increasing antibiotic use. Good hygiene, both in healthcare settings and in the community, is crucial in preventing the rise of AR (Aspenberg *et al*., 2023).

Breastfeeding is widely recognised as the optimal source of nutrition for infants, offering a range of immunological and developmental benefits. It provides essential maternal immune cells and antibodies, as well as commensal and probiotic bacteria that support the maturation of the infant's gut microbiota and immune system (Nadimpalli *et al.,* 2020). Human milk contains both nutritional and microbial antigens, alongside immune effectors such as antibodies, antimicrobial peptides, and live immune cells, which contribute to the exclusion and elimination of harmful pathogens (Robertson *et al.,* 2019). These components are not present in infant formula, which, although nutritionally adequate, lacks the bioactive and immunological elements found in breast milk. The early introduction of formula feeding has been linked to alterations in gut microbiota composition and an increased risk of AR, possibly due to the absence of protective microbial and immune factors present in human milk (Patangia *et al*., 2024).

Inappropriate infant feeding practices compromise optimal growth, development, and survival in early childhood by weakening immunity and increasing vulnerability to infections (WHO and UNICEF, 2023$_a$). This heightened infection burden often results in frequent and sometimes inappropriate antibiotic use, which not only undermines child health outcomes but also contributes to the growing challenge of AMR (Murray *et al*., 2022). Addressing suboptimal feeding practices is therefore critical, not only for improving child growth and development but also for reducing unnecessary antibiotic exposure and mitigating the spread of AMR.

The WHO and UNICEF (2003) recommend initiating breastfeeding within the first hour after birth, followed by exclusive breastfeeding for the first six months of life, with continued breastfeeding alongside appropriate complementary feeding for up to 2 years or beyond. Exclusive breastfeeding remains the preferred infant feeding practice because of its well-documented benefits, including enhanced immune protection, reduced susceptibility to infectious diseases, and positive effects on cognitive development (Victora *et al*., 2016). Although infant formula is designed to substitute breast milk when breastfeeding is not possible or medically contraindicated, it does not confer comparable immunological or developmental advantages. Despite strong global policy endorsement of exclusive breastfeeding, its uptake remains suboptimal. Globally, only 44% of infants under six months were exclusively breastfed between 2015 and 2020 (WHO and UNICEF, 2023a), while a study by Mafhungo *et al*. (2025) in Thabazimbi sub-district, Limpopo province, reports that lower prevalence rates in specific contexts, with exclusive breastfeeding observed in only 27% of children under two years of age. These findings illustrate persistent gaps between recommended infant feeding practices and real-world implementation, underscoring the continued reliance on suboptimal feeding methods.

Newborns are particularly vulnerable to AR due to their immature immune systems (Moraes-Pinto *et al.,* 2021). Infections and the development of antimicrobial resistance (AMR) during early childhood can have serious implications for neonatal health. According to Woll *et al*. (2018), newborns and young children face an increased risk of bacterial infections due to underdeveloped immune systems, greater exposure to bacteria, and limited preventive measures. This susceptibility contributes to the higher rates of antibiotic use among children under five compared to adults (Hsia *et al*., 2019). The WHO (2016) estimated that infections caused by multidrug-resistant (MDR) bacteria lead to approximately 700 000 deaths globally each year, with about 200 000 of these occurring in newborns.

As of 2017, the World Bank projected that AMR could reduce global gross domestic product (GDP) by up to 3.8% by 2050, with the most severe economic consequences expected in low-income countries (World Bank, 2017). Gandra *et al*. (2019) further noted that nations with high poverty rates are already experiencing the economic and health burdens associated with AMR. According to the Organisation for Economic Co-operation and Development (OECD, 2019), resistance to commonly used antibiotics may exceed 40–60% in certain countries by 2030. O'Neill (2016) projected that AMR could result in 10 million deaths annually by 2050, with approximately 9 million of these deaths expected to occur in Asia and Africa.

Africa bears a disproportionately high share of the global AMR burden. A recent report by the Africa Centres for Disease Control and Prevention (CDC) (2022) revealed that

AMR is now responsible for more deaths on the continent than HIV/AIDS, malaria, or tuberculosis, with an estimated 255 000 direct AMR-related deaths in 2019. Sub-Saharan Africa has the highest regional mortality rate from AMR, at 27.3 deaths per 100,000 population (Murray *et al*., 2022). Contributing factors include widespread misuse of antibiotics, limited laboratory capacity, and poor WASH infrastructure (CDC, 2022).

In South Africa, the situation is similarly concerning. According to the Institute for Health Metrics and Evaluation (IHME, 2022), approximately 9,500 deaths were directly attributable to AMR in 2019, with a further 39,000 deaths associated with resistant infections. The most prevalent AMR pathogens include Klebsiella pneumoniae, Staphylococcus aureus, Streptococcus pneumoniae, Escherichia coli, and Acinetobacter baumannii (IHME, 2022). In response, South Africa has developed a National AMR Strategy Framework (2018-2024) and continues to strengthen surveillance through the National Institute for Communicable Diseases (NICD, 2022). Hence, this study aims to explore the influence of WASH and feeding practices on growth status and AR in infants aged 0-6 months in the Dzimauli rural villages. The results are expected to provide evidence-based insights to inform policy and programmatic interventions aimed at improving child health and nutrition outcomes in rural South African contexts.

1.2 Problem statement

Malnutrition in low-resource settings is a critical public health issue closely aligned with several United Nations Sustainable Development Goals (SDGs). For instance, SDG 2 (Zero Hunger) targets malnutrition and stunting, SDG 3 (Good Health and Well-being) seeks to improve health outcomes for infants and children, and SDG 6 (Clean Water and Sanitation) emphasises the role of hygiene and sanitation in preventing diseases and reducing AR, particularly in vulnerable populations such as infants.

In Limpopo Province, several studies have documented persistent levels of infant undernutrition alongside suboptimal feeding practices. Modjadji *et al.* (2019) reported early introduction of complementary foods in rural communities, largely driven by perceptions of insufficient breast milk. Modjadji and Mashishi (2020) subsequently identified high prevalence rates of stunting and underweight among children under five, reflecting significant early-life nutritional vulnerability and the coexistence of overweight. Makwela *et al.* (2024) highlighted ongoing barriers to exclusive breastfeeding in Polokwane, including maternal employment and social influences that contribute to early supplementation. Similarly, Mafhungo *et al.* (2025) documented substantial levels of stunting and underweight in the Thabazimbi sub-district. However, these studies have largely overlooked the potential role of AR as a contributing risk factor for recurrent infections, treatment failure, and prolonged illness, which may further exacerbate growth faltering and undernutrition in early childhood. While both global and national action plans on AMR have been established, their focus has predominantly centred on healthcare facilities, with limited attention given to household and community-level prevention strategies (Maillard *et al*., 2020). Moreover, Fuhrmeister *et al*. (2023) highlight the need for further research to establish causal links between improved WASH practices and a reduced AMR burden. Given these gaps, there is a critical need to understand how infant feeding and WASH practices contribute not only to infant health but also to the development and spread of AR, particularly in rural communities such as Dzimauli. Generating this knowledge is essential to inform context-specific interventions that improve immediate health outcomes and promote sustainable, long-term public health solutions in the region.

Undernutrition and AR have serious economic consequences. Undernutrition in early life can affect cognitive development, educational achievement, and future productivity, limiting long-term economic opportunities. At household and provincial levels, recurrent infections and treatment failures increase healthcare costs and strain limited health services. In vulnerable rural communities such as Dzimauli, the combined burden of undernutrition and AR may therefore sustain cycles of poverty and poor health.

1.3 Research aim

The study aims to explore the influence of WASH and feeding practices on growth and antibiotic resistance in infants aged 0-6 months in Dzimauli rural villages.

1.4 Objectives of the study

1.4.1 To determine the WASH practices of mothers of infants aged 0-6 months in Dzimauli villages, through

- Water availability, sources, and collection;
- Sanitation (toilet) facilities; and
- Hygiene practices, including hand washing and food handling practices.

1.4.2 To determine infant feeding practices in infants aged 0-6 months, focusing on breastfeeding.

1.4.3 To assess the growth status of infants aged 0-6 months by measuring weight and length.

1.4.4 To identify the prevalence of antibiotic resistance in infants aged 0-6 months using stool samples.

1.4.5 To examine the associations between socio-demographic characteristics, WASH practices, infant feeding practices, growth status, and antibiotic resistance.

1.5 Research questions

The main research question is**:** What is the influence of WASH and feeding practices on growth and antibiotic resistance in infants aged 0-6 months in Dzimauli rural villages?

Subsequent questions:

- What are the WASH practices among mothers of infants aged 0-6 months in Dzimauli rural villages?
- What are the feeding practices of infants aged 0-6 months in Dzimauli rural villages?
- What is the growth status of infants aged 0-6 months in Dzimauli rural villages?
- What is the prevalence of antibiotic resistance in infants aged 0-6 months in Dzimauli rural villages?

- What is the association of demographic characteristics, WASH practices, and infant feeding practices with growth status and antibiotic resistance in the infants aged 0-6 months in Dzimauli rural villages?

1.6 Conceptual framework of the study

The framework depicted in Figure 1.1 is grounded in conceptual models and empirical literature on the links between WASH conditions and child nutrition and health outcomes. This relationship is well-documented, showing that poor WASH conditions heighten exposure to enteric pathogens, leading to infections, reduced nutrient absorption, and ultimately adverse growth outcomes such as stunting and wasting (Humphrey, 2009; Ngure *et al.,* 2014; Zavala *et al*., 2021; Gizaw and Worku, 2019). This framework is an adaptation of the conceptual model proposed by Zavala *et al*. (2021), developed through an extensive literature review to illustrate the various pathways through which WASH impacts nutritional outcomes in low- and middle-income countries. The model created by Zavala *et al*. (2021) includes environmental, behavioural, biological, and systemic factors, demonstrating how inadequate WASH contributes to enteric infections, environmental enteric dysfunction, reduced nutrient absorption and ultimately poor growth in children.

For the scope of this study, the original framework by Zavala *et al*. (2021) was revised to align with the study's goals, population, and available data, specifically concentrating on early infancy (0-6 months) and the use of repeated longitudinal measurements. Socio-demographic factors such as maternal age and education were included in the revised framework because they impact household behaviours and access to critical resources. These socio-demographic elements, in turn, shape WASH practices, including water sources, sanitation facilities, and hygiene behaviours, as well as infant feeding methods, which encompass exclusive breastfeeding, mixed feeding, formula feeding, and the timing of early complementary feeding.

Moreover, the adjusted framework incorporates connections between WASH conditions, exposure to infections, antibiotic use, and the development of antimicrobial AR. Although these pathways are not explicitly highlighted in the original Zavala *et al*.

(2021) model, they are essential to the current study and reflect emerging evidence that early-life exposure to infections and antibiotic treatment contributes to the acquisition and spread of resistant organisms. By integrating AR as an outcome, the revised framework expands the existing model to encompass the broader ecological impacts of inadequate WASH and early-life infection burdens. As a result, this framework enhances and builds upon the WASH-nutrition model proposed by Zavala *et al*. (2021) It adds significance by (i) elaborating on the exposure mechanisms at the infant level, (ii) giving greater emphasis to feeding hygiene, and (iii) presenting antimicrobial resistance as a subsequent outcome, thereby broadening the conceptual relationship between WASH, infection, and child health outcomes.

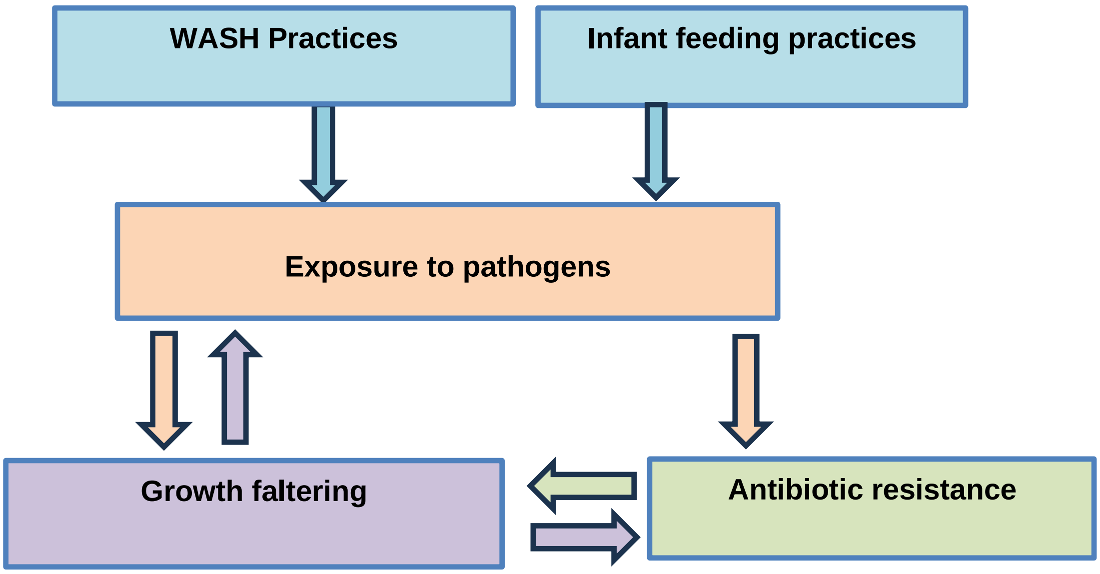

Figure 1.1: Conceptual framework of how WASH practices and feeding practices influence growth status and AR

1.7 Significance of the study

This study aims to generate baseline data on the associations between WASH practices, infant feeding practices, growth status, and AR in infants aged 0-6 months in Dzimauli rural villages. Conducting this study is necessary because there is limited evidence at the community level, particularly in rural Limpopo Province. By identifying the strength and direction of these relationships during a critical period of infant development, the study aims to inform integrated public health strategies to improve early-life nutrition and reduce unnecessary antibiotic exposure. The results may contribute to evidence-based policy development and guide context-specific interventions to improve WASH conditions and infant feeding practices in Limpopo Province and beyond. Moreover, the results may offer baseline information to support provincial health authorities in interpreting AR patterns among infants within the broader context of child health and nutrition.

This research forms part of a broader project led by the SAMRC and the University of Venda Institute for Pathogen and Global Health Research titled *“Acquisition of antibiotic-resistant gut bacteria and impact of microbiota (2023-2027).”* Within this framework, the present study contributes context-specific evidence from rural Limpopo by examining AR patterns in relation to early growth indicators during the first six months of life. Furthermore, the study may enhance understanding of the observed associations between the selected variables. In doing so, it supports the overarching objective of elucidating the timing, composition, and evolution of the gut resistome, with *Escherichia coli* serving as a model organism. Again, the information from this study will serve as a reference for those interested.

1.8 Structure of the dissertation

Chapter 1 presents the introduction, including the study's background information, problem statement, aim, objectives, research questions, conceptual framework, and the study's significance. The dissertation's structure is also included.

Chapter 2 reviews empirical literature relevant to the study objectives and concludes by identifying key gaps that justify the present investigation.

Chapter 3 presents the methods used during the study, including the study design, population and area, sampling, instrument development, data collection procedures, data analysis, ethical consideration, and dissemination of results.

Chapter 4 presents the study's results, following the objectives.

Chapter 5 discusses the results in relation to the literature.

Chapter 6 presents the study's conclusions and recommendations.

CHAPTER 2: LITERATURE REVIEW

2.1 Overview

The literature reviewed highlights WASH practices, infant feeding practices, and the critical interconnections between WASH and infant feeding methods, and their combined influence on infant growth and AR.

2.2 WASH practices

The WASH practices refer to the collective behaviours, infrastructures, and interventions that ensure the safe handling of drinking water, access to adequate sanitation facilities, and the adoption of effective hygiene behaviours, such as handwashing with soap (WHO, 2018; UNICEF and WHO, 2020). These practices are critical for preventing waterborne diseases and promoting health, especially among vulnerable populations like infants and young children (WHO, 2018). Inadequate WASH services disproportionately increase environmental health risks in low-resource settings, where children under five and older adults bear the greatest burden (Howard *et al*., 2020).

According to the WHO (2019a), safe WASH practices not only prevent disease transmission but also enhance personal dignity, support school attendance, and improve economic productivity by reducing illness. Despite its importance, access to safe water and sanitation remains limited globally. A 2023 joint report by WHO and UNICEF estimated that nearly 2 billion people lack safely managed drinking water, while over 3 billion lack access to basic sanitation services (WHO and UNICEF, 2023). These deficits hinder progress toward Sustainable Development Goal 6, which aims for universal access to clean water and sanitation.

In South Africa, disparities in access to WASH services persist. Statistics South Africa (2019) reported that 44.9% of households had piped water inside their homes, with many others relying on communal or neighbouring taps. Although Census 2022 indicated that 91.3% of households had access to piped water, the data does not specify whether the water source was within the household or from communal sources, raising concerns about water safety (Stats SA, 2022). Access to improved sanitation rose from 61.7% in 2002 to 82.1% in 2019; however, regional gaps remain, particularly in Limpopo and Eastern Cape provinces (Stats SA, 2022).

Understanding infant growth and antibiotic resistance in Dzimauli rural villages requires consideration of the broader water, sanitation and hygiene (WASH) context within the Vhembe District. Research consistently shows that rural communities in Vhembe district experience substantial challenges related to water access, quality, and hygiene infrastructure. Murei *et al*. (2022) reported that a large proportion of rural households in Vhembe rely on unimproved or intermittently available water sources, with persistent risks of microbial contamination arising from inadequate infrastructure, limited monitoring, and weak implementation of Water and Sanitation Safety Plans. The study further highlights that more than half of the communities assessed faced frequent water interruptions and unsafe storage practices, increasing the likelihood of exposure to waterborne pathogens. Complementing the findings of Murei *et al*. (2022), Masia *et al*. (2025) show that the majority of households in rural Vhembe perceive the water supply as unreliable and insufficient for daily hygiene practices, including regular handwashing. The latter study indicates that a substantial number of households lack improved sanitation facilities, while hygiene behaviours are often constrained by water scarcity rather than knowledge deficits.

The consequences of unsafe water and poor sanitation are significant, contributing to health risks such as diarrhoeal disease, which remains a leading cause of death in children under five worldwide. In 2019, diarrhoea caused approximately 370,000 deaths in this group (WHO, 2019a). Pathogens like Escherichia coli and rotavirus are common in low-resource settings with poor sanitation and contribute to severe diarrhoeal illness among infants (WHO, 2017).

Cloete and Fourie (2020) have linked untreated water consumption in South Africa to increased diarrhoeal cases among children. Black *et al*. (2021) demonstrated that recurrent diarrhoea episodes disrupt nutrient absorption and contribute to stunting, creating a cycle in which malnutrition heightens infection risk, which, in turn, worsens nutritional status. Addressing WASH deficits is therefore critical to improving child health outcomes, especially in rural settings such as Vhembe District, where infrastructural limitations, poverty, and reduced access to safe water and sanitation increase vulnerability to infection and associated nutritional consequences.

2.3 Infant feeding practices

Feeding practices in the first six months of life are vital for shaping an infant's growth and overall health. Exclusive breastfeeding (EBF), formula feeding, a combination of both, or the introduction of complementary foods significantly influence infants' physical development, nutrient intake, and immune system function.

2.3.1 Exclusive breastfeeding

Exclusive breastfeeding is defined as the practice where an infant receives only breast milk, without any additional liquids or solids, including water (WHO, 2019a). The only exceptions are oral rehydration solutions and drops and syrups containing vitamins, minerals, or medications. The WHO (2019b) asserts that EBF for the first six months of life is the optimal feeding practice for infant health and survival. Breast milk provides all the essential nutrients, including proteins, fats, and vitamins, in proportions perfectly suited to support an infant's development (Victora *et al.*, 2021). It protects infants from viral and chronic illnesses while promoting sensory and cognitive development (Oddy, 2001).

Breast milk provides essential immune protection through antibodies and antimicrobial components that reduce infection risk and support immune development (Baker *et al.,* 2021a). Key antibodies, particularly secretory immunoglobulin A (sIgA), along with IgG and IgM, protect infants by preventing pathogen attachment and enhancing early immune defense in a developmentally appropriate, non-inflammatory manner (Verhasselt *et al.*, 2024). Furthermore, antimicrobial proteins such as lactoferrin and lysozyme inhibit microbial growth and strengthen protection against enteric and respiratory infections (Verhasselt *et al.*, 2024). Together with breast milk oligosaccharides, which promote a beneficial gut microbiota, these components help regulate immune responses, support immune tolerance, and reduce the risk of infections and immune-mediated diseases, highlighting the critical role of exclusive breastfeeding in healthy infant immune development (Alotiby, 2023).

The EBF rate has increased globally but remains below the WHO's 2025 target of 50% (UNICEF and WHO, 2023). In 2018, globally, only 41% of infants under six months of age were exclusively breastfed (WHO, 2018). By 2021, this percentage had increased to 47.7%, marking a significant rise from the 2012 baseline of 37.0% (UNICEF, 2021). Projections for 2025 indicate that 53.4% of infants under six months are expected to be exclusively breastfed. In sub-Saharan Africa, EBF rates are among the highest globally but vary widely between countries (UNICEF, 2021). East African nations like Rwanda and Burundi have achieved EBF rates above 80%, while West African countries, including Nigeria and Chad, have EBF rates closer to 20-30% (Victora *et al*., 2021).

Despite the well-documented benefits of EBF, South Africa ranks among the lowest countries worldwide for EBF rates. The 2016 SADHS reported that approximately 31.6% of infants under six months were exclusively breastfed. Compared to the 2003 national estimates, exclusive breastfeeding rates have increased following intensified policy efforts, including the Tshwane Declaration of 2011, which reinforced breastfeeding promotion within public health facilities (NDoH, 2011; NDoH *et al*., 2019). In 2017, statistics from a health centre in Limpopo Province revealed that only 10.7% of infants who received the third dose of the hexavalent vaccine were exclusively breastfed. The hexavalent vaccine is given at 3 months of age. Muleka *et al*. (2023) in Seshego, Limpopo, found that 94.2% of participants-initiated breastfeeding within an hour of delivery at a healthcare facility. However, only 43.6% of the children were exclusively breastfed. Another study in Limpopo found that many mothers believe breast milk alone is inadequate for their infants' growth, prompting them to introduce porridge and other solid foods early (Modjadji *et al*., 2019).

Studies conducted in the Vhembe District indicate that EBF practices remain suboptimal despite relatively high levels of maternal awareness. Mandiwana (2017) found that although most lactating mothers in rural Vhembe villages demonstrated good knowledge of exclusive breastfeeding recommendations, compliance was considerably lower, with many mothers introducing water, soft foods, or formula before six months. Consistent with these findings, Mugware *et al*. (2022) reported that only 7.6% of infants in the Vhembe District were exclusively breastfed for the recommended six-month period. Similarly, Mudau (2022) found that just 27.1**%** of mothers in Vhembe district practiced exclusive breastfeeding.

Multiple factors contribute to the low rates of exclusive breastfeeding observed in many regions, including the premature introduction of complementary foods, socio-cultural influences, socioeconomic inequalities, inadequate maternity leave leading to early return to work, and insufficient support systems for breastfeeding mothers (Rollins *et al*., 2021; Shisana *et al*., 2019). In addition, cultural beliefs that breast milk alone is insufficient for infant growth often promote mixed feeding practices, further undermining adherence to exclusive breastfeeding recommendations (Shisana *et al*., 2019).

Exclusive breastfeeding (EBF) practices in the Vhembe District are influenced by a complex combination of individual, socio-cultural, health system, and structural factors. Socio-cultural pressures, particularly advice from older female relatives and entrenched community norms favouring early supplementation, further undermine adherence to EBF (Mandiwana, 2017; Mudau, 2022). From a health system perspective, professional nurses report constraints such as staff shortages, heavy workloads, and limited time for postnatal breastfeeding counselling, resulting in inadequate follow-up support once mothers leave health facilities (Mudau *et al*., 2023). Environmental and household-level challenges, including poverty, food insecurity, and unreliable access to clean water, also contribute to early introduction of complementary foods, as evidenced by the very low EBF prevalence reported in Thulamela Municipality (7.6%) (Mugware *et al*., 2022). Additionally, maternal employment in informal or unsupportive work settings and early return to work limit mothers' ability to maintain exclusive breastfeeding (Mudau, 2022; Mudau et al., 2023). Collectively, these interconnected factors highlight the need for context-specific interventions in Vhembe that extend beyond knowledge-based education to address social support, health system capacity, and broader structural constraints.

2.3.2 Formula feeding

Formula feeding refers to the practice of feeding infants with manufactured infant formula, a breast milk substitute designed to provide the nutrients necessary for infant growth and development when breastfeeding is not possible or chosen (WHO, 2023a). Infant formulas are typically made from cow's milk or soy protein that is modified to resemble breast milk and fortified with vitamins, minerals, and essential fatty acids (Moreira *et al*.,2023). Infant formula serves as a substitute for breast milk, meticulously designed to fulfil the nutritional needs of infants during their initial months, until they are ready for complementary foods (Codex Alimentarius Commission, 2007). The Codex Alimentarius Commission distinguishes between a regular formula for healthy infants and specialized formulas for specific medical conditions, which should be used under medical supervision. While infant formulas aim to replicate breast milk's nutrient composition, notable differences exist, potentially affecting infant growth.

Formulas often lack the protective antibodies and enzymes found in breast milk, which are crucial for immune system development (Baker *et al.,* 2021a). As more women enter the workforce, caregivers often rely on commercial or ready-to-use milk powder administered in bottles (Hunde *et al*., 2023). Infants who do not receive breast milk require high-quality infant formulas to ensure adequate intake of essential nutrients. This is particularly crucial for preterm and very low-birth-weight infants, who are at higher risk of inadequate growth and development (Ahmad *et al*., 2021). However, in low socioeconomic settings, access to high-quality formula may be limited due to financial constraints, leading caregivers to dilute formula to extend its use, substitute it with inappropriate milk products, or introduce low-cost complementary foods prematurely (WHO, 2023a). Such practices may compromise nutritional adequacy and increase the risk of infections, particularly in environments with inadequate water and sanitation (Arikpo *et al*., 2020).

Globally, approximately 40% of mothers introduce breast milk substitutes (BMS) by the time their infants are eight weeks old, with many combining breast milk and BMS before six months (UNICEF, 2020). South Africa has a particularly high formula-feeding rate in the first 6 months, with over 60% of infants introduced to formula during this period, contrary to the World Health Organization's recommendation for exclusive breastfeeding (WHO, 2023a). A study by Mugware *et al*. (2022) in Thulamela Municipality, Limpopo, found that over one-third (36.4%) of children aged zero to 36 months were given infant formula.

Formula feeding can negatively affect fundamental oral functions such as chewing, sucking, and swallowing, potentially leading to dental malocclusion (Melo *et al.,* 2023). Bottle use during formula feeding is also linked to morbidity and mortality from diarrhoeal diseases due to hygiene challenges, especially in developing nations with poor sanitation (Kebebe and Assaye, 2017). The prevalence of inappropriate or poor-quality feeding bottles and nipples in developing countries exacerbates this issue (Seid *et al*., 2019). Globally, failure to adhere to breastfeeding guidelines and reliance on BMS contribute to over 800,000 infant deaths and cognitive impairments (Wondim *et al*., 2023). Infants exposed to formula and who stop breastfeeding early face higher risks of illness, obesity, allergies, sudden infant death syndrome (SIDS), and impaired cognitive development (Alemu *et al*., 2023).

Infant formula plays an important role in providing nutrition to infants who cannot be breastfed (WHO, 2023b). However, it is essential to recognise the compositional differences between infant formula and breast milk, as well as the potential health risks associated with formula feeding, particularly in settings with limited sanitation and safe water access (Victora *et al*., 2021). Promoting breastfeeding while ensuring access to high-quality infant formula when medically necessary is critical for supporting optimal infant health and development.

2.3.3 Complementary feeding

Complementary feeding refers to the transition from exclusive milk consumption to the introduction of solid, semi-solid, and soft foods and liquids other than milk, typically occurring between six and 24 months of age (Chehab *et al*., 2021). Historically, these foods were often referred to as "weaning foods" (Acevedo *et al*., 2021). However, the term "complementary foods" is now preferred, as "weaning" implies the cessation of breastfeeding, whereas the purpose of CF is to supplement human milk or infant formula rather than replace them. Complementary foods can be prepared at home or purchased as ready-to-eat or ready-to-mix commercial products (Saavedra *et al*., 2022). Muleka *et al*. (2023) agreed that sufficient and safe CF, delivered in a timely and correct manner, is essential for optimal infant growth and development. They further emphasise the significance of the timely introduction of CF, as it should occur when the infant's nutritional and energy needs exceed what can be provided by breastfeeding alone.

Despite these recommendations, CF practices in many African countries, including South Africa, often deviate from WHO guidelines, with caregivers frequently introducing solids too early or offering foods lacking in essential nutrients (Labadarios *et al*., 2018). For instance, a study by Chakona (2020) among mothers in the Eastern Cape revealed that 56% perceived mealie meal porridge as the primary food for child growth, while only 35%, 32%, and 12% recognised the importance of vegetables, fruits, and meat, respectively.

