UNIVERSITEIT VAN PRETORIA
UNIVERSITY OF PRETORIA
YUNIBESITHI YA PRETORIA

Epidemiological and molecular approach to determine factors associated with diarrheagenic *E. coli* in food for children under five in Mozambique

By

Sara Lino Faife

Student number: 19406632

Supervisor: Professor Elna M. Buys

Co-supervisor: Professor Custódia Macuamule

Co-Superviso: Dr Josphat Gichure

Submitted in partial fulfilment of the requirements for the degree

PhD (Microbiology)

In the Faculty of Natural & Agricultural Sciences

University of Pretoria

30 May 2025

DECLARATION

I, Sara Lino Faife declare that the dissertation, which I hereby submit for the degree PhD (Microbiology) at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

Signature: 

Date: 30 May 2025

DEDICATION

I dedicate this work to my husband Paulino Armando Timana, my kids Adner Paulino Timana, Megan Paulino Timana and Nolan Paulino Timana, my mother Elisabeth Joaquim Comé, my father Lino Faife (in momory), and my brothers, Nelson Augusto Nhanombe (in memory), Sidónio Augusto Nhanombe, Januário Lino Faife and Cláudio Lino Faife.

ACKNOWLEDGEMENTS

I would like to express my sincere gratitude to:

My supervisor, Prof. Elna Buys, who selflessly and patiently guided me throughout this journey, for her support and the supervisory strategy she adopted, which made me confident in the research process;

My co-supervisor, Prof. Custódia Macuamule, for her patience, dedication, and zeal in guiding me throughout my training;

My co-supervisor, Dr. Josphat Gichure, for patiently guiding me in scientific writing, especially during data analysis;

Dr. Rodney Owusu-Darko, for his guidance at the beginning of my doctoral programme;

Prof. Elsa Maria Salvador, for the ideas shared during the preparation of the articles;

Prof. Belisário Moiane, for his technical assistance during the microbiological analyses;

Dr. Joaquim Maguele, Dr. Gedeão Macandza, Dr. Pascoal Rafael, and António Moisés Guambe, for their support during the microbiological analyses;

The Health Centres of Marracuene and Primeiro de Maio, for allowing the study to be conducted at their facilities;

The staff and postgraduate students of the Department of Consumer and Food Sciences, for their support and encouragement throughout my research stay;

The Bill and Melinda Gates Foundation and the Foreign, Commonwealth & Development Office (FCDO) of the United Kingdom Government, for their financial support;

The Instituto Superior de Ciências de Saúde (ISCISA), my place of work, for authorising me to pursue my studies.

ABSTRACT

Epidemiological and molecular approach to determine factors associated with diarrheagenic *E. coli* in food for children under five in Mozambique

Diarrhoeagenic *Escherichia coli* (DEC) is associated with diarrhoea and is responsible for around 200,000 deaths worldwide annually, with children under five being the most affected. Children under five from Mozambique are at constant risk for developing diarrhoea due to factors. These include lack of improved drinking water access or sanitation and poor literacy of caregivers, coupled with the fact that their immune systems are under development. In 2018, approximately 500,000 cases of diarrhoea in Mozambique were caused by foodborne pathogens. Recent studies have shown that DEC strains are among the pathogens that cause enteric infection. This study aims to determine sources (food and water) and factors associated with foodborne pathogens causing diarrhoea in children under five years old in rural and urban areas of Maputo province, Mozambique.

A total of three hundred children under five years of age with diarrhoea were selected in two Health Care Centres of Maputo province in Mozambique as study cases. The caregivers of the children completed a semi-structured questionnaire. This allowed obtaining information related to demographics, housing conditions, food consumed a week before the children's diarrhoeagenic episodes, presence of domestic animals, and the general health of the child. Faecal (n =300), food (n = 167) and water (n = 100) samples were collected for diarrhoeagenic bacterial identification. *Escherichia coli* strains were identified from food and water samples using the Bacteriological Analytical Manual (BAM) protocol developed by the U.S. Food and Drug Administration (FDA). A technical reference guide produced by the Ministério da Saúde de Moçambique (Mozambique's Ministry of Health) was also used. This analysis was conducted at the Hygiene and Food Technology Laboratory of Eduardo Mondlane University. On the other hand, *E. coli* strains in faecal samples were identified based on colony morphology characteristics and biochemical tests, performed at the National Institute of Health. The confirmed *E. coli* strains were whole genome sequenced at the Agricultural Research Council, Pretoria, South Africa. The fastq files of the identified DEC strains were molecularly characterised, and their relatedness was evaluated using the EnteroBase platform.

Explanatory variables associated with DEC in children and DEC/*Salmonella* in food were analysed using chi-square tests and binomial logistic regression. Antimicrobial resistance (AMR) gene profiles were explored through genome analysis using hierarchical clustering, Pearson correlation, and network analysis in R.

Only Enteropathogenic *E. coli* (EPEC) pathotypes (2.0%) were detected in faecal samples of children involved in this study, representing a low prevalence compared to the overall burden of childhood diarrhoea in Mozambique (9.0%). Feeding children with yoghurt was seen as a protective factor against diarrhoea by EPEC. Food and drinking water consumed by the children under five with diarrhoea from Maputo were contaminated with enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC) and EPEC strains, with a prevalence of 13.0%. *Salmonella* spp. were also detected, with a prevalence of 2.2%. Feeding children with infant formula, fruit puree, ready-to-eat meals, and bottled water was associated with DEC.

There was an abundance of DEC strains belonging to sequence types (STs) ST40 (40.0%), ST206 (20.0%), and ST301 (20.0%) and serotypes O109:H21 (40.0%), O88:H5 (20.0%) and O80:H2. These strains harboured antimicrobial resistance genes conferring resistance to aminoglycosides, fosfomycin, polymyxins, beta-lactam, fluoroquinolone, trimethoprim, tetracycline and sulphonamides.

Phylogenetic analysis revealed the relatedness of the DEC strains detected in food, drinking water and childrens's faecal samples. This suggests that food and drinking water are the potential sources of DEC contributing to diarrhoea in the children under five years old in Maputo.

With the present study, we conclude that diarrhoeagenic pathogens were present in faecal samples from children under five with diarrhoea (EPEC) and in food and water they consume (ETEC, EIEC, EPEC and *Salmonella* spp.). Foods were the main factors associated with these pathogens (yoghurt, infant formula, fruit puree, ready-to-eat meals, and bottled water). The DEC strains belonged to ST and serotypes known to cause diarrhoea, and they carry AMR genes that confer resistance to antimicrobials that are important for clinical practices. Food and drinking water are the potential sources of DEC in children under five years involved in this study.

The results of this study provide valuable data on the contribution of food and water to diarrhoea in children, addressing a gap in existing research that has primarily focused on detecting diarrhoeagenic pathogens in faecal samples. In addition, the study demonstrates that DEC strains present in food and water consumed by children in Maputo harbour

AMR genes, underscoring the importance of preventing contamination by these pathogens.

So, the results of this study highlight the need for education on proper food preparation, storage, and water treatment practices, as well as the adoption of a One Health approach to monitor, prevent, and control the spread of antimicrobial resistance across sectors.
The study also underscores the need for further research on caregivers' attitudes and practices related to food preparation. It also calls for investigation of additional sources contributing to diarrhoea in children under five years old.

Keywords: Children; diarrhoeagenic *E. coli*; *Salmonella* spp.; food; drinking water and molecular characterisation.

CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

aEPEC – atypical enteropathogenic *Escherichia coli*

AMR – Antimicrobial resistance

BPW – Buffered peptone water

CDC – Centers for disease control and prevention

CFU – Colony-forming unit

CGE – Center of genomic epidemiology

DAEC – Diffusely adherent *Escherichia coli*

DALYs – Disability-adjusted life years

DEC – Diarrhoeagenic *Escherichia coli*

DNA – Deoxyribonucleic acid

EAEC – Enteroaggregative *Escherichia coli*

EIEC – Enteroinvasive *Escherichia coli*

EPEC – Enteropathogenic *Escherichia coli*

ESBL – Extended-spectrum beta-lactamase

ETEC – Enterotoxigenic *Escherichia coli*

FDA – BAM - Food and drug administration-bacteriological analytical manual

H – Flagellar antigen

HE – Hektoen enteric agar

LT – Heat-labile

MAC – MacConkey agar

MIO – Motility indole ornithine medium

MKTTn – Muller-Kauffman tetrathionate-novobiocin broth

MLVA –Multilocus variable-number tandem-repeat analysis

mTSB – Modified tryptic soy broth

NGS – Next-generation sequencing

O – Somatic antigen

PCR – Polymerase chain reaction

PFGE – Pulsed-field gel electrophoresis

qPCR – Quantitative polymerase chain reaction

RNA – Ribonucleic acid

RVS – Rappaport-vassiliadis with soya

ST – Heat-stable

ST – Sequence type

STEC – Shiga toxin-producing *Escherichia coli*

Stx – Shiga toxins

tEPEC – typical enteropathogenic *Escherichia coli*

TGS – Third-generation sequencing

TSI – Triple sugar iron agar

TTSS – Type III secretion system

WGS – Whole genome sequencing

XLD – Xylose lysine deoxycholate agar

PUBLICATIONS AND PARTICIPATION IN SCIENTIFIC EVENTS

Publication

Published: International Journal of Environmental Research and Public Health: 2024, 21(9), 1122; https://doi.org/10.3390/ijerph21091122.

Participation in scientific event

Participation in Scientific Events

Participated in the annual meeting on *Foodborne Disease Epidemiology, Surveillance, and Control in African LMICs (FOCAL)*, where I presented preliminary data on the identification, characterization, and transmission routes of foodborne pathogens causing diarrhoea in children under five years old in Maputo Province, Mozambique.

Participated in a scientific event at the Higher Institute of Health Sciences, where I delivered an oral presentation on the contamination of food and water by diarrhoeagenic *Escherichia coli* and *Salmonella spp.* consumed by children with diarrhoea in Maputo, Mozambique.

CHAPTER I: INTRODUCTION

Foodborne illness is a major public health concern caused mainly by bacteria and other agents such as viruses, parasites or chemical substances (WHO, 2024a). The illness occurs due to consuming contaminated food or water, which frequently causes diarrhoea, resulting in morbidity and mortality due to this disease, especially in children (WHO, 2024b). Diarrhoeal disease is considered the third leading cause of death in children under five years old and is responsible for killing around 443,832 children every year (WHO, 2024b).

Sub-Saharan Africa region has the highest mortality rate globally, which is fifteen times higher than in high-income countries, with one child in 13 dying before their fifth birthday (WHO, 2018a). The high rates of foodborne illness in low and middle-income countries are attributed to several factors, which include the high prevalence of foodborne pathogens in people with diarrhoea, the use of human sewage or animal waste for irrigation and lack of clean water for washing utensils and food (Grace, 2015).

Viral pathogens such as rotavirus, norovirus, adenovirus and astrovirus, bacteria like *Escherichia coli*, *Salmonella* spp., *Shigella* spp., and *Campylobacter* spp., and parasites such as *Cryptosporidium*, *Giardia*, and *Entamoeba* spp. are among the foodborne pathogens causing diarrhoea in children under five years of age (WHO, 2024b).

Mozambique is a low-income country where recurring climatic disasters, inadequate housing, poor water and sanitation infrastructure, and seasonal droughts and floods contribute to public health challenges that increase the country's vulnerability to diarrhoeal diseases (AFRIKAIA, 2022). In 2018, about 500,000 cases of diarrhoea were caused by foodborne pathogens in Mozambique (ONU, 2019). A review of the epidemiology of diarrhoea in children under five years showed an incidence of 11.0% high disease burden and an associated case fatality rate of nearly 10% (Chissaque *et al.*, 2018). Demographic survey and health data from 2022 to 2023 indicated that 9.0% of children experienced diarrhoea two weeks before the survey (INE, 2024), reflecting a 2.0% reduction compared to a previous study on the burden of diarrhoea in Mozambique. From 2015 to 2019, 9041 cases of diarrhoea were reported in children under five years old in Maputo, of which 25.2% were in rural areas and 74.8% were in urban areas (Machava *et al.*, 2022a). These data highlight the need for a better understanding of the

epidemiology of foodborne diarrhoea in order to better inform strategic health interventions for its prevention.

Despite several studies reporting diarrhoea, the majority of the existing literature focuses on the detection of diarrhoeagenic pathogens in humans, which include Rotavirus, Adenovirus, *Shigella* spp., *E. coli*, *Vibrio cholerae*, and *Cryptosporidium* spp. (Nhampossa *et al.*, 2015; Chissaque *et al.*, 2018; Dall, 2023). Diarrhoeagenic pathogens are organisms that cause diarrhoeal infections, primarily including bacteria, viruses, and parasites, most of which are transmitted through faecally contaminated water (WHO, 2024a). Limited studies identified the diarrhoeagenic pathogens in food and water, and the factors associated with their contamination, which may result in these sources not being recognised as potential contributors to diarrhoeal disease. Moreover, limited understanding of the factors contributing to childhood diarrhoea hampers the development and implementation of targeted interventions to effectively address its causes.

A study conducted by Bick *et al*, (2020), indicated that 53.0% of infant food from low-income Maputo neighbourhoods was contaminated with faecal bacteria. In addition, this contamination was associated with the food type, preparation and hygiene practices. In a population-based study, factors such as bottle feeding and untreated water used for drinking were associated with foodborne diarrhoea in children younger than five years in urban and rural areas of Maputo (Machava *et al*., 2022b). Several other factors associated with diarrhoea in vulnerable communities have been reported in countries such as Namibia, Rwanda and Ethiopia, which include the child's age, number of people per household, mothers' age and education, residential area, household income, unemployment, access to information, type of toilet facilities, and child immunisation and nutritional status (Woldu *et al*., 2016; Bauleth *et al*., 2020).

This study aimed to ascertain the contribution of infant food to diarrhoeal cases in Maputo's urban and rural areas by identifying the prevalence of diarrhoeagenic pathogens and determining the factors associated with these pathogens in food and water consumed by children under five years. The study also aimed to assess the presence of antimicrobial resistance genes and to determine if the food and water consumed by children under five years contribute to diarrhoea in these children. The findings from this study will allow

the establishment of control systems and to address underlying causes, thereby reducing the prevalence of diarrhoeagenic agents among vulnerable groups in the communities.

This is the first study of its kind to be conducted in Mozambique. It fills a gap in existing research, which has primarily focused on detecting diarrhoeagenic pathogens in faecal samples, by highlighting the contribution of food and water to diarrhoea in children. Additionally, it identifies factors associated with the presence of these pathogens in food and water, providing valuable insights for the development of effective diarrhoea prevention strategies.

CHAPTER II: LITERATURE REVIEW

2.1. The burden of foodborne disease in low and middle-income countries

The global burden of foodborne disease is a significant public health issue, carried disproportionately by low- and middle-income countries (LMICs) and by children under five years of age (WHO, 2025).

LMICs bear 53% of all foodborne illnesses and 75% of related deaths, despite comprising only 41% of the global population (World Bank, 2018). However, experts believe that they are the most affected regions due to the high prevalence of potentially foodborne registered in hospital and community surveys of people with diarrhoea, inadequate water supplies, poor environmental hygiene and sanitation, and insufficient education, use of human sewage or animal waste for horticulture production is common (Croxen *et al*., 2013; Grace, 2015; Komarulzaman, *et al*., 2017; Nguyen *et al.*, 2021). Factors such as lack of maternal education and sanitation infrastructure, urban living conditions, and inconsistent maternal hygiene practices have been associated with childhood diarrhoea (Alebel *et al*., 2018).

Countries from Africa and South-East Asia bear the highest burden of foodborne diseases, including among children under five years (Havelaar *et al.*, 2015). In African regions, foodborne diseases are a significant cause of morbidity, where 91 million people consume contaminated food that makes them ill, resulting in about 137,000 people dying from it, and children under five years of age being the most affected (WHO, 2022).

In Mozambique, the available data indicate that from 2010 to 2015, deaths due to diarrhoeal diseases reduced by almost half from 2010 (13,105) to 2015 (7,340) in children under five years old (Chissaque *et al.*, 2018). This reduction was attributed to the introduction of rotavirus vaccination, increased access to safe water, and improved sanitation and hygiene (Chissaque *et al.*, 2018). However, this continues to be among the most crucial causes of morbimortality, especially in children under five years old (UNICEF, 2021). A five-year study conducted in Manhiça involving children with moderate-to-severe diarrhoea showed a death rate of 10.2 deaths per 1000 persons-week at risk (Acácio *et al.*, 2019). Data from 2019 to 2020 indicated that diarrhoea was among the leading cause of death in children under five, with a mortality rate of 11.0% (Macicame *et al.*, 2023).

Foodborne diseases are caused by a variety of microorganisms, including bacteria, moulds, protozoa and viruses (WHO, 2015). Bacteria mostly involved in foodborne diseases in LMIC include *Salmonella* sp. *Shigella* spp., Diarrhoeagenic *E. coli* (DEC) and *V. cholerae*, and the first three pathogens are the focus of this study so they will be further characterised. DEC are *E. coli* strains that cause diarrhoea and are caracterised based on the group of virulence determinants they have acquired (Gomes *et al*., 2016).

2.1.1. Prevalence of Diarrhoeagenic *E. coli* in under five children in low and middle-income countries

Escherichia coli is a member of the intestinal microbiota and Gram-negative bacilli that belongs to the Enterobacteriaceae family (Gomes *et al.*, 2016; Ekici and Dumen, 2019). *Escherichia coli* is associated with diarrhoeal diseases, and its classification is based on the characteristics of its virulence properties, each group causing disease by a different mechanism, characteristics that allowed the classification of this strain in different DEC pathotypes (Gomes *et al.*, 2016; Ekici and Dumen, 2019). Genes in the plasmids encode adherence properties to the epithelial cells of the small or large intestine. Similarly, toxins produced by DEC are often mediated by plasmids or phage (Brooks *et al.*, 2013).

The DEC pathotypes are classified as enteropathogenic *E. coli* (EPEC), enteroaggregative *E. coli* (EAEC), diffusely adherent *E. coli* (DAEC), enterotoxigenic *E. coli* (ETEC), enteroinvasive *E. coli* (EIEC) and enterohemorrhagic *E. coli* (EHEC) (Figure 2.1). All pathotypes indicated above are considered diarrhoeagenic because the main symptom in patients infected by any of them is diarrhoea (Brooks *et al.*, 2013; Ekici and Dumen, 2019).

EPEC strains are characterised by producing attaching and effacing lesions and non-production of Shiga toxins (Stx) and heat-labile (LT) or heat-stable (ST) enterotoxins (Gomes *et al.*, 2016). Additionally, they are classified as typical EPEC (tEPEC) and atypical EPEC (aEPEC) based on the presence of plasmid adherence factor (pEAF), which encodes the type IV fimbriae called the bundle-forming pilus necessary for adherence to epithelium cells. This plasmid is present on tEPEC but absent on aEPEC (Gomes *et al.*, 2016).

Virulence factors such as adhesins, cytotoxins, enterotoxins and secreted proteins have been identified in EAEC (Meza-Segura *et al.*, 2020). EAEC adheres to the intestinal

mucosa for disease development and then releases enterotoxins and cytotoxins, leading to mucosal inflammation (Gomes *et al.*, 2016; Ekici and Dumen, 2019). EAEC is the DEC pathotype characterised by the presence of AA pattern on epithelial cells in culture encoded by the *aggR* gene, which allowed the classification of this strain as typical (tEAEC) and atypical (aEAEC) based on the presence and absence of this gene, respectively (Meza-Segura *et al.*, 2020).

DAEC pathotypes are classified based on their characteristic diffuse adherence pattern on HEp-2 cells, which is mediated by adhesins such as Afa, F1845, and Dr. DAEC strains bind to proteins degrading the intestinal epithelium (Ekici and Dumen, 2019; Meza-Segura *et al.*, 2020).

ETEC acts producing colonisation factors (CFs) and LT e/o ST enterotoxins. The CF induce bacterial adherence to the intestinal mucosa and confers host specificity to the different strains, while LT and ST toxins act by deregulating membrane ion channels in the epithelial membrane, leading to water and ions loss, causing watery diarrhoea (Gomes *et al.*, 2016).