Evidence from Limpopo Province consistently demonstrates a widespread pattern of early CF that undermines recommended infant feeding practices and may compromise child health outcomes. In Thulamela Municipality, Mugware *et al*. (2022) reported that 87.5% of children were introduced to complementary foods, predominantly maize-meal soft porridge, before six months of age, reflecting a strong departure from exclusive breastfeeding guidelines. Comparable findings were observed in Seshego, where Muleka *et al*. (2023) found that more than half of caregivers (52.3%) initiated CF prematurely, with a substantial proportion introducing foods as early as three months (43.2%) or even before two months (15%). Furthermore, a study by Mafhungo *et a*l. (2025) highlighted broader nutritional challenges across Limpopo Province, linking inappropriate CF practices to poor dietary diversity and increased nutritional vulnerability among children under five. Together, these studies suggest that early CF introduction in the province is not an isolated behaviour but part of a broader pattern of suboptimal infant and young child feeding.

Inappropriate CF practices are associated with adverse long-term health outcomes, including obesity, type 2 diabetes, and functional impairments in later life (WHO, 2016). Failure to adhere to WHO recommendations regarding the timing, quality, and quantity of complementary foods may increase the risk of undernutrition, impair linear growth, and compromise child development and survival. Conversely, delayed initiation of CF has also been linked to increased childhood morbidity and mortality (Chehab *et al*., 2021; Hamer *et al*., 2022). Therefore, adherence to WHO guidelines regarding the timing and nutritional content of complementary foods is crucial for promoting optimal child health and development.

2.3.4 Mixed feeding

Mixed feeding is the practice of supplementing breast milk with formula or complementary solid foods and is a common infant feeding strategy worldwide (WHO, 2023$_a$). The global prevalence of mixed feeding during the first year of life ranges from 23% to 32%, with the highest rates observed in infants aged four to six months (Monge-Montero *et al*., 2020). This period often coincides with mothers returning to work or facing perceived or real challenges with breast milk supply (Kowalski, 2025). Kowalski (2025) states that mixed feeding is often recommended for families who cannot exclusively breastfeed for personal, medical, or logistical reasons. Common reasons include maternal health issues, infant feeding difficulties, and societal pressures (WHO, 2023$_b$).

In South Africa, mixed feeding is a prevalent practice often adopted in response to various challenges, including mothers returning to work, perceived insufficient breast milk, and maternal health concerns (Makwela, 2019). The Makwela study (2019) revealed that 39% of mothers with infants aged zero to six months in the Polokwane Municipality of Limpopo practiced mixed feeding, with 85.7% citing perceived insufficient breast milk, often influenced by infant crying and concerns about milk adequacy, as the primary reason, underscoring the need to address maternal concerns and strengthen support and education on breastfeeding techniques and milk production.

A study by Ngure *et al.* (2019) indicates that mixed feeding can diminish the protective benefits of exclusive breastfeeding against infections such as diarrhoea and respiratory illnesses, potentially hindering infant growth. These infections are significant contributors to infant morbidity and mortality, particularly in resource-limited settings. Although Mixed feeding may serve as a practical alternative for mothers who are unable to exclusively breastfeed and can support adequate weight gain, provided it is implemented safely (Victora *et al*., 2021). However, its safety depends largely on appropriate formula preparation and hygienic practices, and in settings with limited access to clean water and sanitation, improper preparation may increase the risk of infection (WHO, 2023$_b$**)**. Therefore, comprehensive education on safe formula handling and hygiene practices is essential to minimise the potential health risks associated with mixed feeding.

One of the primary risks associated with mixed feeding is the potential exposure of infants to pathogens, especially in environments with limited access to clean water and sanitation (Arikpo *et al*., 2020). Formula milk, when improperly prepared or stored, can significantly increase the risk of gastrointestinal infections. Studies have consistently shown that exclusive breastfeeding reduces the incidence of respiratory and gastrointestinal infections in infants, whereas mixed feeding does not offer the same level of protection (Weinfield *et al*., 2005; Duijts *et al*., 2010; Rosas-Salazar *et al*., 2022; WHO, 2023$_b$). This is because breast milk contains antibodies and other bioactive components that strengthen the infant's immune system and reduce susceptibility to infection. Furthermore, the introduction of formula can alter the infant's gut microbiome, potentially increasing vulnerability to infections and allergic conditions (Berdnikovs *et al*., 2024). The gut microbiome plays a crucial role in immune system development and overall health, and disruptions caused by formula feeding can have long-term consequences.

Despite the risks, mixed feeding can offer certain benefits, particularly when exclusive breastfeeding is not feasible. It can ensure adequate nutrition and weight gain in infants, especially when maternal milk supply is insufficient or when mothers face challenges such as returning to work (Makwela, 2019). Moreover, mixed feeding can offer psychological benefits to mothers by reducing stress and anxiety associated with breastfeeding challenges. Research shows that breastfeeding is linked to improved mood and reduced physiological stress responses, such as lower heart rate and lower cortisol levels, compared to formula feeding (Dennis and Ross, 2023; Ahluwalia *et al.*, 2022). The flexibility of mixed feeding allows mothers to share feeding duties with other caregivers, helping alleviate the emotional and physical burden of exclusive breastfeeding (Thomson and Downe, 2023). Mixed feeding is therefore a complex and multifaceted practice with both potential benefits and risks. While exclusive breastfeeding remains the gold standard for infant nutrition (WHO, 2023a), mixed feeding provides a viable alternative for families encountering barriers to exclusivity (Victora et al., 2021). By understanding maternal motivations, risks, and benefits associated with mixed feeding, healthcare providers can deliver evidence-based support tailored to diverse family circumstances. Further research is needed to examine the long-term effects of mixed feeding on infant health and development, particularly across varied settings such as rural South Africa (Madiba and Langa, 2023).

2.4 Interplay between wash practices and feeding practices

WASH practices are foundational components of infant health, critically influencing infection prevention, safe feeding, and overall growth (Michaelsen *et al.,* 2021). The integrity of these practices is especially vital during infancy, as inadequate WASH conditions significantly elevate the risk of malnutrition and stunted growth (Dube *et al.*, 2021). Contaminated water and poor sanitation can compromise the safety of breast milk, formula, and complementary foods, thereby exposing infants to pathogens that increase infection risk (Michaelsen *et al.*, 2021; Pongou *et al.*, 2020). Studies emphasize that inadequate hygiene during breastfeeding can lead to contamination, making infants more vulnerable to infectious diseases (Dube *et al.*, 2021). Moreover, improper hygiene of feeding bottles further escalates the likelihood of contamination and infection (Agarwal and Roy, 2022).

Complementary feeding practices introduce additional risks if food preparation and storage are conducted under unsanitary conditions (Njie *et al.,* 2023). Research in South Africa shows that infants receiving complementary foods in environments with poor hygiene are at higher risk of gastrointestinal infections, which contribute to malnutrition and growth faltering (Njie *et al*., 2023; Michaelsen *et al*., 2021). These infections hinder nutrient absorption and result in nutrient loss, factors that play a critical role in the development of stunting (Kosek *et al*., 2020). Furthermore, recurrent infections such as diarrhoea deplete essential nutrients**,** including sodium and potassium, exacerbating malnutrition and impairing infant growth (Baker *et al*., 2021b).

Stunting is one of the most significant outcomes of poor WASH conditions. Research by Mungube *et al*. (2022) shows that insufficient sanitation in rural South Africa substantially contributes to stunting by perpetuating cycles of infection and malnutrition. Consistent with these results, Sekgala *et al*. (2022) demonstrate that improving access to clean water and sanitation reduces stunting and malnutrition rates. They argue that enhanced hygiene and sanitation are critical to disrupting the cyclical relationship between infection and undernutrition.

Addressing the identified challenges requires integrated interventions to improve WASH conditions. Njie *et al*. (2023) stress that improving water quality and sanitation services is essential not only for reducing infection rates but also for supporting infant nutrition and preventing stunting. However, they emphasise that WASH improvements alone are insufficient without concurrent nutritional support (Dube *et al*., 2021). Public health strategies must therefore highlight the intertwined importance of safe water, sanitation, and adequate nutrition to ensure that infants receive safe, nourishing food (Pongou *et al*., 2020). The collective evidence supports the implementation of comprehensive policies that tackle both environmental and nutritional determinants to prevent long-term adverse health outcomes in infants (Michaelsen *et al*., 2021).

2.5 Interplay between WASH, feeding practices and antibiotic resistance

WASH practices are fundamental to protecting infant health, particularly in settings characterised by high infection and malnutrition rates (Michaelsen *et al*., 2021). Limited access to clean water, adequate sanitation, and proper hygiene compromises the safety of all infant feeding practices, including breastfeeding, formula feeding, mixed feeding, and CF (Pongou *et al*., 2020. In low-resource environments, contamination may occur through caregivers' hands, feeding equipment, water used for formula preparation, or improperly handled complementary foods, increasing infants' exposure to environmental pathogens (Njie *et al*., 2023).

Infections arising from poor WASH conditions are frequently treated with antibiotics, and in many cases, these treatments may be unnecessary or inappropriate, particularly for self-limiting diarrhoeal illnesses (Kosek *et al*., 2020). Repeated antibiotic exposure promotes the selection and spread of antibiotic-resistant bacteria, including common enteric pathogens such as *Escherichia coli* and *Salmonella* (Baker *et al*., 2021b). Evidence from rural South African communities indicates that poor sanitation and hygiene are associated with increased infection burden and higher prevalence of resistant bacterial strains (Dube *et al*., 2021; Mungube *et al*., 2022).

The antibiotics tested in this study included gentamicin, cefotaxime, ceftriaxone, cefoxitin, cefepime, aztreonam, ertapenem, and tetracycline, which represent a clinically and epidemiologically relevant range of agents used in the management of infant infections and antimicrobial-resistance surveillance in South Africa (Koopmans *et al*., 2018). Gentamicin and third-generation cephalosporins such as cefotaxime and ceftriaxone are widely used as first-line empirical therapy for neonatal and infant sepsis and severe bacterial infections, making resistance to these antibiotics a critical indicator of early-life antimicrobial exposure (Koopmans *et al*., 2018). Cefoxitin and cefepime are commonly included in susceptibility testing as markers of more advanced β-lactam resistance mechanisms, including AmpC and extended-spectrum β-lactamase production, which have important implications for treatment effectiveness (Pillay, 2022). Aztreonam is relevant for monitoring resistance among Gram-negative pathogens, particularly in settings where alternatives are required due to β-lactam resistance or hypersensitivity (Koopmans et al., 2018). Ertapenem, a carbapenem antibiotic, was included to detect resistance to last-line therapies used for infections caused by multidrug-resistant organisms, the emergence of which is considered a major public health concern in South Africa (Pillay, 2022). Although tetracycline is not routinely prescribed to young infants, resistance to this antibiotic is widely used as an indicator of broader community and environmental antimicrobial pressure, which may, in turn, indirectly influence infant microbial exposure and resistance patterns (Pillay, 2022).

The interaction among inadequate WASH infrastructure, infection risk, and antibiotic use, therefore, represents a critical pathway in the emergence of AR in early life. Overuse and misuse of antibiotics to prevent infections further accelerate the development of resistance (Collignon *et al*., 2018; Laxminarayan *et al*., 2020). Strengthening WASH systems alongside promoting antibiotic stewardship is essential to reducing infection incidence, limiting unnecessary antibiotic exposure, and mitigating the development of AR among infants.

2.6 Growth status

Growth status refers to the assessment of an infant's physical growth and development using standardised anthropometric measurements that are compared against internationally accepted reference values established by the World Health Organization (WHO, 2006). Growth status is assessed using standardized anthropometric indices such as weight-for-age, length-for-age, and weight-for-length, which reflect different forms of undernutrition, including underweight, stunting, and wasting (WHO, 2006). These indicators are widely applied in both research and public health practice to evaluate whether infants are growing adequately relative to international growth standards and to identify early signs of growth faltering that may have short- and long-term consequences for health and development (UNICEF, WHO, and World Bank, 2023).

2.6.1 Length-for-age

Length-for-age is an anthropometric indicator used to assess an infant's linear growth in relation to age by comparing a child's measured length with that of healthy children of the same age and sex, based on the WHO Child Growth Standards (WHO, 2006). It is expressed as a LAZ, which indicates how far an infant's length deviates from the median of the WHO reference population. A LAZ below −2 standard deviations is classified as stunting and reflects chronic malnutrition resulting from prolonged inadequate nutrient intake, recurrent infections, or adverse environmental conditions (UNICEF, WHO, and World Bank Group, 2023). Unlike other growth indicators, length-for-age captures long-term growth trajectories rather than short-term nutritional fluctuations, making it a reliable measure for identifying sustained growth faltering that often originates in early life (Victora *et al*., 2010).

The National Food and Nutrition Security Survey National report (2023) indicates that approximately 28.8% of children under five years in South Africa were stunted, confirming that chronic undernutrition remains a major public health challenge in South Africa (Department of Agriculture, Land Reform and Rural Development (DALRRD) et al., 2023). At the provincial level, Limpopo Province reports a stunting prevalence of about 18% among children under five, meaning nearly one in five children experiences impaired linear growth (DALRRD *et al*., 2023). A study by Mugware *et al*. (2022) in the Vhembe district reported normal HAZ in 90.0% of children aged zero to 36 months, while stunting prevalence was 10.0%. Another study conducted in the Thabazimbi sub-district of Limpopo Province found that 38% of children aged 12-24 months were stunted, indicating substantial growth challenges that might have originated in early infancy if feeding and health conditions were not optimal (Mafhungo *et al*., 2025).

Stunting among children is influenced by a combination of nutritional, health, environmental, and socio-economic factors that interact during early childhood (UNICEF, WHO and World Bank, 2023). Inadequate dietary intake, particularly poor breastfeeding and complementary feeding practices, contributes significantly to stunting because insufficient nutrient intake during the first 1000 days of life limits optimal growth and development (WHO, 2023). Recurrent infections, especially diarrhoeal diseases and respiratory illnesses, further contribute to stunting by reducing nutrient absorption and increasing nutritional requirements (Prendergast and Humphrey, 2014).

2.6.2 Weight-for-age

Weight-for-age is an anthropometric indicator used to assess an infant's body mass in relation to age by comparing the child's weight with that of healthy children of the same age and sex, based on the WHO Child Growth Standards (WHO, 2006). It is expressed as a WAZ, which indicates how far an infant's weight deviates from the median of the reference population. A WAZ below −2 standard deviations is classified as underweight and reflects a composite measure of both acute and chronic malnutrition (WHO, 2006). This condition may result from inadequate dietary intake, recurrent infections, or a combination of these factors (UNICEF, WHO, and World Bank Group, 2023). Unlike length-for-age, weight-for-age does not differentiate between short-term and long-term nutritional deficits but provides an overall indication of poor nutritional status and increased vulnerability to morbidity in early life (Victora *et a*l., 2010).

The UNICEF, WHO, and World Bank Joint Child Malnutrition Estimates (2023) report that approximately 12% of children under five worldwide are underweight, with many cases linked to poor feeding practices and repeated illness episodes that compromise weight gain in early childhood. These global trends reinforce the importance of monitoring weight changes in early infancy as indicators of potential nutritional vulnerability. Underweight remains a significant problem among South African children, with 13% of children under five being underweight, highlighting persistent challenges related to child nutrition and household food security (DALRRD *et al*., 2023).

Provincial evidence from Limpopo Province similarly indicates that underweight remains a significant constraint to optimal child growth across multiple districts. In the Waterberg District, Modjadji and Mashishi (2020) reported a high prevalence of underweight, affecting 29% of children, highlighting substantial nutritional vulnerability in the province. Subsequent findings from the Vhembe District by Mugware *et al*. (2022) showed that 17.2% of children aged 0–36 months were underweight, demonstrating the persistence of poor weight-for-age outcomes in rural settings. More recently, Mafhungo *et al*. (2025) reported that 13% of children aged 12–24 months in the Thabazimbi sub-district were underweight, suggesting continued but variable burden across districts. Collectively, these studies indicate that underweight remains a widespread challenge in Limpopo Province, reflecting enduring influences of suboptimal infant feeding, recurrent infections, and underlying socio-economic and environmental constraints

2.6.3 Weight-for-length

Weight-for-length is an anthropometric indicator used to assess an infant's body weight in relation to length by comparing the child's measurements with those of healthy children of the same length and sex, based on the World Health Organization (WHO) Child Growth Standards (WHO, 2006). It is expressed as a WLZ, which indicates how far an infant's weight deviates from the median of the reference population. A WLZ below −2 standard deviations is classified as wasting and reflects acute malnutrition, often resulting from recent and severe weight loss associated with illness, insufficient food intake, or both (UNICEF, WHO, and World Bank Group, 2023). Unlike length-for-age, weight-for-length is sensitive to short-term changes in nutritional status and is therefore particularly useful for identifying infants at immediate risk of morbidity and mortality (Victora *et al*., 2010).

Globally, the WHO has reported that rapid weight gain during infancy is becoming increasingly common and is a key driver of the rising prevalence of childhood overweight and obesity, particularly in LMICs undergoing rapid nutrition, economic, and lifestyle transitions (WHO, 2024). The MAL-ED birth cohort study indicated that although wasting was present during early infancy, many children also experienced periods of rapid weight gain associated with feeding practices and exposure to infections, resulting in varying growth trajectories across infancy (MAL-ED Network Investigators, 2017). The South Africa Demographic and Health Survey reported that wasting among children under five was approximately 2.5%, while about 13% were overweight, reflecting the country's growing double burden of malnutrition, in which undernutrition and excess weight coexist (NDoH *et a*l., 2019). Modjadji and Mashishi (2020) reported a wasting prevalence ranging between 5% and 7% among children under five years of age in Limpopo, reflecting the burden of acute malnutrition at the time of their study. More recently, Mugware et al. (2022), in a facility-based study conducted in Thulamela Municipality, found that 60.0% of children aged 0-36 months had a normal weight-for-length z-score, while a substantially higher proportion (33.6%)

were classified as wasted. The marked difference between these estimates may be attributed not only to differences in study design and age groups, but also to the different years in which the studies were conducted, potentially reflecting changes in underlying nutritional, epidemiological, and socio-economic conditions over time.

2.7 Growth status and antibiotic resistance

Stunting and developmental delays significantly compromise an infant's immune system. Shisana *et al*. (2018) observed a strong association between undernutrition and higher rates of infant morbidity due to infectious diseases in South Africa. The combination of undernutrition and frequent infections creates a self-perpetuating cycle of poor growth and weakened immunity. Mokoena *et al*. (2022) emphasised that recurrent infections worsen undernutrition and impair the absorption of essential nutrients, particularly among children without access to clean water and proper sanitation. Chibinga *et al*. (2020) found that waterborne diseases, such as diarrhoea, significantly contribute to a vicious cycle of infection and chronic undernutrition in rural South Africa, further weakening the child's immune system**,** rendering irreversible growth stunting.

The overuse of antibiotics in malnourished children is a growing concern, particularly in low-resource settings. Van der Merwe *et al*. (2021) highlighted that frequent antibiotic use can contribute to AR. Steyn et al. (2020) found that antibiotics are often prescribed for infections that could resolve on their own, exacerbating AR in South Africa. Mungube *et al*. (2022) noted that poor sanitation and hygiene practices raise the risk of infections, leading to unnecessary antibiotic use and further promoting resistance. This over-prescription, coupled with inadequate sanitation and nutrition, creates a breeding ground for antibiotic-resistant bacteria. Undernutrition may compromise antibiotic effectiveness by impairing immune function, altering drug pharmacokinetics, and reducing intestinal absorption, thereby increasing the risk of prolonged infection and treatment failure (Bourke *et al*., 2016). Smit *et al*. (2016) further explain that alterations in T cells compromise the body's ability to clear pathogens, resulting in prolonged or recurrent Chibinga *et al*. (2020) found that frequent antibiotic exposure accelerates the emergence of AR in malnourished children, particularly in areas with high rates of undernutrition and limited access to healthcare.

Addressing AR in infants requires a multifaceted approach that addresses the root causes of undernutrition and infection. Swanepoel *et al*. (2018) emphasised the importance of improving nutrition, particularly through supplementation with essential micronutrients such as vitamin A and zinc. Mokoena *et al*. (2022) stressed that providing clean water and improving sanitation are crucial for reducing the incidence of infections, thereby decreasing the reliance on antibiotics.

2.8 Strategies to promote WASH and feeding practices

Access to adequate, clean, safe water, adequate sanitation, and hygiene practices is widely recognised as essential for child health and survival, as inadequate conditions increase exposure to waterborne pathogens, contributing to diarrhoeal disease, undernutrition, and preventable mortality among infants and young children (WHO and UNICEF, 2023). Globally, these risks are addressed through SDG 6, which aims to ensure universal and equitable access to safe water and sanitation by 2030 (UN, 2015). In South Africa, this commitment is historically grounded in the White Paper on Water Supply and Sanitation Policy (1994), which prioritised universal access in the post-apartheid era and laid the foundation for subsequent reforms (Department of Water Affairs and Forestry, 1994). Contemporary governance is overseen by the Department of Water and Sanitation (DWS), with the Blue Drop Certification Programme serving as a key accountability mechanism for assessing municipal compliance with national drinking water standards and promoting improved risk management and infrastructure maintenance (DWS, 2023). The programme is further integrated into broader reforms such as Operation Vulindlela, aimed at addressing systemic service delivery constraints and accelerating progress toward national and global WASH targets (DWS, 2021).

South Africa has taken cognisance of the strong global evidence supporting exclusive breastfeeding by adopting and localising several international frameworks designed to promote, protect, and support optimal infant feeding practices. These include the

Baby-Friendly Hospital Initiative (BFHI), launched by WHO and UNICEF to institutionalise the "Ten Steps to Successful Breastfeeding" within maternity facilities (WHO and UNICEF, 2018); the International Code of Marketing of Breast-Milk Substitutes, which seeks to restrict unethical marketing practices that undermine breastfeeding (WHO, 1981); and national regulations that domesticate the Code within South Africa's legal framework, notably Regulation R991 under the Foodstuffs, Cosmetics and Disinfectants Act (NDoH, 2012). In addition, maternity protection provisions aligned with International Labour Organization standards support breastfeeding by providing time and income security for postpartum mothers (ILO, 2022). Complementing these structural measures, digital health initiatives such as MomConnect, introduced in 2014, provide breastfeeding information and support directly to mothers through mobile messaging platforms (NDoH, 2014).

2.9 Conclusion

The reviewed literature clearly establishes that infant health outcomes are shaped by the interrelated influences of WASH conditions, infant feeding practices, nutritional status, and antibiotic exposure. Limited access to safe water, adequate sanitation, and proper hygiene significantly increases infants' exposure to enteric pathogens, contributing to diarrhoeal disease, impaired nutrient absorption, and growth faltering. In this context, feeding practices play a critical role, either protective or risk-enhancing. Exclusive breastfeeding during the first six months provides complete nutrition and immunological protection, reducing vulnerability to infection. Conversely, formula feeding, mixed feeding, and the premature introduction of complementary foods may increase infection risk, particularly in environments with inadequate water quality and sanitation.

The interaction between poor WASH conditions, suboptimal feeding practices, recurrent infections, and frequent antibiotic use creates a reinforcing cycle that undermines child health. Repeated infections often lead to unnecessary antibiotic treatment, thereby accelerating the development of antimicrobial resistance. Undernutrition further compounds this cycle by weakening immune function and increasing susceptibility to prolonged or recurrent illness. Together, the evidence underscores the need for integrated, multisectoral strategies that simultaneously strengthen WASH infrastructure, promote optimal feeding practices, address nutritional deficiencies, and support responsible antibiotic stewardship to improve infant growth, survival, and long-term health outcomes.

CHAPTER 3: RESEARCH METHODOLOGY

3.1 Introduction

The study aimed to explore the influence of the two independent variables (WASH and feeding practices) on the two outcome measures, growth and AR in infants aged 0-6 months in Dzimauli rural villages. Figure 3.1 depicts the intended bivariate associations between the independent (Wash and feeding practices) and dependent variables (growth status and AR). This section describes the methods used to carry out the research study. The study design, study area, population, sampling, inclusion and exclusion criteria, subject recruitment, validity and reliability, measured variables, pilot study, data collection, data analysis, ethical considerations, and results dissemination were all thoroughly explained.

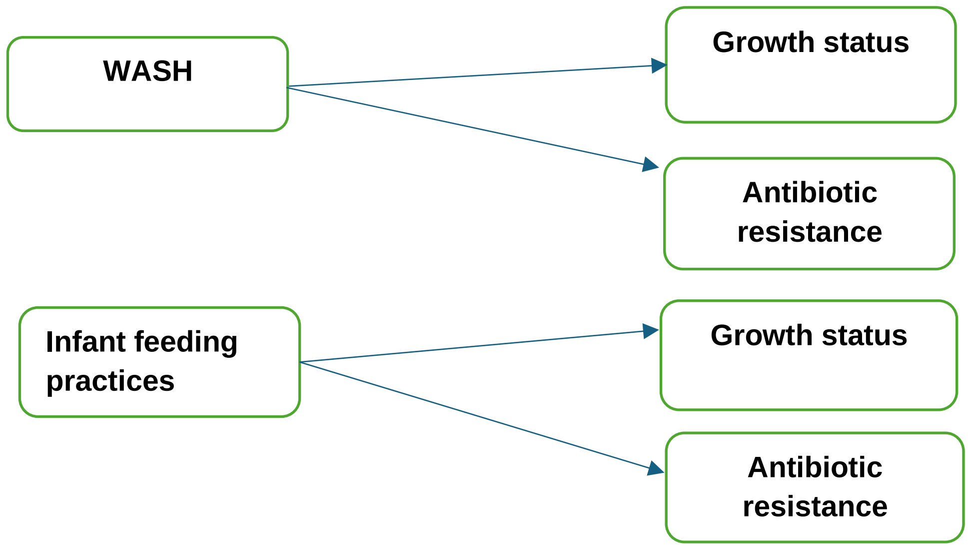

Figure 3.1: Intended associations between independent and dependent variables

This study is part of a broader research study hosted by the SAMRC and the University of Venda Antimicrobial Resistance Global Health Research Institute, titled "Acquisition of AR gut bacteria and impact of microbiota". One of the broader research study aims is to conduct community-based surveillance to understand the time of acquisition, composition, and evolution of the gut resistome, using *E. coli* as a model. This aim will be achieved by determining the *E. coli* resistome in the first 24 months of life. The broader research study will evaluate this monthly from zero to 12 months and then at 15, 18, 21, and 24 months. However, the current study focused only on infants aged

0-6 months to phenotypically describe AR in E. *coli* from monthly-collected normal and diarrhoeal stools.

3.2 Study design

A prospective cohort study design was used in this study. As primary longitudinal studies, prospective cohort investigations follow a defined population over time to establish temporal relationships to track progression from exposure to outcome. In this study, this design was chosen because it observed and collected data on infant-mother pairs, following them from birth to 6 months of age, to investigate relationships among WASH, feeding practices, growth status, and AR (Wang and Kattan, 2020).

This study used descriptive and exploratory research types. A descriptive research design was used to describe WASH, infant feeding practices, growth status, and AR in infants aged 0-6 months (Aggarwal and Ranganathan, 2019). The study explored associations between independent variables, WASH and infant feeding practices, and dependent variables, growth status, and AR in the first six months of life. A quantitative approach was used for data collection and analysis. A quantitative approach is a form of research that draws on the natural sciences, producing numerical data and hard facts (Ahmad *et al*., 2019).

3.3 Study area

The study was conducted in the rural area of Dzimauli, located in the Vhembe region of the Limpopo province of South Africa. The study area included 13 villages. These villages were Mbahela, Mukondeni, Thongwe, Madadani, Muledani, Matshavhawe, Tshibvumo, Tshapasha, Pile, Tshandama, Mutodani, Tshitopeni, and Tshagwa. The terrain of Dzimauli is predominantly hilly and densely vegetated. It is approximately 45 km north of Thohoyandou, the nearest large town and the capital of the Vhembe region. The cohort study conducted by Bessong *et al*. (2014) in the Dzimauli rural area found that only 12% of households treated drinking water, with 46% (~5) of these households using bleach or chlorine for treatment. The study further revealed that the majority of households (95%) relied on pit toilets. Regarding cooking practices, about

61% of participants prepared meals over open fires, and 48% cooked indoors using electric stoves (Bessong *et al*., 2014).

3.4 Population

In this study, the target population consisted of mother-infant pairs. The accessible population was mother-infant pairs with infants aged 0 to 17 days at the time of recruitment, residing in the Dzimauli rural villages.

3.5 Inclusion and exclusion criteria

The criteria for selecting eligible participants in this study are explained below.

3.5.1 Inclusion criteria

The eligibility criteria required that each infant-mother pair reside in the Dzimauli villages to ensure that the study focused on a specific geographic area and its associated environmental and socio-economic factors. Only mothers aged 16 years or older who could provide informed consent were eligible to participate (MAL-ED Network investigators, 2014). This criterion ensured that ethical standards were met and that participants were legally and cognitively capable of understanding the study's purpose and procedures.

Infants were required to have a birth weight of at least 1.8 kg to exclude those with very low birth weights, as such infants may follow distinct health trajectories and require specialised care that could confound the study outcomes. Additionally, infants had to be healthy at the time of recruitment, with no congenital abnormalities or severe neonatal illnesses requiring prolonged hospitalisation. All participating infants were to be born between July 2023 and June 2024 to establish a well-defined cohort, enabling consistent data collection and analysis within a specific time frame.

3.5.2 Exclusion criteria

Mothers who planned to move outside the community within six months of recruitment were excluded. This criterion ensured that participants remained accessible for follow-up assessments and data collection throughout the study period. Mothers who would not be accessible for follow-up or data collection were excluded. This criterion ensured that the study team maintained contact with participants and gathered the necessary data to meet the study objectives.

3.5 Sampling

A convenience sampling strategy was employed in this study because mother-infant pairs were recruited based on their availability and accessibility at selected health facilities during routine clinic visits, rather than through random selection from the entire target population. This approach was considered appropriate given the logistical constraints of working with a vulnerable population, the need to align recruitment with routine postnatal and infant health services, and the absence of a comprehensive sampling frame of all eligible infant-mother pairs within the study area.

A total of 194 mother-infant pairs were approached, of whom 170 were successfully enrolled after eligibility confirmation between July 2023 and June 2024. 156 mother-infant pairs provided complete data suitable for descriptive analyses, and of these, a final analytical sample of 108 infant-mother pairs was used for inferential analyses examining growth outcomes, feeding practices, WASH exposure, and antimicrobial resistance indicators. The final sample size was considered sufficient to achieve adequate statistical power for the primary analyses, as samples of approximately 100-120 observations are generally adequate to detect moderate effect sizes at a 5% significance level with a statistical power of at least 80% in observational studies using regression-based analyses, while allowing for adjustment for potential confounding variables (VanVoorhis and Morgan, 2007).

3.6 Training of field workers

Prior to data collection, seven field workers received comprehensive training to ensure the accurate, consistent, and ethical implementation of study procedures. Field workers were recruited based on predefined inclusion criteria, which required a minimum educational qualification of Grade 12 (matric). Preference was given to individuals with prior experience in community-based research or data collection, particularly in health-related studies. Additional inclusion criteria included fluency in

English and Tshivenda, familiarity with the study area, and the ability to engage respectfully with mothers and infants in a rural setting. The training was facilitated by the principal investigator, with support from subject-matter experts, including nutritionists and microbiologists, and aimed to equip field workers with the necessary knowledge and skills to administer research instruments accurately and interact appropriately with study participants. Its primary aim was to equip the field workers with the necessary knowledge and skills to administer the research instruments effectively and engage appropriately with study participants.

The training began with an overview of the study objectives and the importance of adhering to ethical research standards. Topics such as obtaining informed consent, maintaining participant confidentiality, and respectful interaction were emphasised. Fieldworkers were also introduced to all questionnaires used in the study, namely, the child assessment form (CAF) (Appendix A), the monthly assessment (MOA) (Appendix B), the follow-up socioeconomic status form (FSE) (Appendix C), and the stool collection form (SCF) (Appendix D). Each questionnaire was reviewed in detail to ensure the fieldworkers understood the purpose and proper use of every item.

In addition to theoretical instruction, practical sessions were conducted to reinforce learning. Fieldworkers engaged in role-playing exercises and mock interviews to simulate real-life field conditions. This hands-on approach allowed them to practice accurate anthropometric measurements (including weight and length) and proper procedures for collecting, labelling, and preserving stool samples using Cary-Blair transport medium. At the end of the training, an assessment was administered to evaluate the fieldworkers' comprehension and readiness to begin data collection. The training played a critical role in standardising procedures, minimising data collection errors, and upholding the ethical integrity of the study.

3.7 Subject recruitment

Approval to conduct the study in the Dzimauli villages was first obtained from the DoH through a formal written request (Appendix E). Additional permission was sought and granted by the Dzimauli Royal Authorities (Appendix F) during a community meeting, during which the study's purpose, aims, objectives, and procedures were explained. After receiving the necessary approvals, communication was established with clinic managers in the area to support the recruitment process.

Infant-mother pairs were identified through local clinics and home visits. As the clinics no longer offered delivery services, clinic personnel assisted in identifying women who gave birth in hospitals or at home during the three-day postnatal visit. These mothers were recorded, and their information was shared weekly with the field supervisor for follow-up and home-based recruitment.

Trained field assistants residing in the villages further supported the recruitment process. They used community referrals and conducted door-to-door visits to introduce the study and identify pregnant women. For the present study, new recruitment was not necessary, as data were obtained from the broader research study.

3.8 Measured variables

This section presents the variables measured in this study. In this study, the variables measured included socio-demographic characteristics, infant feeding practices, WASH practices, growth status, and resistome.

3.8.1 Socio-demographic characteristics

Socio-demographic characteristics for the mother and infant were collected. For the mother, the collected data included age, marital status, educational level, monthly income, and the household head's identification. For the infant, the collected data included date of birth, gender, and place of birth.

3.8.2 WASH Practices

The study collected data on water, sanitation and hygiene variables. Hygiene practices data included the mothers' handwashing and food-handling practices. Water data included the availability, source, and utilisation of water within the household. Sanitation data focused on the types of toilet facilities used and the number of individuals sharing these facilities.

3.8.3 Infant feeding practices

The study measured initiation of breastfeeding and exclusive breastfeeding. Measuring infant feeding practices helped the researcher determine whether they aligned with the WHO recommendations.

3.8.4 Growth status

Infant growth status in this study was defined using standard anthropometric measurements, including weight and length, obtained by a trained field worker using calibrated equipment. Weight and length measurements were used to calculate weight-for-age (WAZ), length-for-age (LAZ), weight-for-length (WLZ), and BMI-for-age (BMIZ) z-scores, which were interpreted according to the World Health Organization (WHO) Child Growth Standards (WHO, 2006; 2009). See Table 3.1 for the WHO growth standard classifications and interpretations.

Table 3.1: WHO growth standard classifications (WHO, 2009)

<table>
<tr><th>Z-score</th><th>WAZ</th><th>LAZ</th><th>WLZ</th><th>BMIZ</th></tr>
<tr><td>>+3</td><td rowspan="3">Not suitable for determining overweight</td><td>Extreme tallness</td><td>Obesity</td><td>Obesity</td></tr>
<tr><td>>+2 to ≤ +3</td><td rowspan="4">Normal</td><td>Overweight</td><td>Overweight</td></tr>
<tr><td>≥-1 to ≥ +2</td><td>Possible risk of being overweight</td><td>Possible risk of being overweight</td></tr>
<tr><td>>-1 to ≤ +1</td><td rowspan="2">Normal</td><td rowspan="2">Normal</td><td rowspan="2">Normal</td></tr>
<tr><td>≥ -2 to < -1</td></tr>
<tr><td>≥ -3 to <-2</td><td>Moderate underweight</td><td>Moderate stunting</td><td>Moderate wasting/MAM</td><td>Moderate wasting/MAM</td></tr>
<tr><td><-3</td><td>Severe underweight</td><td>Severe stunting</td><td>Severe wasting/SAM</td><td>Severe wasting/SAM</td></tr>
</table>

MAM: Moderate Acute Malnutrition; SAM: Severe Acute Malnutrition

3.8.5 Resistome

The resistome, representing the collection of antibiotic-resistant genes (ARGs) present in the infants' gut microbiota, was measured using stool samples collected from the infants. Stool collection was conducted monthly from birth until six months of age. In this study, the focus was on assessing the overall abundance and diversity of antibiotic resistance genes rather than targeting individual ARGs, thereby providing a composite measure of the resistome burden across early infancy. This approach is consistent with previous studies demonstrating that early antibiotic exposure and environmental factors are associated with a general increase in faecal ARG load, even when specific resistance genes are not individually characterised (Fadeyi *et al*., 2023).

3.8.6 Instrument development

Data for the study were collected using questionnaires that consisted primarily of closed-ended questions (Appendix A, B, C, and D). The development of the questionnaires was guided by a comprehensive review of relevant literature to ensure both content relevance and validity. To enhance accuracy and reliability, the instruments were reviewed by subject matter experts, including nutritionists and microbiologists. Furthermore, the questionnaires underwent pretesting to identify and address any ambiguities or potential challenges prior to full-scale data collection.

- The CAF (Appendix A) is a structured questionnaire developed to collect baseline information on infants during the first six months of life. The instrument consists of approximately 20 items, covering four domains: mother and infant demographic characteristics, birth-related information, early feeding practices, and anthropometric measurements (weight, length and head circumference). Most items are descriptive and categorical (e.g., sex, place of delivery, birth weight, breastfeeding initiation) and are therefore not scored. As the CAF primarily serves a descriptive and measurement function, no composite score was generated for this instrument.
- The MOA (Appendix B) is a structured questionnaire used to monitor infant feeding, hygiene practices, and anthropometric growth indicators from one to six months of age. The MOA consists of approximately 62 items and is administered monthly by

trained fieldworkers. Feeding practice items include breastfeeding status, frequency of feeds, use of formula or animal milk, introduction of solid or semi-solid foods, and liquid intake.

- The FSE (Appendix C) questionnaire is a structured instrument administered once every six months to assess changes in household and maternal socio-economic conditions. The questionnaire contains 50 questions covering maternal demographic characteristics, reproductive history, household composition, income sources, housing characteristics, water source, sanitation facilities, and hygiene practices. Most items are categorical and descriptive; however, selected WASH and hygiene items were scored to generate composite indicators of household conditions. Where scoring was applied, items were coded so that higher scores indicated better socio-economic or WASH conditions, with reverse-scored items recoded accordingly. Composite scores were converted to percentage scores and interpreted using the same poor-to-excellent classification applied to hygiene practices. Hygiene practices (handwashing and food handling) were analysed using the following scores: poor (0-25%), fair (25-50%), good (50-75%), and excellent (75-100%) (WHO and UNICEF, 2023).
- The SCF (Appendix D) is a structured questionnaire used by fieldworkers to document key details during infant stool sample collection. It has 08 questions. It records information such as participant ID, visit date and time, stool collection status, sample type, preservation time, and fieldworker ID. The form ensured accurate and traceable handling of stool samples for health research. The SCF is a non-scored instrument used exclusively to ensure accurate tracking, traceability, and quality control of biological samples for antimicrobial resistance analysis.

3.8.7 Pre-testing of instruments

All questionnaires were pre-tested at Dzimauli Health Centre. A total of 26 mothers (10% of the intended sample size) who attended the health centre for three-day postnatal check-ups participated in the pre-testing. The data collected during this phase were not included in the final analysis but were instrumental in refining the research instruments. The pre-testing process focused on evaluating the wording, sequencing, and comprehension of questionnaire items, as well as the time required to complete each instrument.

Feedback from participants and fieldworkers' observations were used to refine question phrasing, improve skip patterns, and ensure cultural and contextual appropriateness. Minor modifications were made to enhance clarity and reduce ambiguity, particularly in items related to infant feeding, hygiene, and household conditions. The pre-testing also provided an opportunity to standardise fieldworker administration procedures and ensure consistency in anthropometric measurements. Based on the feedback and observations from the pre-test, the following improvements were made to the questionnaire:

- The inclusion of the month of data collection, and
- The addition of a question to determine whether a stool sample was collected during that specific month.

3.9 Data collection

Data were collected by trained field workers during scheduled home visits to participating mother-infant pairs to maximise convenience and minimise loss to follow-up. Prior to any data collection, contact was established with eligible mothers either through the collaborating health facilities or via previously obtained contact information, and appointments were arranged at a time suitable for the participant. Upon arrival at the household, field workers introduced themselves, explained the study's purpose, and verified participant eligibility. Mothers were then provided with detailed information about the study objectives, procedures, potential risks and benefits, confidentiality measures, and their right to withdraw from the study at any time without consequence. Written informed consent was obtained from all participating mothers before any data collection commenced.

Following consent, the field worker requested a quiet, private, and comfortable space within the home to ensure confidentiality and minimise distractions during the data collection process. Anthropometric measurements of the infants, including weight and recumbent length, were taken using standardised equipment and protocols. Infant weight was measured using a calibrated digital infant scale, with the infant lightly clothed or undressed, while length was measured using a portable infant length board with the infant positioned according to WHO-recommended techniques. All measurements were taken by trained field workers to ensure accuracy and consistency, and repeated where necessary to minimise measurement error. This standardised approach was employed to ensure the reliability and comparability of growth data across participants and across repeated monthly visits throughout the study period.

Data accuracy and completeness were ensured through multiple quality-control measures implemented throughout the data collection and management process. During fieldwork, questionnaires and anthropometric records were reviewed immediately after completion by the field workers to confirm that all sections were fully completed and that responses were legible and logically consistent. Where missing or unclear responses were identified, clarification was sought from the participant during the same visit to minimise recall bias and missing data.

The stool samples were collected from the infants into sterile containers on the day of collection. The trained field worker returned the following day if the infant had not defecated the previous day of stool collecyion. The stool samples were then transported the same day to the laboratory in a biosafety transport box and homogenised there to ensure even distribution of bacteria. The homogenised stool samples were plated onto selective and differential agar plates to isolate and identify potential antibiotic-resistant bacteria. The plates were incubated under conditions suitable for the growth of the target bacteria (typically 37°C for 18-24 hours). A Mueller-Hinton agar plate was inoculated with a bacterial suspension, antibiotic-impregnated disks were placed on the surface, and the plates were incubated. The inhibition zones around the disks were measured to determine susceptibility or resistance from the stool samples.

3.10 Data collection procedures

Infant weight was measured using a Seca 354 pan infant scale placed on a flat, stable surface. The scale was checked for zero balance before each measurement. Infants were weighed with minimal clothing (usually only a dry diaper) to reduce variability caused by clothing weight. Each infant was gently placed in the centre of the scale tray in a supine position, ensuring they remained still and did not touch any other surface. The weight was recorded to the nearest 0.01 kg once the reading stabilised. To enhance reliability, two measurements were taken for each infant. If the difference between the two readings exceeded 100 grams, a third measurement was taken, and the average of the two closest readings was used as the final weight. The procedure followed the standardised anthropometric guidelines recommended by Lee and Nieman (2010).

A Seca 417 baby measuring board (0-100 cm) was used to measure the infant's recumbent length once per month from birth to 6 months. The measuring board was placed on a flat surface, and the infants lay face-up on it with the crown of the head touching the headboard. A head position was maintained during measurement. The infant's legs were extended at the hips and knees, and the infant was made to remain flat on the board while the movable board was adjusted against the heels. Two length measurements were taken, and the average length was recorded to the nearest 0.1cm. The procedure followed the standardised anthropometric guidelines recommended by Lee and Nieman (2010).

Monthly stool sample collection was synchronised with the completion of the MOA (Appendix B) and took place on or around the monthly anniversary of the child's birth (±2 days). For example, if a child was born on February 17th, the scheduled visits occurred between the 15th and 19th of each subsequent month. It was critical that both the stool sample and the MOA (Appendix B) form were completed within this ±2-day window to maintain protocol fidelity and data consistency. However, if it was not possible to conduct the visit within this timeframe despite best efforts, the fieldworker was permitted to conduct it up to 7 days after the child's birthdate. Stool samples were collected for six months.

3.11 Validity and reliability

Validity is the degree to which the study accurately measures what it intends to measure (Middleton, 2019). Reliability refers to the extent to which consistent results can be obtained when a study is repeated under the same conditions (Middleton, 2019).

3.11.1 Validity

The researchers meticulously developed questionnaires that aligned with the study's objectives and the relevant literature. Before collecting data, pre-testing was done in the Dzimauli Health Centre to ensure the questionnaire was valid. Nutrition specialists and the research supervisors evaluated the questionnaire's content to assess its relevance and coverage.

3.11.2 Reliability

In this study, several measures were taken to ensure the reliability of data collection. All fieldworkers were thoroughly trained in the proper administration of questionnaires and in standardised procedures for collecting stool samples and anthropometric measurements. Anthropometric data such as weight and length were measured twice for each infant to reduce measurement error and enhance accuracy. Stool samples were collected, labelled, stored, and transported in accordance with strict protocols, using Cary-Blair medium to ensure consistency across all samples. Furthermore, a biotechnologist conducted routine quality assurance checks throughout the data collection process to identify and correct any inconsistencies or errors. These measures helped to ensure that the data collected were consistent, repeatable, and dependable across different time points and among different fieldworkers.

3.12 Data analysis

The data was cleaned, coded, and entered into the Microsoft Excel 2022 spreadsheet. Descriptive statistics were calculated to describe participants' demographics and water and sanitation using the Statistical Package for the Social Sciences (SPSS) version 27. Hygiene practices (handwashing and food handling) were analysed using the following scores: poor (0-25%), fair (25-50%), good (50-75%), and excellent (75-100%) (WHO and UNICEF, 2023). Infant growth status was calculated using the WHO reference growth standards z-scores in WHO Anthro version 3.2.2 (2009).

Significance levels were set at $p < 0.05$ and $p < 0.01$. Resistome was determined by measuring the minimum inhibitory concentration (MIC) of antibiotics. Bacteria were inoculated into broth containing serial dilutions of antibiotics, and growth was observed. The results were compared against clinical breakpoints provided by guidelines to determine if the bacteria are resistant or susceptible to the antibiotics tested (Bakthavatchalam *et al*., 2024).

A total of 40 imputed datasets were generated under the assumption of missing-at-random (MAR). Associations between WASH practices and infant growth outcomes were analysed using Stata version 18. To assess the association between WASH practices and infant growth outcomes, generalized estimating equations (GEE) were applied using an independent working correlation structure across four progressively adjusted models, allowing for appropriate handling of repeated measurements within infants over time. The growth outcomes included LAZ, WAZ, weight-for-length z-score WLZ, and BMIZ. Possible predictor variables included hygiene and food-handling scores, infant sex, mothers' marital status, and age. Four GEE models with robust standard errors clustered by infant were specified to account for repeated measures. Model 1 included the hygiene score and the number of months. Model 2 included the food-handling score. Model 3 adjusted for infant sex, and Model 4 further included maternal age and marital status.

A multivariable logistic regression was conducted to determine factors associated with MDR among children. Several variables could not be estimated (NE) due to complete separation or sparse data resulting from the limited analytical sample and were acknowledged as limitations. Due to time and financial constraints, laboratory analysis could only be conducted on 55 isolates obtained from infants. Among these, 33 had complete WASH practice data and were included for inferential statistics. From 55, there was a further reduction to 23-28 observations in multivariable models due to missing covariate data and complete separation in sparse subgroups.

3.13 Ethical considerations

This study was conducted in accordance with the ethical principles for medical research involving human participants as outlined in the Declaration of Helsinki, including respect for persons, beneficence, non-maleficence, and justice. The broader project first received ethical approval from the University of Venda Research Ethics Committee, in accordance with all institutional procedures (certificate number: FSEA/22/MBY/04/0302). Thereafter, ethical permission to conduct the study was obtained from the Limpopo DoH (Appendix E) and the Dzimauli traditional authorities (Appendix F). The research proposal for this study was first submitted to the Departmental Research Committee for review and approval. Thereafter, the proposal was submitted to the Higher Degrees and Ethics Committee for ethical review. Final ethical clearance was subsequently granted under certificate number FHS/24/NUT/21/1610 (Appendix G).

Privacy and confidentiality were strictly maintained throughout the study period. Data were collected in the privacy of the participants' homes, and interviews were conducted in a manner that ensured confidentiality and minimised discomfort or intrusion. Completed questionnaires were stored in a locked room, accessible only to the research team, while electronic data were securely stored in a password-protected computer application. Personal identifiers were removed and replaced with unique study codes, and all analyses and reports were conducted using anonymised data.

Informed consent (Appendix H) was obtained from all participating mothers before enrolment. Participants were provided with clear verbal and written information about the study objectives, procedures, potential risks and benefits, and the voluntary nature of participation, in a language they understood. Participants were informed that they could withdraw from the study at any time without any penalty or loss of access to healthcare services, and this right was reiterated during follow-up visits.

The principle of justice was upheld by ensuring fair and equitable selection of participants. All eligible mother-infant pairs within the study area were given an equal opportunity to participate, and no group was unfairly excluded or overburdened.

Recruitment was based solely on predefined inclusion criteria, not on socioeconomic status, ethnicity, or perceived vulnerability. The study posed minimal risk to participants, and no invasive procedures beyond routine anthropometric measurements and stool sample collection were conducted. Participants did not receive financial incentives that could unduly influence participation.

Participants were informed about how the study findings would be used and disseminated. The results will be shared with local clinics and health centres in Dzimauli, district health authorities, and the broader scientific community through reports, presentations, and academic publications. Importantly, all disseminated findings will be presented in aggregate form, ensuring that individual participants and households remain unidentifiable. Although participants did not receive direct clinical treatment as part of the study, they benefited from regular growth monitoring, increased awareness of infant feeding and hygiene practices, and referrals to health services where concerns were identified.

3.14 Dissemination of results

The results of this study will be shared with clinics and health centres in rural villages of Dzimauli. A written summary report highlighting key findings, implications for infant growth, WASH practices, and antibiotic resistance will be prepared in clear, non-technical language and shared with clinic managers and professional nurses at participating health facilities. Where feasible, printed copies of the summary will be distributed, and electronic versions will be shared via email or messaging platforms commonly used by clinic staff. The results will also be shared with traditional leaders and the Dzimauli community members at large. The researcher will present the results at local, national, and international conferences. Additionally, the results will be shared with the SAMRC-UNIVEN Antimicrobial and Global Health Research Unit. Efforts will be made to publish the results in accredited, peer-reviewed journals.

CHAPTER 4: RESULTS

4.1 Introduction

This chapter presents results derived from data collected from mother-infant pairs residing in the Dzimauli rural villages. The study aimed to explore the influence of water, sanitation and hygiene (WASH) and infant feeding practices on infant growth and AR during the first six months of life. The results include the socio-demographic characteristics of mother-infant pairs, WASH and infant feeding practices, infant growth status, AR patterns, and associations among selected study variables.

4.2 Socio-demographic characteristics

Table 4.1 presents the socio-demographic characteristics of mother-infant pairs included in the study. The results indicate that over half of the infants in the study were girls (80; 51.3%), while boys accounted for 48.7% (76). Almost all infants (150; 96.1%) were born at full term, and 150 (96.1%) were delivered in health facilities. Most of the mothers (68; 43.6%) were aged 25-35 years, while 39.1% (61) were aged 16-24 years. Over half of the mothers (92; 59.0%) were never married, while 62 (39.7%) were married. Three-quarters of the mothers (117; 75.0%) had secondary education (Grades 8-12), while a few (30; 19.2%) had attained tertiary education from colleges and universities.

Table 4.1: Socio-demographic characteristics of mother-infant pairs (n=156)

Variable	n	%
Infants		
Gender		
Boy	76	48.7
Girl	80	51.3
Duration of pregnancy		
Full-term	150	96.1
Preterm	6	3.9
Place of birth		
Health facility	150	96.1
Home	6	3.9
Mothers		
Age (years)		
16 to 24	61	39.1
25 to 35	68	43.6
36 to 45	27	17.3
Marital status		
Never married	92	59.0

Variable	n	%
Married	62	39.8
Divorced	1	0.6
Widowed	1	0.6
Level of education		
Primary (Grades R to 7)	9	5.8
Secondary (Grades 8 to 12)	117	75.0
Tertiary (College and university qualifications)	30	19.2

4.3 WASH practices of mothers

This section presents results on mothers' WASH practices. The WASH components assessed in the study include household water sources, availability, and collection; sanitation, focusing on toilet facilities; and handwashing practices, which focused on washing and food-handling practices.

4.3.1 Household water sources, availability, and collection

Household water sources, availability, and collection practices reported by 156 participating mothers are presented in Table 4.2. More than half of the households (88; 56.4%) relied on piped water to the yard or plot as their main source, followed by surface water sources like rivers, dams, lakes, ponds, or streams (38; 24.4%). Most (109; 69.9%) of the mothers reported experiencing water interruption, of whom fewer than half (54; 49.5%) reported interruption for 1 to 3 days in succession. More than half of the households (85; 54.5%) relied on piped water to the yard or plot for other purposes, including cleaning, washing, bathing, and handwashing, while surface water sources were used by 25.7% (40). When mothers were asked about the time spent collecting water, 73.1% (38) spent 10 minutes or less, while 19.2% (10) spent 11 to 20 minutes. Mothers reported that adult women in the households were responsible for water collection (151; 96.8%).

Table 4.2: Household water sources, availability, and collection

Variable	n	%
Main source of drinking water (n=156)		
Household borehole	8	5.1
Piped into the household	1	0.6
Piped to yard/plot	88	56.4
Protected well	2	1.3
Public tap	3	1.9
River/dam/lake/pond/stream	38	24.4
Unprotected well	17	11.0
Piped water availability (n=156)		
Always available	47	30.1
Sometimes interrupted	109	69.9
Duration of interruptions to the piped water supply (n=109)		
One to three days	22	20.1
Four to six days	54	49.5
A week	26	23.9
More than a week	7	0.6
Main source of water for other purposes (n=156)		
Piped to yard/plot	85	54.5
Public tap	3	1.9
Borehole	9	5.8
Protected well	3	1.9
Unprotected well	16	10.3
Surface water (river/dam/lake/pond/stream	40	25.7
Time taken to collect water (minutes) (n=52)		
≤ 10 minutes	38	73.1
11–20 minutes	10	19.2
21–30 minutes	1	1.9
> 30 minutes	3	5.8
Person responsible for water collection (n=156)		
Adult woman	151	96.8
Adult man	4	2.6
Children	1	0.6

4.3.2 Sanitation practices

The sanitation results, focusing on toilet facilities, are presented in Table 4.3. The main source of toilet facilities reported by 90.4% (141) of the mothers is a pit latrine, 7.7% (12) used a flush toilet, and 1.9% (3) reported that they did not have a toilet in their households. Two-thirds of the mothers (106; 68.0%) always used toilet paper in their households, while 28.2% (44) used it sometimes. The majority of the mothers (143; 91.7%) indicated that they do not share toilet facilities with other households.

Table 4.3: Sanitation practices (n=156)

Variable	n	%
Type of toilet facility used		
No facility/bush/field	3	1.9
Pit latrine	141	90.4
Flush to piped sewer system	4	2.6
Flush to septic tank	8	5.1
Use of toilet paper		
Never	6	3.9
Sometimes	44	28.1
Always	106	68.0
Sharing of the toilet facilities with other households		
No	143	91.7
Yes	13	8.3

4.3.3 Handwashing practices

This section presents the results of mothers' handwashing and food-handling practices.

4.3.3.1 Handwashing practices

Table 4.4 presents the results on the frequency of handwashing practices at critical points during months 1, 2 and 6 among mothers in the study. Over two-thirds of the mothers reported always washing their hands before eating at month 1 (119; 76.3%), month 2 (121;77.6 %), and month 3 (82; 78.1%). More than half of the mothers reported always washing their hands before and after feeding their infants in month 1 (86; 55.1%), month 2 (78; 50.0%), and month 3 (61; 58.1%). Always washing hands before and after nappy changing was highest in month 1 (81; 51.9%) but declined to 44.8% (47) by month 6, while sometimes washing hands became the most common practice in month 3 (87; 55.8%) and month 6 (56; 53.3%). Water remained the most used handwashing method across the study period, reported by 50.0% (78) of mothers in month 1, increasing to 56.1% (87) in month 3, and slightly declining to 48.6% (51) in month 6.

Table 4.4: Frequency of handwashing practices

Handwashing practices	Month 1	Month 3	Month 6
Handwashing before eating (n=156)			
Never	4 (2.6)	3 (1.9)	1 (1.0)
Rarely	1 (0.6)	0 (0.0)	1 (1.0)
Sometimes	32 (20.5)	31 (19.9)	21 (20.0)
Always	119 (76.3)	121 (77.6)	82 (78.1)
Handwashing before and after feeding (n=156)			
Never	10 (6.4)	5 (3.2)	3 (2.9)
Rarely	2 (1.3)	2 (1.3)	2 (1.9)
Sometimes	58 (37.2)	70 (44.9)	39 (37.1)
Always	86 (55.1)	78 (50.0)	61 (58.1)
Handwashing before and after nappy changing (n=156)			
Never	7 (4.5)	1 (0.6)	1 (1.0)
Rarely	0 (0.0)	1 (0.6)	1 (1.0)
Sometimes	68 (43.6)	87 (55.8)	56 (53.3)
Always	81 (51.9)	66 (42.3)	47 (44.8)
Methods of handwashing	**n=156**	**n=155**	**n=105**
Water only	78 (50.0)	87 (56.1)	51 (48.6)
Water and soap	61 (39.1)	52 (33.5)	46 (43.8)
Warm water and soap	17 (10.9)	16 (10.3)	8 (7.6)

Figure 4.1 illustrates handwashing practice scores for months 1 (n=156), 3 (n=155), and 6 (n=104). Overall, most mothers reported good to excellent practices, while only a few reported excellent practices throughout the study period.