EIEC strains invade and penetrate the enterocytes, inducing inflammatory damage in intestinal mucosa and submucosa, leading to cell destruction, similar to *Shigella* spp. The virulence factors of EIEC are due to the proteins encoded by a 31-kb fragment from plasmid pInv, containing 38 genes responsible for bacterial invasion and escape by cell spreading, inhibition of autophagy, regulation of immune response of the host apparatus and type III secretion system (TTSS). The effects of EIEC's pathogenesis into the host allow these microorganisms' intracellular survival (Gomes *et al.*, 2016).

EHEC, also named Stx-producing *E. coli* (STEC) and verotoxin-producing *E. coli* (VTEC), produce Stx that destroys vero cells similarly to Stx produced *by Shigella dysenteriae* type 1 (Brooks *et al.*, 2013; Ekici and Dumen, 2019).

The Stx are divided into two main groups, Stx1 and Stx2. These toxins inhibit protein synthesis by removing an adenine residue from the 28S rRNA of the 60S ribosome. They are involved in cell signal transduction and immune modulation, inducing pro-inflammatory and pro-apoptotic responses (Gomes *et al.*, 2016).

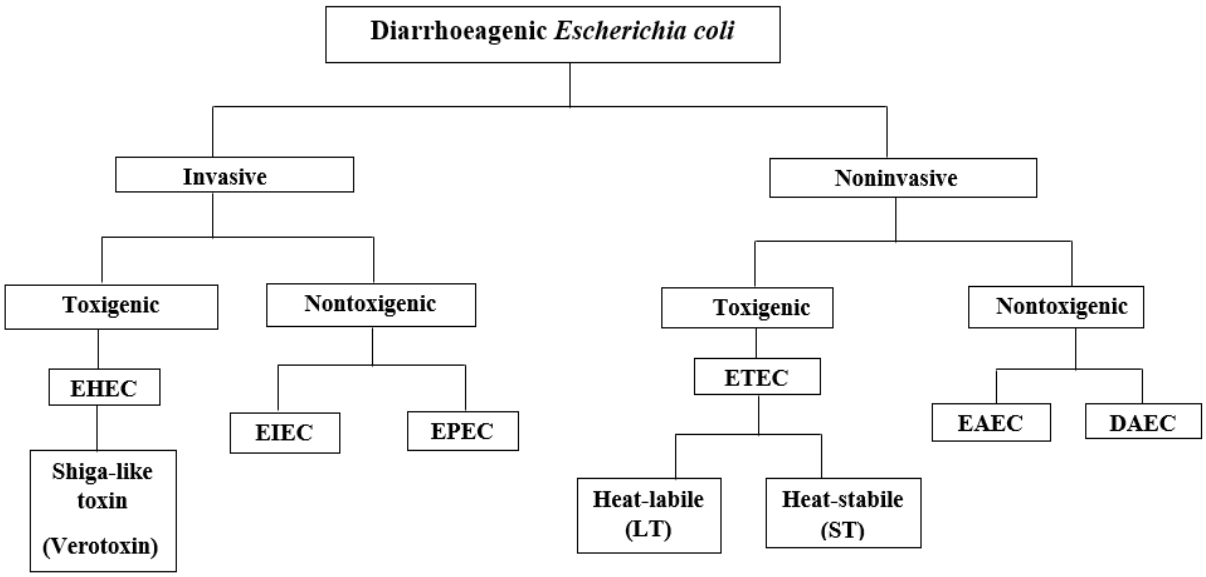

Figure 2.1. Diarrhoeagenic *E. coli* pathotypes and summary of their pathogenicity (adapted from Gerba, 2015).

EPEC - Enteropathogenic *E. coli*
EAEC - Enteroaggregative *E. coli*
DAEC - Diffusely adherent *E. coli*
ETEC - Enterotoxigenic *E. coli*
EIEC - Enteroinvasive *E. coli*
EHEC - Enterohemorrhagic *E. coli*

EPEC strains are associated with diarrhoea mortality, mainly among children under 12 months in LMICs (Das *et al*., 2022). Animal contact and contaminated foods, including raw meat, unpasteurised milk, vegetables and water, are among the sources of EPEC for humans (Gomes *et al.*, 2016). EPEC pathotype was present in faecal samples from children under five with diarrhoea in Kenya (Yeda *et al.*, 2024). It was the most abundant pathotype (13.0%) isolated in cattle present in children's homesteads (Yeda *et al.*, 2024). In Mozambique, EPEC was identified in children under five years of age with and without diarrhoea (1.7%). In another study from Mozambique, EPEC was present in faecal samples from children with acute diarrhoea in three provinces (Manhique-Coutinho *et al.*, 2022). In peri-urban areas of Lusaka, Zambia, children between zero and 36 months harboured DEC strains and EPEC was among the most prevalent (43.0%) (Mwape *et al.*, 2023). These strains were detected in both diarrhoeal and non-diarrhoeal children aged zero to 36 months (Dow *et al*., 2009). Humans are seen as reservoirs of typical EPEC pathotypes as these have not been found in animals (Trabulsi *et al*., 2002). At the same

time, atypical EPEC pathotypes have been isolated from animals such as cattle and calves (Trabulsi *et al*., 2002).

The EAEC strains are responsible for acute and chronic diarrhoea in children, mainly in low-income countries (Jenkins, 2018). They are associated with travellers' diarrhoea in children and adults in middle and high-income countries (Jenkins, 2018). Water, cheese made from unpasteurised milk and fresh produce are among the vehicles of EAEC pathotypes (EFSA, 2015). In Kenya, a study by Yeda *et al*. (2024) showed that EAEC was the most prevalent DEC pathotype (12.0%) among children below five years with diarrhoea and close contact with food animals. This pathotype was also the most abundant in Mozambican children under five with diarrhoea (66.3%) (Manhique-Coutinho *et al.*, 2022). In Mozambique, an epidemiological study by Chissaque *et al.* (2018) found that EAEC was among bacterial pathogens associated with infantile diarrhoea. Additionally, EAEC was the most prevalent *E. coli* pathotype in under-five children's diarrhoea outbreaks in Kenya at three outpatient facilities in an informal settlement (Jepleting *et al.*, 2023). Further, this pathotype has also been identified in children under five with acute diarrhoea in Ethiopia (Mulu *et al.*, 2024).

ETEC is one of the major pathogens that cause moderate-to-severe diarrhoea in children under five years (Anderson *et al.*, 2019). Estimations from the Global Burden of Disease Study (GBD 2016) indicate that ETEC is responsible for about 220 million diarrhoea cases globally, where around 75 million cases were registered in children under five years of age, resulting in about 18,700 deaths, with 42,000 deaths in children under five years (WHO, 2022). Although diarrhoeal mortality rates for ETEC are decreasing due to economic development, availability of safe water and sanitation, these reductions were registered in many LMICs (WHO, 2022). Estimations from a modelling analysis study involving 89 low-income and LMIC indicated that from a total of 119 million ETEC diarrhoea episodes registered per year, 84.4 million were from LMIC (Anderson *et al.*, 2019). In an epidemiology study of ETEC among children and adults in two rural areas in Bangladesh, 19.7% of children under five had diarrhoea caused by this strain (Chakraborty *et al.*, 2024). In Zambia, the prevalence of ETEC in children under three years was 2.47%. In Nigeria, ETEC was also present in children below six months without diarrhoea and in children with diarrhoea aged over nine months (Akinlabi *et al.*, 2023). In Mozambique, 2.7% of ETEC was recovered in children under five who suffered from mild diarrhoea (Rappelli *et al.*, 2005). In another study involving children below 15 years

of age with diarrhoea, ETEC was among the most prevalent strains, with a 13.5% prevalence (Manhique-Coutinho *et al.*, 2022). In Kenya, the presence of ETEC was evidenced in asymptomatic children aged six to 24 months (3.1%) (Okumu *et al.*, 2023).

EIEC strains cause a disease similar to shigellosis, which is more common in children. EIEC infections occur worldwide, although they are most common in low-income countries and people travelling to these countries (Gomes *et al.*, 2016; Makarova, 2023). This pathotype is transmitted mainly through person-to-person contact (Bhavnani *et al.*, 2016). EIEC strains were among diarrhoeagenic pathogens recovered from children between zero to 36 months with diarrhoea in Zambia (20.2%) (Mwape *et al.*, 2023). In Ethiopia, this pathotype was present in 13% of children with and without diarrhoea (Zelelie *et al.*, 2023). The low frequency of EIEC was registered in children below five with diarrhoea from Mozambique (7.7%) and the same age children without diarrhoea in Nigeria (0.2%) (Garrine *et al.*, 2020; Akinlabi *et al.*, 2023).

STEC causes infections varying from unapparent diarrhoea to more serious manifestations such as hemorrhagic colitis (HC) and hemolytic uremic syndrome (HUS), mainly in infants and children. However, the incidence of infection varies in different regions, including in LMIC (Gomes *et al.*, 2016). World Health Organization (WHO), in 2010, indicated that STEC was responsible for more than one million illnesses, resulting in more than 100 deaths and approximately 13,000 disability-adjusted life years (DALYs) (Pires *et al.*, 2019).

The serotype *E. coli* O157:H7 is the most relevant among the STEC strains regarding public health (WHO, 2018b). A study in Ethiopia showed that the prevalence of *E. coli* O157:H7-associated was 15.3% (Getaneh *et al.*, 2021). In another study carried out also in Ethiopia, *E. coli* O157:H7 was found in 6.1% of the children (Balew and Kibret, 2023).

DAEC, the less-studied DEC pathotype, causes chronic diarrhoea in children between the ages of one and five, mainly in LMIC (Ocampo *et al.*, 2021). According to the Centers for Disease Control and Prevention (CDC), its primary source is uncertain (CDC, 2024a).

Available studies show that the prevalence of DEC strains in children under five years of age across LMICs ranges widely, from 1.5% to 66.3%. Among the DEC pathotypes, DAEC is the least studied, with no available data on its prevalence.

The presented studies primarily applied cross-sectional or observational epidemiological designs. While the predominance of cross-sectional methods provides useful prevalence data, it limits the ability to infer causality or fully compare results across different populations and settings. In contrast, observational epidemiological studies that included both symptomatic and asymptomatic children offered a more comprehensive picture of pathogen presence. Limitations of these study designs include susceptibility to confounding factors, potential selection bias, difficulty in controlling exposure variables, and challenges in establishing causality despite providing valuable associations.

2.1.2. Prevalence o *Salmonella* spp. in under five years children in low and middle-income countries

Salmonella is the most complex group of Enterobacteriaceae, with more than 2,200 serotypes. The Kaufmann-White scheme classifies *Salmonella* serovars based on somatic (O) antigens and phase 1 and phase 2 flagellar (H) antigens (Winn *et al.*, 2010). Chattaway and collaborators proposed a new nomenclature for *Salmonella*, based on a MAC-type name derived from multilocus sequence typing (MLST) and the genetic antigenic profile, aligning with the traditional Kaufmann–White scheme (Chattaway *et al*., 2021). They recommend that *Salmonella* strains should be named first by *Salmonella* species and subspecies and then the MAC type (ST or provisional ST plus historical serovarname or Serogene) scheme scheme (Chattaway *et al*., 2021).

The *Salmonella* genus is divided into *S. enterica* and *S. bongori* (formerly subspecies V), with multiple subspecies and serotypes (Brooks *et al.*, 2013; Kuria, 2024). *Salmonella enterica* contains six subspecies, which are subspecies enterica (subspecies I), subspecies salamae (subspecies II), subspecies arizonae (subspecies IIIa), subspecies diarizonae (subspecies IIIb), subspecies houtenae (subspecies IV), and subspecies indica (subspecies VI) (Brooks *et al.*, 2013; Kuria, 2024). Most human illnesses are caused by *S. enterica* subspecies enterica (Brooks *et al.*, 2013).

Four *Salmonella* serotypes were identified using biochemical and serological tests: *S. Paratyphi* (serogroup A), *S. Paratyphi* (serogroup B), *S. Choleraesuis* (serogroup C1), and *S. Typhi* (serogroup D) (Brooks *et al.*, 2013). These serotypes are associated with distinct clinical syndromes: *S. Typhi* and *S. Paratyphi* are primarily responsible for enteric

fever, while *S. Choleraesuis* is often linked to septicemia and focal infections (Brooks *et al.*, 2013).

Based on the *Salmonella* species and serotypes presented above, MAC types should be assigned as in the following examples: S.enterica ST43—S. Java and S. enterica ST86—S. Paratyphi B scheme (Chattaway *et al*., 2021).

Many *Salmonella* serotypes are pathogenic for humans or animals when taken orally. They are transmitted from animals and animal products such as poultry, poultry products, beef, pork and fish as well as non-animal-derived foods such as fruits and vegetables to humans, causing enteritis, systemic infection, and enteric fever (Brooks *et al.*, 2013; Lamichhane *et al.*, 2024).

Salmonella infects many mammals, birds, reptiles, amphibians, fish and invertebrates, and these hosts carry the bacteria in their tissues (meat), manure, or eggs, constituting reservoirs for human infection (Kuria, 2024). The microorganisms usually enter orally, usually with contaminated food or drink, which in turn is contaminated by persons who have unsuspected subclinical diseases or are carriers when they handle food (Brooks *et al.*, 2013). The infective dose to produce clinical or subclinical infection in humans is 10^5–10^8 colony-forming units (CFU)/ml (Brooks *et al.*, 2013).

Salmonella is among the most important bacterial pathogens causing diarrhoea in children under five, especially in LMICs, although low prevalence is reported (Kasumba *et al.*, 2023; Hugho *et al.*, 2024; WHO, 2024a). A study carried out in Mali, Gambia, and Kenya in children between zero to 59 months of age with moderate-to-severe diarrhoea reported *Salmonella* prevalence of 1.6%, 4.0%, and 1.9%, respectively (Kasumba *et al.*, 2023). In a recent study from Tanzania, *Salmonella* was recovered in 2.6% of children under five years with diarrhoea (Hugho *et al.*, 2024). In Ethiopia, 3.15% of diarrhoeic children under five years old attending public health facilities in Debre Markos town harboured *Salmonella* (Dessale *et al*., 2023). In another study from Ethiopia, in Ambo town, a lower prevalence of *Salmonella* was found in children below five years with diarrhoea, which was 1.3% (Tosisa *et al.*, 2020). A recent study, also conducted in Ethiopia, reported a prevalence of 6.3% for *Salmonella* among children under five years of age with diarrhoea attending Bule Hora University Teaching Hospital (Dade *et al.*, 2025). In Botswana, *Salmonella* was recovered in 3.0% of children under five with diarrhoea (Urio *et al.*,

2001). Nontyphoidal *Salmonella* was associated with mortality among rural children with diarrhoea who accessed a hospital in Kenya (O'Reilly *et al.*, 2012). In the same study, nontyphoidal *Salmonella* was detected in 22.0% of faecal samples of children who died (O'Reilly *et al.*, 2012). More than 10 years ago, the burden of diarrhoeal disease among children between zero to 59 months seeking care at health facilities in rural Mozambique indicated that *Salmonella* was not among the most prevalent pathogens involved in the cause of diarrhoea, being detected at a frequency of 1.0 % in children aged zero to 11 months and the same frequency in children between 12 to 23 months (Nhampossa *et al.*, 2015).

Studies on the prevalence of *Salmonella* spp. in children under five years of age in LMICs indicate that it typically ranges from 1.0% to 22.0%.This prevalence is low compared to that of DEC strains. Nontyphoidal *Salmonella* has been associated with mortality in children, particularly in Kenya, highlighting its pathogenic potential.

2.1.3. Prevalence of *Shigella* spp. in under five years children in low and middle-income countries

Shigella is part of the Enterobacteriaceae family characterised as Gram-negative, straight rods and similar in morphology to other Enterobacteriaceae (Schneider *et al.*, 2012; Brooks *et al.*, 2013). They are facultative anaerobes but grow best aerobically (Schneider *et al.*, 2012; Hmar *et al*., 2024). The natural habitat of *Shigella* is limited to the gastrointestinal tract of humans, where it causes bacillary dysentery (Pakbin *et al*., 2023). *Shigella* has a complex antigenic pattern. There is a significant overlap in the serologic behaviour of different species, and most share O antigens with other enteric bacilli (Brooks *et al.*, 2013). The classification of *Shigella* is based on biochemical and antigenic characteristics, and they are classified as *S. dysenteriae* (group and type A), *S. flexneri* (group and type B), *S. boydii* (group and type C) and *S. sonnei* (group and type D) (Brooks *et al.*, 2013).

In 2015, shigellosis accounted for at least 54,900 deaths in children younger than five years globally (Collaborators, 2017). *Shigella* is transmitted from person to person by food, fingers, faeces, and flies (Brooks *et al.*, 2013). Most cases of *Shigella* infection occur in children younger than ten years of age (Brooks *et al.*, 2013). Shigellosis is highly contagious and its infectious dose is on the order of 10^3 CFU per g/ml (Brooks *et al.*, 2013; Pakbin *et al*., 2023). The pathological process consists of invasion of mucosal cells

by induced phagocytosis, escape from the phagocytic vacuole, multiplication and dissemination in the cytoplasm of epithelial cells, and passage to the adjacent cells (Brooks *et al.*, 2013).

Shigella dysenteriae type 1 causes the most severe disease and is the only serotype that produces the Shiga toxin, which may be partially responsible for cases in which haemolytic uremic syndrome develops (Cody and Dixon, 2019). By acting as a neurotoxin, it can contribute to the extreme severity and fatal nature of *S. dysenteriae* infections and to central nervous system reactions such as meningismus and coma (Brooks *et al.*, 2013). *Shigella dysenteriae* serotype 1 (Sd1) is the agent of epidemic dysentery and often causes severe illness, while *S. sonnei* produces the mildest form of shigellosis, usually watery diarrhoea. *Shigella flexneri* and *S. boydii* infections can be mild or severe (CDC, 2024b). Other *Shigella* species can cause severe illness in people with compromised immune systems (CDC, 2024b).

In a study involving 28 LMICs, *Shigella* was the second leading cause of diarrhoea in children under five years (Cohen *et al.*, 2022). *Shigella dysenteriae* and other *Shigella* species were recovered in children under five attending public health facilities in Northwest Ethiopia, with a prevalence of 3.60% and 4.95%, respectively (Dessale *et al*., 2023). In the same country, in another study involving children with diarrhoea from Ambo town, three *Shigella* species were detected, namely, *S. flexneri* with 1.3% prevalence, 0.8% of *S. boydii*, and 0.4% of *S. sonnei* (Dessale *et al*., 2023). A recent study conducted in Ethiopia among children under five years of age with diarrhoea attending Bule Hora University Teaching Hospital, reported a prevalence of 4.9% for *Shigella* (Dade *et al.*, 2025). *Shigella* was associated with mortality among rural Kenyan children with diarrhoea, and this strain was present in 11% of faecal samples of children who died (O'Reilly *et al.*, 2012). In Botswana, *Shigella* prevalence in children under five years with diarrhoea was 21%, and the most abundant species were *S. boydii*, *S. flexneri* and *S. sonnei* (Urio *et al.*, 2001). A study of the epidemiology of diarrhoea in children under five years in Mozambique showed that *Shigella* strains were among the diarrhoeagenic pathogens associated with diarrhoeal fatalities, which was approximately 10% (Chissaque *et al.*, 2018). Still in Mozambique, a study conducted in a rural area by Vubil *et al.* (2018) demonstrated the presence of *Shigella* in children under five with moderate-to-severe diarrhoea (3.9%) and less severe diarrhoea (6.1%).

The presented studies show that the prevance of *Shigella* spp. varies from 0.4% to 21%. *Shigella dysenteriae*, *S. boydii*, *S. flexneri* and *S. sonnei* were the most frequent species. Avaliables studies in Kenya and Mozambique have associated *Shigella* infections with child mortality.