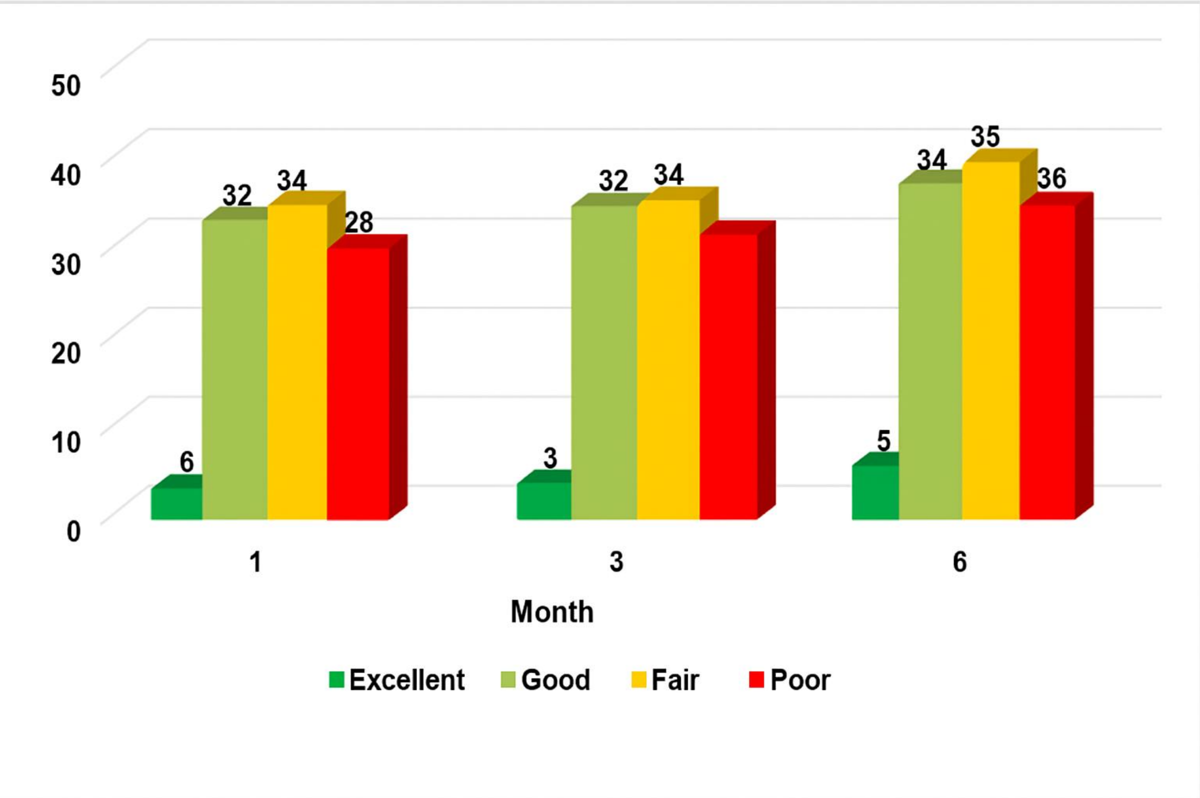

Figure 4.1: Handwashing practices scores by months

4.3.3.2 Food handling practices

Mothers were asked about handwashing practices during food handling, and the results are presented in Table 4.5. The percentage of mothers who always washed their hands when handling raw food increased from 32.1% (50) in month 1 to 44.2% (46) in month 6. The prevalence of mothers who always washed their hands before cooking increased from 37.1% (58) in month 1 to 47.2% (49) in month 6. Most mothers sometimes washed fruits and vegetables before eating, increasing from 61.5% (96) in month 1 to 71.2% (74) in month 6. The practice of always separating raw and cooked foods increased from 53.2% (83) in month 1 to 68.3% (71) in month 6. Most mothers reported storing leftover food in the refrigerator within two hours, from 47.4% (74) in month 1 to 64.4% (76) in month 6.

Table 4.5: Food handling practices

Food handling practices	Month 1 (n= 156)	Month 3 (n=155)	Month 6 (n=104)
Handwashing when handling raw food			
Always	50 (32.1)	59 (38.1)	46 (44.2)
Sometimes	94 (60.3)	91 (58.7)	53 (51.0)
Rarely	12 (7.7)	5 (3.2)	5 (4.8)
Handwashing before cooking			
Always	58 (37.2)	59 (38.1)	49 (47.1)
Sometimes	88 (56.4)	94 (60.6)	51 (49.0)
Rarely	10 (6.4)	2 (1.3)	5 (4.8)
Use separate utensils for raw and ready-to-eat foods			
Always	75 (48.1)	83 (53.5)	64 (61.5)
Sometimes	65 (41.7)	53 (34.2)	29 (27.9)
Rarely	16 (10.3)	19 (12.3)	12 (11.5)
Cleaning food preparation areas, equipment and utensils			
Always	95 (60.9)	98 (63.2)	80 (76.9)
Sometimes	59 (37.8)	56 (36.1)	25 (24.0)
Rarely	1 (0.6)	1 (0.6)	-
Washing fruits and vegetables before eating			
Always	56 (35.9)	49 (31.6)	29 (27.9)
Sometimes	96 (61.5)	100 (64.5)	74 (71.2)
Rarely	4 (2.6)	5 (3.2)	2 (1.9)
Separating raw and cooked foods			
Always	83 (53.2)	83 (53.5)	71 (68.3)
Sometimes	67 (42.9)	68 (43.9)	33 (31.7)
Rarely	6 (3.8)	4 (2.6)	1 (1.0)
Storing leftovers for two hours in the refrigerator			
Always	69 (44.2)	80 (51.6)	-
Sometimes	74 (47.4)	67 (43.2)	67 (64.4)
Rarely	5 (3.2)	5 (3.2)	36 (34.6)

Figure 4.2 illustrates food handling practice scores for months 1 (n = 156), 3 (n = 155), and 6 (n = 104). Poor food handling practice scores were observed in month 1 (69; 44.2%), month 3 (56; 36.1%), and month 6 (47; 44.7%), while less than a quarter of mothers reported excellent food handling practices in month 1 (25; 16.0%), month 3 (19; 12.2%), and month 6 (14; 13.3%).

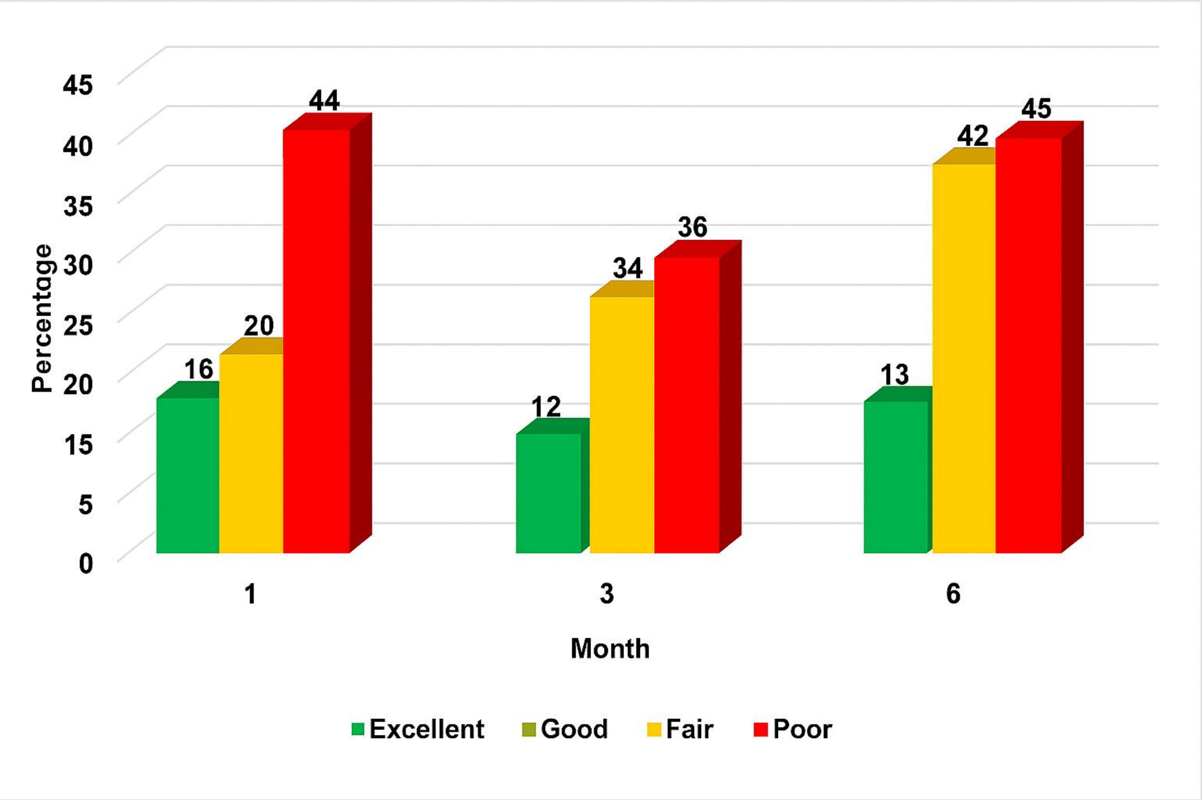

Figure 4.2: Food handling practices scores by month

4.4 Infant feeding practices

This section presents results on infant feeding practices, focusing on breastfeeding initiation and exclusive breastfeeding.

4.4.1 Breastfeeding initiation

Table 4.6 presents breastfeeding initiation practices among mothers in the study. Most of the mothers (95; 61.7%) reported initiating breastfeeding within an hour of birth. Almost all infants (149; 95.5%) received colostrum, while fewer than half (56; 41.7%) received prelacteal feeds.

Table 4.6: Breastfeeding initiation

Variable	n	%
Breastfeeding initiation time (n=154)		
Within one hour	95	61.7
One to 24 hours	53	34.4
One to three days	5	3.2
More than three days	1	0.6
Colostrum (n=156)		
No	7	4.5
Yes	149	95.5
Pre-lacteal feeding (n=156)		
No	91	58.3
Yes	65	41.7

4.4.2 Exclusive breastfeeding practices

The results on exclusive breastfeeding practices show a decline from 71.0% (111) in month 1 to 2.0% (3) in month 6. Figure 4.3 illustrates exclusive breastfeeding (EBF) practices over the period of six months of life.

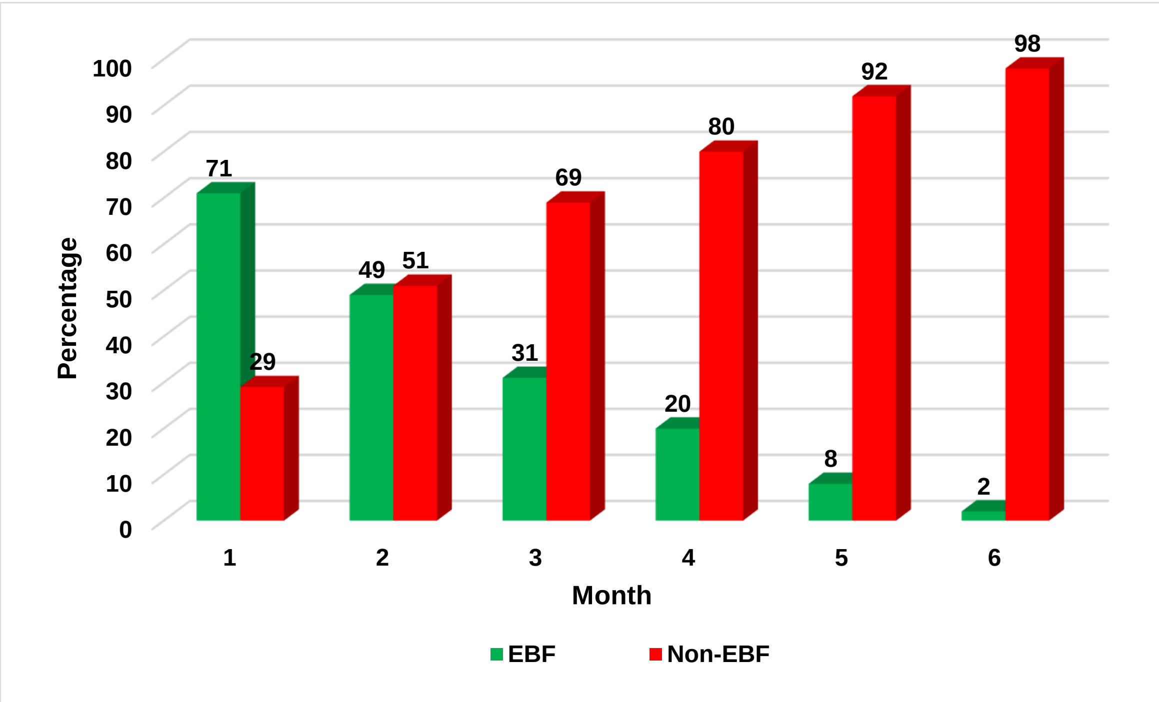

Figure 4.3: Exclusive Breastfeeding duration by month

EBF: Exclusively breastfed; Non-EBF: Not exclusively breastfed

4.5 Growth status of the infants

Infant growth status in the first six months of life was measured using the WHO growth indicators (2006), including length-for-age, weight-for-age, length-for-weight, and BMIZ. The number of infants measured varied by month: 156 in months 1 and 2, 155 in month 3, 150 in month 4, 141 in month 5, and 105 in month 6.

4.5.1 Length-for-age

The LAZ results for infants are presented in Figure 4.4. Most infants across all months had normal length-for-age; however, it fluctuated from 85.0% (132) in Month 1 to 71.0% (110) in Month 6. The stunting rate was below 20.0% in all months.

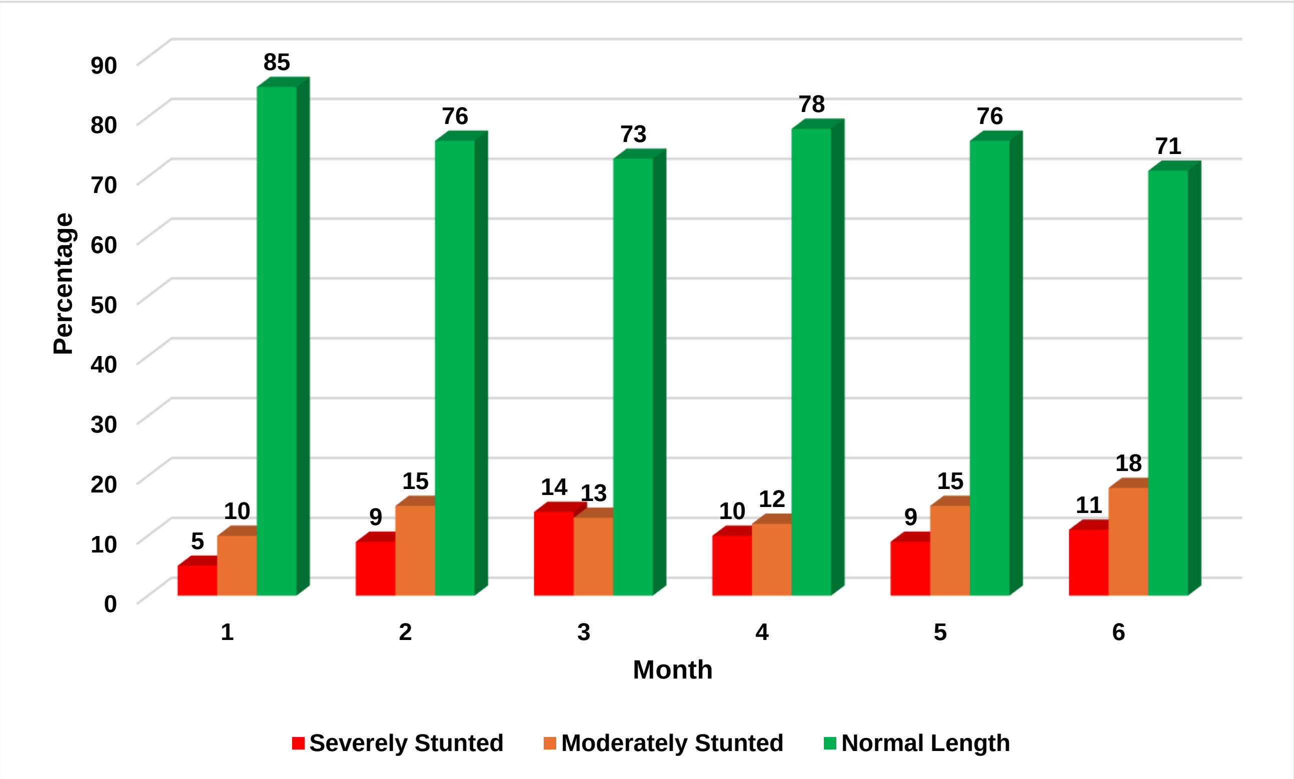

Figure 4.4: Length-for-age by month

Length-for-age classification (WHO, 2009): LAZ <-3 SD = severely stunted; LAZ <-2 SD to ≥ −3 SD = moderately stunted; and LAZ ≥ −2 SD to ≤ +2 SD = normal.

4.5.2 Weight-for-age

Figure 4.5 presents the results for WAZ categories among infants across six months. The results show that most infants maintained normal weight-for-age throughout the six-month period; however, a slight decrease was observed at month 6 to 85.0% (89). Moderate underweight fluctuated over time, increasing from 8.0% (13) in month 1 to 11.0% (12) in month 6, while severe underweight remained low throughout the study period, ranging between 2.0% and 4.0%.

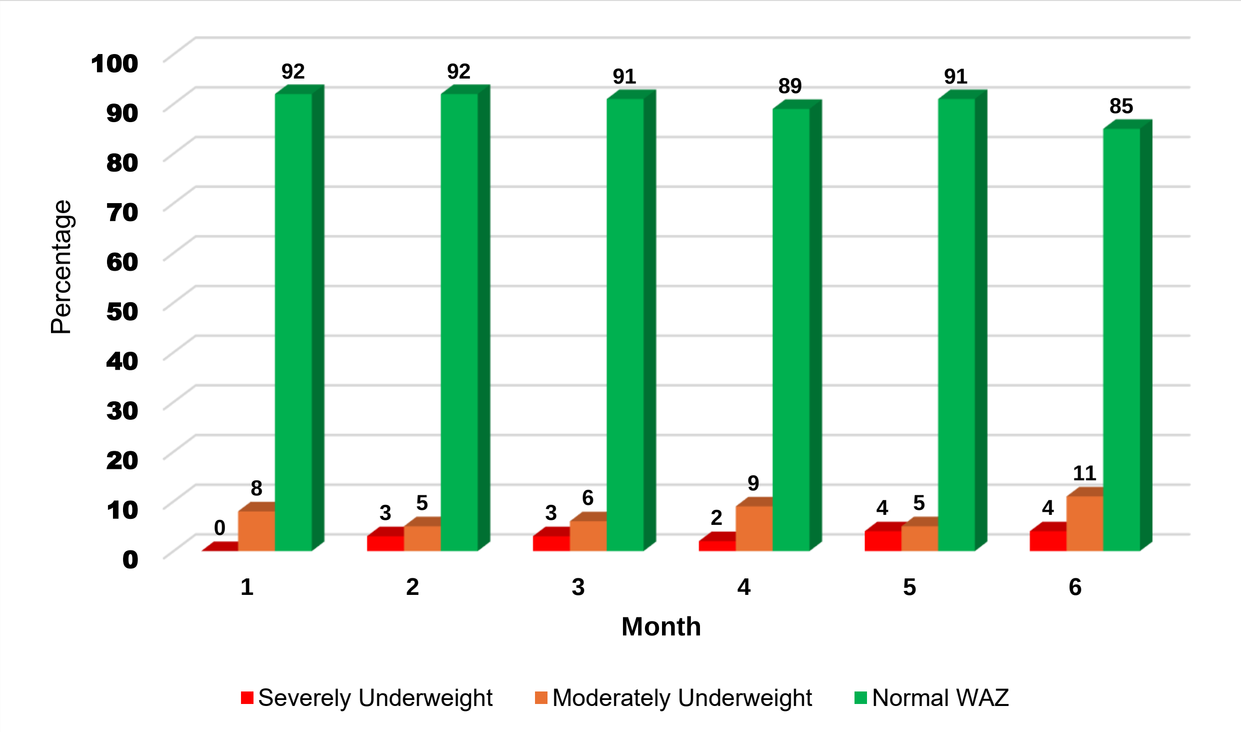

Figure 4.5: Weight-for-age by month

Weight-for-age classification (WHO,2009): WAZ < −3 SD = severely underweight; WAZ < −2 SD to ≥ −3 SD = moderately underweight; WAZ ≥ −2 SD to ≤ +2 SD = normal

4.5.3 Weight-for-length

Figure 4.6 shows that infants with normal weight-for-length had the highest percentages across the six months, with the lowest percentage in month 1 (76; 43.0%) and the highest in month 6 (63; 60.0%). Most infants were at risk of being overweight in month 3 (47; 31.0%). The percentage of infants who were overweight ranged from 20.0% (31) in month 1 to 13.0% (20) in month 3, while obesity was observed in less than 10.0% of infants across the six-month period.

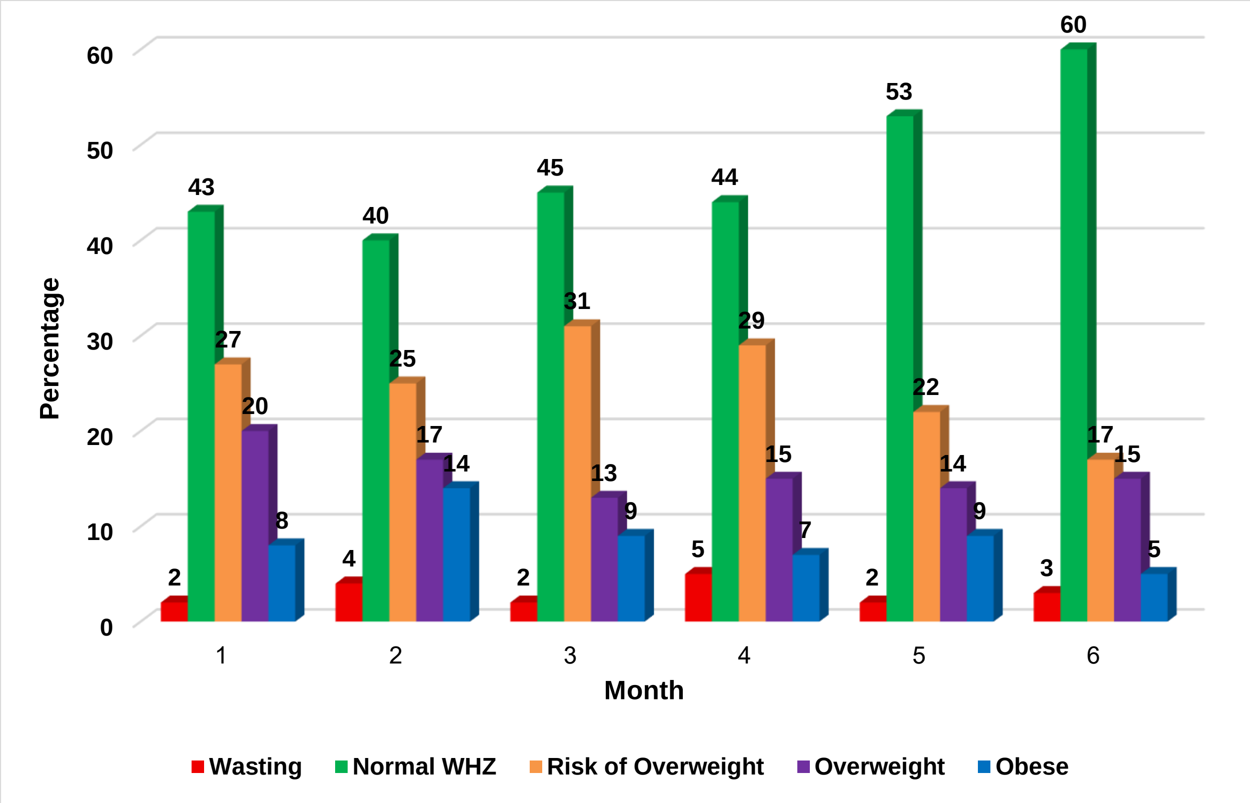

Figure 4.6: Weight-for-length by month

Weight-for-length classification based on WHO Child Growth Standards: wasting (includes moderate wasting: BAZ < −2 SD to ≥ −3 SD, and severe wasting: BAZ < −3 SD); WLZ ≥ −2 SD to ≤ +1 SD = normal; WLZ > +1 SD to ≤ +2 SD = risk of overweight; WLZ > +2 SD to ≤ +3 SD = overweight; WLZ > +3 SD = obese.

4.5.4 BMI-for-age

Figure 4.7 shows the BMIZ results of infants. The results indicate that most infants had normal BMIZ, ranging from 46.0% (72) in month 1 to 60.0% (63) in month 6, followed by those at risk of overweight, which fluctuated from 31.0% (49) in month 1 to 21.0% (22) in month 6. The prevalence of obesity among these infants was very low, below 10.0% in all the months.

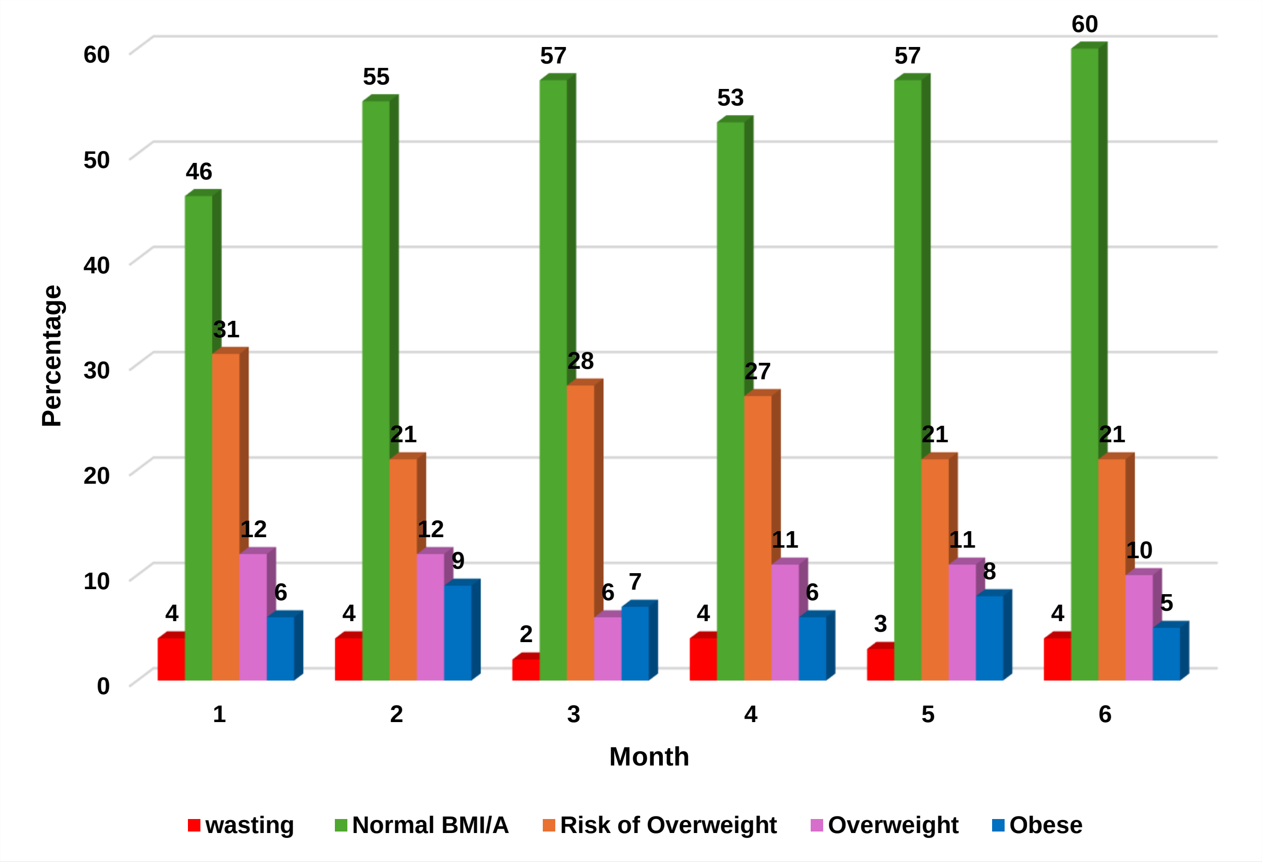

Figure 4.7: BMI-for-age by month

BMI-for-age classification based on WHO Child Growth Standards: wasting (includes moderate wasting: BAZ < −2 SD to ≥ −3 SD, and severe wasting: BAZ < −3 SD); BAZ ≥ −2 SD to ≤ +1 SD = normal; BAZ > +1 SD to ≤ +2 SD = risk of overweight; BAZ > +2 SD to ≤ +3 SD = overweight; BAZ > +3 SD = obese.

4.6 Prevalence of antibiotic resistance among infants

The prevalence of AR among 55 infants, determined using faecal samples, is presented in Table 4.7. The results show varying resistance patterns across antibiotics, with several antibiotics showing high levels of resistance or intermediate susceptibility. High resistance was observed for tetracycline and cefepime, each affecting 70.9% (39) of infants, while resistance to cefoxitin was recorded in 43.6% (24) and gentamicin in 34.5% (19) of infants. Lower resistance levels were observed for aztreonam (23.6%; 13), ertapenem (21.8%; 12), ciprofloxacin (16.4%; 9), ceftriaxone (9.1%; 5), and cefotaxime (5.5%; 3). Overall, multidrug resistance (MDR) was detected in 23.6% (13) of infants, while 67.3% (37) showed no multidrug resistance.