2.2. Environmental transmission routes of foodborne pathogens affecting under five years children in low and middle-income countries

Low-income countries are more affected by diarrhoea, and children under three years old experience an average of three episodes of diarrhoea annually (WHO, 2024a). Contaminated food, water sources, and poor sanitation are the main factors for widespread diarrhoeagenic pathogens (WHO, 2024a). The environment plays a crucial role in the transmission of foodborne pathogens (Lake and Barker, 2018). These pathogens infect humans through the faecal-oral route, and the environment serves as the medium where they circulate before infecting a new host (Graaf *et al.*, 2020).

The excrement of animals and humans is eliminated in the environment, posing a risk for diarrhoeal diseases, especially in countries with poor environmental sanitation (Penakalapati *et al.*, 2017). The excreted faeces may contain diarrhoeagenic pathogens. These pathogens may be transmitted to the five environmental reservoirs represented by the F diagram: food, fluids (water), fingers (hands), fields (soil), and flies (Ijaz and Rubino, 2012) (Figure 2.2). The contamination of the new host may be prevented in two phases. First, by implementing good sanitation to limit the spread of diarrhoeagenic agents through water, soil, fingers and flies. Second, by following good handling practices during food preparation and conservation (Ijaz and Rubino, 2012).

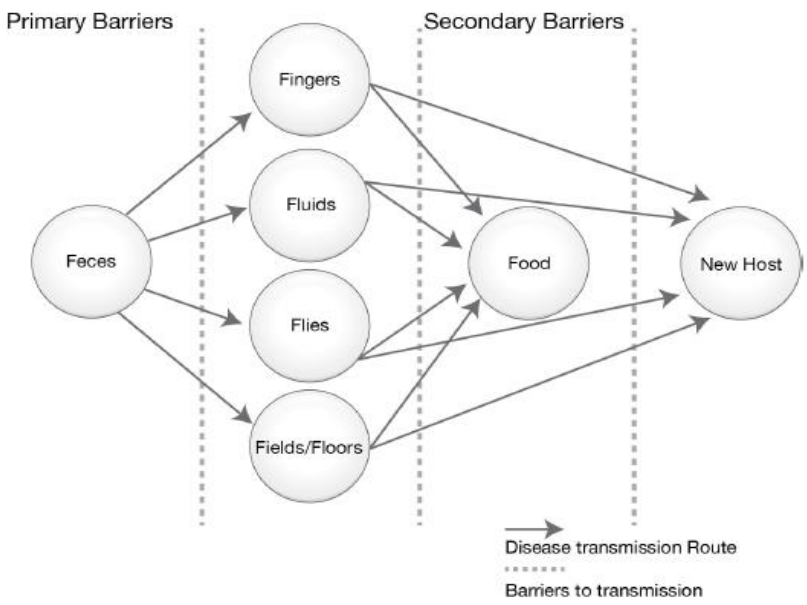

Figure 2.2. F diagram representing the source of diarrhoeagenic pathogens (Ijaz and Rubino, 2012).

2.2.1. Food as a source of diarrhoeagenic bacteria for children under five years old in low and middle-income countries

Foods such as meat, vegetables, poultry, and dairy products have been indicated as common sources of foodborne diseases, such as *E. coli*, *Salmonella* spp. and *Shigella* spp. (Table 2.1). The presence of contaminants in children's foods can be attributed to factors related to the food production and processing phase, which include the use of manure as fertiliser, the use of contaminated water for irrigation, contact with animal faeces during slaughter, and processing of colonised animals (Croxen *et al.*, 2013). Post-production contamination may result from household-level factors, which include children feeding themselves, animal exposure, type of food, food preparation, hygiene behaviour, and food storage and preparation practices (Bick *et al.*, 2020; Wells *et al.*, 2023). Studies reporting the food as a source of *E. coli* pathotypes causing diarrhoea in children under five years old are available. In Kenya, EPEC (17.0%) was among the most detected pathogens in infant weaning food, such as porridge, milk, tea, water, flour bread, mashed potatoes and beans (Tsai *et al.*, 2019). A recent study in Kenya demonstrated the presence of EPEC in food consumed by children in Dagoretti South, Nairobi (Okumu *et al.*, 2025). EPEC and EAEC were identified in Bangladesh, cereal-based children's food such as rice, suji (a preparation of semolina with milk or water), khichuri (Rice prepared with pulse and vegetables) that had been stored for about three hours at room temperature, which may

endanger the health of the child who will consume it (Doza *et al.*, 2018). EAEC was present in food consumed consumed by children in Dagoretti South, Nairobi, Kenya (Okumu *et al.*, 2025).

Matoke (mashed bananas) and broth consumption were associated with ETEC carriage in Kenya's children aged six to 24 months (Okumu *et al.*, 2023). A recent study, also conducted in Kenya, demonstrated the presence of ETEC in food consumed by children in Dagoretti South, Nairobi (Okumu *et al.*, 2025). The primary sources of STEC strains include raw or undercooked ground meat products, raw milk, and faecal contamination of vegetables (WHO, 2018b). Raw milk from around Bahir Dar town dairy farms in Ethiopia was shown to harbour STEC strains (Yihunie *et al.*, 2024). In Nigeria, *E. coli* O157:H7 was present in meat, fufu, pumpkin, meat pie, yoghurt, cucumber, cabbage, garden egg, okra, chicken, unpasteurised milk and salad from local market, which are part of the foods given to children under five years of age (Lennox *et al.*, 2020). In Ethiopia, *E. coli* O157:H7 has been related to undercooked meat, raw vegetables and/or fruit juice consumption by children under five (Getaneh *et al.*, 2021).

Many cases of salmonellosis in humans are associated with consuming contaminated food products such as beef, pork, poultry meat, undercooked or raw eggs, vegetables, juices and other foods (Bintsis, 2017). Complementary foods given to children below or at two years of age in Zambia include maize meal, nshima (very thick porridge), boiled rice, bread, porridge mixed with ground nuts, pounded ground nuts, cooked beans, chicken soup, fresh milk, chicken meat, fried egg, peanut butter, sour milk, cooked pumpkin leaves, cooked vegetables and cabbage (Mwisha Kinkese *et al.*, 2018). These foods were found to be contaminated with *Salmonella* (Mwisha Kinkese *et al.*, 2018). In Tanzania, consumption of raw milk, use of infant formula and purchasing eggs directly from the farms were identified as risk factors for *Salmonella*-associated diarrhoea in children under five years (Hugho *et al.*, 2024). Cow milk and cereal blend samples in bottle-fed babies were shown to harbour *Salmonella* strains in Ethiopia (Marege *et al.*, 2023).

Shigella foodborne transmission is mainly due to vegetables, lettuce, cold salads, herbs, meat and dairy items and hot dishes (CDC, 2024b). Kenyan infant food prepared with unpackaged (59.0%), fresh pasteurised (3.6%), and ultra-high temperature (UHT) treated milk (0.7%) was shown to harbour *S. sonnei* strains (Hoffmann *et al.*, 2022). In the same

countries, the presence of *Shigella* was demonstrated in infant foods such as porridge, milk, tea, bread, mashed potatoes, and beans (Tsai *et al.*, 2019).

Foods such as cooked pork, meat pie, cooked meal, vegetables, fruits, market raw milk, ready-to-eat salad, raw salad, and poultry have been shown as the source of *Shigella*, mainly of *S. sonnei*, *S. flexneri* and *S. dysenteriae* in LMIC such as Nigeria, Cameroon, India, Tunisia and Pakistan (Mokhtari *et al.*, 2012; Samad *et al.*, 2018; Makinde *et al.*, 2020; Nisa *et al.*, 2021).

Available studies show that rice, milk, and vegetables are the main food groups associated with diarrhoea caused by EPEC, EIEC, STEC, *Salmonella* spp., and *Shigella* spp. in children under five years of age in LMICs. This highlights the role of these foods as potential vehicles for the transmission of foodborne pathogens.

Table 2.1. Food associated with diarrhoea caused by Diarrhoeagenic *E. coli*, *Salmonella* spp. and *Shigella* spp. in under five years children's from low and middle-income countries

Food Group	EPEC	EAEC	ETEC	STEC	*Salmonella* spp.	Shigella spp.	Reference
Cereals	Rice, porridge, milk, flour bread	Rice		Fufu	Nshima***, boiled rice, bread	Porridge and bread	
Milk and Milk Products	Milk			Yoghurt, unpasteurized milk	fresh milk, raw milk, infant formula	Milk	
Meat				Meat, meat pie, undercooked meat	Chicken meat		
Vegetables	Mashed potatoes			Pumpkin, cucumber, cabbage, garden egg, okra and raw vegetables	cooked beans	Mashed potatoe and beans	
Fruits			Mashed banana				
Combined foods	Suji*, khichuri*	Suji*, khichuri**	Broth	Salad	Porridge mixed with ground nuts, chicken soup		
Other foods	Tea				pounded ground nuts	Tea	

*Suji - a preparation of semolina with milk or water
**Khichuri - ice prepared with pulse and vegetables
***Nshima - very thick porridge

2.2.2. Drinking water as a source of diarrhoeagenic bacteria for children under five years old in low and middle-income countries

Drinking water is the most potent vehicle of foodborne pathogens, mainly in low-income countries due to lack of safe water. In Vietnam, lack of fresh water supply and unhygienic septic tanks were seen as factors that could contribute to the morbidity of diarrhoea in children (Vu Nguyen *et al.*, 2006).

Studies reporting drinking water as a source of *E. coli* pathotypes causing diarrhoea in children under five years old are available (Chukwu *et al.*, 2019; Suge *et al*., 2023). Water plays a role in the transmission of EPEC as evidenced in South Africa (Chukwu *et al.*, 2019). EPEC strains recovered from municipal taps and water storage containers in homes of children under five, with and without diarrhoea, were similar to those detected in the children (Chukwu *et al.*, 2019). In Kenya, EPEC was among the dominant strains (32.0%) identified in water samples collected in households of children under five years old (Suge *et al*., 2023).

ETEC was shown to survive for up to three months in freshwater and form biofilms in drinking water sources; however, its ability to survive in these environments is mostly unknown (Croxen *et al.*, 2013). In Zambia, children whose source of drinking water was a public tap/borehole/well were at high risk of developing diarrhoea caused by the ETEC pathotype (Sukwa *et al*., 2024). In a study in Kenya, drinking water from households of children under five years harboured EIEC strains (4.0%), which were correlated with those present in children's faecal samples (Suge *et al*., 2023). In the same study, EAEC was the most prevalent strain (44.0%) in collected water (Suge *et al*., 2023).

Water can be contaminated by different strains of *Salmonella*, which poses a risk for those who consume it. In a study in Kenya, a significant proportion of children infected with *S.* Typhimurium/ Enteriditis was observed in households that used water pots as water storage containers and water from the tap (Mbae *et al.*, 2020). In Ethiopia, drinking from unimproved water sources contributed to a higher prevalence of *Salmonella* and *Shigella* infection in children (Assefa and Girma, 2019).

Shigella is a diarrhoeagenic pathogen that can also contaminate water, which is worrying as it is very infectious, requiring a small dose to establish the infection (Brooks *et al.*, 2013). A study conducted in Ethiopia reported the presence of Shigella in 5% of

household drinking water samples collected from homes of children under five years of age with diarrhoea (Gobena *et al.*, 2024).

Drinking water is a majour transmission route of DEC, *Salmonella* spp., and *Shigella* spp. in children in LMICs, due to the lack of access to safe water. Multiple studies across Africa and Asia have shown a direct link between contaminated household water and the presence of these diarrhoeagenic strains in children's faecal samples.

2.2.3. Children's hands as a vehicle of diarrhoeagenic bacteria in low and middle-income countries

Hands are an essential vehicle of diarrhoeagenic bacteria, mainly in children, and their presence is strongly associated with subsequent diarrhoeal disease (Pickering *et al.*, 2018). The hand-to-mouth contact in children can occur three to 28 times per hour, allowing the passage of microbes from the hands to the mouth when they are present (EPA, 2011).

In a systematic review by Cantrell *et al.* (2023), *E. coli* and *Salmonella* were among the pathogens and indicators identified on hands. The contamination was higher in low-income countries than in high-income countries, possibly due to a lack of safe drinking water, poor sanitation and hygiene. In tropical low-income countries, hand washing does not guarantee they are free of contaminants for an extended period. Recontamination can occur within minutes due to high levels of faecal contamination on surfaces and soils in the domestic environment (Cantrell *et al.*, 2023). In rural Bangladesh, *E. coli* was detected in 43% of young children aged one to 14 months (Parvez *et al.*, 2019).

Lack of care for a child's nails can be a source of diarrhoeagenic pathogens; this has been evidenced in children under five in South Ethiopia, where untrimmed fingernails were associated with *Salmonella* and *Shigella* infection (Ameya *et al.*, 2018).

Hand washing is an essential factor in preventing diarrhoea. When Covid-19 broke out, one of the biggest appeals to avoid contamination was frequent hand washing with water and soap or ash. This practice helped reduce cases of diarrhoea, as evidenced in some studies. For example, in Ethiopia, the prevalence of childhood diarrhoea was more common among households with poor practices of COVID-19 prevention (Alemayehu *et*

al., 2024). A study in Ghana highlighted the effectiveness of COVID-19 hand hygiene protocols in diarrhoeal reduction (Adu *et al.*, 2024).

Hands are a major route for transmitting diarrhoeagenic bacteria in children due to frequent hand-to-mouth contact and poor hygiene, which increases the risk of infection, especially in low-income settings. Effective handwashing has been a key factor in reducing diarrhoeal infections, as evidenced during the COVID-19 pandemic.

2.2.4. Soil as a source of diarrhoeagenic bacteria for children under five years old in low and middle-income countries

The soil harbours numerous microbes, and children are vulnerable as the soil is one of the places they prefer to play. Children's average daily soil ingestion (200 mg) poses a health risk, especilly when pathogenic agents are present (EPA, 2011). The microbes in the soil are attributed to the presence of animal excreta in the living environment and open defecation (Gizaw *et al.*, 2022). In the rural Democratic Republic of the Congo, 75% of soil samples collected in child play spaces were contaminated by *E. coli* (George *et al.*, 2022). Diarrhoea in children under five from urban areas of Kenya has been associated with soil ingestion (Bauza *et al.*, 2017). The same study demonstrated *E. coli* (100.0%) in soil samples (Bauza *et al.*, 2017). DEC strains such as EAEC (11.0%), EPEC (17.0%), EHEC (23.0%), EIEC (51.0%) and ETEC (28.0%) were present in soil samples from Tanzanian households with and without improved sanitation (Pickering *et al.*, 2012).

Animals that carry *Salmonella* can eliminate them through manure, which can be used as fertiliser, and this pathogen can survive in fertilised soil (Winfield and Groisman, 2003). For example, in Nigeria, *Salmonella* was present in manure-treated soil, which may lead to produce contamination, risking the health of those who will consume it (Abakpa *et al.*, 2015). In rural Mozambique, the annual infection risk by ingesting fecally contaminated domestic soils by *Shigella*/EIEC was estimated at 40%, meaning that children from this area are constantly exposed to this pathogen (Capone *et al.*, 2021).

Soil is a significant source of diarrhoeagenic pathogens, such as DEC strains, *Salmonella*, and *Shigella* due to contamination from animal excreta and open defecation. The presence of these pathogens poses a high risk for children to develop diarrhoeal infections, as they frequently ingest soil while playing.

2.2.5. Flies as vectors of diarrhoeagenic bacteria affecting children under five years old in low and middle-income countries

Insects are vectors responsible for diseases, as they can land in contaminated environments carrying pathogenic agents that can be placed in foods, especially when they are not properly preserved. When it comes to children's food, the risk of children is even more significant as often, during feeding, part of it can become contaminated when it is poorly preserved. For example, in Bangladesh, it was detected in flies in households of children under five with a concentration of 2.9 log10 Most Probable Number (MPN) *E. coli*/fly, where one sample contained EPEC-specific pathogenic genes (eae and bfp). Additionally, for each log10 MPN *E. coli* increase in flies, a 0.31 log10 MPN *E. coli* increase in children stored food, evidencing the role of flies in pathogens dissemination (Doza *et al.*, 2018).

A significant relation between *E. coli* in the Musca domestica house flies and the incidence of diarrhoea in children under five years old was demonstrated in Indonesia (Siregar and Susanna, 2020). *Salmonella* was among the diarrhoeagenic pathogens identified in flies collected between the abattoir, the dump and the market in Benin (Tokponnon *et al.*, 2023). The circulation of flies in these places may lead to food contamination and diseases occurrence when the food bought from this market is prepared not following good hygiene practices. *Salmonella* and *Shigella* strains were recovered from Musca domestica house flies in a refuse dump site in a market, a two-storey apartment, eating places and in a resident building in Nigeria (Ugbogu *et al*., 2006). In Bangladesh, springtime peaks in housefly density in 2009 and 2010 were followed one to two months later by *Shigella*-associated moderate-to-severe diarrhoea at zero to 11 months and 12 to 23 months of age (Farag *et al.*, 2013).

Insects, particularly houseflies, are important vectors of diarrhoeagenic pathogens such as DEC, *Salmonella*, and *Shigella*, as they transfer microbes from contaminated environments to food, especially when it is inadequately stored. Studies LMICs have demonstrated strong associations between fly contamination and increased diarrhoeal infections in children under five.

2.3. Sources of antimicrobial resistant Diarrhoeagenic *E. coli* in children under five in low and middle-income countries

Antimicrobial resistance (AMR) within various infectious agents is a growing public health concern worldwide. Estimations indicate that in 2019, AMR was responsible for 1.27 million global deaths (WHO, 2023). The use of antibiotics for infection prevention in farm animals and as growth promoters rather than to cure infections is partly responsible for antibiotic-resistant bacteria emerging on farms. These resistant bacteria can subsequently reach the population through the food chain (Todar, 2012).

Antibiotic resistance is one of the biggest threats to global health, food security, and development. Antibiotic resistance occurs naturally but can be accelerated by the misuse of antibiotics in humans and animals, resulting in more extended hospital stays, higher medical costs and increased mortality (WHO, 2023).

Escherichia coli is one of the bacteria of interest in terms of antimicrobial resistance. Although it is intrinsically susceptible to most clinically relevant antimicrobials, it can accumulate several resistance genes, mainly through horizontal gene transfer (Poirel *et al.*, 2018).

Extended-spectrum beta-lactamase (ESBL) - producing Enterobacteriaceae is the most studied resistance mechanism in different samples, which include food and water (Doi *et al.*, 2017). ESBLs producing Enterobacteriaceae act by hydrolysing penicillins and cephalosporins, and they become resistant to these classes of beta-lactam agents when they acquire an ESBL gene and produce the enzyme (Doi *et al*., 2017).

ESBL genes such as $bla_{\text{CTX–M}}$ ($bla_{\text{CTX–M–1}}$ group, $bla_{\text{CTX–M–15}}$), bla_{TEM}, and bla_{SHV} gene have been detected in water samples, including in drinking water collected in households from children under five years old who had reported diarrhoea (Mahmud *et al.*, 2020; Suge *et al.*, 2023).

Previous studies in Mozambique demonstrated the presence of antimicrobial resistance genes in water and food samples like poultry and ready-to-eat street food. Beta-lactamase genes were among the antimicrobial resistance (AMR) genes detected, including bla_{TEM}, bla_{OXA}, bla_{SHV} and $bla_{\text{CTX–M}}$ variants (Faife *et al.*, 2020; Salamandane *et al.*, 2022).

In Nigeria, DEC pathotypes such as ETEC, EPEC, STEC and EIEC were detected in faecal samples from children under five years old with diarrhoea and from food and drinking water collected in their households. These strains showed very high resistance to commonly used antibiotics such as augmentin (94%), ceftazidime (86%), cefuroxime (86%), and cefixime (84%) (Ogunbiyi *et al.*, 2023).

EAEC, EPEC, ETEC and EIEC were identified in drinking water collected in households from children under five who had reported diarrhoea in a paediatric unit from a general hospital in Kenya. These strains harboured *bla*$_{\text{CTX–M}}$, *bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$ AMR genes, which were correlated with the ones detected in children's faecal samples (Suge *et al.*, 2023).