Table 4.7: Prevalence of antibiotic resistance among infants (n=55)

Antibiotic	n	%
Gentamicin (GM)		
Resistant	19	34.5
Intermediate	18	32.7
Susceptible	18	32.7
Tetracycline (TS)		
Resistant	39	70.9
Intermediate	11	20.0
Susceptible	5	9.1
Aztreonam (ATM)		
Resistant	13	23.6
Intermediate	6	10.9
Susceptible	17	30.9
Ertapenem (ETP)		
Resistant	12	21.8
Intermediate	25	45.5
Susceptible	18	32.7
Cefoxitin (FOX)		
Resistant	24	43.6
Intermediate	23	41.8
Susceptible	7	12.7
Ciprofloxacin (CIP)		
Resistant	9	16.4
Intermediate	7	12.7
Susceptible	35	63.6
Cefotaxime (CTX)		
Resistant	3	5.5
Intermediate	42	76.4
Susceptible	10	18.2
Cefepime (CPM)		
Resistant	39	70.9
Intermediate	19	34.5
Susceptible	18	32.7
Ceftriaxone (CRO)		
Resistant	5	9.1
Intermediate	39	70.9
Susceptible	11	20.0
Multidrug resistance (MDR) status		
No	37	67.3
Yes	13	23.6

4.7 Associations of selected variables

This section presents the association of selected variables, including socio-demographic characteristics, WASH practices, infant feeding practices, infant growth status, and AR.

4.7.1 Infants' growth status at six months by breastfeeding

Table 4.8 indicates that at six months of age, most infants who were not exclusively breastfed had normal growth indicators. Among the 103 non-exclusively breastfed infants, 18.4% were moderately stunted, and 10.7% were severely stunted, while 11.7% were moderately underweight and 3.9% were severely underweight. Although wasting was relatively uncommon, some infants were classified as overweight (15.5%) or obese (4.9%) based on weight-for-length. BMI-for-age was 21.4% were at risk of overweight, with smaller proportions classified as overweight (10.7%) or obese (4.9%). Exclusively breastfed infants (n = 2) had normal values across all growth indicators.

Table 4.8: Infants' growth status at six months by breastfeeding

Interpretation	Cut-off points	Not exclusively breastfed (n=103)	Exclusively breastfed (n=2)
Length-for-age (LAZ)			
Moderately stunted	< -2 to ≥ -3 SD	19 (18.4)	-
Severely stunted	< -3 SD	11 (10.7)	-
Normal length-for-age	≥ -2 to ≤ +2 SD	73 (70.9)	2 (100.0)
Weight-for-age			
Moderately underweight	< -2 to ≥ -3 SD	12 (11.7)	-
Severely underweight	<-3 SD	4 (3.9)	-
Normal weight-for-age	≥ -2 to ≤ +2 SD	87 (84.5)	2 (100.0)
Weight-for-length			
Severely wasted	< -3 SD	1 (1.0)	-
Moderately wasted	< -2 to ≥ -3 SD	2 (1.9)	-
Normal weight-for-length	≥ -2 to ≤ +1 SD	61 (59.2)	2 (100.0)
Risk of overweight	> +1 to ≤ +2 SD	18 (17.5)	-
Overweight	+2 to ≤ +3 SD	16 (15.5)	-
Obese	> +3 SD	5 (4.9)	-
BMI-for-age (BMIZ)			
Severely wasted	< -3 SD	1 (1.0)	-
Moderately wasted	< -2 to ≥ -3 SD	3 (2.9)	-
Normal BMI-for-age	≥ -2 to ≤ +1 SD	61 (59.2)	2 (100.0)
Risk of overweight	> +1 to ≤ +2 SD	22 (21.4)	-
Overweight	> +2 to ≤ +3 SD	11 (10.7)	-
Obese	> +3 SD	5 (4.9)	-

4.7.2 Handwashing practices score of mothers by infants' growth status

Table 4.9 indicates that normal growth classifications were most common across all indicators. The highest proportion of infants with normal BMIZ was observed among infants whose mothers had good handwashing practice scores (26; 24.8%), while normal length-for-age was also most common among infants whose mothers had good handwashing practice scores (29; 27.6%). In addition, normal weight-for-age was highest among infants whose mothers had fair handwashing practice scores (34; 32.4%), whereas normal weight-for-length was most frequent among infants whose mothers had good handwashing practice scores (25; 23.8%).

Table 4.9: Handwashing practices scores of mothers by infants' growth status

Interpretation	Cut-off points (SD)	Handwashing practices n (%)			
		Poor	Fair	Good	Excellent
Length-for-age (LAZ)					
Severely stunted	<-3	3 (2.9)	2 (1.9)	5 (4.8)	1 (1.0)
Moderately stunted	< -2 to ≥ -3	8 (7.6)	7 (6.7)	3 (2.9)	1 (1.0)
Normal length-for-age	≥ -2 to ≤ +2	16 (15.2)	27 (25.7)	29 (27.6)	3 (2.9)
Weight-for-age (WAZ)					
Severely underweight	<-3	2 (1.9)	-	2 (1.9)	-
Moderately underweight	< -2 to ≥ -3	3 (2.9)	2 (1.9)	5 (4.8)	2 (1.9)
Normal weight-for-age	≥ -2 to ≤ +2	22 (21.0)	34 (32.4)	30 (28.6)	3 (2.9)
Weight-for-length (WLZ)					
Severely wasted	<-3	1 (1.0)	-	-	-
Moderately wasted	< -2 to ≥ -3	1 (1.0)	-	1 (1.0)	-
Normal weight-for-length	≥ -2 to ≤ +1	13 (12.4)	21 (20.0)	25 (23.8)	4 (3.8)
Risk of overweight	> +1 to ≤ +2	5 (4.8)	5 (4.8)	8 (7.6)	-
Overweight	> +2 to ≤ +3	5 (4.8)	8 (7.6)	2 (1.9)	1 (1.0)
Obese	> +3	2 (1.9)	2 (1.9)	1 (1.0)	-
BMI-for-age (BMIZ)					
Severely wasted	< -3	1 (1.0)	-	-	-
Moderately wasted	< -2 to ≥ -3	1 (1.0)	1 (1.0)	1 (1.0)	-
Normal BMI-for-age	≥ -2 to ≤ +1	13 (12.4)	20 (19.1)	26 (24.8)	4 (3.8)
Risk of overweight	> +1 to ≤ +2	7 (6.7)	8 (7.6)	7 (6.7)	-
Overweight	> +2 to ≤ +3	3 (2.9)	5 (4.8)	2 (1.9)	1 (1.0)
Obese	> +3	2 (1.9)	2 (1.9)	1 (1.0)	-

4.7.3 Food handling practices of mothers by infants' growth status

The results in Table 4.10 show that normal growth outcomes were most common among infants whose mothers had fair and poor food-handling practice scores across all growth indicators. For length-for-age, normal length was highest among infants whose mothers had fair food handling practice scores (31; 29.5%), followed by those with poor practice scores (28; 26.7%). Normal weight was highest among infants whose mothers had fair (37; 35.2%) or poor (37; 35.2%) food-handling practice scores. Normal WLZ was most common among infants whose mothers had fair food-handling practice scores (26; 24.8%), followed by those with poor practice scores (25; 23.8%). Normal BMIZ was highest among infants whose mothers had poor food handling practice scores (26; 24.8%), followed by those with fair practice scores (25; 23.8%).

Table 4:10: Food handling practices scores of mothers by infants' growth status

Interpretation	Cut-off points (SD)	Food handling practices n (%)		
		Excellent	Fair	Poor
Length-for-age (LAZ)				
Severely stunted	< -3	-	3 (2.9)	8 (7.6)
Moderately stunted	< -2 to ≥ -3	-	9 (8.6)	10 (9.5)
Normal length	≥ -2 to ≤ +2	14 (13.3)	31 (29.5)	28 (26.7)
Weight-for-age (WAZ)				
Severely underweight	< -3	-	2 (1.9)	2 (1.9)
Moderately underweight	< -2 to ≥ -3	-	4 (3.8)	7 (6.7)
Normal WAZ	≥ -2 to ≤ +2	14 (13.3)	37 (35.2)	37 (35.2)
Weight-for-length (WLZ)				
Severely wasted	< -3	-	-	1 (1.0)
Moderately wasted	< -2 to ≥ -3	-	1 (1.0)	1 (1.0)
Normal	≥ -2 to ≤ +1	10 (9.5)	26 (24.8)	25 (23.8)
Risk of overweight	> +1 to ≤ +2	2 (1.9)	7 (6.7)	9 (8.6)
Overweight	> +2 to ≤ +3	2 (1.9)	6 (5.7)	8 (7.6)
Obese	> +3	-	3 (2.9)	2 (1.9)
BMI-for-age (BMIZ)				
Severely wasted	< -3	-	-	1 (1.0)
Moderately wasted	< -2 to ≥ -3	-	2 (1.9)	1 (1.0)
Normal BMIZ	≥ -2 to ≤ +1	10 (9.5)	25 (23.8)	26 (24.8)
Risk of overweight	> +1 to ≤ +2	2 (1.9)	10 (9.5)	10 (9.5)
Overweight	> +2 to ≤ +3	2 (1.9)	3 (2.9)	6 (5.7)
Obese	> +3	-	3 (2.9)	2 (1.9)

4.7.4 Association of WASH practices and length-for-age z-score

The results for the correlation between WASH practices and length-for-age are presented in Table 4.11. The unadjusted models for handwashing scores (Model 1: $\beta=0.015$, SE=0.036, $p>0.10$) and food handling scores (Model 2: $\beta=0.027$, SE=0.054, $p>0.10$) were not significantly associated with LAZ. When both WASH indicators were included simultaneously with infant gender (Model 3), the associations remained non-significant (handwashing: $\beta=0.022$, SE=0.034; food handling: $\beta=-0.013$, SE=0.045; $p>0.10$). In the fully adjusted model controlling for maternal age and marital status (Model 4), neither handwashing practices ($\beta=0.013$, SE=0.035, $p>0.10$) nor food-handling practices ($\beta=-0.021$, SE=0.053, $p>0.10$) showed a statistically significant association with infant linear growth.

Across all models, significant temporal trends in LAZ were observed. Compared to month 1, infants showed statistically significant declines in LAZ by month 4 (Model 4: β = -0.227, SE = 0.135, $p < 0.10$) and month 6 (Model 4: β = -0.344, SE = 0.139, $p < 0.01$), suggesting progressive linear growth faltering over the study period. Infant gender emerged as a strong predictor of LAZ in adjusted models. Male infants had significantly higher LAZ than females (Model 4: β=1.308, SE=0.198, $p<0.01$), representing 1.3 standard deviations (SD) higher height-for-age after controlling for WASH practices, time, and maternal characteristics. Neither maternal age nor marital status was significantly associated with infant LAZ in the fully adjusted model.

Table 4.11: Association of WASH practices and length-for-age z-scores (LAZ)

	Model 1	Model 2	Model 3	Model 4
Handwashing practices scores	0.015	-	0.022	0.013
	(0.036)	-	(0.034)	(0.035)
Food handling scores	-	0.027	-0.013	-0.021
	-	(0.054)	(0.045)	(0.053)
LAZ				
Month 1 (reference)	-	-	-	-
Month 2	-0.166 (0.112)	-0.161 (0.113)	-0.160 (0.111)	-0.122 (0.117)
Month 3	-0.161 (0.116)	-0.134 (0.116)	-0.138 (0.116)	-0.107 (0.126)
Month 4	-0.211 (0.124)	-0.211 (0.125)	-0.212 (0.125)	-0.227 (0.135)
Month 5	-0.084 (0.130)	-0.084 (0.135)	-0.066 (0.135)	-0.016 (0.144)
Month 6	-0.412** (0.134)	-0.416** (0.137)	-0.346** (0.130)	-0.344** (0.139)
Gender				
Female (reference)	-	-	-	-
Male	-	-	1.366**	1.308**
	-	-	(0.185)	(0.198)
Age				
14 to 24 years (reference)	-	-	-	-
25 to 35 years	-	-	-	0.029
	-	-	-	(0.206)
36 to 45 years	-	-	-	-0.072
	-	-	-	(0.329)
Marital status				
Divorced	-	-	-	0.000
Married	-	-	-	-0.168
	-	-	-	(0.351)
Never married	-	-	-	0.031
	-	-	-	(0.368)

	Model 1	Model 2	Model 3	Model 4
Widowed	-	-	-	-0.423
	-	-	-	(0.361)

The values outside the parentheses indicate the β coefficient, and standard errors are shown in parentheses. A dash (–) indicates that the variable was not included in the model. *= $p < 0.05$, ** = $p < 0.01$. *Model 1 included the hygiene score and the number of months. Model 2 included the food-handling score. Model 3 adjusted for infant sex, and Model 4 further included maternal age and marital status.*

4.7.5 Association of WASH practices and weight-for-age z-scores (WAZ)

Table 4.12 presents the results for the association of WASH Practices and WAZ. The results showed no significant associations between handwashing practices, food-handling practices, and infant WAZ across all, consistent with the LAZ results. In unadjusted analyses, handwashing scores showed no association with WAZ (Model 1: β = -0.020, SE = 0.034, p > 0.10), and food-handling scores similarly showed no significant association (Model 2: β = 0.055, SE = 0.048, p > 0.10). When both WASH indicators were included simultaneously with infant gender (Model 3), the associations remained non-significant (handwashing: β=-0.022, SE=0.027; food handling: β=0.024, SE=0.040; p>0.10). In the fully adjusted model controlling for maternal characteristics (Model 4), neither handwashing practices (β = -0.003, SE = 0.027, p > 0.10) nor food-handling practices (β = 0.055, SE = 0.047, p > 0.10) were significantly associated with infant weight status.

Infants experienced significant weight faltering over time. Relative to month 1, WAZ declined significantly by month 4 (Model 4: β = -0.187, SE = 0.108, p < 0.10) and month 6 (Model 4: β = -0.322, SE = 0.130, p < 0.01), indicating progressive underweight over the study period. The magnitude of decline in the final month was equivalent to one-third of a standard deviation in WAZ. Infant gender was the strongest predictor of WAZ across adjusted models. Male infants had significantly higher WAZ than females (Model 4: β=1.535, SE=0.165, p<0.01), representing a 1.5 standard deviation difference in weight-for-age after controlling for all covariates. Maternal age and marital status were not significantly associated with infant WAZ in the fully adjusted model.

Table 4.12: Associations of WASH practices and weight-for-age z-scores (WAZ)

	Model 1	Model 2	Model 3	Model 4
Handwashing scores	-0.020	-	-0.022	-0.003
	(0.034)	-	(0.027)	(0.027)
Food handling scores	-	0.055	0.024	0.055
	-	(0.048)	(0.040)	(0.047)
WAZ				
Month 1 (reference)	-	-	-	-
Month 2	-0.138 (0.094)	-0.147 (0.096)	-0.151 (0.096)	-0.101 (0.103)
Month 3	-0.159 (0.089)	-0.136 (0.091)	-0.143 (0.092)	-0.122 (0.098)
Month 4	-0.224* (0.103)	-0.230* (0.102)	-0.245* (0.103)	-0.187 (0.108)
Month 5	-0.100 (0.111)	-0.124 (0.114)	-0.109 (0.113)	-0.064 (0.119)
Month 6	-0.419** (0.139)	-0.445** (0.136)	-0.359** (0.121)	-0.322* (0.130)
Gender				
Female	-	-	0.000	0.000
Male	-	-	1.560**	1.535**
	-	-	(0.163)	(0.165)
Age				
16 to 24 years (reference)	-	-	-	-
25 to 35 years	-	-	-	-0.060
	-	-	-	(0.179)
36 to 45 years	-	-	-	0.262
	-	-	-	(0.237)
Marital status				
Divorced (reference)	-	-	-	-
Married	-	-	-	0.139
	-	-	-	(0.291)
Never married	-	-	-	-0.135
	-	-	-	(0.304)
Widowed	-	-	-	0.382
	-	-	-	(0.351)

The values outside the parentheses indicate the β coefficient, and standard errors are shown in parentheses. A dash (–) indicates that the variable was not included in the model. ***= $p < 0.05$, *** = $p < 0.01$. Model 1 included the hygiene score and the number of months. Model 2 included the food-handling score. Model 3 adjusted for infant sex, and Model 4 further included maternal age and marital status.*

4.7.6 Associations of WASH practices and weight-for-length z-scores (WLZ)

The results for the association of WASH Practices and WLZ are presented in Table 4.13. Handwashing practices showed a marginally significant negative association with WLZ in unadjusted and partially adjusted models. In Model 1, higher handwashing scores were associated with lower WLZ (β=-0.055, SE=0.029, p<0.01), and this relationship persisted when both WASH indicators and infant gender were included

(Model 3: β=-0.055, SE=0.030, p<0.01). However, this association lost statistical significance in the fully adjusted model controlling for maternal characteristics (Model 4: β = -0.024, SE = 0.031, p > 0.10). Food-handling practices showed no significant association with WLZ across any model specification (Model 4: β = 0.035, SE = 0.061, p > 0.10).

Infants experienced progressive wasting over the study period, with the most pronounced declines at later months. Compared to Month 1, WLZ declined significantly by month 5 (Model 4: β=-0.338, SE=0.166, p<0.05) and month 6 (Model 4: β=-0.467, SE=0.180, p<0.01). The magnitude of decline at the final assessment was nearly half a standard deviation, indicating substantial acute malnutrition over time.

Infant sex remained a significant predictor; male infants had significantly higher WLZ than females (Model 4: β=0.572, SE=0.164, p<0.01), representing a 0.6 standard deviation difference in weight-for-height. Maternal age was not significantly associated with infant WLZ. However, an unexpected finding emerged for maternal marital status: infants of widowed mothers had significantly higher WLZ compared to divorced mothers (β=0.947, SE=0.348, p<0.01), but these results should be interpreted cautiously because of the small reference group.

Table 4.13: Associations of WASH practices and weight-for-length z-scores (WLZ)

	Model 1	**Model 2**	**Model 3**	**Model 4**
Handwashing scores	-0.055*	-	-0.055*	-0.024
	(0.029)	-	(0.030)	(0.031)
Food handling scores	-	-0.014	-0.008	0.035
	-	(0.052)	(0.054)	(0.061)
WLZ				
Month 1 (reference)	-	-	-	-
Month 2	0.103 (0.138)	0.080 (0.137)	0.069 (0.139)	0.084 (0.146)
Month 3	-0.106 (0.134)	-0.105 (0.132)	-0.113 (0.134)	-0.123 (0.141)
Month 4	-0.260* (0.149)	-0.265* (0.151)	-0.288* (0.149)	-0.225 (0.149)
Month 5	-0.342** (0.156)	-0.352** (0.160)	-0.355** (0.162)	-0.338** (0.166)

	Model 1	Model 2	Model 3	Model 4
Month 6	-0.535** (0.170)	-0.534** (0.170)	-0.512** (0.170)	-0.467** (0.180)
Gender				
Female (reference)	-	-	-	-
Male	-	-	0.568**	0.572**
	-	-	(0.167)	(0.164)
Age				
14 to 24 years (reference)	-	-	-	-
25 to 35 years	-	-	-	0.062
	-	-	-	(0.183)
36 to 45 years	-	-	-	0.361
	-	-	-	(0.267)
Marital status				
Divorced (reference)	-	-	-	-
Married	-	-	-	0.418
	-	-	-	(0.301)
Never married	-	-	-	0.063
	-	-	-	(0.326)
Widowed	-	-	-	0.947***
	-	-	-	(0.348)

The values outside the parentheses indicate the β coefficient, and standard errors are shown in parentheses. A dash (–) indicates that the variable was not included in the model. FMI = Fraction of Missing Information. **= p < 0.05, ** = p < 0.01. Model 1 included the hygiene score and the number of months. Model 2 included the food-handling score. Model 3 adjusted for infant sex, and Model 4 further included maternal age and marital status. female," "mother's age 14–24 years," and "divorced" are reference categories in the models*

4.7.7 Associations of WASH practices and BMI-for-age z-scores (BMIZ)

The results for the association of WASH Practices and BMIZ are presented in Table 4.14**.** Handwashing practices showed a marginally significant negative association with BMIZ in unadjusted and partially adjusted models. Higher handwashing scores were associated with lower BMIZ in Model 1 (β = -0.054, SE = 0.031, p < 0.01) and Model 3 (β = -0.055, SE = 0.028, p < 0.01). However, this association lost statistical significance when maternal characteristics were added to the model (Model 4: β = -0.024, SE = 0.029, p > 0.10), suggesting potential confounding by unmeasured maternal or household factors. Food handling practices showed no significant association with BMIZ across any model specification (Model 4: β = 0.052, SE = 0.065, p > 0.10).

Infants showed a pattern of declining BMIZ over time, though the temporal effects were less consistent than those observed for other anthropometric indicators. Only the final month showed marginal significance in the unadjusted models, with BMIZ declining by month 6 compared to month 1 (Model 1: $\beta = -0.302$, SE = 0.166, $p < 0.10$; Model 2: $\beta = -0.313$, SE = 0.164, $p < 0.10$). However, this temporal decline lost statistical significance in the fully adjusted model (Model 4: $\beta = -0.230$, SE = 0.170, $p > 0.10$), suggesting that maternal characteristics may partially explain the observed changes in infant BMI over time. Infant gender emerged as a significant predictor of BMIZ, with male infants having substantially higher BMIZ than females (Model 4: $\beta=0.947$, SE=0.169, $p<0.01$), representing a nearly one-standard-deviation difference. This sex differential falls between the smaller effect observed for WLZ (0.6 SD) and the larger effects for LAZ and WAZ (1.3-1.5 SD). Maternal age was not significantly associated with BMIZ. Notably, infants of widowed mothers had significantly higher BMIZ compared to infants of divorced mothers ($\beta=0.804$, SE=0.397, $p<0.05$), showing again the pattern observed for WLZ.

Table 4.14: Associations of WASH practices and BMI-for-age (BMIZ)

	Model 1	Model 2	Model 3	Model 4
Handwashing scores	-0.054	-	-0.055	-0.024
	(0.031)	-	(0.028)	(0.029)
Food handling score	-	0.012	0.009	0.052
	-	(0.055)	(0.057)	(0.065)
BMIZ				
Month 1 (reference)	-	-	-	-
Month 2	-0.063 (0.128)	-0.082 (0.129)	-0.096 (0.129)	-0.060 (0.139)
Month 3	-0.064 (0.126)	-0.049 (0.126)	-0.055 (0.127)	-0.050 (0.134)
Month 4	-0.154 (0.140)	-0.158 (0.142)	-0.183 (0.139)	-0.116 (0.142)
Month 5	-0.143 (0.153)	-0.161 (0.156)	-0.161 (0.156)	-0.141 (0.163)
Month 6	-0.302 (0.166)	-0.313 (0.164)	-0.274 (0.160)	-0.230 (0.170)
Gender				
Female (reference)	-	-	-	-
Male	-	-	0.955**	0.947**
	-	-	(0.176)	(0.169)
Age				
16 to 24 years (reference)	-	-	-	-
25 to 35 years	-	-	-	-0.042
	-	-	-	(0.190)
36 to 45 years	-	-	-	0.347
	-	-	-	(0.261)
Marital status				
Divorced (reference)	-	-	-	-
Married	-	-	-	0.307
	-	-	-	(0.332)

Never married	-	-	-	-0.107
	-	-	-	(0.366)
Widowed	-	-	-	0.804*
	-	-	-	(0.397)

The values outside the parentheses indicate the β coefficient, and standard errors are shown in parentheses. A dash (–) indicates that the variable was not included in the model. FMI = Fraction of Missing Information. **= *p < 0.05, *** = p < 0.01. Model 1 included the hygiene score and the number of months. Model 2 included the food-handling score. Model 3 adjusted for infant sex, and Model 4 further included maternal age and marital status. Female," mother's age 14–24 years," and "divorced" are reference categories in the models*

4.7.8 Factors associated with MDR model 1: food handling

As shown in Table 4.15, Model 1, which examines food-handling practices and other covariates in relation to MDR, did not identify any statistically significant associations. Male infants had lower odds of MDR than females (aOR = 0.52; 95% CI: 0.05–5.43; p = 0.587), though this difference was not significant. Compared to poor food handling practices, fair practices were associated with increased odds (aOR = 1.76; 95% CI: 0.16–19.43; p = 0.643), while excellent practices showed reduced odds (aOR = 0.74; 95% CI: 0.07–7.90; p = 0.807), but neither reached statistical significance. Infants classified as at risk of overweight had higher odds of MDR (aOR = 5.02; 95% CI: 0.36–68.90; p = 0.227), although this association was also not significant. Several variables, including maternal age, anthropometric indicators, obesity, and infant feeding practices, were not estimable due to sparse data. Overall, Table 4.15 indicates no independent predictors of MDR in this adjusted model, with wide confidence intervals suggesting limited statistical power.

Table 4.15: Factors associated with multi-drug resistance model 1 - food handling

Variable(s)	Logistic regression		
	Multivariable		
	aOR	p-value	CI 95%
Sex			
Female	**Ref**		
Male	0.52	0.587	0.05 - 5.43
Mother's age			
14 to 24 years	NE	-	-
25 to 35 years	NE	-	-
36 to 45 years	NE	-	-
Food handling			
Poor	**Ref**		
Fair	1.76	0.643	0.16 - 19.43
Good	-	-	-
Excellent	0.74	0.807	0.07 - 7.90
Weight-for-age			
Severely Underweight	NE	-	-
Moderately Underweight	NE	-	-
Normal WAZ	NE	-	-
Height-for-age			
Severely stunted	NE	-	-
Moderately Stunted	NE	-	-
Normal length	NE	-	-
BMI-for-age			
Normal		-	-
Severely wasted	NE	-	-
Risk of Overweight	5.02	0.227	0.36 - 68.90
Overweight	1.80	0.686	0.10 - 31.7
Obese	NE	-	-
Infant feeding practices			
Non EBF	NE	-	-
Exclusive breastfeeding	NE	-	-

CI: Confidence Interval, aOR: adjusted odds ratio, NE: Not Estimable. *Model 1 included the hygiene score and the number of months.*

4.7.9 Factors associated with multi-drug resistance model 2: food handling

As shown in Table 4.16, none of the variables included in Model 2 was statistically significantly associated with MDR. Although higher odds were observed for infants in households with good food handling (aOR = 7.03) and those classified as at risk of overweight (aOR = 18.1), these associations did not reach statistical significance and were accompanied by wide confidence intervals, indicating imprecision. Sex and maternal age were also not significantly associated with MDR, and several variables were not estimable due to sparse data. Overall, Table 4.16 indicates that no independent predictors of MDR were identified in this adjusted model.

Table 4.16: Factors associated with multi-drug resistance model 2: food handling

Variable(s)	Logistic regression Multivariable		
	aOR	p-value	CI 95%
Sex			
Female	Ref	-	-
Male	0.31	0.466	0.01 - 7.04
Mother's age			
14 to 24 years	Ref	-	-
25 to 35 years	0.92	0.949	0.08 - 9.99
36 to 45 years	1.69	0.881	0.01 - 81.20
Food handling			
Poor	-	-	-
Fair	2.19	0.583	0.13 - 36.6
Good	7.03	0.101	0.68 - 72.41
Excellent	-	-	-
Weight-for-age			
Severely Underweight	NE	-	-
Moderately Underweight	NE	-	-
Normal WAZ	NE	-	-
Height-for-age			
Severely stunted	NE	-	-
Moderately Stunted	NE	-	-
Normal length	NE	-	-
BMI-for-age			
Normal BMIZ	-	-	-
Severely wasted	NE	-	-
Risk of Overweight	18.1	0.058	0.90 - 365.6
Overweight	3.02	0.398	0.23 - 39.3
Obese	NE	-	-
Infant feeding practices			
Non EBF	NE	-	-
Exclusive breastfeeding	NE	-	-

*CI: Confidence Interval, aOR: adjusted odds ratio, NE: Not Estimable. *Model 2 included the food-handling score.*

CHAPTER 5: DISCUSSION OF RESULTS

5.1 Introduction

This chapter discusses the study's results in relation to the objectives and the relevant existing literature. The study aimed to examine the influence of WASH and infant feeding practices on infant growth and AR among mother-infant pairs in the Dzimauli rural villages during the first six months of life. The discussion focuses on WASH practices, infant feeding practices, infant growth status, AR, and the relationships among selected study variables.

5.2 WASH practices

The WASH components included household water sources, availability, and collection; sanitation, such as toilet facilities; and hygiene practices, focusing on handwashing and food-handling practices.

5.2.1 Water sources, availability, and collection

The high prevalence of piped water in participants' households suggests a gradual improvement in access to improved water sources in the study area. More than half of the households in the study relied on piped water for cleaning, bathing, and handwashing. The study conducted by Mudau *et al.* (2019) in the Vhembe District found that households often relied on wells and rivers because piped water was unreliable. Another study conducted in Limpopo Province showed that rural households frequently reverted to untreated water sources when piped systems failed (Edokpayi *et al.*, 2018). Howard (2024), on the other hand, indicated the widespread use of multiple water sources, including groundwater and untreated surface water, among rural Limpopo households. In other areas of South Africa, such as the rural communities of the Free State province, Mokoena *et al.* (2020) found that households commonly relied on surface water and boreholes due to limited access to piped water. In KwaZulu-Natal, Kunene *et al.* (2021) reported that many households continued to source water from streams and rivers due to inadequate piped water infrastructure. These studies demonstrate that unimproved water sources remain an alternative across rural South Africa.

The continued reliance on surface water and unprotected wells indicates that most households still use unsafe or less reliable sources, reflecting the persistent global inequalities in rural access to safe drinking water (WHO and UNICEF, 2021). The United Nations (2023) reported that global access to safely managed drinking water has improved, rising from 69% in 2015 to 73% in 2022. Despite this global progress, a substantial gap remains, where 2.2 billion people still lack safely managed drinking water services, including 703 million who lack access to a basic drinking water source.