The consumption of water and food-contaminated *E. coli* strains harbouring genes that encode antimicrobial resistance can lead to treatment failure (WHO, 2023). For example, *bla*$_{\text{CTX–M}}$, when present in DEC, can confer resistance to antibiotics such as ceftriaxone and aztreonam (Suge *et al*., 2023). When carried by DEC strains, the *bla*$_{\text{TEM}}$ can result in antibiotic resistance to ampicillin and cefotaxime, while *bla*$_{\text{SHV}}$ may lead to resistance to ampicillin and cefotaxime (Suge *et al*., 2023).

2.4. Source attribution of foodborne pathogens

Source attribution of foodborne pathogens estimates the number of illnesses associated with specific food sources (CDC, 2018). Different methods are used for source attribution of foodborne pathogens, including microbial subtyping, outbreak summary data, epidemiological studies, comparative exposure assessment, and structured expert opinion (Andreoletti *et al.*, 2008; Pires *et al.*, 2014; Falcao *et al.*, 2024).

2.4.1. Microbial subtyping method

The microbial subtyping method is based on accurately matching and distributing the cases of human illness caused by a particular subtype over the possible sources where that subtype is found (Andreoletti *et al.*, 2008). The results from surveillance programs are compared to the cases of human illness, reducing uncertainty due to cross-contamination and the risk of attributing to a possible accidental source (Pires *et al.*, 2014; Falcao *et al.*, 2024). Microbial subtyping is based on characterising isolates of a specific pathogen by

different phenotypic or genotypic subtyping methods, and the results are comparable between laboratories, regions, and countries (Andreoletti *et al.*, 2008).

The technique has several limitations. First, it is restricted to pathogens that are heterogeneously distributed among the reservoirs. Second, there is lack of information on different transmission pathways from reservoirs to humans. Third, the technique needs a standardised subtyping methods with a suitable level of discrimination. Finally, it requires an extensive collection of representative isolates from humans and all significant sources (Pires *et al.*, 2014).

2.4.2. Outbreak investigations

An outbreak is the occurrence of two or more related cases of an identical disease or an increase in the observed incidence of disease above the expected incidence. Outbreaks can occur when food safety measures fail at any point along the chain from primary production to the consumer (Andreoletti *et al.*, 2008). The definition of an outbreak is based on the calculation of the number of cases of infectious intestinal disease by pathogen and the calculation of the percentage of foodborne transmission for each pathogen (Andreoletti *et al.*, 2008). The limitations of this method include the lack of harmonisation in the quality of evidence and food classification schemes. Additionally, not all foodborne illnesses may be represented in the outbreak data used. Finally, certain food vehicles are more likely to be associated with reported outbreaks than others, which can introduce bias (Pires *et al.*, 2014).

2.4.3. Epidemiological studies for source attribution of sporadic cases

Epidemiology studies include case-control or cohort studies, but the second one is less used for source attribution of sporadic foodborne infections because it requires interviewing many people, most of whom would probably not be infected. Epidemiology studies involve interviewing persons with and without the infection (Andreoletti *et al.*, 2008).

A case-control study is an analytical epidemiologic study design that compares individuals with the disease under study (also called cases) with asymptomatic individuals (controls) (Andreoletti *et al.*, 2008). This method can be used to identify relevant risk factors for human foodborne infections, including behaviour, sources of

exposure and predisposing or seasonal factors (Tenny *et al*., 2023). If the exposure is found more often in the cases than in the controls, it can be hypothesised that the exposure may be linked to the outcome of interest (Tenny *et al*., 2023). The relative exposures are estimated by comparing the frequency of exposures among cases and controls (Andreoletti *et al.*, 2008). The risk factor can be estimated by calculating the population-attributable fractions (Andreoletti *et al.*, 2008). In this method, the exposure is often identified based on personal recall, using either a self-administered questionnaire or an interview (Andreoletti *et al.*, 2008). Sometimes the exposure can be ascertained and established according to historical records of interest (Tenny *et al*., 2023). A primary limitation of the method is the accuracy of the recall about exposures from interviewed participants, which can lead to either an over- or underestimation of the contribution of specific sources (Pires *et al.*, 2014). In addition, many participants will need to be enrolled to have sufficient statistical power to determine the importance of common exposures c

2.4.4. Source attribution by comparative exposure or risk assessment

The risk assessment methodology focuses on the point of exposure and transmission routes rather than animal reservoirs, quantifying exposure to pathogens from a multitude of sources (Andreoletti *et al.*, 2008; Falcao *et al.*, 2024). The estimated exposure can be per person per day or averaged over a specified population for all relevant specific sources such as food, animal contact and environment (Andreoletti *et al.*, 2008; Falcao *et al.*, 2024). The results of all exposures can be used to calculate the total exposure or to identify the most significant exposure sources (Andreoletti *et al.*, 2008). The major limitation of this method is the lack of data, which may lead to significant uncertainties in the estimates (Pires *et al.*, 2014).

2.4.5. Source attribution by expert opinion

The expert opinion method uses a broad set of information from pathology, microbiology, epidemiology, technology, ecology, and consumer behaviour to obtain estimates of the proportion of cases transmitted by a particular pathway (Andreoletti *et al.*, 2008). This method explores sources of uncertainty and answers questions where data are expensive or difficult to collect (Butler *et al*., 2015).

The data analysis consists of aggregating individual expert's assessments, possibly applying performance-based weights, robustness and discrepancy analysis, and reporting

(Andreoletti *et al.*, 2008). The conclusions are based on the individual experts' judgment, which constitutes a limitation of the method (Pires *et al.*, 2014).

2.5. Molecular diagnostic methods for foodborne pathogens

Molecular techniques are among the diagnostic methods frequently used for detecting foodborne pathogens, based on the detection of nucleic acids (Hadi *et al.*, 2023). Polymerase chain reaction (PCR) and DNA sequencing are among the most used molecular techniques for enterobacterial pathogens (Ammari *et al.*, 2009; Pornsukarom *et al.*, 2018; Taşkale Karatuğ *et al.*, 2018).

2.5.1.Polymerase chain reaction

Polymerase chain reaction (PCR) is a technique that amplifies small quantities of target nucleic acid sequences, yielding an amount of product that is detectable by downstream methods, such as visualisation of nucleic acid on an agarose gel (Walker-Daniels, 2012)

PCR is one of the most commonly used molecular methods, enabling faster identification and increasing food safety and quality (Jay *et al.*, 2005; Hadi *et al.*, 2023). The PCR technique, due to its high sensitivity, specificity and availability in several formats, has been successfully applied for identification of bacteria and viruses in foods (Jay *et al.*, 2005).

PCR can be used as a tracking device for epidemiological investigations of food sources of microbial contamination and strain confirmation (Staley *et al.*, 2018). The quantitative PCR method has been used to track human sewage from microbial sources in recreational waters, coral reef waters, and groundwater (Staley *et al.*, 2018; Sinigalliano *et al.*, 2021). A high-throughput quantitative PCR was applied for microbial source tracking markers in environmental water (Raya *et al.*, 2024). Microbial source tracking markers were detected in infant food prepared by caregivers in Kenya using qPCR, indicating contamination during food preparation (Tsai *et al.*, 2022). These studies highlight the potential of PCR technology in detecting microbial source tracking markers in faecal and water samples. This allows a better understanding of the microbial risk assessment from different sources and strategies to prevent future outbreaks (Tsai *et al.*, 2022).

In general, PCR provides fast identification, high sensitivity and specificity and is available in different formats (Jay *et al.*, 2005; Grohmann *et al.*, 2021). However, the high

cost of equipment and reagents and the need for skilled people to perform may constitute a limitation (Jay *et al*., 2005).

2.5.2. DNA sequencing

Deoxyribonucleic acid (DNA) sequencing techniques involve technologies used to determine the order of the nucleotide bases (namely adenine, cytosine, guanine and thymine) in a DNA molecule (Adzitey *et al*., 2013). Recently, DNA sequencing has been used widely and routinely in the identification, typing, characterization and/or taxonomic classification of unknown or novel pathogen isolates by many researchers (Adzitey *et al*., 2013).

Since its appearance, the DNA sequencing technique has evolved from the first to the third generation; the last two are also called next-generation sequencing (NGS) (Besser *et al.*, 2019). The first generation includes Sanger, Maxam and Gilbert's techniques, which produce relatively long (500 to 1000 bp) high-quality DNA sequences and have been considered the reference standard for sequencing DNA (Besser *et al.*, 2019). The emergence of pyrosequencing technology by 454 Life Sciences in 2005 marked a major advancement in sequencing. This high-throughput technology enabled the generation and detection of thousands to millions of short sequencing reads in a single machine run without the need for cloning; this was considered the beginning of NGS (Heather and Chain, 2016; Besser *et al.*, 2019).

According to Vincent *et al.* (2017), most NGS technologies currently available are based on the following principles: i) DNA extraction and purification, ii) library preparation, iii) sequencing and iv) data analysis.

The main characteristics of second-generation sequencing technology are: i) generation of many millions of short reads in parallel, ii) increased speed of the sequencing process compared to the first generation, iii) low cost of sequencing and iv) sequencing output detection without the need for electrophoresis (Kchouk *et al*., 2017).

Illumina is the leading next-generation technology for DNA sequencing, where all the technology’s enzymatic processes and imaging steps occur in a flow cell (Buermans and den Dunnen, 2014). The Illumina platform uses bridge amplification for polony generation and a sequencing-by-synthesis approach (Buermans and den Dunnen, 2014).

Third-generation sequencing (TGS), also called long-read technologies, is now revolutionizing genomics research because it enables researchers to explore genomes at an unprecedented resolution (van Dijk *et al.*, 2018). TGS also provides metagenomics analyses that benefit from long-read sequencing, enhancing the quality of genome assembly and the analysis of genomic structures. This technology allowed for the first time, the resolution of microbial communities at the species level (van Dijk *et al.*, 2018; Scarano *et al.*, 2024).

Among different sequencing methods, Whole Genome Sequencing (WGS) is one of the most used in foodborne pathogen diagnosis (Pornsukarom *et al*., 2018). WGS is a cutting-edge molecular technology that identifies bacteria isolated from food or environmental samples (Pornsukarom *et al*., 2018). Several studies are available in which these methods were used for different molecular characteristics of foodborne pathogens (Pornsukarom *et al*., 2018; Fiedoruk *et al.*, 2019; Nouws *et al.*, 2020; Schadron *et al.*, 2024).

PCR technology detects a small portion of the microbial genome, while WGS, at the same time, captures the entire genome and provides information for serotyping, virulence and AMR genotyping. This allows a more accurately describes the genetic relatedness of strains from outbreaks as well as the food production chain, assuring that they could be part of the same transmission chain (Jagadeesan *et al.*, 2019; Nouws *et al.*, 2020).

Since the WGS was introduced into public health, it has been considered an essential tool for surveillance and response to foodborne diseases (Jagadeesan *et al.*, 2019). When associated with other information, WGS enhances the determination of the source of infection and the transmission route by tracking and tracing the pathogen through the food chain (Jagadeesan *et al.*, 2019).

WGS can be used to investigate food-related outbreaks and perform surveillance to identify pathogens' local, regional, and global genomic epidemiology (Moran-Gilad, 2017; Schadron *et al.*, 2024). It can also attribute the infection source to determine virulence and antimicrobial resistance (Allard *et al.*, 2018). WGS provides information to help distinguish between the new and existing organisms in the environment's production, thus supporting risk assessment and guiding interventions for preventing and controlling infections (Moran-Gilad, 2017; Jagadeesan *et al.*, 2019).

In general, the advantages of WGS are high discriminatory power, 100 % typeability and good reproducibility; however, it requires two to three days to complete a test and has limited availability and higher costs (Adzitey *et al.*, 2013).

PCR is a widely used molecular diagnostic tool known for its speed, high sensitivity, and specificity. It is effective for targeted pathogen detection and microbial source tracking in food and environmental samples. However, its main limitation is that it can only detect known genetic targets.

In contrast, DNA sequencing, particularly WGS, provides a comprehensive and high-resolution approach. It enables complete genomic characterization, including strain typing, virulence profiling, and AMR detection, in a single run. This technology relies on specialized bioinformatics tools to identify specific genetic characteristics. However, WGS is resource-intensive, requiring high costs, advanced data analysis, and is often less accessible in low-resource settings.

2.5.3. Whole genome sequencing for source attribution and risk assessment

Whole genome sequencing is a technology adopted for source attribution and risk assessment. It offers advantages over other available methods, such as pulsed-field gel electrophoresis (PFGE) or multilocus variable-number tandem-repeat analysis (MLVA) (Koutsoumanis *et al.*, 2019). The use of WGS technology for source attribution allows for obtaining the highest level of bacterial strain discrimination. Additionally, this technology permits pathogen typing to establish the risk assessment of specific pathogenic agents (Koutsoumanis *et al.*, 2019).

WGS is more effective in the source attribution approach as it improves transmission pathway identification by integrating factors related to place and time, detecting different transmission routes and interactions between pathogen and host (Koutsoumanis *et al.*, 2019). Due to the high level of discriminatory power associated with WGS, challenges arise with this technology as novel modelling approaches are needed to handle the large amount of data (Franz *et al.*, 2016).

Some authors suggested incorporating WGS into risk assessment to set the priority of high-risk phenotypes and link genome sequences with phenotypic properties related to persistence in the food chain and in vitro or in vivo virulence assessments.

2.6. Control of contamination of children's food by enteric bacteria

The microbial population in food can be controlled or reduced to prevent the risk of diarrhoeagenic infections. The World Health Organization (WHO) has established five keys to safer food, which are essential for reducing foodborne illness caused by pathogens such as *Salmonella*, *Shigella*, and DEC. The first key is to keep clean, which involves washing hands before handling food and regularly cleaning kitchen surfaces and utensils to prevent the spread of microorganisms. The second key is to separate raw and cooked foods to avoid cross-contamination, since raw foods such as meat, poultry, vegetables, and their juices often carry harmful microbes. The third key is to cook thoroughly, as proper cooking destroys most pathogenic microorganisms and ensures food is safe for consumption. The fourth key is to keep food at safe temperatures, specifically below 5°C or above 60°C, to prevent microbial proliferation. Finally, the fifth key is to use safe water and raw materials, meaning that water used in food preparation should be treated by boiling, chlorination, or filtration and raw ingredients should be fresh and uncontaminated. Applying these five keys is critical for reducing the transmission of diarrhoeagenic pathogens through food.

Adherence to the WHO's five feys to safer food by caregivers is a critical component in preventing diarrhoea in children. Additional preventive measures include the use of improved sanitation, exclusive breastfeeding during the first six months of life, good personal and food hygiene, and health education on how infections are transmitted children (WHO, 2024b).

2.7. Aim and objectives

2.7.1 Aim

To investigate the role of food and water as sources of foodborne pathogens, and the associated risk factors contributing to diarrhoea among children under five in Maputo Province, Mozambique

2.7.2. Objectives

1. To perform whole genome sequencing (WGS) on enterobacteria isolated from children under five years old's faecal samples using the Illumina Miseq platform with the aim of

identifying diarrhoeagenic bacteria and factors associated with these pathogens in in these children, in urban and rural areas of Maputo;

2. To isolate and identify enterobacteria isolates from food and water using biochemical techniques, Matrix-assisted laser desorption ionisation–time-of-flight mass spectrometry (MALDI-TOF MS) and Polymerase Chain Reaction (PCR) with the aim of identifying diarrhoeagenic bacteria and factors associated with contamination of food and water consumed by children under five years old from urban and rural areas of Maputo province;

3. To characterise diarrhoeagenic bacteria molecularly isolated from from food, water and children's faecal samples, with the aim of identifying antimicrobial resistance and determining the sources and transmission routes of foodborne pathogens.

2.8. Hypotheses

2.8.1. Hypothesis 1

Whole-genome sequencing of *E. coli* strains isolated from faecal samples of children under five in urban and rural areas will reveal the presence of pathogenic variants. EAEC, EPEC, DAEC, EIEC, STEC, and ETEC pathotypes are among the foodborne pathogens implicated in childhood diarrhoea. These pathotypes have been detected in children's faecal samples, and their association with diarrhoeal disease has been demonstrated. Food has also been shown to be contaminated with DEC strains, highlighting its role in the transmission of these pathogens to humans. Food can be contaminated by DEC pathotypes through the faecal-oral pathway, mainly when good hygiene practices are not followed, and the food is not properly conserved. Food contamination factors include hygiene practices, food preparation and storage, children feeding themselves, and exposure to infected animals (Rúgeles *et al.*, 2010; Varela *et al.*, 2015; Bick *et al.*, 2020; Wells *et al.*, 2023). Children who eat contaminated food are likely to have diarrhoea due to their weak immune system. Other factors for diarrhoea include early weaning, low maternal education, lack of piped water supply, poor water-storage practices, younger maternal age, poor sanitation, visible faeces in the yard, unsatisfactory garbage disposal, and not treating water for drinking (George *et al.*, 2014).

2.8.2. Hypothesis 2

Food and drinking water in urban and rural areas of Maputo are likely to be contaminated with *Salmonella* spp., *Shigella* spp. and DEC agents, contributing to diarrhoea in children under five years old. Enteric bacteria, such as *Salmonella* spp., *Shigella* spp., and DEC, can contaminate food and water due to poor hygienic conditions during processing, either from sick people or animals or from faeces from infected individuals, leading to the occurrence of diarrhoeal disease, mainly in children under five years old (Pinto, 1996; Mensah *et al.*, 2012; WHO, 2019). A study conducted in Colombia observed that DEC (STEC and EAEC) strains isolated from food retail stores could be the source of infection in the community (Rúgeles *et al.*, 2010). In a study by Varela *et al.* (2015), acute diarrhoea in children was associated with EPEC, STEC, and *Salmonella* spp. foodborne pathogens.

2.8.3. Hypothesis 3

Molecular characterisation of DEC isolated from food, water, and children's faecal samples will reveal the presence of antimicrobial resistance and help identify the sources and transmission routes of foodborne pathogens. Enterobacterial strains isolated from food and drinking water can carry AMR genes affecting antimicrobial efficacy (Faife *et al.*, 2020; Suge *et al.*, 2023). Enterobacteriaceae isolated from different sources will likely harbour the same resistance gene group (Suge *et al.*, 2023). Water samples consumed by children under five years old were shown to harbour DEC that carry extended-spectrum β-lactamases (ESBL)/AmpC genes, namely $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{SHV} and these genes were correlated with the ones detected in children's faeces (Suge *et al.*, 2023). Chicken is a type of food incorporated in the feeding of children under five years old and can carry AMR genes, as was demonstrated in a study by Faife *et al.* (2020) in Mozambique, where plasmid-mediated resistance to β-lactam such as bla_{SHV}, bla_{TEM}, $bla_{\text{CTX-M-55/-79/-101/-164}}$, bla_{CMY}, bla_{MOX}, bla_{FOX}, bla_{DHA} was detected in *E. coli* isolates from retail frozen chickens. The presence of β-lactamase genes such as SHV, CTX-M and OXA in ready-to-eat foods has been demonstrated (Liu *et al.*, 2023). DEC pathotypes in children's faecal samples have been shown to harbour ESBL genes such as bla_{TEM}, $bla_{\text{CTX-M}}$ (Heine *et al.*, 2024; Mahamat *et al.*, 2024). Contaminated food and water are the main sources of diarrhoeagenic pathogens, including DEC strains.These pathogens are transmited via faecal-oral route (WHO, 2024b).

CHAPTER 3: FOOD AND ANIMALS ASSOCIATED WITH DIARRHOEAGENIC *E. COLI* IN CHILDREN UNDER FIVE WITH DIARRHOEA IN MAPUTO, MOZAMBIQUE

3.1. ABSTRACT

Diarrhoeagenic *E. coli* strains are among the major cause of diarrhoeal disease, a public health concern worldwide, particularly in low- and middle-income countries, including Mozambique, due to limited access to portable drinking water, inadequate sanitation, and poor knowledge. This study aimed to identify DEC in stool samples from children with diarrhoea and risk factors associated with this infection. Three hundred children under five years of age with diarrhoea were selected from the Health Care Center of Marracuene (n=156), rural area, and Health Care Center of Primeiro de Maio (n=144), urban area. The data were collected using a semi-structured questionnaire, completed by the caregivers of the children. The questionnaire allowed obtaining information related to demographics, food consumed a week before the occurence of diarrhoea in children, domestic animals present in the households, and the general health of the children. Faecal samples were collected from children with diarrhoea. Explanatory variables were screened using chi-square tests and then analysed with Lasso logistic regression in R to identify factors linked to DEC in children.