Nearly half of the households (49.5%) in the study experienced a piped water interruption lasting four to six days. A study conducted in the Vhembe District of Limpopo Province found that unreliable water supply often leads to unsafe water storage and reversion to contaminated sources, increasing microbial risks for infants and caregivers (Edokpayi *et al.*, 2018). In the Lepelle Nkumpi Municipality in Limpopo Province, a persistent water service interruption compelled households to depend on alternative water sources (Bazaanah and Mothapo, 2023). According to the WHO and UNICEF (2023), piped water in rural areas does not necessarily guarantee safety or reliability, as many households experience frequent water interruptions, limiting their ability to practise good hygiene. Frequent water interruptions affect several determinants of infant health, including consistent handwashing, appropriate food preparation, and cleaning of infant feeding utensils, all of which are crucial for preventing diarrhoeal, respiratory, and parasitic infections (WHO, 2019). To address water interruptions, WHO and UNICEF (2023) recommended strengthening the reliability of rural water systems by improving water quality monitoring and promoting safe household water handling and storage practices. This would assist in achieving SDG target 6.1, which aims to achieve the universal and equitable access to safe and affordable drinking water for all by 2030 (UN, 2015).

The study shows that women are mainly responsible for collecting water, aligning with the UNICEF and WHO (2021) report and the study by Bazaanah and Mothapo (2023), indicating that water collection responsibilities are typically assigned to women. Since women are responsible for caring for children, they face constraints that reduce their time and resources available for key nutrition-related tasks, such as preparing appropriate meals, breastfeeding on demand, and maintaining hygienic household conditions (Bazaanah and Mothapo, 2023). This is often the case when water sources are distant, unreliable, or unsafe.

5.2.2 Sanitation practices

Sanitation practices in this study were described by types of toilet facilities used, households' sharing of toilet facilities, and the use of toilet paper.

- ***Types of toilet facilities***

The study found widespread use of pit latrines and a few households with flush toilet facilities. According to Hlongwane *et al.* (2022), the use of pit latrines is due to ongoing sanitation inequalities across rural communities in the Limpopo province. The latter study found that in the Greater Letaba Municipality, most households relied on pit latrines because they had no option of flush toilets. Lack of flush toilet use in Dzimauli areas is due to the absence of sewer-connected systems and limited investment in sanitation infrastructure. Similar concerns were highlighted by Masia *et al.* (2025), who reported that pit latrines remain the dominant sanitation option in rural Vhembe District. The 2023 report of the WHO-UNICEF Joint Monitoring Program (JMP) indicated that there is a persistent reliance on pit latrines and open defecation in rural settings, despite national and global commitments to improve sanitation infrastructure (WHO and UNICEF, 2023). The widespread use of pit latrines in Dzimauli reflects a broader sanitation pattern seen across the district.

The proposition is that the progress toward safely managed sanitation is slow. In the Vhembe District, Masia *et al.* (2025) reported that rural communities perceive sanitation services as inadequate due to poor maintenance, limited government support, and the health risks associated with basic facilities. The 2023-2024 National State of Water Reports in South Africa are quite resourceful in documenting persistent rural sanitation challenges (DWS, 2024). The reports underscore that access to acceptable sanitation has improved over time, but basic sanitation options continue to dominate in rural provinces.

On the contrary, properly constructed pit latrines are classified as “improved” sanitation under JMP standards, but they do not eliminate exposure pathways to faecal contamination. Risks increase when latrines are poorly maintained, located too close to dwellings, or lack handwashing facilities (WHO and UNICEF, 2021). Infants are particularly vulnerable because they frequently explore their environment orally and rely entirely on caregivers to maintain hygienic conditions (Wolf *et al.*, 2022). Repeated exposure to faecal pathogens in early life is associated with increased risk of diarrhoeal disease, environmental enteric dysfunction, and growth faltering (Prüss-Ustün *et al.*, 2019). The continued reliance on improved sanitation, such as pit latrines in Dzimauli, therefore, poses significant infection-control challenges for households with infants and young children.

- ***Household sharing of toilet facilities***

Regarding household toilet sharing, this study found that most households did not share their toilet facilities, thereby ensuring adequate maintenance, reducing user traffic and exposure to faecal contamination (WHO, 2023). A study conducted across South Africa found that most households (75.6%) do not share toilet facilities (Tota *et al.*, 2024). Conversely, shared sanitation facilities, especially in low-resource settings, are often unclean due to high user loads and inadequate maintenance (Tota *et al.*,2024). Ramlal *et al.* (2022) found that shared toilet facilities in informal settlements of eThekwini Municipality were frequently contaminated with faecal bacteria, indicating their potential to serve as hotspots for pathogen transmission. The WHO and UNICEF (2023) emphasised that shared toilet facilities are generally less hygienic and pose a higher risk of infection.

- ***Use of toilet paper***

Access to sanitation facilities does not guarantee optimal hygiene. The 2023 WHO-UNICEF JMP report emphasises that the availability of hygiene materials such as toilet paper, water for cleaning, and soap strongly influences household sanitation behaviour. The current study found consistent toilet paper use in most households;

however, the practice may vary over time depending availability of toilet paper in the household.

The type of toilet facility, the extent to which facilities are shared, and the consistent availability of hygiene materials, such as toilet paper, constitute key dimensions of household sanitation (WHO, 2018). These factors collectively determine whether sanitation infrastructure effectively reduces exposure to faecal contamination (Wolf *et al.*, 2022). Even with improved facilities, shared use and limited access to hygiene materials may compromise cleanliness and increase pathogen transmission. In rural South Africa, resource constraints and unreliable water supply continue to constrain sanitation maintenance and hygiene practices (Stats SA, 2022). These structural limitations reflect ongoing challenges in achieving SDG 6, particularly Target 6.2, which emphasises equitable access to adequate sanitation and hygiene for all, especially vulnerable groups such as children (UN, 2015). Progress toward this target requires not only infrastructure provision but also private access and sustained hygiene practices to minimise faecal–oral transmission risks among infants.

5.2.3 Hygiene practices

Hygiene practices, as one of the components of WASH, include handwashing and food-handling practices in the study. The section below presents the findings on mothers' handwashing and food-handling practices in Dzimauli villages.

- ***Handwashing practices***

The handwashing practices determined in this study focused on mothers' handwashing at critical moments and the use of soap. The critical moments studied were before eating, before and after feeding their infants, and after a nappy change in months 1, 3 and 6. Overall, the results showed that handwashing behaviour was common among mothers, which is commendable. A systematic review found that the global pooled daily handwashing rate was approximately 72%, though this varied considerably by region and population subgroup (Zhou *et al.*, 2025). In Africa, the lowest handwashing rate was 59.5%, while in South America it was 94.5%. In 2024, South Africa had a rate of 56.5% (Tota *et al.*, 2025). However, in 2025, the rate increased to 67.7% (Zhou *et al*., 2025). A study conducted by Johnstone *et al*. (2022) in Soweto, Johannesburg found that most respondents reported always washing their hands after using the toilet (88.5%), before preparing food (80.6%), before eating (78.6%), before feeding babies and young children (77.2%), and after changing children's nappies or diapers (79.5%). These handwashing practices are similar to those in this study.

Most of the mothers in the study reported washing their hands at critical times, especially before and after eating and feeding their infants. The current results are similar to the one reported by Johnstone *et al.* (2022) in Soweto, Johannesburg, where most respondents were always washing their hands after using the toilet (88.5%), before preparing food (80.6%), before eating (78.6%), before feeding babies and young children (77.2%), and after changing children's nappies or diapers (79.5%). Based on the customs of the current study participants, the practice of handwashing before and after meals is influenced by the water provided with the food. These results indicate that hand hygiene during food handling is a well-established practice among the mothers in this study, regardless of the reported water interruption.

The study also showed fluctuations in handwashing practices before and after feeding infants. While most mothers reported always washing hands at these critical times, the proportion declined in some months before improving again. Handwashing after feeding and changing nappies was less consistent, with a notable proportion of mothers reporting that they washed their hands only "sometimes." At times, handwashing practices are prompted by threatening outbreaks or situational circumstances. For instance, a report by WaterAid (2021) indicated that 58% of people worldwide washed their hands at least five times a day during the COVID-19 pandemic. These hygienic gaps point to areas for improvement, especially given that infant feeding and nappy-changing activities pose a high risk of faecal–oral transmission.

Given the inconsistent use of soap observed in this study and other studies in Limpopo Province, alternative handwashing agents such as wood ash or sand, which are sometimes used in low-resource settings, warrant consideration. Wood ash has been reported to possess some pathogen-reducing capacity due to its alkaline nature and abrasive action and has been used as a coping alternative when soap is unavailable or unaffordable (WHO and UNICEF, 2020). However, evidence indicates that its effectiveness is highly variable and depends on factors such as the source and cleanliness of the ash, the availability of sufficient water, and correct handwashing technique (Zambrana *et al.,* 2022). Unlike soap, ash lacks surfactant properties, is less effective at removing grease and microorganisms, and may itself be contaminated, potentially increasing the risk of pathogen transmission if improperly used (Hilton *et al*., 2025). Similar limitations apply to the use of sand or soil, which may introduce additional microbial contamination rather than reduce it (WHO and UNICEF, 2020). In the study setting, where intermittent water supply and limited household resources have been reported, such alternatives likely reflect constrained coping practices rather than effective hygiene solutions. Consequently, while locally available materials like ash may offer a temporary option in emergency or extreme scarcity contexts, they cannot substitute for soap, reinforcing the need for interventions that ensure sustained access to soap and water, particularly for high-risk activities such as infant feeding and nappy changing.

The lower age range (younger than 35 years) of the study participants may help explain the observed handwashing patterns, as younger mothers are typically more actively involved in daily infant care than their counterparts. Their generally higher levels of education further support the observed study pattern, as education is known to enhance understanding of hygiene recommendations and to increase the likelihood of translating handwashing knowledge into consistent practice (Machava, 2023). The study's results further show that mothers are not only aware of recommended handwashing practices but also apply this knowledge at key moments during food preparation and feeding. This differs from national results reported by Stats SA (2023), which indicate that although handwashing awareness is generally high among caregivers in South Africa, this does not always translate into consistent practice.

A study conducted in low-resource communities in South Africa indicates that inadequate handwashing after nappy changes contributes significantly to diarrhoeal disease in young children (WHO, 2022). Similar results have been reported in rural areas of South Africa, where inconsistent handwashing after child faecal contact remains common due to limited access to water and sanitation facilities (Stats SA, 2023; DWS, 2022). Studies conducted in Limpopo Province (Munyaka *et al.*, 2022; DoH, 2021) have shown that inadequate handwashing practices contribute to recurrent episodes of diarrhoeal disease among young children, particularly in rural communities with limited sanitation infrastructure. It implies that improving hygiene practices after nappy changes remains critical for reducing infection risk among infants in rural settings.

- ***Food-handling practices***

Food-handling practices measured in this study included handwashing when handling raw food, separating raw and cooked food, cleaning food-preparation areas and utensils, washing fruits and vegetables, and storing leftovers. The study results showed gradual but uneven improvements in food handling practices among mothers over the three-month period. Handwashing practices, particularly when handling raw food and before cooking, improved steadily, with "always" responses increasing from 32.1% to 44.2% and 37.2% to 47.1%, respectively. The observed increase in handwashing practices during raw food handling and prior to cooking may be attributed to several plausible factors. One explanation is participant sensitization or reactivity, whereby repeated exposure to study questions increases awareness and reinforces recommended behaviours over time, a phenomenon increasingly recognized in longitudinal public health research (Hilton *et al.*, 2025). Although no formal WASH intervention was implemented as part of this study, participants may have been indirectly exposed to routine hygiene promotion activities, including counselling at health facilities, interactions with community health workers, school-based messaging, or mass media campaigns, which continue to prioritise food hygiene and handwashing practices in many low-resource settings (UNICEF, 2023).

Improvements may also reflect a heightened perception of risk associated with food preparation, as handwashing before cooking and handling raw foods are closely linked to preventing foodborne and enteric illnesses, particularly in households with infant and young children (Curtis *et al*., 2021). Contextual factors, such as seasonal changes in food preparation patterns, shifts in household routines, or intermittent improvements in access to water or soap, may have further facilitated better adherence to these practices (Hilton *et al*., 2025). Finally, increased familiarity with socially desirable hygiene norms may have influenced self-reported practices over time. While the relative contribution of each factor cannot be determined from the available data, it is likely that the observed improvement reflects an interaction of increased awareness, contextual reinforcement, and gradual internalisation of recommended food hygiene behaviours rather than exposure to a single identifiable intervention. The South Africa National Hand Hygiene Behaviour Change Strategy (2016–2020) emphasised the importance of soap use and behavioural reinforcement in food handling (DoH, 2020). A study conducted in the Waterberg District of Limpopo Province showed that mothers and caregivers often improved hygiene practices when exposed to structured health education programmes (Mokgatle and Madiba, 2017).

The practice of separating raw and cooked foods showed even a greater improvement of 15% (53.2%-68.3%) in the “always” response. These results align with food safety guidelines under the Foodstuffs, Cosmetics and Disinfectants Act, which stress the prevention of cross-contamination (DoH, 2018). Comparable results in Limpopo schools and households suggest that awareness campaigns have increased recognition of the dangers of mixing raw and ready-to-eat foods (Mashaba *et al.*, 2019). Food separation is a highly visible and procedural behaviour that does not rely heavily on consumable resources, such as soap or water, making it easier to adopt and sustain compared to other hygiene practices (FAO and WHO, 2023). Separation of raw and cooked foods is also consistently emphasized in food hygiene guidance as a critical measure for preventing cross-contamination, and households may perceive this practice as directly linked to illness prevention, especially in the context of caring for young children (Hilton *et al*., 2025). In addition, repeated engagement with study instruments may have led to gradual internalization of safe food-handling norms, resulting in improved adherence through heightened awareness and habit formation.

Contextual factors, such as increased food preparation responsibilities or seasonal variations influencing cooking practices, may have further reinforced the need to maintain clearer distinctions between raw and cooked foods within the household.

Cleaning food preparation areas and utensils was another area of strength, with “always” responses reaching 76.9% by month six. This aspect reflects a growing adherence to hygiene standards, which is encouraging given that poor sanitation has been linked to outbreaks of diarrheal diseases and various infections, particularly respiratory and parasitic, in rural South African communities (DoH, 2020). The study reported a lower proportion of mothers who reported always washing fruits and vegetables before eating. This inconsistency is concerning, as fresh produce is a common source of contamination (WHO, 2020a). Studies conducted in rural Limpopo Province have similarly reported that although many caregivers understand the importance of washing fruits and vegetables before eating, limited access to clean water and other resources often prevents consistent practice of these hygiene behaviours (Marutha and Chelule, 2020; Howard, 2024).

The reported practice of safely storing food leftovers showed a clear decline over time. “Always” responses decreased to none by month six, while approximately one-third of participants reported “rarely” adhering to safe storage practices at that point. The decline in the reported practice of safe food storage over time can plausibly be attributed to a range of structural, environmental, and socioeconomic constraints affecting households in the study area. Safe storage of cooked leftovers relies heavily on consistent access to refrigeration, which may be undermined by unreliable electricity supply, household energy-coping strategies, and efforts to minimise costs associated with appliance use. Where electricity is unstable, households may limit refrigerator use or avoid storing leftovers altogether due to concerns about spoilage, particularly during warmer periods when food deteriorates more quickly (Motjoadi, 2022). In addition, socioeconomic pressures, such as food insecurity and limited household resources, may encourage meal preparation patterns focused on immediate consumption rather than on storage. Compared to other food hygiene behaviours that depend primarily on knowledge and routine actions, safe storage practices are more infrastructure-dependent and therefore more difficult to sustain in resource-constrained rural settings. These factors together suggest that the observed decline reflects practical limitations rather than reduced awareness, underscoring the importance of considering enabling household conditions when promoting food safety behaviours.

Improper food storage is a significant risk factor for microbial growth, and local health authorities have repeatedly warned about food poisoning linked to poor refrigeration practices in rural households (Tshwane DoH, 2021). Mothers with higher levels of education are generally better informed about health and more likely to adopt safe food-handling practices (Wrottesley *et al.*, 2020). The improvements observed in handwashing when handling raw food (from 32.1% to 44.2%) and before cooking (from 37.2% to 47.1%) may be partly explained by the relatively high proportion of mothers with secondary education (75.0%). Mokgatle and Madiba (2017) indicate that caregivers with higher education are more likely to understand the risks of cross-contamination and foodborne illness and therefore adopt better hygiene practices. Similarly, research in Limpopo has shown that education enhances awareness of food safety regulations and guidelines, which can translate into improved household practices (Mashaba *et al.*, 2019). The overall food handling practice scores reinforce these mixed results. Poor practices remained high (44.2% in month one, 44.7% in month six), while excellent practices stayed below 20% across all months. The results suggests that while some food-handling practices improved, overall adherence to safe food-handling practices remains low.

5.3 Infant feeding practices

This section discusses the results of breastfeeding initiation, prelacteal feeding, and exclusive breastfeeding.

5.3.1 Breastfeeding initiation and prelacteal feeding

Breastfeeding initiation and colostrum feeding practices observed in this study are generally commendable and reflect adherence to national and global breastfeeding recommendations, including the Tshwane Declaration of Support for Breastfeeding and WHO guidelines, which emphasise early initiation of breastfeeding as critical for neonatal survival and immune protection (DoH, 2011; WHO, 2023). Globally, only about 48% of newborns are breastfed within the first hour of life (UNICEF and WHO, 2023), and the early initiation rate observed in this study (61.7%) therefore exceeds the global average, suggesting relatively positive practices within the study population. However, this rate remains substantially lower than national estimates for South Africa, where early initiation has consistently been reported at around 89%, with recent evidence indicating a slight improvement to 89.3% between 2018 and 2023 (UNICEF, 2018; Simelane *et al*., 2023).

Evidence from other South African provinces further supports generally high early initiation rates; for example, a longitudinal study in KwaZulu-Natal reported initiation rates exceeding 60% alongside high levels of colostrum feeding among mothers receiving routine postnatal counselling (Naicker, 2024). Similarly, studies conducted in Limpopo Province have consistently reported relatively high early breastfeeding initiation, with approximately two-thirds of mothers initiating breastfeeding within the first hour in Vhembe District (Motadi *et al*., 2019; Mphasha *et al*., 2023) and rates of up to 70.3% reported elsewhere in the province (Simelane *et al*., 2023). Taken together, these findings suggest that while early breastfeeding initiation remains common in Limpopo and nationally, the lower rate observed in the present study indicates a relative decline within the Vhembe District, highlighting the need to strengthen facility- and community-based support for early initiation to sustain progress achieved at provincial and national levels.

The relatively high prevalence of prelacteal feeding observed in this study is concerning. Prelacteal feeding interrupts exclusive breastfeeding and increases the risk of infection among newborns. Prelacteal feeding remains common worldwide despite global efforts to promote optimal breastfeeding practices. Global analyses across LMIC indicate that between 30% and 50% of newborns receive prelacteal feeds within the first three days after birth, often in the form of water, sugar solutions, formula milk, or other liquids given before breastfeeding is fully established (Neves *et al*., 2022). Another pooled analysis across multiple countries further estimates that about 25% of infants receive water-based prelacteal feeds and around 18% receive milk-based prelacteal feeds shortly after birth (Smith *et al*., 2022). The MAL-ED study (2017) reported that early introduction of foods and liquids increases exposure to pathogens and undermines optimal infant feeding practices.

In Limpopo Province, studies show that prelacteal feeding remains a notable infant feeding practice despite ongoing efforts to promote exclusive breastfeeding. A study conducted by Mugware *et al*. (2022) in Vhembe District found that 90.8% of mothers reported not giving their infants prelacteal feeding. However, a study by Mphasha *et al*. (2023) in the same district as the latter study reported that prelacteal feeds were given to approximately one in three newborns, including water, sugar water, and traditional herbal mixtures. Another study in rural parts of the province found that about 29% of infants received prelacteal feeds (Letsoalo *et al*., 2024). Together, these results indicate that, although early initiation of breastfeeding is relatively common in parts of Limpopo, prelacteal feeding practices continue to undermine exclusive breastfeeding and optimal infant nutrition in the province.

Early initiation of breastfeeding and prelacteal feeding practices are influenced by a combination of socioeconomic, cultural, and health system factors (WHO, 2019; Makwela *et al*., 2024). Socioeconomic challenges and limited access to reliable maternal health services further affect feeding decisions, particularly in rural settings (WHO, 2019). Health system practices, including implementation of the BFHI, have been shown to improve early breastfeeding initiation and reduce prelacteal feeding through counselling and immediate post-delivery support (WHO, 2023). Cultural beliefs and advice from older family members often lead to the early introduction of liquids or foods before breastfeeding is fully established, despite ongoing health education efforts (Makwela *et al*., 2024).

Delayed initiation prevents infants from receiving colostrum, which is rich in antibodies and essential nutrients that protect against infections and support immune development (Jozsa and Thistle, 2025). As a result, infants who do not begin breastfeeding within the first hour after birth are at higher risk of neonatal infections, diarrhoeal diseases, and respiratory illnesses, which contribute significantly to neonatal morbidity and mortality (WHO, 2023). Prelacteal feeding further increases these risks by exposing newborns to potentially contaminated foods or liquids and interfering with the establishment of exclusive breastfeeding. Studies also show that infants who receive prelacteal feeds are more likely to experience breastfeeding difficulties and are less likely to remain exclusively breastfed during the first six months of life, increasing vulnerability to malnutrition and infection (UNICEF and WHO, 2023).

5.3.2 Exclusive Breastfeeding

Exclusive breastfeeding in this study declined sharply across the six months, dropping from high levels in the first month to very low levels by month six. This decline indicates that while breastfeeding initiation is intense, sustaining exclusive breastfeeding remains a significant challenge in the study area. Similar sentiments were stated by Makhavhu *et al.* (2025) from another study conducted in Limpopo Province. Makhavhu *et al.* reported that, despite good early initiation practices, exclusive breastfeeding declines rapidly in the months following birth, suggesting challenges in maintaining optimal feeding practices beyond the early postnatal period. Evidence indicates that exclusive breastfeeding is not only discontinued but is often interrupted, where infants temporarily receive other foods or liquids before mothers attempt to resume breastfeeding, resulting in unstable feeding patterns during the first six months of life (Patil *et al.*, 2016). The same pattern was observed in this study, indicating a continued practice, as both studies were conducted in the same setting. The MAL-ED (2017) study further demonstrated that the rapid decline in exclusive breastfeeding over the months is not only in SA but in many other LMICs (India, Pakistan, Brazil, Peru, Tanzania, Nepal, and Bangladesh). While Simelane *et al.* (2023) reported that 22.2% of infants in SA were exclusively breastfed for 6 months, a study by Mugware *et al.* (2022) in the Vhembe District found that only 7.6% infants were exclusively breastfed for 6 months.

The longitudinal study by Naicker (2024) in KwaZulu-Natal found that although many mothers initially practised exclusive breastfeeding, rates declined substantially within the first six months. A study conducted by Makwela *et al.* (2024) in Polokwane found that only 39% of infants aged 0 to 6 months were exclusively breastfed for 6 months. This early introduction of complementary foods and liquids practice is reportedly often driven by maternal workload, cultural practices, and perceptions that breast milk alone is insufficient for infant growth (MAL-ED Network Investigators, 2017). Kubeka (2024) reported similar factors as identified in South African studies, including maternal return to work or school, lack of workplace support for breastfeeding, and pressure from family members to introduce complementary foods early. These factors are particularly relevant in rural communities where informal employment and limited maternity support structures may force mothers to introduce other foods prematurely. Although most mothers in the study had secondary schooling, which likely contributed to high rates of early initiation and colostrum feeding, education alone did not prevent early cessation of exclusive breastfeeding. Similar results have been reported in KwaZulu-Natal and Gauteng, where even educated mothers struggled to maintain exclusive breastfeeding due to economic pressures, social expectations, and insufficient postnatal support (Naicker, 2024). A positive association between exclusive breastfeeding practices and the age of the mother was observed, with older mothers more likely to exclusively breastfeed their infants (Makwela *et al.*,2024).

Improving exclusive breastfeeding practices is critical for achieving global and national development goals, particularly the Sustainable Development Goals (SDGs). Optimal infant feeding contributes directly to SDG 2 (Zero Hunger) by improving child nutrition and reducing malnutrition, and SDG 3 (Good Health and Well-being) by reducing infant morbidity and mortality through protection against infections and poor growth outcomes. Supporting breastfeeding practices also indirectly contributes to SDG 1 (No Poverty) and SDG 5 (Gender Equality) by reducing healthcare costs and supporting women's health and economic participation.

At national level, the Infant and Young Child Feeding Policy emphasises the protection, promotion, and support of exclusive breastfeeding for the first six months of life, followed by continued breastfeeding up to two years and beyond (NDoH, 2013). The policy seeks to re-establish breastfeeding as the biological norm in South Africa and provides guidance to health facilities, communities, and workplaces to create enabling environments for mothers, while reinforcing compliance with the International Code of Marketing of Breast-milk Substitutes to prevent inappropriate promotion of formula feeding (WHO, 2018). Similarly, the National Development Plan 2030 positions maternal and child health as central to strengthening the health system and building human capital, recognising early childhood nutrition as foundational to cognitive development, school readiness, and long-term economic productivity (Chigova, 2021). By promoting exclusive breastfeeding, South Africa advances its national targets to reduce child mortality, improve nutritional status, and break intergenerational cycles of poverty.

5.4 Growth status of the infant

This section discusses the results for infant growth status using length-for-age, weight-for-age, length-for-weight, and BMI-for-age z-scores.

5.4.1 Length-for-age z-scores (LAZ)

The study found that most infants maintained normal LAZ throughout the follow-up period, although the proportion declined from 85.0% in month one to 71.0% in month six. Although overall stunting remained below 20% at all time points, it is important to note the increase in the proportion of children who were moderately to severely stunted over the study period. The trend in this study indicates that while most infants still experienced normal growth, some began to show slower length growth as they approached six months of age. A recent longitudinal study conducted in rural Vijayapura, India, by Hulamani *et al*. (2025) reported that LAZ tend to decline gradually within the first six months of life, particularly as infants transition through feeding changes and increased exposure to infections. Similar results were reported in the MAL-ED multi-country birth cohort study, which followed children from birth in eight low- and middle-income settings, including Bangladesh, Brazil, India, Nepal, Pakistan, Peru, South Africa (Venda, Limpopo Province), and Tanzania (MAL-ED Network Investigators, 2017). The latter study showed that declines in length-for-age commonly begin in early infancy and are strongly associated with repeated infections, poor sanitation conditions, and suboptimal feeding practices. Infants are starting to show slower growth, and if conditions do not improve, more infants may become stunted later.

The National Food and Nutrition Security Survey National report (2023) indicates that approximately 28.8% of children under five years in South Africa were stunted, confirming that chronic undernutrition remains a major public health challenge (Department of Agriculture, Land Reform and Rural Development (DALRRD) et al., 2023). At the provincial level, Limpopo Province reports a stunting prevalence of about 18% among children under five, meaning nearly one in five children experiences impaired linear growth (DALRRD *et al*., 2023). Mugware *et al*. (2022) study reported normal HAZ in 90.0% of children aged zero to 36 months, while stunting prevalence was 10.0%. Another study conducted in the Thabazimbi sub-district of Limpopo Province found that 38% of children aged 12-24 months were stunted, indicating substantial growth challenges that might have originated in early infancy if feeding and health conditions were not optimal (Mafhungo *et al*., 2025).

Stunting among children is influenced by a combination of nutritional, health, environmental, and socio-economic factors that interact during early childhood (UNICEF, WHO and World Bank, 2023). Inadequate dietary intake, particularly poor breastfeeding and complementary feeding practices, contributes significantly to stunting because insufficient nutrient intake during the first 1000 days of life limits optimal growth and development (WHO, 2023). Recurrent infections, especially diarrhoeal diseases and respiratory illnesses, further contribute to stunting by reducing nutrient absorption and increasing nutritional requirements (Prendergast and Humphrey, 2014). Poor WASH conditions increase children’s exposure to infections and environmental enteric dysfunction, impairing nutrient uptake and contributing to growth faltering (Rahut *et al*., 2022). Household poverty, food insecurity, low maternal education, and limited access to health services further increase vulnerability to chronic undernutrition and long-term growth failure among children (UNICEF, WHO and World Bank, 2023).

5.4.2 Weight-for-age z-scores (WAZ)

Most infants maintained normal weight-for-age throughout the six-month follow-up period, with a slight decline observed by month 6. The pattern is similar to the results for length-for-age. However, in this case, moderate underweight increased slightly from 8.0% in month one to 11.0% in month six. Severe underweight, on the other hand, remained consistently low, fluctuating between 2.0% and 4.0% over the study period. These results suggest that although underweight was not widespread in the cohort, a small proportion of infants experienced weight faltering as they approached six months of age.

Weight-for-age is a composite indicator reflecting both acute and chronic nutritional status. Changes in WAZ during early infancy often signal challenges related to feeding adequacy and exposure to illness (WHO, 2009). The slight increase in moderate underweight observed in this study as infants approached six months is consistent with global evidence showing that infant weight gain may slow when exclusive breastfeeding is interrupted or when complementary foods are introduced prematurely or inadequately. The MAL-ED multi-country birth cohort study demonstrated that infant growth trajectories are strongly influenced by recurrent infections and suboptimal feeding practices, particularly during the transition from exclusive breastfeeding (MAL-ED Network Investigators, 2017).

Global estimates further confirm that underweight remains a significant contributor to poor child growth outcomes, particularly in LMICs where feeding and infection risks persist. The UNICEF, WHO, and World Bank Joint Child Malnutrition Estimates (2023) report that approximately 12% of children under five worldwide are underweight, with many cases linked to poor feeding practices and repeated illness episodes that compromise weight gain in early childhood. These global trends reinforce the importance of monitoring weight changes in early infancy as indicators of potential nutritional vulnerability.

The results in this study also align with national patterns in South Africa. Underweight remains a significant problem among South African children, with 13% of children under five being underweight, highlighting persistent challenges related to child nutrition and household food security (DALRRD *et al.*, 2023). Compared with these national figures, the prevalence of underweight observed in the present study remains relatively lower, which may reflect the younger age group studied, as weight deficits often become more pronounced after infancy if nutritional risks persist.

Provincial evidence from Limpopo similarly indicates that underweight continues to affect child growth. The Mugware *et al*. (2022) study found that underweight amongst the 0-36 months age group was 17.2% in the Vhembe district. Mafhungo *et al*. (2025), on the other hand, in the Thabazimbi sub-district found that 13% of children aged 12-24 months were underweight. The low levels of underweight prevalence remained low in the early months of follow-up, a notable increase in moderate underweight by month six was observed, indicating that growth vulnerability emerged later in infancy rather than at birth. This temporal pattern suggests that early protective factors, such as breastfeeding and limited environmental exposure, may diminish over time, increasing susceptibility to inadequate dietary intake and infection as infants grow older. The high coverage of health facility births in this study suggests that most newborns receive essential care such as weighing, early health checks, and counselling on breastfeeding soon after birth, which supports improved infant health indicators (Wan *et al*., 2023).

5.4.3 Weight-for-length z-scores (WLZ)

The results of this study show that normal WLZ increased from 43.0% in month one to 60.0% in month six. Weight-for-length reflects an infant's weight in relation to their length, and the observed increase therefore indicates that more infants achieved a healthy weight relative to their length as they grew over time, suggesting improvement in short-term nutritional status (WHO, 2006). Similar trends have been observed globally, with improvements in infant growth during the first months of life often linked to better feeding and caregiving practices (Victora *et al*., 2021). However, this study also shows that 5% in month four are wasted, which is a public health concern (WHO, 2019_b). Furthermore, a considerable proportion of infants were at risk of overweight, with the highest rate observed in month three (31.0%). The results for overweight in month three indicate that nearly one-third of infants at that time had a weight-to-length ratio above the expected range. Global evidence (WHO, 2024) indicates that rapid weight gain in infancy is increasingly common and contributes to rising levels of childhood overweight, particularly in LMICs undergoing dietary and lifestyle transitions.

The results of this study further indicate that the percentage of infants classified as overweight decreased from 20.0% in month one to 13.0% in month three, while obesity remained below 10.0% throughout the study period. Obesity levels fluctuated from 8% in month one to 5% at month six. The month with the highest prevalence was month two (14%). Early excess weight gain remains concerning because higher weight-for-length during infancy has been associated with increased risk of overweight and obesity later in childhood (Woo Baidal *et al*., 2016). The overweight/obesity results in this study indicate a public concern. The WHO global target is to reduce and maintain overweight and obesity at less than 5% by 2030 (WHO, 2024). The patterns in this study are consistent with national South African data showing that overweight among young children coexists with relatively low wasting levels. The MAL-ED birth cohort study showed that although wasting was present during early infancy, many children also experienced periods of rapid weight gain associated with feeding practices and infection exposure, resulting in varying growth trajectories across infancy (MAL-ED Network Investigators, 2017).

The South Africa Demographic and Health Survey reported that wasting among children under five was approximately 2.5%, while about 13% were overweight, reflecting the country's growing double burden of malnutrition, in which undernutrition and excess weight coexist (NDoH *et a*l., 2019). Mugware et al. (2022) further indicate that 60.0% children had normal WLZ, and wasting was at 33.6%. Overall, wasting was not a dominant growth problem in this study, as prevalence remained very low to low at most measurement points, with only a moderate increase observed at month four, suggesting a short-term period of vulnerability rather than sustained acute undernutrition. In contrast, evidence of growth faltering in older infants alongside emerging overweight indicates that the primary challenge is suboptimal growth, likely driven by inappropriate feeding transitions and increased infection exposure rather than inadequate energy intake.

5.4.4 BMI-for-age z-scores (BMIZ)

The BMI-for-age results show that most infants in the study maintained a healthy body mass index relative to their age, and the percentage within the normal BMI-for-age category increased over the first six months. The results suggest that many infants experienced appropriate growth during early infancy. Despite the predominance of normal BMI-for-age, a notable proportion of infants remained at risk of overweight, a trend that declined over time. Monitoring BMI-for-age in infancy remains important because rapid increases in BMI early in life are associated with greater risk of overweight and obesity later in childhood, particularly in settings undergoing nutritional transition (Woo Baidal *et al.,* 2016; WHO, 2024).

South African national data reflect a pattern in which stunting persists alongside increasing childhood overweight, illustrating the country's growing double burden of malnutrition (NDoH *et al*., 2019). Importantly, stunting and overweight are not mutually exclusive and can coexist within the same child. Evidence shows that children who experience chronic undernutrition and repeated infections early in life may develop impaired linear growth, while subsequent exposure to energy-dense, nutrient-poor diets promotes excess weight gain without corresponding gains in height (Sebsbie *et al*., 2022). This results in children who are short for their age but relatively heavy for their height. Metabolic adaptations to early undernutrition, including reduced fat oxidation and altered appetite regulation, may further predispose stunted children to overweight when food environments shift towards high-calorie diets (Steyn and Nel, 2022). In South Africa, this phenomenon is reinforced by feeding practices characterised by early introduction of refined carbohydrates, limited dietary diversity, and ongoing exposure to infection, explaining why stunting can coexist with overweight even in settings where wasting is relatively uncommon.

5.4.5 Summary of the growth status of infants in Dzimauli villages

The results that most infants maintained normal growth during early infancy support progress toward SDG 2, Zero Hunger, particularly Target 2.2, which seeks to end all forms of malnutrition, including stunting and wasting in children under five years of age. However, a slight decline in growth indicators over the past six months suggests that some infants were beginning to face growth risks as they grew older, underscoring the need for continued interventions to prevent later growth faltering and to ensure progress toward eliminating malnutrition.

5.4 Prevalence of antibiotic resistance

The findings of this study indicate that AR is already present among infants, with resistance patterns varying across the antibiotics tested. From a clinical and public-health perspective, infants, particularly within the first six months of life, would be expected to have limited direct exposure to antibiotics and, consequently, minimal colonization with resistant organisms. However, growing evidence demonstrates that resistant bacteria can be acquired very early in life through indirect pathways, including vertical transmission from mothers during delivery, postnatal household contact, environmental exposure, and interactions with healthcare settings (WHO, 2023; Essack, 2024). Studies have shown that neonates may become colonised with resistant Enterobacteriaceae shortly after birth, even in the absence of direct antibiotic treatment, due to maternal carriage or exposure within healthcare facilities (NICD, 2023). In contexts characterised by high background antimicrobial use and environmental contamination, infants may therefore acquire resistant organisms indirectly, highlighting the ecological nature of AR transmission.

The resistance patterns observed in this study, particularly the high levels of resistance to tetracycline and cefepime, suggest early exposure to community-circulating resistant strains. In sub-Saharan Africa, resistant organisms have been widely detected in community water sources, household environments, livestock, and healthcare settings, creating multiple opportunities for early colonisation (WHO, 2023; Essack, 2024). Frequent antibiotic prescribing in both outpatient and inpatient settings further contributes to selective pressure, increasing the prevalence of resistant strains within communities (Mthombeni *et al*., 2023). Moreover, even in the absence of direct infant antibiotic use, exposure may occur through maternal antibiotic use during pregnancy or breastfeeding, contaminated food and water, or close contact with colonised caregivers (WHO, 2023).

The substantial proportion of intermediate susceptibility observed for cefotaxime and ceftriaxone is particularly concerning, as these third-generation cephalosporins are widely used in neonatal and paediatric care. Intermediate susceptibility is often regarded as an early warning signal of evolving resistance under sustained antibiotic pressure (NICD, 2023; WHO, 2023). The presence of such resistance patterns in early infancy underscores that antimicrobial resistance is driven not only by individual-level antibiotic exposure but also by community-level transmission dynamics and environmental reservoirs. These findings reinforce the need for integrated interventions that strengthen infection prevention and control, antimicrobial stewardship, and environmental health measures to reduce early-life colonisation and limit the further spread of resistant bacteria (Essack, 2024; WHO, 2023; Laxminarayan *et al*., 2020).

Recent evidence from around the world indicates that approximately 25% of people worldwide harbour extended-spectrum beta-lactamase (ESBL)-producing bacteria in the gut, demonstrating widespread exposure to resistant organisms within communities (Karanika *et al.*, 2024). In sub-Saharan Africa, resistance levels appear even higher among children, with a recent review reporting that 32.2% of children carry extended-spectrum cephalosporin-resistant Enterobacterales, while carbapenem-resistant organisms were detected in about 3.6% of children (Afolabi *et al.*, 2024). These results indicate that carriage of AR is becoming common in childhood across many low-resource settings, consistent with the resistance levels detected in infants in this study.

In South Africa, AR among children has similarly become a growing concern. Studies conducted in paediatric hospital settings (Gauteng, KwaZulu-Natal, Western Cape and Free State) have reported that 8% of children admitted to intensive care units already carry carbapenem-resistant bacteria, while over 10% acquire resistant organisms during hospital admission, demonstrating ongoing transmission in healthcare environments (Perovic *et al.*, 2020). Although these results relate mainly to hospitalised children, they show that resistant bacteria are increasingly encountered among young children in South Africa.

Evidence from Limpopo Province further confirms the presence of AR in children. Studies conducted among children under five years presenting with diarrhoea in Limpopo reported extremely high levels of resistance, with over 90% of bacterial isolates showing resistance to multiple antibiotics, indicating widespread circulation of resistant organisms in community and clinical settings (Monyama *et al.*, 2024). Similarly, results from the MAL-ED birth cohort conducted in Venda, Limpopo, among infants aged 4-12 months demonstrated substantial resistance to commonly used antibiotics, including co-trimoxazole (54.0%), penicillin-class antibiotics (47.0%), and tetracycline (45.0%), indicating that resistant gut bacteria are already present in early childhood (MAL-ED Network Investigators, 2017).

Infants living in environments with inadequate WASH are more likely to experience recurrent infections that impair nutrient absorption and suppress appetite, contributing to poor growth outcomes such as underweight and stunting (Prüss-Ustün *et al.*, 2019). These recurrent illness episodes frequently lead to antibiotic treatment, sometimes unnecessary or incomplete, thereby increasing selective pressure for AR (Laxminarayan *et al.*, 2020). Global evidence shows that improvements in WASH conditions substantially reduce childhood diarrhoeal disease, associated malnutrition, and the need for antibiotics, which, in turn, lower community-level antimicrobial resistance (WHO and UNICEF, 2023). In the present study, although most infants maintained normal growth during early infancy, the emergence of moderate underweight and stunting by six months suggests increasing growth vulnerability as infants age and face cumulative nutritional and health challenges, a pattern well documented in the literature (Prendergast and Humphrey, 2014; UNICEF, WHO, and World Bank, 2023). Concurrently, the detection of antibiotic-resistant bacteria among infants indicates that resistant organisms are already circulating within households and communities, likely facilitated by environmental exposure and suboptimal WASH conditions, reinforcing the interconnected pathways linking WASH, infection, growth faltering, and antimicrobial resistance.

5.5 Associations of selected variables

This section discusses the associations observed between selected maternal and household factors and infant growth and health outcomes at six months of age. The analysis focuses on associations between breastfeeding practices, mothers' hygiene and food-handling practices, and infant growth status, including LAZ, WAZ, WLZ, and BMIZ. In addition, the section examines factors associated with AR among infants.

5.6.1 Infants' growth status at six months by breastfeeding

The UNICEF and WHO (2023) emphasise that early cessation of exclusive breastfeeding remains a major global challenge linked to persistent infant growth deficits in many low- and middle-income countries. Consistent with this, the present study found that although some non-breastfed infants maintained normal growth at six months, a notable proportion showed stunting and underweight, suggesting early growth vulnerability. Evidence across Africa similarly indicates that infants introduced to foods before six months face increased risks of infection, inadequate feeding, and subsequent growth faltering. In South Africa, despite good breastfeeding initiation, exclusive breastfeeding declines rapidly, contributing to the country's double burden of malnutrition, where persistent stunting coexists with rising childhood overweight (NDoH *et al.*, 2019; Simelane *et al.*, 2023). Studies from Limpopo Province mirror these patterns, reporting early introduction of complementary foods and low exclusive breastfeeding rates associated with poorer growth outcomes (Mbhenyane *et al.*, 2023; Makwela *et al.*, 2024). The presence of overweight among some non-exclusively breastfed infants in the current study further reflects broader global trends in which early feeding transitions contribute simultaneously to undernutrition and excess weight gain in early childhood.

5.6.2 Handwashing practices score of mothers by infants' growth status

A positive association between handwashing behaviour and infant growth was observed. The results indicate that normal growth classifications were most common across all indicators, with slightly better growth outcomes observed among infants whose mothers reported good handwashing practices. Similar patterns have been reported globally, showing that improved household handwashing practices are associated with improved child growth outcomes. Thus, effective hygiene reduces diarrhoeal disease and supports better nutrient absorption during early childhood (WHO, 2023). A study conducted by Rahut *et al.* (2022). Also demonstrate that infants and children living in households with improved sanitation and hygiene conditions experience lower rates of growth faltering than those living in households with poor hygiene. This was also evident in Africa, as reported by Headey and Palloni (2019), Headey and Palloni (2019) where they showed that improved household hygiene behaviours contribute to better nutritional outcomes by reducing infection-related growth interruptions among children in their study.

In South Africa, national data demonstrate that inadequate sanitation and hygiene are associated with increased infection risks, negatively influencing child nutritional status and growth (NDoH *et al.*, 2019). Provincial results from Limpopo also support this pattern, showing that households with improved WASH access report fewer diarrhoeal episodes among children, contributing to improved growth outcomes when hygiene practices are consistently maintained (Munyai *et al.*, 2023; Bazaanah and Mothapo, 2023). The patterns in this study suggest that strengthening handwashing practices may help create healthier environments that support optimal infant growth, highlighting. In Limpopo, it was also reported that WASH improvements, along with nutrition and child health interventions in rural communities (Sello *et al.,* 2023).

5.6.3 Scores of food handling practices of mothers by infants' growth status

The findings of this study indicate that exclusive breastfeeding was not consistently practised during the first six months of life, as some mothers introduced complementary foods as early as the first month. Despite this early introduction, normal growth outcomes were most common among infants whose mothers had fair and poor food-handling practice scores across all growth indicators. These findings suggest that infant growth may not directly correspond with reported household food-handling practices. Similar observations have been reported in international literature, where child growth outcomes are influenced by multiple, interrelated factors, including breastfeeding practices, dietary intake, exposure to infection, and household living conditions, rather than food handling practices alone (UNICEF, WHO and World Bank, 2023). Furthermore, although poor food handling increases the risk of foodborne infections, growth outcomes in early infancy may remain normal when breastfeeding remains the primary food source and provides protective immunological benefits (WHO, 2023).

At a broader contextual level, evidence from African settings indicates that food-handling practices alone may not fully predict infant growth outcomes, as access to safe water, sanitation, adequate complementary feeding, and overall socioeconomic conditions play substantial roles in child nutrition and health (Rahut *et al*., 2022). South African research similarly demonstrates that growth faltering is often driven by poverty, recurrent infections, and suboptimal complementary feeding practices rather than food handling practices in isolation (NDoH *et al*., 2019). Studies conducted in Limpopo further support this perspective, showing that while households may demonstrate varying food handling practices, child growth outcomes are more strongly shaped by feeding practices, socioeconomic conditions, and patterns of disease exposure within communities (Munyai *et al*., 2023; Bazaanah and Mothapo, 2023). Nonetheless, maintaining good food-handling practices remains important for preventing diarrhoeal diseases and protecting infant health, particularly as complementary feeding increases during later infancy (WHO, 2023).

5.4.6 Association Between WASH Practices and Infant growth Indicators

Across all anthropometric indicators, LAZ, WAZ, WLZ, and BMIZ, household WASH practices, including maternal handwashing and food-handling behaviours, were not significantly associated with infant growth outcomes after full adjustment. These findings are consistent with evidence from large multi-country analyses demonstrating that although improved WASH conditions reduce diarrhoeal disease, their independent effect on linear growth and other anthropometric indicators is often modest unless combined with improvements in nutrition, caregiving, and living conditions (Headey and Palloni, 2019; Cumming *et al*., 2019). Similarly, literature shows that stunting, underweight, and wasting are influenced by multiple interacting determinants, including dietary adequacy, recurrent infections, household food security, and broader environmental conditions, rather than WASH practices alone (Prendergast and Humphrey, 2014; Ngure *et al*., 2014; UNICEF, WHO and World Bank, 2023). Large trials further indicate that WASH interventions reduce diarrhoeal morbidity but do not consistently translate into measurable improvements in LAZ, WAZ, or WLZ in the absence of concurrent nutrition interventions (Luby *et al*., 2018; Cumming *et al*., 2019).

The absence of association with BMIZ in the present study aligns with evidence suggesting that BMI-related outcomes in early infancy are more strongly shaped by feeding adequacy and socio-demographic factors than by hygiene practices alone. Notably, significant declines in LAZ, WAZ, and WLZ were observed between month one and month six, indicating that linear growth faltered and weight deficits emerged during early infancy. This trajectory reflects patterns widely reported in low- and middle-income countries, where growth deficits become more pronounced as infants transition from exclusive breastfeeding to complementary feeding and encounter cumulative nutritional and infectious exposures (Jones *et al*., 2016; UNICEF, WHO and World Bank, 2023). South African national data similarly show increasing risks of underweight and wasting during infancy, particularly in socio-economically disadvantaged communities (Shisana *et al*., 2013; NDoH *et al*., 2019). Provincial evidence from Limpopo further documents persistent growth challenges in rural settings where complementary feeding practices, food security, and environmental health conditions remain suboptimal (Mugware *et al*., 2022; Mphasha *et al*., 2023; Makhavhu *et al*., 2025).

Infant sex emerged as a significant predictor across several indicators, with male infants showing higher LAZ, WAZ, WLZ, and BMIZ compared with females. While some international literature reports greater biological vulnerability to growth faltering among boys (Victora *et al*., 2016; Prendergast and Humphrey, 2014), Sex differences may vary by context and reflect local caregiving and feeding dynamics. Although strengthening WASH practices remains essential for infection prevention, meaningful improvements in infant growth require integrated strategies that combine WASH, optimal infant feeding, improved household food security, and broader socio-economic support (UNICEF, WHO and World Bank, 2023; Cumming *et al.*, 2019).

5.6.4 Factors associated with MDR

In both adjusted models examining factors associated with MDR, no statistically significant independent predictors were identified after accounting for food-handling and hygiene practices and other covariates. Although some estimates suggested directional differences, such as lower odds of MDR among male infants and higher odds among infants classified as at risk of overweight, none reached statistical significance, and wide confidence intervals indicated substantial imprecision. These findings likely reflect limited statistical power due to the small number of MDR cases, a challenge commonly reported in antimicrobial resistance research in low-resource settings where sparse data restrict the detection of meaningful associations (WHO, 2023).

The absence of significant sex differences aligns with evidence suggesting that early childhood infection risk is more strongly influenced by exposure patterns and healthcare access than by biological sex alone (UNICEF, 2023). Similarly, the non-significant association observed among infants at risk of overweight may reflect underlying illness or prior antibiotic exposure rather than a direct relationship between nutritional status and MDR, particularly given emerging, though inconclusive, evidence linking early antibiotic exposure, alterations in gut microbiota, and growth patterns (UNICEF, 2023).

Across both models, neither food-handling nor food handling practices were significantly associated with MDR. Although fair food-handling practices showed higher odds and excellent practices lower odds of MDR in Model 1, and good food handling practices showed higher odds in Model 2, these estimates were imprecise and not statistically significant. This pattern is consistent with growing evidence that antimicrobial resistance transmission occurs through complex, multi-level pathways, including environmental contamination, antibiotic misuse, healthcare contact, and community transmission, rather than through household food handling practices alone (Essack, 2024; WHO, 2023).

In South Africa, resistant organisms have been documented in both community and healthcare environments, with high levels of antibiotic prescribing in public hospitals and increasing burdens of MDR infections among neonates and young children, underscoring the role of healthcare exposure and the challenges of stewardship (Mthombeni *et al*., 2023; NICD, 2023). Poor WASH conditions further contribute to the environmental persistence and spread of resistant bacteria in sub-Saharan Africa (WHO, 2023). Together, these findings suggest that broader structural and healthcare-related factors may overshadow the measurable effects of individual household practices in community-based analyses, highlighting the need for larger, integrated studies that incorporate antibiotic use patterns, environmental contamination, and sanitation conditions to better understand MDR transmission dynamics (Essack, 2024; WHO, 2023).

CHAPTER 6: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

This longitudinal study provides a comprehensive assessment of how WASH conditions, infant feeding practices, and household socio-demographic factors interact to influence infant growth and AR in infants aged 0-6 months in Dzimauli rural villages. The findings depict a community with partial access to improved water and sanitation infrastructure, yet persistent structural and behavioural gaps remain. Although many households reported improved water sources and toilet access, frequent water interruptions and reliance on alternative sources may compromise consistent hygiene practices. Similarly, while self-reported handwashing was common, the frequent use of water alone suggests incomplete adherence to optimal hygiene standards. Food-handling practices also varied across households and over time, indicating inconsistent protective behaviours during early infancy.

Infant feeding practices reflected both strengths and vulnerabilities. Early initiation of breastfeeding and the provision of colostrum were widely practised. However, exclusive breastfeeding was not sustained for most infants beyond six months. Although most infants remained within normal growth ranges, significant shifts in anthropometric indicators over time point to emerging risks of linear growth faltering alongside early signs of overweight within the same population. These patterns illustrate the dual burden of malnutrition in early infancy and highlight the complexity of growth trajectories in rural settings.

Importantly, the study demonstrates that infant growth outcomes are shaped by multiple interacting determinants rather than by a single household practice. While hygiene and food-handling behaviours play an important role in infection prevention, WASH practice scores alone were not consistently associated with anthropometric indicators after accounting for maternal and household characteristics. Infant sex emerged as a consistent predictor of growth, with male infants showing higher anthropometric scores across models. Correlation analyses further underscored that non-exclusive breastfeeding was associated with both undernutrition and emerging overweight risks, reflecting the need for balanced feeding support.

This study found no statistically significant associations between household WASH practices (including handwashing, food-handling, and food handling behaviours) and infant growth outcomes (LAZ, WAZ, WLZ, and BMIZ) after adjusting for maternal and socio-demographic characteristics. Growth declines observed between one and six months across LAZ, WAZ, and WLZ suggest emerging growth faltering during early infancy, a pattern widely documented in low- and middle-income countries as infants transition from exclusive breastfeeding to increased environmental and dietary exposure (Jones *et al*., 2016; NDoH *et al*., 2019).

Although WASH improvements are well established in reducing diarrhoeal disease, their independent effect on anthropometric outcomes in early infancy appears limited in this setting, consistent with multi-country evidence indicating that hygiene interventions alone rarely produce substantial improvements in linear growth or weight status without concurrent nutrition and socio-economic support (Headey and Palloni, 2019; Luby *et al.,* 2018; Cumming *et al*., 2019; UNICEF, WHO and World Bank, 2023).

Similarly, no independent predictors of AR were identified in the adjusted models examining food-handling and food handling practices. The absence of statistically significant associations likely reflects limited statistical power due to the sparse number of MDR cases and underscores the complexity of antimicrobial resistance transmission pathways. Current evidence indicates that MDR in early life is shaped by interactions among antibiotic exposure, healthcare contact, environmental contamination, and sanitation conditions rather than by household hygiene practices alone (WHO, 2023; Essack, 2024; NICD, 2023). The findings highlight that infant growth and MDR risk in this rural South African context are influenced by multifactorial and interconnected determinants that extend beyond household-level hygiene behaviours.

6.2 Limitations of the study

The study employed a longitudinal design and had attrition over time, resulting in variations in sample size across data collection points. Some participants discontinued their involvement due to relocation, loss to follow-up, or other personal reasons, which reduced the number of complete data available for analysis. Due to time and financial constraints, laboratory analysis could only be conducted on 55 isolates obtained from infants, rather than the total sample of 156 infants who participated in the study. As a result, AR testing was limited to a smaller subset of participants, potentially reducing the generalisability of the results to the entire study population.

6.3 Recommendations

Based on the study findings, improving infant growth outcomes and reducing AR in Dzimauli rural villages requires coordinated, multisectoral action. The following recommendations identify both priority interventions and the key stakeholders responsible for implementation.

To strengthen exclusive breastfeeding and infant feeding support the following recommendations are made:

- The DoH and local clinics should strengthen breastfeeding counselling during antenatal, postnatal, and immunisation visits.
- CHWs should conduct home visits to provide continuous breastfeeding and complementary feeding support.
- Dietitians and nutrition advisors should develop culturally appropriate feeding guidelines.
- Community and traditional leaders should support social norm change initiatives that promote exclusive breastfeeding for six months.

To implement Integrated WASH Nutrition Programmes

- Government departments should design integrated programmes that combine hygiene promotion with IYCF education.

- Municipalities should ensure a consistent water supply and support safe sanitation infrastructure.
- CHWs and environmental health practitioners should promote correct handwashing with soap and safe food preparation practices, especially during complementary feeding.
- Schools and community platforms should reinforce hygiene education at the household level.

To address the Dual Burden of Malnutrition observed in the current study:

- There should be routine growth monitoring and promotion activities implemented and strengthened to detect both undernutrition and emerging overweight risks early.
- Further studies exploring the association between non-exclusive breastfeeding and overweight/obesity should be conducted.
- Healthcare providers should offer tailored counselling for caregivers of infants showing growth deviations.
- The Department of Social Development should support food security initiatives targeting vulnerable households.

Stakeholders should strengthen infection prevention and antibiotic stewardship. Thus:

- Healthcare facilities should strengthen infection prevention and control (IPC) measures.
- Antibiotic stewardship programmes should be reinforced to ensure rational prescribing practices.
- Pharmacists and clinicians should educate caregivers on appropriate antibiotic use and risks of misuse.
- National surveillance bodies such as NICD should continue monitoring antimicrobial resistance trends and guide policy responses.

More research and surveillance should be strengthened to:

- Conduct larger longitudinal studies integrating WASH, feeding practices, environmental sampling, and antibiotic exposure data.
- Strengthen rural surveillance systems to monitor growth trends and MDR patterns.
- Facilitate partnerships between academic institutions and local health authorities to ensure evidence informs policy and practice.

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APPENDICES

Appendix A: Child assessment form

Child study ID: □ □ □

CHILD ASSESSMENT FORM (CAF)			
If no response to any question, write NA as the response.			
#	**Question**	**Code**	**Response**
01	Study researcher/Nurse/Fieldworker ID		□ □ □
02	Today's date (DD/MMM/YY)		□ □ / □ □ □ / □ □
03	Screen complete and Consent obtained	Yes = 01 No = 00	□ □
04	Date of birth (DD/MMM/YY)		□ □ / □ □ □ / □ □
05	Gender of child	Male=01 Female=02	□ □
05	Age of birth	Full term=00 Preterm=01	□ □
06	Place of birth	Home=00 Health facility=01	□ □
07	Birthweight (kg) * (from birth record, if available)		□ □.□ .□
08	Birth length (cm) from birth record		□ □ □ . □
09	Head circumference (cm). From birth records		□ □.□ .□
08	How long after childbirth did the mother begin breastfeeding him/her?	= within 1 hour = 1-24 hours. = 1-3 days = 4+ days later NA = never breastfed	□ □
10	Was the child fed the first milk (colostrum)?	Yes = 01 No=00	□ □
11	Was there pre-lacteal feeding of the child?	Yes = 01 No = 00	□ □
12	Current weight (kg) *		□ □.□ . □
13	Current length (cm)		□ □ □ . □

14	Current head circumference (cm)		□ □.□ . □
15	Did the child receive Vaccination at Birth?	Yes=01 No=02	□ □
16	Did the child receive any medical treatment after birth?	Yes=01 No=02	□ □
17	Medication given (Names of the medication)		
18	Record all medication given to the child after birth	....	