The majority of children in the study were males (55.3%) and most were aged 7 to 24 months (59.7%). From the faecal samples collected, 2.0% had EPEC, 1.0% from rural and also 1.0% from urban areas.

Yoghurt consumption was found to be protective against EPEC-associated diarrhoea in children, in contrast to the consumption of fruit, vegetables, or juice and the presence of domestic animals such as dogs and chickens, which were identified as risk factors. The findings underscore the necessity for educating caregivers on good hygiene practices in food preparation and storage, and on how to handle animals and their excrement.

Keywords: Children, diarrhoea, diarrhoeagenic *E. coli*, food, animals.

3.2. INTRODUCTION

Diarrhoeal disease is a public health concern in most parts of the world and is considered the third leading cause of mortality in children under five years of age (WHO, 2024a). Low-income countries are likely to have more cases of diarrhoea due to factors such as limited access to potable water, lack of improved sanitation, and poor education of the caregivers (He *et al*., 2023; WHO, 2024a). Risk factors for diarrhoeal mortality in children include lack of sanitation, poor hygiene, not breastfeeding or early cessation of breastfeeding, malnutrition, wasting, non-vaccination or not receiving the full dose of rotavirus vaccines and inadequate access to health care services (Hartman *et al.*, 2023).

In Mozambique, diarrhoea is among the three primary diseases for seeking care and treating childhood illness. Additionally, an epidemiological study showed a high diarrhoeal disease burden and an associated case fatality rate of nearly 10% (Nhampossa *et al.*, 2015; INE, 2024). *Escherichia coli*, Rotavirus, *Shigella* spp., *V. cholerae*, and *Cryptosporidium* spp. are among the pathogens associated with childhood diarrhoea (Nhampossa *et al.*, 2015; Chissaque *et al.*, 2018). In rural and urban areas of Maputo, household factors such as the use of traditional medication, bottle feeding and use of unsafe drinking water have been identified as being associated with diarrhoea in children under five (Machava *et al*., 2022b). However, there is a lack of studies on the contribution of foods and domestic animals to childhood diarrhoea, although there is evidence of an association with this illness.

Domestic animals can carry pathogenic agents including diarrhoeagenic pathogens, which can be spread in the household environment through animal faeces, resulting in contamination of food and water, which may lead to diarrhoea in children (Delahoy *et al.*, 2018). Animal ownership and observations of domestic animal waste in households have been associated with household drinking water contamination (Barnes *et al.*, 2018). Food can also act as a vehicle for pathogens, leading to enteric infections especially if good hygiene practices are not followed during preparation (Bintsis, 2017).

This study aimed to identify diarrhoeagenic *E. coli* (DEC) in stool samples from children under five years of age with diarrhoea and the risk factors associated with DEC diarrhoea. The study's results provide knowledge on the type of foods and animals that influence the occurrence of *E. coli* diarrhoea in children under five. This knowledge will allow the

development of prevention strategies for diarrhoea management in the Maputo province community.

3.3. METHODOLOGY

3.3.1. Study design and sampling site

This is a community-based quantitative and qualitative cross-sectional study conducted in Marracuene and Kamaxakeni, districts of Maputo in Mozambique. Kamaxakeni is an urban district of Maputo, while Marracuene is a rural district in the eastern part of Maputo.

The children included in the study were sampled from the Marracuene and Primeiro de Maio Health Care Centres from Kamaxakeni and Marracuene districts, respectively. These healthcare centres provide primary healthcare services.

3.3.2. Sampling and semi-structured interviews

Three hundred children under five with diarrhoea were selected at the Health Care Centres of Marracuene (n=156) and Primeiro de Maio (n=144) from 2021 to 2022. The sample size was calculated using EpiTool (http://epitools.ausvet.com.au/), based on an assumption of an average prevalence of less than 10%, an assumed sensitivity and specificity of 60% and 99%, respectively, and with a precision of 5% at 95% confidence. The sample size calculation was based on a binomial distribution, where 300 (rounded up from 284) samples/data from children allow estimation of the true prevalence of any of the investigated pathogens.

Children under five years of age with diarrhoea were selected for the study as they arrived at the health care centres. Prior to selection, consent was obtained by explaining the purpose of the research to the caregivers, after which faecal samples were collected from the children.

Faecal samples were obtained using rectal swabs. After collection, each swab was placed in Cary-Blair transport medium, stored in a cooler box, and transported to the laboratory for analysis.

Caregivers were interviewed in their households based on a semi-structured questionnaire. The questionnaire obtained information on demographics, the children's symptoms and treatments, the caregivers' suspected source of diarrhoea, the source of

water supply, animals present in the house, and foods consumed in the household prior to the children's diarrhoeal episodes. The questionnaire was organised to obtain information on the food consumed by children under five years old, in general, and particularly food consumed by children below two years old (Appendix 1).

3.3.3. Diarrhoeagenic *E. coli*

3.3.3.1. *Escherichia coli* identification

The identification of *E. coli* in faecal samples was carried out at the National Institute of Health in Maputo, Mozambique. Faecal samples were plated on MacConkey agar medium (Liofilchem Diagnostici-610028, Italy), and after 24 hours of incubation, *E. coli* was presumptively identified based on the growth of pink colonies due to lactose fermentation (Basavaraju and Gunashree, 2023). Biochemical tests were done using sugars with triple sugar iron agar (Liofilchem Diagnostici-360620055), motility-indole-ornithine agar (HIMEDIA –M378-500G, USA) and lysine ion agar (HIMEDIA-M377-100G). Isolates were identified as *E. coli* after overnight incubation at 37°C if they exhibited the following characteristics: glucose and lactose fermenters, gas producers, indole-positive, lysine and ornithine-positive and motile (Roy *et al*., 2023). From the 300 faecal samples analysed (rural=156 and urban = 144), 72 *E. coli* strains were detected in rural and 76 in urban areas.

3.3.3.2. Whole genome sequencing and diarrhoeagenic *E. coli* identification

DNA extraction

The *E. coli* DNA was extracted after overnight bacterial culture in brain heart infusion agar (HIMEDI), using the quick-DNATM Fungal/Bacterial Miniprep kit (Zymo Research, California, USA). *Escherichia coli* strains were added directly to a ZR BashingBead™ Lysis Tube (0.1 & 0.5 mm) and lysed by bead-beating without the use of organic denaturants or proteinases. After washing the DNA was eluted to obtain ultra-pure DNA for whole genome sequencing.

Genome sequencing and analysis

Whole genome sequencing was carried out at the Agricultural Research Council, Onderstepoort, South Africa. Multiplexed paired-end libraries were prepared using the

MGIEasy Universal DNA Library Prep Kit (MGI, China). Genome sequencing was performed on the MGI DNBSEQ-G400 (MGI) sequencing instrument, generating 150 150-base-pair paired-end FASTQ reads. The quality of the sequences was ensured by trimming and de novo assembling the paired-end reads using the CLC genomics workbench version 9 (Qiagen, Netherlands) and by removing all low-quality reads. The fastq files were also uploaded to the EnteroBase web-based platform for pathotype identification, based on the expression of the *fimH*, *stx1*, *stx2*, *ipaH*, pInv, ST, LT and *eae* genes.

3.3.4. Statistical analysis

All explanatory variables were screened for inclusion in a binomial regression using the chi-square test (X^2 test) on Epi info (version 7.2.5.0) as recommended (CDC, 2021). The X^2 was based on the threshold set at $p \leq 0.1$, considering the one-tailed hypothesis of the Fisher's exact test when observations were below five. Lasso binomial logistic regression was performed in R using the glmnet package (version 4.1-8) (Friedman, Hastie and Tibshirani, 2010). The explanatory variables tested for inclusion in the logistic regression were child age, caregiver age, child gender, caregiver gender, region, ready-to-eat meals, fruit puree, yoghurt, cheese, chicken, pork, fermented cereals and bottled water. The outcome variable was whether the child was found positive for DEC or not.

3.3.5. Ethical approval and informed consent to participate

This study is part of the Foodborne Disease Epidemiology, Surveillance and Control in African LMIC (FOCAL) project involving the following countries: Nigeria, Ethiopia, Tanzania, and Mozambique. The ethical clearance (registration number: CIBS FM&HCM/092/2019) was obtained from the institutional health bioethics committee of the Faculty of Medicine/Maputo Central Hospital, harbouring the following registration number: CIBS FM&HCM/092/2019. All the participants gave written consent for the interview and sample collection in their households. The data were stored electronically and transferred to a password-protected database to ensure privacy and confidentiality. Additionally, the data analysis was based on ID codes assigned to each participant.

3.4. RESULTS

3.4.1. Demographics of the participants

Table 3.1 shows the demographic characteristics of children under five and their caregivers. More than half of the children involved in the study were male (55.3%) and aged 7 to 24 months (59.7%). Most caregivers of the children were aged 18 to 25 years (45.0%), single but living with a partner (72.7%) and were female (91.0%).

Table 3.1. Demographic characteristics of the children under five years old and their caregivers

Variable	Category	Rural area (n=156)	Urban area (n=144)	X2	Total (n=300)
		n(%)	n(%)		n(%)
Child gender	Female	75(48.1)	59(40.3)		134 (44.7)
	Male	81(51.9)	85(59.0)	0.216	166 (55.3)
Caregiver gender	Female	143(91.7)	130(90.3)		273 (91.0)
	Male	11(7.1)	14(9.7)	0.35	25 (8.3)
	Not disclosed	2(1.3)	0	0.4	2 (0.7)
Child age (months)	≤6 months	32(20.5)	18(12.5)		50 (16.7)
	7 to 24 months	89(57.1)	90(62.5)	5.14	179 (59.7)
	> 24 months	34(21.8)	36(25)	0.11	70 (23.3)
	Not disclosed	1(0.6)	0	<0.01	1 (0.3)
Caregiver (age years)	18 to 25 years	69(44.2)	66(45.8)		135 (45.0)
	26 to 35 years	63(40.4)	44(30.6)	1.17	107 (35.7)
	>35 years	19(12.2)	16(11.1)	0.02	35 (11.7)
	Not disclosed	5(3.2)	18(12.5)	5.68	23 (7.7)
Marital status	Married	20(12.8)	28(19.4)		48 (16.0)
	Divorced	1(0.6)	1(0.7)	<0.01	2 (0.7)
	Widowed	1(0.6)	0	0.02	1 (0.3)
	Single but living with a partner	122(78.2)	96(66.7)	2.68	218 (72.7)
	Single and living alone	11(7.1)	18(12.5)	0.01	29 (9.7)
	Not disclosed	1(0.6)	1(0.7)	<0.01	2 (0.7)
Relation to the child	Mother	139(89.1)	117(81.3)		256 (85.3)
	Father	11(7.1)	8(5.6)	<0.004	19 (6.3)
	Grandmother/Parents	5(3.2)	10(6.9)	1.73	15 (5.0)
	Not disclosed	1(0.6)	3(2.1)	0.44	10 (3.3)

3.4.2. Symptoms in children under five with diarrhoea

Table 3.2 presents symptoms reported by the caregivers during the period in which the child had diarrhoea. The most common symptoms reported by caregivers were vomiting (32.0%), fever (27.0%), and coughing (23.0%). A high frequency of fever symptoms was mainly reported in rural area, while fever and coughing were more frequent in urban area. Muscle ache, flatulence, eye problems and dysuria were the least reported, with only 0.3%.

Table 3.2. Symptoms presented by children during the period in which the child had diarrhoea

Category	Rural area (n=156)	Urban area (n=144)	Total (n=300)
	n (%)	n (%)	n (%)
Vomiting	61(39.1)	35(24.3)	96(32.0%)
Muscle ache	0	1(0.7)	1(0.3%)
Joint pain	2(1.3)	0	2(0.7%)
Fever	36(23.1)	45(31.3)	81(27.0%)
Abdominal pain	17(10.9)	20(13.9)	37(12.3%)
Flatulence	1(0.6)	0	1(0.3%)
Constipation	1(0.6)	1(0.7)	2(0.7%)
Jaundice	1(0.6)	0	1(0.3%)
Headache	2(1.3)	3(2.1)	5(1.7%)
Eye problems	0	1(0.7)	1(0.3%)
Dysuria	1(0.6)	0	1(0.3%)
Running nose	22(14.1)	23(16.0)	45(15.0%)
Coughing	29(18.6)	40(27.8)	69(23.0%)
Dizziness	2(1.3)	0	2(0.7%)
Loss of appetite	20(12.8)	23(16.0)	43(14.3%)

3.4.3. Caregiver's perception of diarrhoeal causes in children under five

Table 3.3 indicates the causes of diarrhoea as perceived by the caregivers. Most respondents pointed to food (16.7%) and teething (10.7%) as the main causes of diarrhoea, with high frequencies having been observed mainly in the rural area. Climate/moon (5.3%) (in some communities, the waning crescent phase of the moon is seen as a period of low energy and increased susceptibility to illness) was seen as one of

the causes of diarrhoea in children. Most caregivers could not give a probable cause for diarrhoeal episodes in children (20.3%).

Table 3.3. Cause of diarrhoea in children under five as perceived by the caregivers

Categories	Rural area (n = 156)	Urban area (n= 144)	Total (n=300)
	n (%)	n (%)	n (%)
Food	37(23.7)	13(9.0)	50 (16.7%)
Water	3(1.9)	10(6.9)	13 (4.3%)
Fruit/Juice	15(9.6)	3(2.0)	18 (6.0%)
Teething	25(16.0)	7(4.9)	32 (10.7%)
Weaning/Infant formula/Milk	9(5.8)	5(3.5)	14 (4.7%)
Climate/moon	11(7.1)	5(3.5)	16 (5.3%)
Other causes	8(5.1)	4(2.8)	12 (4.0%)
Don't know the reason	37(23.7)	24(16.7)	61 (20.3%)
Not disclosed	11(7.1)	73(50.7)	84 (28.0%)

3.4.4. Household animals and children's contact with them

Table 3.4 highlights animals present in the households and their contact with children with diarrhoea. The results show that chickens (17.3%), cats (14.3%), ducks (9.7%) and dogs (7.0%) were the animals frequently observed in children's households and those with which children most commonly had contact. In the rural area, the presence of animals in the home was more common, especially chickens, ducks and dogs.

Table 3.4. Household animals and children's contact with them

Animal name	Animals in households			Contact with animals		
	Rural area (n=156)	Urban area (n=144)	Total (n=300)	Rural area (n=156)	Urban area (n=144)	Total (n=300)
	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)
Cat	23(14.7)	20(13.9)	43 (14.3%)	15(9.6)	14(9.7)	29 (9.7%)
Chicken	47(30.1)	5(3.5)	52 (17.3%)	15(9.6)	3(2.1)	18 (6.0%)
Dog	14(9.0)	7(4.9)	21 (7.0%)	6(3.8)	1(0.7)	7 (2.3%)
Duck	19(12.2)	10(6.9)	29 (9.7%)	18(11.5)	5(3.5)	23 (7.7%)
Goat	2(1.3)	0	2 (0.7%)	0	0	0 (0.0%)
Pigs	4(2.6)	0	4 (1.3%)	1(0.6)	0	1 (0.3%)

3.4.5. Foods consumed by children before diarrhoea

Table 3.5 shows foods consumed by children before the diarrhoea episode. Foods such as chicken (69.3%), fish (61.7%), eggs (57.0%), beef (54.3%) and pork (36.7%) were among the most commonly consumed animal protein food by children under five years of age, and high levels of consumption were reported mainly in the rural area. Milk and milk products were consumed by less than 20% of children in both sampling areas. Cheese was consumed only in the urban area. The consumption of vegetables was also observed in both sampling areas, with the highest frequency in the urban area (89.6%).

Children under two years of age were mainly fed with fermented cereals (28.0%), biscuits/rusks/cookies (28.0%), breast milk (24.7%), fruit/vegetables/juice (24.3%) and bottled water (22.7%). High consumption of these foods was mainly observed in rural areas, with the exception of fermented cereals and bottled water, which were mainly given to children under two in the urban area.

Table 3.5. Food consumed by children under five in the week before the diarrhoea episodes

Variable	Category	Rural area (n=156)	Urban area (n=144)	Total (n=300)
		n(%)	n(%)	n(%)
Food consumed by children <5 years	Beef	120(76.9)	43(29.9)	163 (54.3%)
	Pork	91(58.3)	19(13.2)	110 (36.7%)
	Lamb	13(8.3)	5(3.5)	18 (6.0%)
	Goat	59(37.8)	7(4.9)	66 (22.0%)
	Chicken	140(89.7)	68(47.2)	208 (69.3%)
	Egg	123(78.8)	48(33.3)	171 (57.0%)
	Fish	133(85.3)	52(36.1)	185 (61.7%)
	Milk	13(8.3)	28(19.4)	41 (13.7%)
	Yoghurt	30(19.2)	23(16.0)	53 (17.7%)
	Cheese	0	5(3.5)	5 (1.7%)
	Vegetables	121(77.6)	129(89.6)	250 (83.3%)
Food consumed by children <2 years	Milk breastfeeding	55(35.3)	19(13.2)	74 (24.7%)
	Infant formula	19(12.2)	15(10.4)	34 (11.3%)
	Fermented cereals	33(21.2)	51(35.4)	84 (28.0%)
	Ready-to-eat meal	13(8.3)	19(13.2)	32 (10.7%)
	Fruit puree	19(12.2)	12(8.3)	31 (10.3%)
	Biscuits/rusks/cookies	66(42.3)	18(12.5)	84 (28.0%)
	Fruit or vegetable juices	60(38.5)	13(9.0)	73 (24.3%)
	Baby bottle	85(54.5)	77(53.5)	162 (54.0%)
	Bottled water	33(21.2)	35(24.3)	68 (22.7%)
	Boiled water	4(2.6)	7(4.9)	11 (3.7%)
	Water not boiled	17(10.9)	14(9.7)	31 (10.3%)

3.4.6. Drinking water and sanitation sources in households of children under five with diarrhoea

Table 3.6 highlights the drinking water and sanitation sources in homes of children under five with diarrhoea. Participants from both sampling areas had drunk tap water (84.3%), with some from the rural areas also drinking water from public/community wells (7.1%), whereas some those from the urban areas having drunk bottled water (16.7%). In addition,

households from both areas reported to having boiled the water prior to consumption by the children (12.3%).

The toiled system consisted mainly of flush or pour toilets connected to sewer pipes (50.0%) and pit latrines with covering slabs (21.0%). Other most commonly used toilet systems were flush or pour toilet with septic tank, including squat toilets and pit latrine without covering slab, which were mainly observed in rural area, with 24.4% and 12.8%, respectively. In the rural area, one household had no toilet system.

Table 3.6. Drinking water and sanitation sources in households of children under five years old with diarrhoea

Variable	Category	Rural area (n=156)	Urban area (n=144)	Total (n=300)
		n(%)	n(%)	n(%)
Water source	Home pipe water	139(89.1)	114(79.2)	253 (84.3%)
	Public/community wells	11(7.1)	0(0.0)	11 (3.7%)
	Bottled water	0	24(16.7)	24 (8.0%)
	Not disclosed	6(3.8)	6(4.1)	12 (4.0%)
Water treatment	Boiling	16(10.2)	21(14.6)	37 (12.3%)
	Other methods	4(2.6)	5(3.5)	9 (3.0%)
	Not disclosed	136(87.2)	118(81.9)	254 (84.7%)
Toilet system	Flush or pour toilet connected to sewer pipe	61(39.1)	89(61.8)	150 (50.0%)
	Flush or pour toilet with septic tank, including squat toilet	35(22.4)	1(0.7)	36 (12.0%)
	Pit latrine with covering slab	28(18.0)	35(24.3)	63 (21.0%)
	Pit latrine without covering slab	20(12.8)	3(2.1)	23 (7.7%)
	Ventilated improved pit latrine (VIP)	6(3.9)	0	6 (2.0%)
	other toilet system	2(1.3)	0	2 (0.7%)
	Without toilet system	1(0.6)	0	1 (0.3%)
	Not disclosed	3(1.9)	16(11.1)	19 (6.3%)

3.4.7. Prevalence of diarrhoeagenic *E. coli* in children under five with diarrhoea

Table 3.7 shows the prevalence of DEC in children under five years old with diarrhoea. Among the 300 faecal samples collected, 148 yielded isolates confirmed as *E. coli* strains, and the whole genome sequencing allowed the identification of *E. coli* pathotypes, which consisted only of EPEC. In total, 6 (2.0 %) of the samples harboured this pathotype, 1.0 % from rural and 1.0 % from urban areas, indicating that the remaining 142 *E. coli* isolates were members of the commensal microbiota.

Table 3.7. Prevalence of DEC in children under five with diarrhoea

Sampling area	Total n° of samples	EPEC (n/%)
Rural	156	3 (1.0)
Urban	144	3 (1.0)
Total	300	6 (2.0)

DEC: Diarrhoeagenic *E. coli*
EPEC: Enteropathogenic *E. coli*

3.4.8. Factors associated with Enteropathogenic *E. coli* in children under five years old with diarrhoea

The Lasso binomial logistic regression model was applied considering EPEC (n=6) as an outcome variable. The model indicated that the relevant factors associated with EPEC in children under five years old were consumption of yoghurt and fruit/vegetables/juices, contact with dogs and chickens. Yoghurt was negatively associated with infection and can be considered a protective factor, whereas fruit/vegetables/juice and contact with dogs and chickens were positively associated with diarrhoea caused by EPEC, representing risk factors (Table 3.8).

Table 3.8. Binomial logistic regression of EPEC present in stool samples from children under five with diarrhoea in Maputo

Variable group	Category	B (SE)	95% CI for Odds Ratio			p-value
			Lower	Odds Ratio	Upper	
	Intercept	-19.57**				
Child gender	Male (n = 166), Female (n = 134)	1.47	0.67	4.33	38.70	0.14
Food	Yoghurt (n = 53)	-2.88*	<0.01	0.06	0.49	0.01
	Ready-to-eat meal (n = 32)	1.45	0.28	4.28	51.90	0.24
	Biscuit/rusks/cookies (n = 84)	0.03	0.10	1.03	9.95	0.98
	Fruit/vegetables/juice (n = 73)	2.62*	1.17	13.70	>100	0.04
	Bottled water (n = 68)	1.66	0.48	5.28	70.70	0.17
Animal contact	Chicken (n = 18)	3.65*	2.55	38.60	>100	0.01
	Dog (n = 7)	4.36*	2.23	78.10	>100	0.01
Sampling area	Rural (n = 156), Urban (n= 144)	-0.57	0.05	0.57	6.68	0.64

B: Estimated coefficient
E: Standard error
CI: Confidence interval
*: Statistically significant at $p < 0.05$
EPEC: Enteropathogenic *E. coli*

3.5. DISCUSSION

This study was conducted in rural and urban areas of Maputo, with more male children being susceptible to diarrhoea. This finding was consistent with other similar studies (Mahmud *et al.*, 2020; Kombat *et al.*, 2024). The reason behind the higher number of diarrhoeal cases in male subjects remains to be elucidated. However, within the Mozambican context, this phenomenon may be explained by cultural beliefs that perceive boys as more vulnerable than girls, leading caregivers to pay closer attention to the health of male children and to seek medical care for them more readily (Jarman *et al.*, 2019).

The majority of children with diarrhoea were aged between seven and 24 months, which may be due to their still-developing immune systems and the fact that most were crawling around and tending to put things like toys and sand in their mouths, exposing them to diarrhoeal bacteria in their environment (Saha *et al.*, 2022).

Vomiting, fever and coughing were the most common symptoms in children with diarrhoea. The observed frequency may be related to the cause of diarrhoea, as this symptom occurs mainly in diarrhoea caused by infectious organisms or toxic substances (Nemeth and Pfleghaar, 2022). Fever is one of the symptoms caused by microorganisms

such as bacteria, viruses and parasites (Akhondi *et al*., 2025). Coughing symptoms may indicate that besides diarrhoea, the children had been affected by other illnesses, such as respiratory infections (Worrall, 2011).

Regarding the cause of diarrhoea, the main causes indicated by the caregivers included food and teething as causes of diarrhoea. Food acts as a vehicle for foodborne pathogens, being contaminated at different stages, from production until it reaches the consumer, where contamination can also occur during its preparation and storage (Fufa *et al.*, 2020). Teething has been reported as a cause of diarrhoea by the mothers of children aged six to 30 months in Iran (Miri-Aliabad *et al*., 2021). However, no association was found between teething and diarrhoea (Miri-Aliabad *et al*., 2021). Some caregivers attributed the cause of diarrhoea in children to factors related to cultural beliefs such as lunar influences. Cultural beliefs have been identified as a significant barrier to preventing diarrhoea (Odo *et al*., 2023).

Chickens (17.3%), cats (14.3%), ducks (9.7%) and dogs (7.0%) were commonly found in children's homes and were the animals with which they most had contact, which may be a risk factor for diarrhoea. Previous studies have shown an association between diarrhoeal diseases and children who had been exposed to domestic animals (Conan *et al.*, 2017; Getachew *et al.*, 2024).

The foods consumed by the children a week before the onset of diarrhoea consisted mainly of animal proteins and vegetables and their consumption differed between the two sampling sites. The consumption of beef, pork, chicken and eggs was more common in the rural area possibly due to farming in these areas. Meat is known to be a potential source of pathogenic bacteria such as *Salmonella* spp., *Staphylococcus aureus*, *Campylobacter* spp. and *Listeria monocytogenes* (Zhang *et al.*, 2016; Abd-El-Malek, 2017).

The types of food consumed by children under two years of age included fermented cereals, biscuits, rusks, cookies, breast milk, fruit, vegetables, juice and bottled water (22.0%). Breastfeeding was common in the rural area, which may be because mothers go to the farm with their children, allowing them to continue to breastfeed. In addition, breastfeeding is considered an important factor in preventing and protecting against diarrhoea in children under two years (Santos *et al.*, 2015). On the other hand, urban

children were more likely to be given fermented cereals, which have been shown to be a protective factor against diarrhoeal diseases (Rohmah *et al*., 2015). Biscuits, rusks and cookies are foods with low water activity, which do not favour microbial growth (Cervenk *et al*., 2006), while fruit, vegetables and juices have been associated with diarrhoeagenic pathogens (Tenea *et al.*, 2023; Zahra *et al.*, 2025). Caregivers gave their children bottled water for drinking because they believed that it was safe. However, previous studies have identified pathogenic bacteria in different brands of bottled water in Kenya (Adam Mohamed *et al.*, 2020).

The water source consisted mainly of tap water in both sampling areas. However, the use of public/community wells in the rural area was observed, which may be related to higher municipal water coverage in urban areas (INE, 2013b), and the use of bottled water was common in the urban area. The lack of a toilet system in the rural area and the use of uncovered pit latrines were also observed, and these have been associated with diarrhoeal diseases (Belay *et al.*, 2022; Mabvouna *et al.*, 2023).

The study found that EPEC was the only *E. coli* pathotype detected in faecal samples from children with diarrhoea, and this is one of the most commonly detected pathotypes in diarrhoea samples (Kralicek *et al.*, 2022; Fellenz *et al.*, 2024). In addition, the regression analysis indicated that feeding children with yoghurt may reduce their risk of EPEC infection ($p < 0.05$), as evidenced in a previous study (Olayanju *et al.*, 2023). Yogurt may promote gut health by modulating the gastrointestinal microbiota and enhancing the immune response (Patro-Gołab *et al*., 2015). It contains *Lactobacillus bulgaricus*, which helps establish a beneficial *Lactobacillus* population in the intestine, thereby inhibiting the growth of proteolytic bacteria and reducing the risk of autointoxication (Nakamura *et al.*, 2022). Furthermore, studies have shown reduced diarrhoea frequency in children on moderately dehydrated hospitalised infants aged six to 24 months with acute non-bloody and non-mucoid diarrhoea (Pashapour and Iou, 2006). Moreover, significantly lower rates of clinical failure, faster resolution of symptoms and reduced antibiotic-associated diarrhoea were observed in children fed with yoghurt (Boudraa *et al.*, 1990; Akram *et al.*, 2023). In this study, fruits, vegetables, juice, and contact with dogs and chickens were seen as risk factors for EPEC diarrhoea ($p < 0.05$). Diarrhoeagenic bacteria such as *Salmonella* spp., *Shigella* spp. and *E. coli* have been linked to fruit, vegetables and juice contamination, making them unsafe for consumption, especially for children under five, so disinfection is essential (Tenea *et al.*,

2023; Zahra *et al.*, 2025). Contact with dogs can be a source of zoonotic infections. A study in Iran demonstrated the presence of DEC, including EPEC, in healthy dogs, and contact with animals carrying this pathogenic bacteria is risky, mainly for children under five with weak immune systems (Askari *et al.*, 2020). Food animals, including chickens, are reservoirs of *E. coli* serotypes (Heredia and García, 2018a). EPEC strains have been recovered from chicken faeces in Burkina Faso, constituting a risk factor for those who come into contact with the animal, especially with its excreta (Kagambèga *et al.*, 2012).

3.6. CONCLUSIONS

In this study, faecal samples from six of 300 children under five with diarrhoea in rural and urban areas of Maputo harboured EPEC pathotypes. Yoghurt consumption during the week prior to disease onset was identified as a protective factor against diarrhoea caused by EPEC, whereas the consumption of fruits, vegetables, and juice, as well as contact with dogs and chickens, were identified as risk factors.

The results of the study highlight the need to educate caregivers about good hygiene practices in food preparation and storage, as well as how to handle animals and their excrement.

CHAPTER 4: DIARRHOEAGENIC *E. COLI* AND *SALMONELLA* SPP. CONTAMINATION OF FOOD AND WATER CONSUMED BY CHILDREN WITH DIARRHOEA IN MAPUTO, MOZAMBIQUE

Published: International Journal of Environmental Research and Public Health: 2024, 21(9), 1122; https://doi.org/10.3390/ijerph21091122.

4.1. ABSTRACT

In Mozambique, about 500,000 cases of diarrhoea were caused by foodborne pathogens in 2018. A review of the epidemiology of diarrhoea in children under five has shown a high disease burden. This study aimed to identify DEC and *Salmonella* spp. contamination of food and water in urban and rural areas of Maputo consumed by children under five with diarrhoea. One hundred and eighty-six children with diarrhoea were selected from Primeiro de Maio and Marracuene Health Care Centres from the Kamaxakeni and Marracuene districts, respectively. Food (n = 167) and water (n = 100) samples were collected in children's households for the identification of diarrhoeagenic bacteria. Interviews were conducted using a semi-structured questionnaire to collect data about demographics and foods consumed a week before the children's diarrhoea episodes. Explanatory variables were screened using chi-square tests and subsequently included in a binomial regression analysis in R to identify factors associated with the presence of DEC/*Salmonella* in food samples. The prevalence of both DEC and *Salmonella* spp. was 9.8% in food and 5.4% in water samples. DEC was most prevalent in cereals (urban = 2.8%; rural = 2.4%) and water samples (urban = 1.4%; rural = 3.3%). *Salmonella* spp. was mainly detected in cereals (urban = 0.7%; rural = 0.8%). Diarrhoeagenic pathogens were associated with the type of food frequently consumed by children under five years of age with diarrhoea (infant formula, fruit puree, ready-to-eat meals, and bottled water), while no association was found with demographic data. We found that the infant foods consumed by children with diarrhoea were associated with DEC and *Salmonella* spp., and the prevalence of these contaminants was higher in the rural (8.9%) than in the urban area (6.3%), showing the need for caregiver education on food handling practices.

Keywords: food; water; diarrhoeagenic *E. coli*; *Salmonella* spp.; Mozambique

4.2. INTRODUCTION

Foodborne illnesses constitute a public health concern, leading to 420,000 deaths yearly worldwide, where low and middle-income countries have the highest burden, accounting for 53% of all foodborne illnesses and 70% of related deaths (WHO, 2020). Children under five years of age are at higher risk, and almost 40% of foodborne diseases and 30% of associated deaths occur in this age group (Havelaar *et al.*, 2015a).

The high foodborne illness rates in low and middle-income countries can be attributed to several factors. These include the lack of clean water for washing utensils and food, unsafe water supply systems, poor sanitation, inadequate hygiene, and the use of human sewage or animal waste for irrigation (Bick *et al.*, 2020). Contaminants in food may be due to poor food preparation and storage, hygiene practices, children feeding themselves, and exposure to infected animals (Grace, 2015; Wells *et al.*, 2023). In Mozambique, about 500,000 cases of diarrhoea were caused by foodborne pathogens in 2018 (ONU, 2019). A review of the epidemiology of diarrhoea in children under five years of age showed a high disease burden and an associated case fatality rate of 10% (Chissaque *et al.*, 2018). From 2015 to 2019, 9041 cases of diarrhoea were reported in children under five years old in Maputo, of which 25.2% were in rural areas and 74.8% were in urban areas *(*Machava *et al.*, 2022a). The majority of the current literature available from Mozambique focuses on the identification of diarrhoeagenic pathogens in humans. These included Rotavirus, Adenovirus, *Shigella* spp., *E. coli*, *V. cholera*, and *Cryptosporidium* spp. (Chissaque *et al.*, 2018; Dall, 2023). Limited existing data showed an association between caregiver hygiene practices during food preparation and storage and infant food contamination (Bick *et al.*, 2020). This study aimed to ascertain the prevalence of diarrhoeagenic pathogens, such as *E. coli* and *Salmonella* spp., in food and water consumed by children under five years of age with diarrhoea.

4.3. MATERIALS AND METHODS

4.3.1. Target population

Children under five were selected from the Primeiro de Maio Health Care Centre in the Kamaxakeni district (urban area) and the Marracuene Health Care Centre in the Marracuene district (rural area) in Maputo, Mozambique. These health care centres offer primary health care services.

A total of 186 children were selected based on convenience sampling method, where they were included because they appeared in the health care centre and if they fulfilled the following inclusion criteria: (i) children were under five years old with diarrhoea, (ii) their caregiver consented to complete the questionnaire, and (iii) consented to provide food and water samples from their respective households for laboratory analyses.

4.3.2. Interviews with caregivers

We conducted face-to-face interviews based on a semi-structured questionnaire to collect demographic data and information on the possible source/vehicle of infection as perceived by the caregivers. We also gathered data on the food consumed in the household a week before the children experienced diarrhoea episodes and the water supply. The questionnaire was designed to obtain information about the food consumed by children under five, especially those under two years old.

4.3.3. Food and water samples collected from households

A total of 267 samples, comprising 167 food and 100 tap water specimens, were collected from selected households. About 100 g of food that was available during sample collection and 1L of water were collected with sterile containers, kept chilled and transported to the laboratory at Eduardo Mondlane University, Mozambique.

The breakdown of food samples included cereal (urban = 57; rural = 26), combined foods (urban = 30; rural = 24), pasteurised cow's milk and milk products (urban = 8; rural = 3), cooked vegetables (urban = 5; rural = 2), and other miscellaneous foods (urban = 2) (Table 4.1). It is important to note that "combined food" typically entailed a blend of pap or rice with curry, or a meat or vegetable stew. This type of meal is prevalent in Mozambican communities as a complementary food for children transitioning to solid foods. Regarding the water samples, 42 were sourced from urban locations, while the remaining 58 were collected from rural settings.

Table 1Table 4.1. Food and water samples collected from households of children with diarrhoea in urban and rural areas of Maputo

Collected samples	Sampling site	
	Urban area n (%)	Rural area n (%)
Cereal	57 (21.3)	36 (13.5)
Combined food	30 (11.2)	24 (9.0)
Milk and milk product	8 (3.0)	3 (1.1)
Vegetable (cooked)	5 (1.9)	2 (0.7)
Other food (fish and fruit puree)	2 (0.7)	0
Water	42 (15.7)	58 (21.7)

4.3.4. Bacteriological analysis of food and water samples

4.3.4.1. Detection of *Salmonella* spp., *Shigella* spp., and *E. coli*

Table 4.2 summarises *Salmonella* spp., *Shigella* spp., and *E. coli* methodology used. *Salmonella* spp. and *Shigella* spp. detection was conducted using the standard operating procedure created based on the U.S. Food and Drug Administration-Bacteriological Analytical Manual (FDA BAM) and ISO 6579-1:2017, while *E. coli* identification was conducted following the FDA-BAM and the technical reference guide produced by the Ministério da Saúde de Moçambique (Mozambique's Ministry of Health). (MISAU, 1997; FDA, 2001; Andrews *et al.*, 2020). From these analyses, 104 food and 37 water samples were found to have *Salmonella* spp./*Shigella* spp. isolates, while 49 food and 38 water samples harboured *E. coli* strains. A single colony of each isolate was further analysed using biochemical tests, confirming the identification of the strains obtained in the culture method.

Table 4.2. Diarrhoeagenic bacteria's identification in food and water samples collected from households of children with diarrhoea

Diarrhoeagenic pathogen	Steps for strain identification	Medium	References
Salmonella spp./*Shigella* spp.	Pre-enrichment and Enrichment	BPW and mTSB broth	(Andrews *et al.*, 2020)
	Strain identification	XLD agar, HE agar, MAC agar,TSI agar, and MIO	
E. coli	Enrichment	BPW	(MISAU, 1997; FDA, 2001)
	Strain identification	MAC agar, TSI agar, and MIO	

BPW (Buffered Peptone Water, Liofilchem Diagnostici-2360611014, Roseto degli Abruzzi, Italy); mTSB (Modified Tryptic Soy Broth, Liofilchem Diagnostici-2360610352, Roseto degli Abruzzi, Italy); RVS (Rappaport-Vassiliadis with Soya, Liofilchem Diagnostici-610175, Roseto degli Abruzzi, Italy); MKTTn (Muller-Kauffman Tetrathionate- novobiocin Broth, Liofilchem Diagnostici-2360610239, Roseto degli Abruzzi, Italy); XLD (Xylose Lysine Deoxycholate agar, Neogen-NCM0021A, Lansing, MI, USA); HE (Hektoen enteric agar, HIMEDIA M377-500G, Kennett Square, PA, USA); MAC (MacConkey agar, Liofilchem Diagnostici-610028, Italy); TSI (Triple Sugar Iron agar, Liofilchem Diagnostici-360620055, Italy); MIO (Motility Indole Ornithine Medium, HIMEDIA–M378-500G, Square, PA, USA).

4.3.4.2. MALDI-TOF MS confirmation of bacterial isolates

For confirmation, 228 isolates were submitted to matrix-assisted laser desorp tion/ionisation time-of-flight mass spectrometry (MALDI-TOF MS) (Rychert, 2019). Single colonies from overnight-grown bacteria isolated from food and water samples were analysed on MALDI Biotyper® sirius RUO (Bruker Daltonics GmbH & Co. KG, 2021; Bremen, Germany) using Bruker Daltonics MALDI-TOF Biotyper FlexControl 3.4 software. A representative spectrum was obtained by collecting 40 shots on six different positions of the sample (240 laser shots in total) in about 15 s per sample, and the results were displayed in real time. The results were obtained by comparing the Bruker Daltonics MALDI-TOF Biotyper Compass RTC 4.1.100 database strains.

4.3.5. DNA Extraction and Diarrhoeagenic *E. coli* PCR

DNA was extracted from confirmed 16-hour *E. coli* cultures using the Quick-DNA Fungal/Bacterial Miniprep kit (Zymo Research, Tustin, CA, USA).

PCR amplification of DEC genes was carried out using SSI Diagnostics protocol (Hillerød, Denmark), which detects EHEC, EIEC, EPEC, and ETEC pathotypes.

The PCR was carried out in a T100^{TM} Thermal cycler (Bio-Rad, Hercules, CA, USA) in a total volume of 20 µL, containing 10 µL PCR ReadyMix, 6 µL Primer Mix, and 4 µL of extracted DNA. The amplification cycle included denaturation for 2 min at 95 ◦C, which was performed in one cycle, followed by 35 cycles of denaturation at 94 ◦C for 50 s, annealing for 40 s at 62 ◦C, extension at 72 ◦C for 50 s, and one cycle of the final annealing at 72 ◦C for 3 min.