** If birth weight OR current weight is less than 1,500 grams (1.5kg), the child is not eligible for the study and the Non-Participant form should be filled out.*

Appendix B: Monthly assessment form (MOA)

MONTHLY ASSESSMENT FORM			
#	**Question**	**Code**	**Response**
01	Study researcher / Nurse / Fieldworker ID		□ □ □
02	Today's date (DD/MMM/YY)	DD/MMM/YY	□ □ / □ □ □ / □ □
Nutrition			
03	Are you breastfeeding <CHILD>? If NO, then skip to Q.6	Yes = 01 No = 00	□ □
04	Last night, how many times did you breastfeed <CHILD> from sunset to sunrise?	00-99	□ □
05	Yesterday, during the day, how many times did you breastfeed <CHILD>?	00-99	□ □
06	Do you give <CHILD> infant formula? If NO, then skip to Q.9	Yes = 01 No = 00	□ □ □
07	Last night, how many times did you feed <CHILD> formula from sunset to sunrise?	00-99	□ □ □
08	Yesterday, during the day, how many times did you feed <CHILD> formula?	00-99	□ □ □
09	Do you give <CHILD> other milks, such as tinned, powdered or fresh animal milk? If NO, then skip to Q.12	Yes = 01 No = 00	□ □ □
10	Last night, how many times did you feed <CHILD> animal milks from sunset to sunrise?	00-99	□ □ □
11	Yesterday, during the day, how many times did you feed <CHILD> animal milk?	00-99	□ □ □
Yesterday, during the day or last night, did <CHILD> have:			
12	Plain water	Yes = 01 No = 00	□ □ □
13	Tea,	Yes = 01 No = 00	□ □ □
	Coffee	Yes = 01 No = 00	□ □□
14	Fruit or vegetable juices?	Yes = 01 No = 00	□ □ □

15	Any other liquids, such as sugar water, thin soup or broth, carbonated drinks <local examples>	Yes = 01 No = 00	□ □ □

16	Is <CHILD> eating any semi-solid, mashed, or solid foods? If NO, go to Q.32	Yes = 01 No = 00	□ □ □
Thinking about yesterday, during the day and at night, did <CHILD> have any of these foods, even if they were in combination with other foods?			
17	Rice, porridge, bread, noodles, or other foods made from grains.	Yes = 01 No = 00	□ □ □
18	White potatoes, white yams, manioc, or other foods made from roots?	Yes = 01 No = 00	□ □ □
19	Carrots, squash, or sweet potatoes that are yellow or orange inside.	Yes = 01 No = 00	□ □ □
19	Do you boil fresh milk before consumption?	Yes = 01 No = 00	□ □ □
21	Any vegetables eaten uncooked, such as lettuce, carrot	Yes = 01 No = 00	□ □ □
22	Any dark green leafy vegetables, such as spinach?	Yes = 01 No = 00	□ □ □
23	Foods made with beans, lentils, peas, corn, and ground nuts.	Yes = 01 No = 00	□ □ □
24	Ripe mangoes, papayas, or other sweet yellow/orange or red fruit?	Yes = 01 No = 00	□ □ □
25	Any other fruits or vegetables, such as bananas, apples, oranges, tomatoes, and avocados?	Yes = 01 No = 00	□ □ □
26	Liver, kidney, heart or other organ meats?	Yes = 01 No = 00	□ □ □
27	Any meat, such as chicken, beef, lamb, goat, duck (others)?	Yes = 01 No = 00	□ □ □
28	Eggs?	Yes = 01 No = 00	□ □ □
29	Fresh or dried fish or shellfish?	Yes = 01 No = 00	□ □ □
30	Cheese, yogurt or other dairy products?	Yes = 01 No = 00	□ □ □

31	Canned foods such as Fish, beans, condensed milk, processed meat e.g. meat balls	Yes = 01 No = 00	□ □□
32	Any sugary foods such as pastries, cakes or biscuits?	Yes = 01 No = 00	□ □ □
33	Any commercially available foods for infants or young children?	Yes = 01 No = 00	□ □ □
34	Yesterday, counting meals and snacks, how many times did you feed <CHILD>?	00-99	□ □ □
35	How would you describe your child's appetite? Would you say it is:	01=poor, 02=fair, 03=good, 04=very good	□ □ □
36	Did your child receive a vitamin A dose in the last month?	Yes = 01 No = 00	□ □ □
37	Do you give your child any vitamin or mineral syrups on a regular basis?	Yes = 01 No = 00	□ □ □
38	Does the salt you cook with contain iodine?	Yes = 01 No = 00	□ □ □
39	Does <CHILD> routinely eat things that are non-food items like ash, clay, paint chips, dirt, starch, cigarettes or cigarette butts?	Yes = 01 No = 00	□ □ □
	Hygiene practices		
40	Do you wash your hand before eating?	Never = 01; Rarely = 02 Sometimes = 03; Always = 04	□ □ □
41	Do you wash your hands before and after feeding the child	Never = 01; Rarely = 02 Sometimes = 03; Always = 04	□ □ □
42	Do you wash your hands before and after changing your child's nappy?	Never = 01; Rarely = 02 Sometimes = 03; Always = 04	□ □ □
43	How do you wash your hands?	Water only=01; water and soap=02 Clean warm water and soap=03	□ □ □
		Food practices	

47	Do you clean the preparation area and equipment for food preparation before using it for other food?	Never = 01; Rarely = 02 Sometimes = 03. Always = 04	□ □□
48	Do you wash fruits and vegetables before eating them?	Never = 01; Rarely = 02 Sometimes = 03. Always = 04	□ □□
49	I separate raw and cooked during storage.	Never = 01; Rarely = 02 Sometimes = 03; Always = 04	□ □□
50	After cooking a meal, I store leftovers in a cool place within two hours.	Never = 01; Rarely = 02 Sometimes = 03; Always = 04	□ □□
51	After handling raw food, do you wash your hands?	Never = 01; Rarely = 02 Sometimes = 03. Always = 04	□ □□
52	Do you wash your hands before cooking food?	Never = 01; Rarely = 02 Sometimes = 03. Always = 04	□ □□
53	Do you use the same kitchen utensils for handling raw and ready-to-eat foods?	Never = 01; Rarely = 02 Sometimes = 03; Always = 04	□ □□
	ANTHROPOMETRY		
54	Weight (kg)		□ □ . □ □
55	Length / height (cm)		□ □ □ . □
56	Head circumference (cm)		□ □ . □
	MONTHLY STOOL SAMPLE COLLECTION		
57	Monthly stool sample collected?	Yes = 01 No = 00	□ □

Appendix C: Follow-up socio-economic status form (FSE)

FOLLOW-UP SOCIO-ECONOMIC STATUS FORM (FSE) **(Administered at 6 months after recruitment)**			
If no response for any question, write NA as response.			
Fieldworker ID		□ □ □	
Date (DD/MMM/YY)		□ □ / □ □ □ / □ □	
DEMOGRAPHIC QUESTIONS			
#	**Question**	**Code**	**Response**
Questions for head of household (If the mother/primary caregiver of the child is the head of household, skip to question 9)			
1	What is your age?	16-99 *(years)*	□ □
2	*(Record sex)*	Male = 01; Female = 02	□ □
3	What is your relationship to [CHILD'S NAME]?	Father = 01; Mother = 02. Grandmother = 03; Grandfather = 04. Sibling = 05; Other, specify........................., = 06	□ □
4	Are you currently married, divorced, widowed, or never married? *If never married, skip to question 6.*	Never married = 01; Married = 02 Divorced = 03; Widowed = 04	□ □
5	How old were you when you got married for the first time?	12-50 *(years)*	□ □
6	Have you ever attended school? *If no, skip to question 9.*	Yes = 01; No = 00	□ □
7	How many years of schooling have you completed?	01-20	□ □
8	*If younger than 25 years old:* Are you currently attending school or college?	Yes = 01; No = 00	□ □
Questions 9-20 are for the child's mother. If the mother is deceased, skip to question 21. If the mother is unavailable, return to the household at another time to complete these questions. Complete these questions even if the mother is not the primary caregiver for the enrolled child.			
9	What is your age?	16-99 *(years)*	□ □
10	Are you currently married, divorced, widowed, or never married? *If never married, skip to question 12.*	Never married = 01; Married = 02 Divorced = 03; Widowed = 04	□ □
11	How old were you when you got married for the first time?	12-50 *(years)*	□ □
12	Have you ever attended school? *If no, skip to question 15.*	Yes = 01; No = 00	□ □
13	How many years of schooling have you have you completed.		□ □

15	How old were you when you first became pregnant?	12-50 (years)	□ □
16	How many pregnancies have you had in your lifetime?	01-20	□ □
17	How many live births have you had in your lifetime?	01-20	□ □
18	Are all of these children still alive? *If yes, skip to question 20.*	Yes = 01; No = 00	□ □
19	How many children have died?	01-10	□ □ □
20	Are you the primary caregiver for [CHILD'S NAME]?	Yes = 01; No = 00	□ □ □
Questions 21-28 should be addressed to the child's primary caregiver. Complete these questions only if the mother is not the primary caregiver. If the mother is the primary caretaker skip to question 29. □			
21	What is your relationship to [CHILD'S NAME]?	Father = 01; Grandmother = 02; Grandfather = 03; Sibling = 04; Other = 05 Specify.................	□ □ □
22	How old are you?	16-70 *(years)*	□ □ □
23	Are you currently married, divorced, widowed, or never married? *If never married, skip to question 25.*	Never married = 01; Married =02; Divorced = 03; Widowed = 04	□ □ □
24	How old were you when you got married for the first time?	12-50 *(years)*	□ □ □
25	What is your relationship to [NAME OF HEAD OF HOUSEHOLD]?	Partner = 01; Daughter = 02; Daughter-in-law = 03; Niece/nephew = 04; Mother/father in-law = 05; Sister = 06; Aunt = 07; Cousin = 08; Granddaughter = 09; Step-daughter or Adopted daughter= 10; Self = 11	□ □ □
26	Have you ever attended school? *If no, skip to question 29.*	Yes = 01; No = 00	□ □ □
27	How many years of schooling have you completed?	01-20	□ □ □
28	*If younger than 25 years old:* Are you currently attending school or college?	Yes = 01; No = 00	□ □ □
31	How many rooms in this household are used for sleeping?	01-10 (rooms)	□ □ □

34	What is the main source of drinking water for members of your household? *If answered 04, 05, 06, 07, or 08 skip to question 37.*	Piped into dwelling = 01 Piped to yard/plot = 02 Public tap= 03; borehole = 04; Protected well = 05 Unprotected well = 06 Surface water (river/ dam/ lake/pond/ stream/canal/irrigation canal) = 07 Other, specify...................= 08	□ □ □
35	Is your piped water supply continuous or is it sometimes interrupted? *If continuous, skip to question 37.*	Continuous = 01; Sometimes interrupted = 02	□ □ □
36	How long do these interruptions usually last?	One to three days=01; Four to six6 days= 02a week= 03; More than a week=04	□ □ □
37	What is the main source of water used by your household for other purposes such as cooking and hand-washing?	Piped into dwelling = 01 Piped to yard/plot = 02 Public tap= 03 Borehole = 04 Protected well = 05 Unprotected well = 06 Surface water (river/ dam/ lake/pond/ stream/canal/irrigation canal) = 07 Other, Specify.........................= 08	□ □ □
38	Do you pay or exchange for water?	Yes = 01; No = 00	□ □ □
39	Where is the water source located?	In own dwelling = 01; In own yard/plot = 02; Elsewhere = 03	□ □ □
40	How long does it take to go there, get water, and come back in one trip? *If water is located on the premises, response is 000.*	000-999 minutes	□ □ □ □
41	Who is the main person in the household who goes to fetch water from this source?	Adult woman = 01; Adult man = 02; Female child under age 15 years = 03; Male child under age 15 years = 04; Other = 05	□ □ □
42	Do you treat your water in any way to make it safer to drink?	Yes = 01 No = 00	□ □ □
43	What do you usually do to the water to make it safer to drink?	Let it stand and settle = 01 Solar disinfection = 02 Use water filter (ceramic /sand/ composite/etc.) = 03 Strain through a cloth = 04 Add bleach/chlorine = 05 Boil = 06 Other = 07	□ □ □
44	Do you wash your hands after helping your child defecate?	Never = 01; Rarely = 02 Sometimes = 03; Always = 04	□ □ □

45	Do you wash your hands before preparing food?	Never = 01; Rarely = 02 Sometimes = 03; Always = 04	□ □ □
46	Do you wash your hands after using the toilet?	Never = 01; Rarely = 02 Sometimes = 03; Always = 04	□ □ □
47	Do you use toilet paper?	Never = 01; Rarely = 02 Sometimes = 03; Always = 04	□ □ □
48	What kind of toilet facility do members of your household usually use?	No facility/bush/field or bucket toilet = 01 Pit latrine without flush = 02 Flush to piped sewer system = 03 Flush to septic tank = 04 Flush to pit latrine = 05 Flush to somewhere else = 06 Other = 07	□ □ □
49	Do you share this toilet facility with other households?	Yes = 01 No = 00	□ □ □
50	How many households use this toilet facility?	00-09 = 00 More than 9 households = 01	□ □ □

Appendix D: Stool collection form (SCF)

STOOL COLLECTION FORM (SCF)

If no response to any question, write NA as the response.

#	**Question**	**Code**	**Response**
01	Study researcher/Nurse/Fieldworker ID	Three letter/figure code	□ □ □
02	Today's date	(DD/MMM/YY)	□ □ / □ □ □ / □ □
03	Time of visit	Time (24 Hr Scale; HH:MM)	□ □□:□□ □□
04	Was a stool sample collected?	No = 00; Yes = 01	□ □
05	Time stool sample was produced	Time (24 Hr Scale; HH:MM)	□ □□:□□ □
06	Type of stool collected	Monthly=M1, Diarrhea=D1, Monthly Recollection=MR, Diarrhea Recollection=DR	□ □
07	Month of stool collection	##	□ □□
08	Is recollection necessary?	No = 00; Yes = 01	□ □□

Observations/Notes:

□

Appendix E: Department of Health approval letter

LIMPOPO
PROVINCIAL GOVERNMENT
REPUBLIC OF SOUTH AFRICA

DEPARTMENT OF
HEALTH

Ref	:	LP_2023-03-014
Enquires	:	Dr Ramalivhana NJ
Tel	:	015-293 6028
Email	:	Phoebe.Mahlokwane@dhsd.limpopo.gov.za

PO Bessong et al., 2023-2027

PERMISSION TO CONDUCT RESEARCH IN DEPARTMENTAL FACILITIES

Your Study Topic as indicated below;

ACQUISITION OF ANTIBIOTIC RESISTANT GUT BACTERIA AND IMPACT OF MICROBIOTA (2023-2027)

1. Permission to conduct research study as per your research proposal is hereby Granted.
2. Kindly note the following:
   a. Present this letter of permission to the Office of District Executive Manager a week before the study is conducted.
   b. This permission is **ONLY for Dzimauli Clinic; Mutale Health Centre and Donald Frazer Hospital**
   c. In the course of your study, there should be no action that disrupts the routine services or incur any cost on the Department.
   d. After completion of study, it is mandatory that the findings should be submitted to the Department to serve as a resource.
   e. The researcher should be prepared to assist in the interpretation and implementation of the study recommendation where possible.
   f. The approval is only valid for a 1-year period.
   g. If the proposal has been amended, a new approval should be sought from the Department of Health
   h. Kindly note that, the Department can withdraw the approval at any time.

   Your cooperation will be highly appreciated.

Ramalivhana

pp **Head of Department** 26/04/2023 **Date**

Private Bag X9302, Polokwane
Fidel Castro Ruz House, 18 College Street, Polokwane 0700. Tel: 015-293 6000/12. Fax: 015 293 6211.
Website: http/www.limpopo.gov.za

The heartland of Southern Africa – Development is about people!

Appendix F: Dzimauli royal authority approval letter

RAMBUDA TRADITIONAL COUNCIL

Enq: NAGELI M.R | Cell: 076 673 7316 | 076 862 2513
Email: nagelirichard72@gmail.com | avhatendirambuda@gmail.com

Ref: CH11/1/4/2/2/5-141
Date: 29/03/2023

Professor Pascal Bessong
Director
SAMRC-UNIVEN Antimicrobial Resistance and Global Health Research Unit

RE: RESEARCH COLLABORATION WITH THE DZIMAULI COMMUNITY

We refer to our meeting of 3 February 2023, during which we discussed the goals of the SAMRC-UNIVEN Antimicrobial Resistance and Global Health Research Unit and how the Dzimauli Community can partner with you to achieve those goals. We understand you will be enrolling babies and their mothers to study how members of the community acquire antibiotic resistant bacteria, as they grow up; to study environmental and animal factors; and to assess our community knowledge and attitudes that may be related to antibiotic resistance.
This letter is to indicate that we are willing to partner with the SAMRC-UNIVEN Unit by permitting access to our community for further engagement with potential study participants appropriately. We would appreciate feedback from the studies to see how the health and wellbeing of the community can be improved.

We look forward to a fruitful collaboration.

Rambuda Traditional Council
Nageli
Senior Administrative Officer

DEPARTMENT OF CO-OPERATIVE GOVERNANCE, HUMAN SETTLEMENTS & TRADITIONAL AFFAIRS
RAMBUDA TRADITIONAL COUNCIL
2023 -03- 29
P.O. BOX 226, DZIMAULI
0975
VHEMBE DISTRICT SUPPORT CENTRE

Appendix G: Ethical Clearance Certificate

ETHICS APPROVAL CERTIFICATE

RESEARCH AND INNOVATION
OFFICE OF THE DIRECTOR

NAME OF RESEARCHER/INVESTIGATOR:
Ms AK Ramalepe

STUDENT NO:
18007610

PROJECT TITLE: **The influence of WASH and feeding practices on growth and antibiotic resistance in the first six months of life at Dzimauli rural villages.**

ETHICAL CLEARANCE NO: **FHS/24/NUT/21/1610**

SUPERVISORS/ CO-RESEARCHERS/ CO-INVESTIGATORS

NAME	INSTITUTION & DEPARTMENT	ROLE
Prof CN Nesamvuni	UNIVEN, Nutrition	Supervisor
Dr TC Mahopo	UNIVEN, Nutrition	Co-Supervisor
Ms TA Masia	UNIVEN, Nutrition	Co-Supervisor
Ms AK Ramalepe	UNIVEN, Nutrition	Investigator – Student

Type: **Masters Research**
Risk: ***Minimal risk to humans, animals, or environment* (Category 2)**
Approval Period: **October 2024 – October 2025**

The Human and Clinical Trials Research Ethics Committee (HCTREC) hereby approves your project as indicated above.

General Conditions
While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, please note the following.

- The project leader (principal investigator) must report in the prescribed format to the REC:
  - Annually (or as otherwise requested) on the progress of the project, and upon completion of the project.
  - Within 48hrs in case of any adverse event (or any matter that interrupts sound ethical principles) during the course of the project.
  - Annually a number of projects may be randomly selected for an external audit.
- The approval applies strictly to the protocol as stipulated in the application form. Would any changes to the protocol be deemed necessary during the course of the project, the project leader must apply for approval of these changes at the REC. Would there be deviated from the project protocol without the necessary approval of such changes, the ethics approval is immediately and automatically forfeited.
- The date of approval indicates the first date that the project may be started. Would the project have to continue after the expiry date; a new application must be made to the REC and new approval received before or on the expiry date.
- In the interest of ethical responsibility, the REC retains the right to:
  - Request access to any information or data at any time during the course or after completion of the project,
  - To ask further questions; Seek additional information; Require further modification or monitor the conduct of your research or the informed consent process.
  - withdraw or postpone approval if:
  - Any unethical principles or practices of the project are revealed or suspected.
  - It becomes apparent that any relevant information was withheld from the REC or that information has been false or misrepresented.
  - The required annual report and reporting of adverse events was not done timely and accurately,
  - New institutional rules, national legislation or international conventions deem it necessary.

ISSUED BY:
UNIVERSITY OF VENDA, RESEARCH ETHICS COMMITTEE
Date Considered: October 2024

Name of the HCTREC Chairperson of the Committee: Prof NS Mashau

Signature:

UNIVERS[illegible] OF VEND[illegible]
OFFICE OF THE DIRECTOR
RESEARCH AND INNOVATION
2024 -10- 16
Private Bag X5050
Thohoyandou 0950

University of Venda
PRIVATE BAG X5050, THOHOYANDOU, 0950), LIMPOPO PROVINCE, SOUTH AFRICA
TELEPHONE (015) 962 8504/8313 FAX (015) 962 9060
"A quality driven financially sustainable, rural-based Comprehensive University"

Appendix H: Consent form

Title of Research Study: Acquisition of antibiotic-resistant gut bacteria and impact of Microbiota

Participant research ID: □ □ □ □ □ □ □ □

LETTER OF INFORMATION

[This information letter and consent form will be administered in the language of choice of the potential participant. The participant may request a copy of the signed informed consent.

Principal Investigator

PO Bessong, PhD

Tel: 073 798 5920 / 015 962 8301

Email: bessong@univen.ac.za

Lead Investigators: Cloupas Mahopo (PHD); Amidou Samie (PhD, MPH); Angelina Maphula (PhD); Rendani Makhwathana (PhD); Lufuno Mavhandu-Ramarumo (PhD); Gloria Selabe (PhD); Teke Apalata (MD, PhD); Winston Garira (PhD); Nontokozo Matume (PhD); and Ramalepe Audrey Kgomotso (BSc).

Brief introduction and purpose of the study: Antimicrobial resistance occurs when germs are no longer killed or inactivated by the medicines that previously killed or inactivated them. One form of antimicrobial resistance is antibiotic resistance. Antibiotic resistance occurs when bacteria are no longer inactivated or killed by antibiotics. Antibiotic resistance is a big public health problem affecting our communities. Antibiotic resistance makes the treatment of bacterial infections much harder; it makes people sick more and makes people die from diseases that could have been managed successfully with antibiotics. Individuals can be infected with bacteria that are already resistant to antibiotics, or the bacteria in them can develop resistance when treated with antibiotics, or through exposure to antibiotics in agricultural produce such as milk, poultry, and other sources of protein.

The SAMRC-UNIVEN Antimicrobial Resistance and Global Health Research Unit is investigating when people in our communities first acquire antibiotic-resistant bacteria in their intestines, the types of antibiotic-resistant bacteria, and the factors that influence the diversity of resistance observed.

Newborns are the most appropriate participants for this study because they offer a unique opportunity to observe the initial colonization of the human gut by bacteria, which begins at birth. Since newborns' intestines are largely sterile at birth, studying newborns allows researchers to accurately determine when and how gut bacteria begin to develop. Additionally, newborns have minimal exposure to antibiotics, food, and environmental influences, providing a clean baseline to track the emergence of both beneficial and antibiotic-resistant bacteria over time. This early stage of life is also critical for examining the potential transmission of resistant bacteria from mother to child, particularly through breastmilk and close physical contact. By starting with newborns and following their development, the study can identify key factors influencing changes in gut bacteria and resistance patterns as children grow. Furthermore, combining these biological insights with information on community knowledge, attitudes, and practices regarding antibiotic use will provide a comprehensive understanding of how resistance emerges and spreads from individuals to the broader population. Newborn babies and their mothers will be recruited by trained personnel from Dzimauli (Limpopo Province), and from Mthatha (Eastern Cape Province). We expect to recruit about 100 newborn babies and their mothers from each province.

Outline of the Procedures: If you consent to participate in this study, we will first conduct a screening to determine whether you qualify for the study. For example, we will check whether you will leave the community soon, whether you are underage, or whether the child is healthy enough to be in the study. If you are selected to be in the study, you and your child will be involved in the following ways:

FOR THE MOTHER

We would collect data about your gender, age, and other socioeconomic data, such as your highest educational qualification and income. This data will be collected at the beginning of your participation in the study and every 6 months for a total of 24 months. This data will be collected through questionnaire administration.

Provide a stool sample every month for 12 months, then at 15, 18, 21, and 24 months. A stool collection container will be provided, and a field worker will pick up the sample when it is available.

Provide a breastmilk sample (8- 10ml) every month, for 12 months; or as long as she is breastfeeding. A sterile container will be provided for this collection, and a field worker will pick up the sample when it is available. A questionnaire will be administered once, by a field worker/investigator, to capture your knowledge, attitude, and practice regarding antibiotics. A discussion may follow with the other to better understand any responses provided.

FOR THE CHILD

We would collect a stool sample (faeces) every month for 12 months, then at 15, 18, 21 and 24 months after recruitment. A stool collection container will be provided, and a field worker will pick up the sample when it is available.

The child's weight, length, and head circumference will be measured every month for 12 months, then at 15, 18, 21, and 24 months. This will be done by a trained field worker using approved and quality-assured balances and length measuring boards.

Once a week, a field worker will administer a questionnaire to capture illnesses, treatments that the child has received, vaccinations received, and dietary habits of the child. The Road to Health Card of the child may be consulted to get relevant data.

At 12 months of age, a mouth swab will be done to get DNA. The DNA will be studied to see how the child processes antibiotics that are administered.

FOR THE UNIVERSITY OF VENDA STUDENTS & THE COMMUNITY MEMBERS

A questionnaire will be administered once, by a field worker/investigator, to capture your knowledge, attitude, and practice regarding antibiotics. A discussion may follow with the other to better understand any responses provided.

Risks or Discomforts to the Participant: We understand you will be in the study for 2 years and will provide monthly samples. This may be uncomfortable to you, but this is the only way

you can work with us to answer the important scientific questions. We are also ready to work with you to address any cultural and religious preferences you may have. Since the samples will be collected from your home and the child assessments will be conducted at home, we will do whatever you require to maintain your privacy at these times.

You will need to sign a consent form to participate in the study. This means your personal details, such as name and signature, will be recorded on the consent form. There is an extremely minimal risk that this personal information will not be kept confidential. We do not see any other potential discomforts or risks associated with your participation in the study.

Benefits: You will not be paid for participating in this study. However, data obtained from your participation in the study will contribute to the development of interventions to reduce the transmission of drug resistance in our communities. The direct benefits of this research to you may be that you gain knowledge about the challenges of antibiotic resistance, which may lead to a change in your attitude toward your personal health.

Withdrawal from the study: Should you agree to participate in this research, you may change your mind at any time without prejudice. Refusal to continue participation shall not be objected.. However, data collected prior to your withdrawal may be included in the analysis.

Remuneration: There is no remuneration for participating in this study.

Costs of the Study: There is no cost to you for participating in this study.

Confidentiality: Should you consent to participate in this research project, your identity will be kept confidential. Samples collected from you will be coded, and the codes will be used for research purposes, without your personal identifiers. As such, the analysis of results will focus only on demographic, clinical, and laboratory data. No identifiable markers linked to your person will be analysed. Consequently, the results obtained may be published, but your identity will not be revealed.

Research-related Injury: We do not foresee any injury to you in this project. Should you experience any discomfort, physical or psychological, health professionals working with you will take care of you. You are free to discontinue participating in research if you choose to do so. You will be asked to provide reasons for your withdrawal, but you are free to decline to give reasons.

Persons to Contact in the Event of Any Problems or Queries:

Please contact the Principal Investigator, Professor P.O. Bessong, on 0159628301 or on 073 798 5920 or send an email to bessong@univen.ac.za. You can also contact the University Research Ethics Committee Secretariat on 015 962 9058. Complaints can be reported to the Director: Research and Innovation, Prof GE Ekosse on 015 962 8313 or Georges Ivo.Ekosse@univen.ac.za

General:

Potential participants must be assured that participation is voluntary and the approximate number of participants to be included should be disclosed. A copy of the information letter should be issued to participants. The information letter and consent form must be translated and provided in the primary spoken language of the research population

CONSENT

Statement of Agreement to Participate in the Research Study:

I hereby confirm that I have been informed by the researcher about the nature, conduct, benefits, and risks of this study (Research Ethics Clearance Number: FSEA/22/MBY/04/0302)

I have also received, read, and understood the above written information (*Participant Letter of Information*) regarding the study.

I am aware that the results of the study, including personal details (of myself and my child) regarding my sex, age, date of birth, initials, and diagnosis, will be anonymously processed into a study report.

I am aware that my clinical records and that of my child will be consulted to extract data on the antibiotics and vaccines received.

In view of the requirements of research, I agree that the samples and data collected during this study can be processed in a computerized system by the researcher and may be reused for other research-related analysis.

I agree that my house can be accessed with my permission to pick up samples, do surveillance for illness and dietary habits, and do anthropometric measurements.

I may, at any stage, without prejudice, withdraw my consent and participation in the study.

I have had sufficient opportunity to ask questions and (of my own free will) declare myself prepared to participate in the study.

I understand that significant new results developed during this research, which may relate to my participation, may be made available to me.

Full Name of Participant Date Time Signature

…………………….. …………… ………… …………………..

I hereby confirm that the above participant has been fully informed of the nature, conduct, and risks of the above study.

Full Name of Researcher:……………………… Date **……………….**

Signature**…………………**

Full Name of Witness/Interpreter (If applicable): …………………………………

Date **……………….** Signature**…………………..**

Full Name of Legal Guardian (If applicable): **…………………**

Date **…………** Signature**……………………..**

Letter of confirmation for proofreading and editing

Office No. 17, C Block

Department of English, Media Studies and Linguistics

University of Venda

P/Bag X 5050

Thohoyandou

0950

23 February 2026

<u>To Whom It May Concern</u>

This serves to confirm that I proofread and edited a dissertation titled "The Influence of Wash and Feeding Practices on Growth Status and Antibiotic Resistance in the First Six Months of Life at Dzimauli Rural Villages" *by Audrey Kgomotso Ramalepe (18007610).*

Regards
confirmation

Dr T.E Sikitime

Lecturer

Department of English, Media Studies and Linguistics

University of Venda

Private Bag X5050

Thohoyandou

0950

Tel: (015) 962 8362

0832561666

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<u>BA (ed), BA (Hons) English, Univen, BA Communication Science UNISA, MA (SLS) Stellenbosch University, PhD (English) Univen</u>

Ramalepe

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