Eighteen microlitres (18 µL) of each completed PCR reaction were visualised in 2% agarose gel for 40 min at 70 V, stained with SYBR green, and detected by Gel Doc^{TM} EZ system (Bio-Rad, Hercules, CA, USA).

4.3.6. Data analysis

Preliminary screening of the demographic characteristics and the caregiver's perception of the cause of diarrhoea in the children was conducted using univariate analyses on Epi info (version 7.2.5.0)(CDC, 2021). The univariate analysis screened the variables for inclusion in the binomial logistic regression model using X^2 with the threshold set at $p \leq 0.2$ with an odd ratio (OR > 1), based on Fisher's test and considering a one-tailed hypothesis. The binomial regression model was performed in the R environment for Windows (version 4.3.1) using the package MASS (Version 7.3-60) (Ripley *et al.*, 2024). For the binomial regression analysis, the presence or absence of DEC/*Salmonella* in food samples was considered an outcome variable, while the explanatory variables included child age, caregiver age, child gender, caregiver gender, marital status, source of thinking, sampling site, baby bottle, bottled water, fruit puree, infant formula, milk breastfeeding, ready-to-eat meals, goat, cassava leaves, water source, water treatment, monthly income, and toilet system. The X^2 test was conducted to detect differences between the urban and rural areas.

4.4. RESULTS

4.4.1. Sociodemographic characteristics of the caregivers and children under five years old

The study included 186 children, most of whom were from the urban area (54.8%) and were mostly male (58.8%). In the rural area, most children were female (51.2%). The majority of the children (53.2%) were between seven and 24 months old, and their caregivers were mainly female (94.1%), with over half of them being between 18 and 25 years old. The majority of the caregivers were single but living with a partner (76.8%) (Table 4.3). The X^2 test indicated a significant difference ($p < 0.05$) in the age distribution of children in the urban and rural areas.

In terms of monthly income, most caregivers in the urban area (33.3%) and the rural area (28.6%) were unaware of the primary breadwinner's income. Among those who did provide information, the majority reported it to be between $71.00 and $140.00 (8.8% urban; 9.5% rural) (Table 4.3).

Concerning water supply, 99.0% of the participants used potable municipal water in the urban area, while in the rural area, they used municipal water and community wells. For water treatment, boiling was the most commonly used method (10.8% urban; 8.3% rural). Flush or pour toilets with septic tanks, including squat toilets, were the most common toilet systems: 56.9% in urban areas and 32.1% in rural areas. The X^2 test indicated a significant difference ($p < 0.05$) in the water source and toilet system used in both sampling areas (Table 4.3).

Table 4.3. Sociodemographic characteristics of the caregivers and children under five years with diarrhoea (n=186)

Variable	Category	Urban area (n=102)	Rural area (n=84)	X^2
		N (%)	N (%)	
Child gender	Female	42(41.2)	43(51.2)	0.22
	Male	60(58.8)	41(48.8)	
Caregiver gender	Female	97(95.1)	78(92.9)	0.74
	Male	5(4.9)	6(7.1)	
Child age (months)	≤6 months	11(10.8)	22(26.2)	0.02
	7 to 24 months	60(58.8)	39(46.4)	
	> 24 months	31(30.4)	22(26.2)	
	Not disclosed	0	1(1.2)	
Caregiver (age years)	18 to 25 years	50(49.0)	42(50)	0.94
	26 to 35 years	33(32.4)	31(36.9)	
	>35 years	10(9.8)	9(10.7)	
	Not disclosed	9(8.8)	2(2.4)	
Marital status	Single but lives with someone	75(73.5)	67(79.8)	0.26
	Single and living alone	10(9.8)	3(3.5)	
	Married	17(16.7)	13(15.5)	
	Widow	0	1(1.2)	
Relation to the child	Mother	91(89.2)	76(90.5)	0.42
	Father	5(4.9)	6(7.1)	
	Grandmother/Parents	6(5.9)	2(2.4)	
Monthly income (USA Dollar)	0 to 70.00 $	6(5.9)	7(8.3)	0.85
	71.00-140.00$	9(8.8)	8(9.5)	
	> 140.00$	2(2.0)	2(2.4)	
	Don't know the income	34(33.3)	24(28.6)	
	Not disclosed	51(50.0)	43(51.2)	
Water source	Home pipe water	101(99.0)	68(81.0)	<0.05
	Public/community wells	0	12(14.3)	
	Not disclosed	1(0.5)	4(4.8)	
Water treatment	Boiling	11(10.8)	7(8.3)	0.37
	Other treatment methods	1(1.0)	1(1.2)	
	Not disclosed	90(88.2)	76(90.5)	
Toilet system	Pit latrine with covering slab	28(27.5)	12(14.3)	<0.05
	Pit latrine without covering slab	3(2.9)	13(15.5)	
	Flush or pour toilet with septic tank, including squat toilet	58(56.9)	27(32.1)	
	Flush or pour toilet connected to sewer pipe	1(1.0)	19(22.6)	
	Other toilet system	0	3(3.6)	
	Not disclosed	12(11.8)	10(11.9)	

4.4.2. Caregiver's perception of the cause of diarrhoea in children under five years old

Table 4.4 shows the cause of diarrhoea as perceived by the caregivers, where the majority of respondents in both urban, 52.0% (53/102), and rural, 25% (23/84), areas could not identify a probable cause for episodes of diarrhoea in the children included in the study. However, some caregivers attributed the cause of diarrhoea to factors such as vegetables, cereals, cookies/cakes/popcorn, weaning/infant formula, teething, and fruits/juice. Interestingly, only a small percentage of respondents in the urban area (2.9%) and the rural area (2.4%) considered water a probable cause of diarrhoea. The X^2 indicated that there was a significant difference ($p < 0.05$) in caregivers' perception of the cause of diarrhoea between the urban and rural areas.

Table 4.4. Perception of the cause of diarrhoea by the caregivers of the children (n=186)

Variables	Categories	Urban area (102)	Rural area (84)	X^2
		n (%)	n (%)	
Cause of diarrhoea in children under five years old	Food	12(11.8)	16(19.0)	<0.05
	Water	3(2.9)	2(2.4)	
	Fruit/Juice	3(2.9)	9(10.7)	
	Dentition	6(5.9)	14(16.7)	
	Weaning/Infant formula	5(4.9)	15(17.9)	
	Other causes	2(2.0)	3(3.5)	
	Don't know the reason	53(52.0)	21(25.0)	
	Not disclosed	18(17.6)	4(4.8)	

4.4.3. One-week food consumption recall for children under five with diarrhoea

Table 4.5 details the food consumed by children with diarrhoea the week before their visit to the health care centre. Based on the one-week food recall, the children had consumed animal protein, vegetables, and fruits (only in rural). Chickens were the most commonly consumed source of animal protein, accounting for 36.3% of cases in the urban area and 90.5% in the rural area. Among dairy products, milk was reported as the most commonly consumed in the urban area, accounting for 12.7% of cases, while in the rural area, yoghurt was the most commonly consumed, accounting for 29.8% of cases. Cheese was only reported to be consumed by the children included in the study in the urban area,

2.9% of cases. Most children consumed green cabbage a week before episodes of diarrhoea in the urban area, 37.3%, and in the rural area, 19.0%. Fruits were only consumed in the rural area, with a frequency of 7.1%.

Regarding food consumption by children under two years old, biscuits/rusks/cookies were the most commonly consumed item in the urban area, accounting for 33.3% of consumption. In the rural area, fermented cereals were the most consumed, representing 33.3% of consumption. Children under two years old were also fed liquid foods using feeding bottles, with 21.6% in urban areas and 29.8% in rural areas (Table 4.5).

Table 4.5. Food consumed by children under five a week before attending the health care centres

Variables	Categories	Urban area (n=102)		Rural area (n=84)		p-value for X^2
		Consumed n (%)	Not consumed n (%)	Consumed n (%)	Not consumed n (%)	
Source of animal protein	Beef	23(22.5)	78(76.5)	64(76.2)	19(22.6)	<0.05
	Pork	11(10.8)	90(88.2)	45(53.6)	38(45.2)	<0.05
	Lamb	1(1.0)	100(98.0)	3(3.6)	80(95.2)	0.48
	Goat	3(2.9)	98(96.1)	27(32.1)	56(66.7)	<0.05
	Chicken	37(36.3)	64(62.7)	76(90.5)	7(8.3)	<0.05
	Egg	23(22.5)	78(76.5)	64(76.2)	19(22.6)	<0.05
	Not disclosed	1(1.0)		1(1.2)		N/A
	Fish	30(29.4)	72(70.6)	69(82.1)	15(17.9)	<0.05
Other sources of animal protein	Milk (pasteurised)	13(12.7)	88(86.3)	11(13.1)	72(85.7)	1
	Yoghurt	6(5.9)	95(93.1)	25(29.8)	58(6.9.0)	<0.05
	Cheese	3(2.9)	98(96.1)	0	83(98.8)	0.32
	Not disclosed	1(1.0)		1(1.2)		N/A
Vegetables	Lettuce	6(5.9)	40(39.2)	9(10.7)	13(15.5)	<0.05
	Cassava leaves	12(11.8)	34(33.3)	10(11.9)	12(14.3)	0.19
	Pumpkin leaves	8(7.8)	38(37.2)	7(8.3)	15(17.9)	0.3
	Cowpea leaves	13(12.7)	33(32.4)	12(14.3)	10(11.9)	0.1
	Green cabbage	38(37.3)	8(7.8)	16(19.0)	6(7.1)	0.53
	Sweet potato leaves	6(5.9)	40(39.2)	3(3.6)	19(22.6)	1
	Cacana	0	46(45.1)	7(8.3)	15(17.9)	<0.05
	Other vegetables	8(7.8)	38(37.3)	5(6.0)	17(20.2)	0.85
	Not disclosed	56(54.9)		62(73.8)		N/A
Fruits	Fruits	0	0	6(7.1)	0	N/A
	Not disclosed	102(100.0)		78(92.9)		
Food consumed by children	Milk breastfeeding	25(24.5)	77(75.4)	19(22.6)	65(77.4)	0.9
	Infant formula	15(14.7)	87(85.3)	6(7.1)	78(92.9)	0.16
	Fermented cereals	22(21.6)	80(78.4)	28(33.3)	56(66.7)	0.1
	Ready-to-eat meals	7(6.9)	95(93.1)	8(9.5)	76(90.5)	0.69
	Fruit puree	12(11.8)	90(88.2)	11(13.1)	73(87.0)	0.96
	Biscuits/rusks/cookies	34(33.3)	68(66.7)	20(23.8)	64(76.2)	0.21
	Fruit/vegetable/juices	28(27.5)	69(67.6)	20(23.8)	63(75.0)	0.58
	Not disclosed	5(4.9)		1(1.2)		N/A
	Baby bottle	22(21.6)	53(52.0)	25(29.8)	50(59.5)	0.72
	Not disclosed	27(26.5)		9(10.7)		N/A
	Bottled water	22(21.6)	79(77.5)	23(27.4)	61(72.6)	0.48
	Not disclosed	1(1.0)		0		N/A
	Boiled water	1(1.0)	91(89.2)	5(6.0)	71(84.5)	0.14
	Not disclosed	10(9.8)		8(9.5)		N/A
	Water not boiled	31(30.4)	61(59.8)	26(31.0)	50(59.5)	1
	Not disclosed	10(9.8)		8(9.5)		N/A

The p-value of the X^2 test indicated a significant difference ($p < 0.05$) in the consumption of certain foods before the occurrence of diarrhoeal episodes in children in both sampling areas. These foods include animal proteins (such as beef, pork, goat, chicken, eggs, fish, and yoghurt) and vegetables like cacana (*Momordica balsamina*), which is used as a food in southern Mozambique and as medicine in other parts of the country (Table 4.5).

4.4.4. Prevalence of foodborne pathogens in food and water samples

Table 4.6 shows the prevalence of foodborne pathogens in samples collected from the children's households. In both rural and urban areas, contamination with DEC and *Salmonella* spp. was found in the food and water collected from the children's homes. The overall prevalence of bacterial contamination was 15.2%, with a higher prevalence in the rural area (8.9%), and water and cereal samples contributed 12.1%. The most prevalent pathogen was DEC, primarily found in cereal and water samples, with a prevalence of 9.9% in both areas. *Salmonella* spp. was only identified in the water samples (0.7%) collected in the urban area and cereal samples (1.5%) from both areas. DEC was the only pathogen detected in combined food (2.4%) and yoghurt (0.7%) from rural and urban areas, respectively.

Table 4.6. Pathogens distribution among food (n = 167) and water (n = 100) samples collected in households of children under five old

Samples	Total Number of Isolates No (%)	Urban Area (n = 144)		Rural Area (n = 123)	
		DEC n (%)	*Salmonella* spp. n (%)	DEC n (%)	*Salmonella* spp. n (%)
Cereal	9 (6.7)	4 (2.8)	1 (0.7)	3 (2.4)	1 (0.8)
Combined food	3 (2.4)	ND	ND	3 (2.4)	ND
Milk and Milk Products	1 (0.7)	1 (0.7)	ND	ND	ND
Vegetable (cooked)	ND	ND	ND	ND	ND
Other food (fish and fruit puree)	ND	ND	ND	ND	ND
Water	7 (5.4)	2 (1.4)	1 (0.7)	4 (3.3)	ND
Total	20 (15.2)	7 (4.9)	2 (1.4)	10 (8.1)	1 (0.8)

ND: Not detected; DEC: Diarrhoeagenic *E. coli*

4.4.5. Prevalence of Diarrhoeagenic *E. coli* in food and water samples

Table 4.7 represents the prevalence of DEC pathotypes among food and water samples collected in households and the sampling sites. The overall frequency of the DEC pathotypes is 13.0% and ETEC was the most prevalent, contributing 11.5% in both rural and urban areas. The other pathotypes consisted of EIEC detected in cereal food from rural areas and EPEC identified in water samples from urban areas.

Table 4.7. Diarrhoeagenic *E. coli* pathotype prevalence in food and water samples collected in rural and urban households from children under five with diarrhoea

Samples	Number of isolates n (%)	Urban area (n=144)			Rural area (n=123)		
		ETEC n (%)	EPEC n (%)	EIEC n (%)	ETEC n (%)	EPEC n (%)	EIEC N (%)
Cereal	7(5.2)	4(2.8)	ND	ND	2(1.6)	ND	1(0.8)
Combined food	3(2.4)	ND	ND	ND	3(2.4)	ND	ND
Yoghurt	1(0.7)	1(0.7)	ND	ND	ND	ND	ND
Vegetable (cooked)	ND	ND	ND	ND	ND	ND	ND
Other food (fish and fruit puree)	ND	ND	ND	ND	ND	ND	ND
Water	6(4.7)	1(0.7)	1(0.7)	ND	4(3.3)	ND	ND
Total	17(13.0)	6(4.2)	1(0.7)	0	9(7.3)	0	1(0.8)

EIEC- Enteroinvasive *E. coli*; ETEC- Enterotoxigenic *E. coli;* EPEC- Enteropathogenic *E. coli*; ND- Not detected

4.4.6. Factors associated with contamination of food and drinking water by Diarrhoeagenic *E. coli*

Table 4.8 presents the results of a binomial logistic regression analysis that aimed to assess the relationship between the prevalence of DEC isolated from the food and water samples and the demographics and food consumed by children participating in the study. However, this analysis was not conducted for *Salmonella* spp. because only three samples were found to be contaminated with this pathogen, which falls below the threshold for conducting binomial logistic regression.

Table 4.8. Binomial logistic regression of Diarrhoeagenic *E. coli* presence on food and water in rural and urban areas of Maputo

Variable group	Category	B (SE)	95% CI for Odds Ratio		
			Lower	Odds Ratio	Upper
Diarrhoeagenic *E. coli*					
Caregiver	18 to 25 years	-3.15(0.45)			
	26 to 35 years	-0.51(0.67)	0.14	0.60	2.07
	>35 years	-0.42(0.90)	0.83	0.66	3.28
Food consumed by children <2years	Fruit puree	1.50(0.61)*	1.31	4.50	14.70
	Infant formula	1.33(0.68)*	0.91	3.81	13.90
	Milk breast	-1.44(0.08)	0.01	0.24	1.32
	Ready-to-eat meals	1.17(0.68)	0.77	3.24	11.80
***Salmonella* spp.**					
Caregiver age	Intercept	-4.60(1.16)***			
	26 to 35 years	-17.9(>100)	NA	<0.01	>100
Source of animal protein	Pork	-0.77(1.43)	0.02	0.46	7.01
Food consumed by children <2years	Baby bottle	-0.03(1.40)	0.13	0.97	14.5
	Bottled water	0.02(1.32)	0.04	1.02	12.6
	Ready-to-eat meals	3.20(1.31)*	2.03	24.6	>100
Kamaxakeni (Urban area)					
Caregiver age	18 to 25 years	-3.32(0.69)***			
	26 to 35 years	-1.04(1.36)	0.01	0.3	3.71
	>35 years	0.09(1.31)	0.04	1.09	11.5
Food consumed by children <2years	Bottled water	-18.67(>100)	NA	<0.01	>100
	Infant formula	2.24(1.08)*	0.04	9.38	83.4
	Ready-to-eat meals	2.03(1.04)*	0.84	7.64	60.7
Marracuene (Rural area)					
Caregiver gender	Male	-3.84(1.41)**			
	Female	-1.16(1.33)	0.02	0.31	7.58
Food consumed by children <2years	Bottled water	3.04(1.22)*	2.79	20.80	>100
	Baby bottle	-0.45(1.21)	0.03	0.64	5.78
	Fruit puree	2.18(0.87)*	1.65	0.80	56.20

Significance level: 0 '***' 0.001 '**' 0.01 '*' 0.0; B: Estimated coefficient; E: Standard error; CI: Confidence interval; N/A: Not applicable

The results of the analysis demonstrated a significant association between DEC contamination and the consumption of fruit puree [(B = 1.50); 95% CI (1.31–14.70), (p < 0.05)] and infant formula [(B = 1.34); 95% CI (0.91–13.90), (p < 0.05)] in children under two years of age. Additionally, the binomial logistic regression analysis conducted on the urban area data revealed associations between the consumption of certain foods by children under two years, such as infant formula [(B = 2.24); 95% CI (0.04–83.40), (p < 0.05)] and ready-to-eat meals [(B = 2.03); 95% (0.84–60.7), (p < 0.05)] with DEC

contamination. Conversely, in the rural area, the consumption of fruit puree [(B = 2.17); 95% CI (1.65–56.2), ($p < 0.05$)] and bottled water [(B = 3.04); 95% CI (2.79–>100), ($p < 0.05$)] by children under five years of age was associated with DEC contamination. Notably, no association was found between demographic characteristics and DEC contamination in this study.

4.5. DISCUSSION

This study assessed the prevalence of diarrhoeagenic bacteria in food and water consumed by children with diarrhoea in urban and rural areas of Maputo and factors associated with children's food contamination.

Most of the selected children were under two years old in urban and rural areas, showing that children of this age group were the most affected by diarrhoea (Saha *et al.*, 2022). The source of water in urban and rural households was different. In the urban area, only tap water was used, while in rural households, tap water and community wells were used as sources of water. This difference may be related to the coverage of municipal water distribution, which is higher in the urban area (INE, 2013a). The type of toilet facility used in the urban and rural areas was also different; more than half of households in the urban area use flush or pour toilets with septic tanks, including squat toilets. This may be related to lower socioeconomic status, as this type of facility is more costly than others.

Although caregivers from urban and rural areas indicated several factors as responsible for the cause of diarrhoea in children under five, most of them did not know the cause. Similar to a study carried out in Ethiopia (Desta *et al.*, 2017), in this study, the percentage of respondents without knowledge of the cause of diarrhoea was higher in the urban area than in the rural area, contradicting the expected association with education level in this area. Chicken consumption in Maputo was lower than that reported in other areas of Africa, which may be a protective factor, as poultry may carry pathogenic agents, including *E. coli*, *L. monocytogenes*, *Campylobacter*, *Salmonella*, and *S. aureus* that can cause diseases, especially in children with weak immune systems (Gonçalves-Tenório *et al.*, 2018; Faife *et al.*, 2020; Rich *et al.*, 2022).

Yoghurt consumption was higher in the rural area compared to a study conducted in Nigeria (Bakare *et al.*, 2023). The consumption of yoghurt has benefits for gastrointestinal conditions such as diarrhoeal disease (Hadjimbei *et al.*, 2022). The

consumption of lettuce and banana was different in urban and rural areas; this fresh produce may constitute a vehicle for diarrhoeagenic pathogens when good hygiene is not appropriately followed (Zhao, 2005).

DEC was the most frequent pathogen isolated in water collected from households in urban and rural areas (5.4%), which was lower than the prevalence reported in another study from Mozambique (Salamandane *et al.*, 2021). The presence of DEC in drinking water may be due to faecal contamination. *Salmonella* spp. was only detected in water samples collected in the urban area of Maputo, while in South Africa, this pathogen was identified in rural areas (Khabo-Mmekoa *et al*., 2022). The presence of *Salmonella* spp. in water samples may be related to the dissemination of these bacteria through the faecal–oral route. Cereal-based foods were the most consumed by children in urban and rural areas, and DEC and *Salmonella* spp. were contaminants of this food group, similar to findings reported in South Africa. The contamination by DEC might have occurred during food preparation and the preservation of leftovers. *Salmonella* spp. contamination may be related to sanitation and poor operational practices during food processing (Potgieter *et al.*, 2005; Podolak *et al.*, 2010). The prevalence of diarrhoeagenic contaminants was higher in the rural area than in the urban area, which may be due to the ability of people in urban areas to purchase and access better quality and safe foods (Dong, 2020). Additionally, the urban area (Kamaxakeni) has benefited from several educational sessions related to food handling practices, as this has been the focus of many studies that include health promotion activities to prevent illness. Contamination of combined food collected in households was observed, similar to a study in Bangladesh that reported contamination of complementary food with *E. coli* (Parvez *et al.*, 2017). The presence of DEC in tested food samples indicates faecal contamination and poor hygiene conditions during food preparation and conservation, which can cause gastroenteritis in children (Ekici and Dumen, 2019).

The present study evaluated the association between diverse types of food and water with DEC contamination. Infant formula and fruit puree were associated with DEC contamination. Infant formula contamination by *E. coli* may occur during preparation, storage, and feeding, making this infant food unacceptable for consumption, so following hygiene practices is essential (López-Mendoza *et al.*, 2023).

Unpasteurised fruit products can contribute to foodborne illness as they can act as vehicles for foodborne pathogens. For example, berry juices and purees have been shown to support pathogens like *E. coli* O157:H7 (Zhao, 2005). In the urban area, factors associated with the presence of contaminants were infant formula and ready-to-eat meals, which is concerning as improper practices during their preparation can introduce diarrhoeagenic pathogens to children.

In the rural area, food contamination was associated with fruit puree and bottled water. The use of bottled water was associated with DEC contamination, which may be related to water counterfeiting. Other studies have also found DEC in bottled water, showing that water that people believe to be safe for consumption can be a risk factor for foodborne illness (Momtaz and Yadollahi, 2013; Hamad *et al.*, 2022).

The present study did not address aspects related to how the caretaker prepares the food and how this is conserved, especially leftover food, which are crucial points where contamination can occur; that is why a study should be carried out to ascertain when and where the contamination takes place in Maputo households.

4.6. CONCLUSIONS

Diarrhoeagenic *E. coli* and *Salmonella* spp. are present in food and water consumed by children under five years with diarrhoea in Kamaxakeni, an urban area, and Marracuene, a rural area of Maputo. The prevalence of these diarrhoeagenic pathogens is higher in rural areas.

Significant disparities are noted between the two study areas in caregivers' perceptions of the causes of diarrhoea, the types of water sources and toilet systems utilized, and the dietary habits of children in the week preceding diarrheal episodes.

These findings underscore the necessity for educating caregivers on proper food handling practices and enhancing water sources, given the evidence of diarrhoeagenic pathogens.

CHAPTER 5: MOLECULAR CHARACTERISATION AND RELATEDNESS OF DIARRHOEAGENIC *E. COLI* DETECTED IN FAECAL SAMPLES FROM CHILDREN UNDER FIVE, IN FOOD AND WATER SAMPLES IN MAPUTO, MOZAMBIQUE

5.1. ABSTRACT

Diarrhoeagenic *E. coli* is associated with diarrhoea and responsible for around 200,000 deaths worldwide yearly, with children under five being most affected. In Mozambique, recent studies have shown that DEC strains are among the pathogens involved in the cause of enteric infection, and they have been identified in food and drinking water samples. This study aimed to characterise the DEC pathotypes isolated from the faeces of children under five years with diarrhoea and to attribute the diarrhoea to food and water consumed by these children within 24 hours.

The study involved 10 DEC, nine EPEC and one EIEC, isolated from faecal samples of these children, as well as from foods and water they consumed, in rural and urban areas of Maputo, Mozambique. Whole Genome sequences of the DEC strains were characterised, and their relatedness evaluated using the Enterobase online platform. The R environment was used for genome analyses and data visualisation. AMR gene analysis was performed using complete linkage hierarchical clustering to construct heat maps. In addition, Pearson correlation and network analysis were applied to assess associations between sample source, sampling area, and sequence type with AMR genes in DEC strains.

There was an abundance of DEC strains belonging to O109:H21/ST40 (40.0%), O88:H5/ST206 (20.0%) and O80:H2/ST301 (20.0%) serogroup and sequence types. The most frequent antimicrobial-resistant (AMR) genes were *glpT*_E448K (16.1%), *pmrB*_Y358N (12.5%), *aadA1* (7.1%), *catA1* (5.4%), *sul1* (5.4%) and *dfrA1* (5.4%). The DEC strains from food, drinking water and human samples were phylogenetically related. The study concluded that the DEC strains isolated from food, drinking water and children faecal samples belonged to serotypes that have been implicated in causing diarrhoea. These strains carry AMR genes that confer resistance to antimicrobials that are important for clinical practices. The relatedness of the DEC strains suggests that food and drinking water are the potential source of DEC causing diarrhoea in children. The results of this study underscore the need for education on good food preparation, conservation, and water treatment practices. In addition, the detection of AMR genes calls for One Health approach against the burden of AMR.

Keywords: diarrhoea, children, food, water, diarrhoeagenic *E. coli*

5.2. INTRODUCTION

Diarrhoeagenic *E. coli* are among the bacterial pathogens associated with diarrhoea, mainly in children under five years, which is widely spread mostly through contaminated food and drinking water. Foodborne illnesses are estimated to be above 300 million, of which approximately 200,000 deaths are caused by DEC worldwide yearly (Havelaar *et al.*, 2015b), and children under five are the most affected (WHO, 2022). Unsafe drinking water, animal contact, and food such as vegetables, raw meat, raw kibbeh, beef, milk and milk products, and milk samples from infant feeding bottles have been indicated as sources of DEC causing diarrhoea (Gomes *et al.*, 2016).

The burden of foodborne illness by DEC in children under five years old in sub-Saharan Africa is unknown. However, there are several studies where *E. coli* pathotypes were identified (Okeke, 2009; Sambe-Ba *et al.*, 2013; Saka *et al.*, 2019; Garrine *et al.*, 2020; Das *et al.*, 2022). Contaminated water, person-to-person transmission, and raw food have been identified as predominant routes for the spread of DEC (Okeke, 2009; Bywater *et al.*, 2024).

In Mozambique, studies have shown that DEC strains are among the pathogens involved in the cause of enteric infection. In urban areas of Maputo, EAEC, DAEC, EIEC, EPEC, ETEC, STEC pathotypes were detected in stool samples of children with diarrhoea (10-13). Additionally, in a rural area of Maputo, Manhiça district, ETEC, EAEC and EPEC were among the pathogens associated with death due to moderate-to-severe diarrhoea in children 0-59 months of age (Acácio *et al.*, 2019). A recent study showed the presence of EPEC and EIEC in food and water samples consumed by children under five in Maputo districts (Faife *et al.*, 2024). However, there is a lack of studies that attribute DEC pathotypes detected in stools to food and water samples using genetic characteristics such as sequence types, serotypes, and AMR genes, allowing the identification of the transmission route of these bacteria.

Previous studies in South Africa, Kenya, and Burkina Faso have reported the presence of AMR in DEC and *Salmonella* strains isolated from foods consumed by children, including milk, ready-to-eat meals, and vegetables (Ngaywa *et al.*, 2019; Soubeiga *et al.*, 2022; Mohapi *et al.*, 2025; Okumu *et al.*, 2025). Resistance to antimicrobials such as ampicillin, trimethoprim/sulfamethoxazole, tetracycline, ceftriaxone, and

amoxicillin/clavulanic acid is commonly observed (Ngyawa et al., 2019; Mohape, et al., 2025). The associated AMR genes include *bla_TEM*, *bla_OXA-1*, *bla_CTX-M*, *bla_SHV*, *tet(A)*, *tet(B)*, *sul1*, *sul2*, and *aadA* (Ngaywa *et al.*, 2019; Soubeiga *et al.*, 2022; Okumu *et al.*, 2025).

This study aimed to characterise DEC pathotypes isolated from the faeces of under-five children with diarrhoea and from food and water samples consumed by these children. This study also aimed to determine if the children's diarrhoea was caused by the food and water they had consumed. The study's results provide knowledge on food and drinking water as probable sources of *E. coli* pathotypes causing diarrhoea in children. Additionally, the study's results will provide information on the presence of AMR genes in the DEC strains. Consequently, this will permit the definition of strategies for preventing diarrhoeagenic transmission among children under five years old in Maputo.

In your introduction, be sure to include relevant literature on the prevalence of antimicrobial-resistant pathogens and genes in foods commonly consumed by Children in Mozambique. If country-specific data is limited or unavailable, expand your scope to include evidence from broader African contexts to provide a solid foundation for your study.

5.3. METHODOLOGY

5.3.1. Study site and sampling

This study analysed ten DEC isolates comprising nine EPEC and one EIEC isolated from the faeces from children with diarrhoea as well as from the foods and water they consumed in Maputo, Mozambique.

The children under five with diarrhoea were selected from Marracuene Health Care Centres and Primeiro de Maio, from Marracuene and Kamaxakeni districts, respectively, which offer primary health care services.

Faecal samples were collected in the health care centre or the children's households after explaining to the caregivers the aim of the study and obtaining their consent. Three hundred stool swabs were collected and sent to the National Institute of Health in Maputo for microbiology analyses. About 100 g of food (n=167) and 1L of tap water (n=100) samples were collected from selected households, where these children resided, in sterile

containers, kept chilled, and transported to the Hygiene and Food Technology Laboratory at Eduardo Mondlane University.

The food samples collected consisted of cereals, combined food (blend of pap or rice with curry, or a meat or vegetable stew) and milk products.

5.3.2. Detection of Diarrhoeagenic *E. coli* in food, water and faecal samples

The detailed methods for *E. coli* detection in children's faecal samples are described in Section 3.2.3.1, and for food and water samples in section 4.3.4. A total of 148 *E. coli* strains were confirmed in children's faecal samples, 72 from rural area and 76 from urban area. Additionally, 40 *E. coli* strains were isolated from food and water samples, equally distributed between urban (n = 20) and rural (n = 20) areas. Of the 148 faecal strains, only 67 were sequenced, as the remaining isolates failed to grow in culture prior to DNA extraction.

5.3.3. Genome sequencing and analyses

Th genome sequencing was preceeded by the DNA extraction of the *E. coli* strains, which was performed as described in section 3.2.3.2. The WGS was conducted at the Agricultural Research Council, Pretoria, South Africa. Genome sequencing was undertaken using MGI DNBSEQ-G400 (MGI, China) sequencing instrument generating 150 bp PE sequencing reads after a multiplexed paired-end libraries preparation using MGIEasy Universal DNA Library Prep Kit (MGI, China). Trimming and de novo assembling of the paired-end reads were done using the Center of Genome Epidemiology (CGE) web-based platform (Qiagen, Netherlands). The fastq files were analysed for pathotype identification on the Enterobase platform, based on the expression of *fimH*, *stx1*, *stx2*, *ipaH*, pInv, ST, LT and *eae* genes, resulting in the detection of nine EPEC and one EIEC strains. Six EPEC strains were detected in children's faecal samples, two in drinking water and one in cereal samples. The EIEC strain was identified in cereal-based food.

Multilocus sequence typing (MLST), core genome multilocus sequence typing (cgMLST), and serotyping of the DEC sequences were performed on the EnteroBase platform (Zhou *et al.*, 2020). The phylogenetic clustering was done on the EnteroBase platform using the neighbour-joining algorithm, comparing sequences of DEC isolated in food, water and children's faecal samples with reference genome K12MG165 obtained

from the National Centre for Biotechnology Information (NCBI). Visualisation of the phylogenetic tree was performed on FigTree v. 1.4.4 (Edinburgh, UK) (Rambaut, 2018).

The Antimicrobial Resistance (AMR) genes were identified using the CGE pipeline ResFinder (version 4.6.0) at default settings (Camacho *et al.*, 2009; Bortolaia *et al.*, 2020). The identified AMR genes were analysed in the R environment for Windows (version 4.4.2) for complete linkage hierarchical clustering using the ComplexHeatmap package (version 2.22.0) (Gu *et al*., 2016). Pearson correlation analysis assessed the direction and strength of association between the sample source, AMR genes, the sampling area with the AMR genes on the DEC strains.

Network analysis was performed to evaluate the correlation between sequence type and AMR genes, where an absolute correlation value of ≥ 0.5 was set as the threshold, which was chosen to reflect moderate to strong correlation strength.

5.4. RESULTS

5.4.1. Sequence types and serotypes of Diarrhoeagenic *E. coli* strains isolated from food, water and children's faecal samples

Table 5.1 shows the sequence types (ST) and serotypes of the DEC isolates from food, water and children's faecal samples from rural and urban areas of Maputo. The results show that the strains belonged to A and B1 lineages and exhibited heterogeneity of ST complex. The strains O109:H21/ST40 (40.0%), O88:H5/ST206 (20.0%) and O80:H2/ST301 (20.0%) were the most abundant serogroups and sequence types. O109:H21/ST40 was identified in two drinking water samples from both sampling areas, one cereal sample and one children's faecal sample from the urban area. O88:H5/ST206 and O80:H2/ST301 were identified in two faecal samples in the rural and urban areas. The only EIEC strain isolated in the study was categorised as ST11715 and O121:H30 serotype.

Table 5.1. Sequence and serotyping of DEC isolated from food, water and children's faecal samples from the urban and rural areas of Maputo

Serogroup and sequence type (O:H/ST)	n (%) of O:H/ST	ST Complex (n)	Lineage (n)	DEC (n)	Sampling source	Sampling area
O109:H21/ST40	4(40.0%)	40(4)	B1(4)	EPEC(4)	Cereal, drinking water and children's faeces	Rural and urban
O88:H5/ST206	2(20.0%)	206(2)	AxB1(2)	EPEC(2)	Children's faeces	Rural and urban
O80:H2/ST301	2(20.0%)	165(2)	-	EPEC(2)	Children's faeces	Rural and urban
O121:H30/ST11715	1(10.0%)		-	EIEC(1)	Cereal	Rural
O127:H45/ST2356	1(10.0%)		-	EPEC(1)	Children's faeces	Urban

DEC - Diarrhoeagenic *E. coli;* EIEC- Enteroinvasive *E. coli;* EPEC- Enteropathogenic *E. coli*

5.4.2. Frequency of AMR genes present in the Diarrhoeagenic *E. coli* strains isolated in faeces of children under five and in food and drinking water consumed by them

Figure 5.1 shows the frequency of AMR genes present in the DEC strains isolated in faecal samples of children under five and in food and drinking water consumed by them. The most dominant AMR genes were *glpT*_E448K (16.1%), *pmrB*_Y358N (12.5%), *aadA1* (7.1%), *catA1* (5.4%), *sul1* (5.4%), and *dfrA1* (5.4%), which confer resistance to fosfomycin, polymyxins, aminoglycosides, phenicols, sulfonamides, and trimethoprim, respectively.

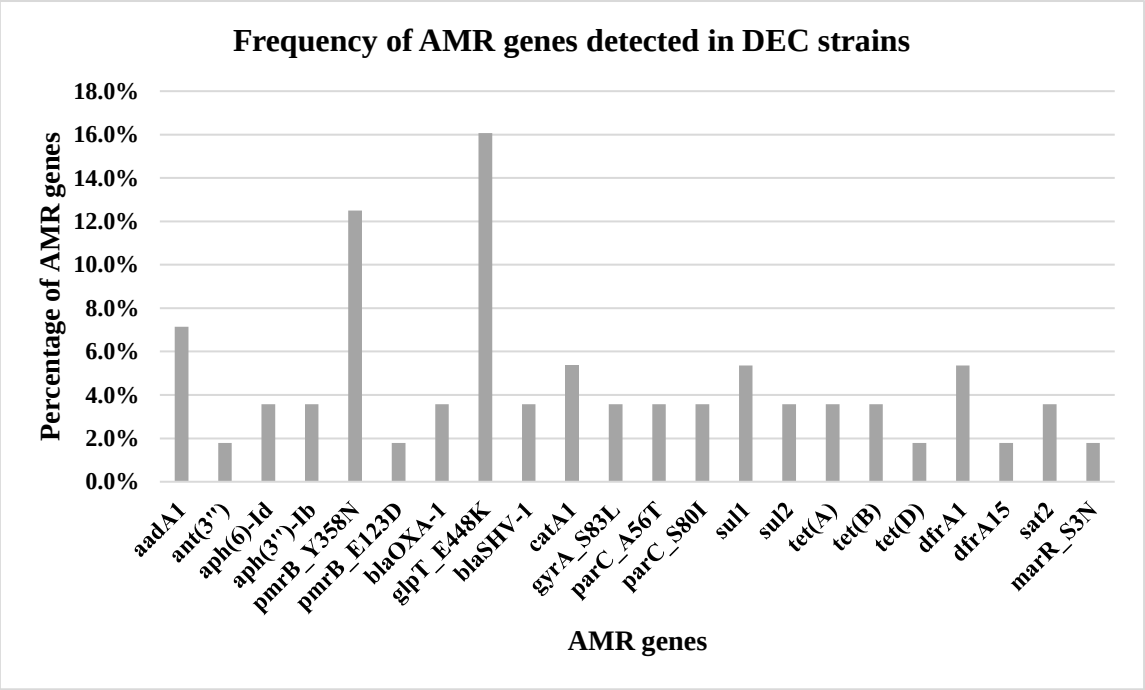

Figure 5.1. Frequency of antimicrobial resistance (AMR) genes detected in Diarrhoeagenic *E. coli* (DEC) strains isolated from faecal samples of children under five with diarrhoea, and from food and drinking water consumed by these children, in rural and urban areas of Maputo.

5.4.3. Diarrhoeagenic *E. coli* AMR genes distribution through the sample source and sampling area

Figure 5.2 represents the heat map of DEC antimicrobial resistance genes distribution through the sample source and sampling area. Overall, the results indicate the presence of diverse AMR gene profiles conferring multidrug resistance to various antimicrobials. Some of the AMR genes detected confer resistance to important antimicrobial classes used in medical practice, including aminoglycosides, polymyxins, fosfomycin, β-lactams, fluoroquinolones, phenicols, trimethoprim, tetracyclines, and sulphonamides. The results show the presence of *bla*$_{\text{OXA-1}}$ and *bla*$_{\text{SHV-1}}$ genes, characterised as encoding ESBLs. Genes encoding polymyxins and fosfomycin resistance, *pmrB*_Y358N and *glpT*_E448K, respectively, were present in the three sample groups in both rural and urban areas. Some genes were restricted to children's faecal samples, which include *bla*$_{\text{OXA-1}}$, *aph(6)-Id*, *aph(3'')-Ib*, *tet(B)*, *sul2*, *sat2*, *marR*_S3N, *dfrA15*, *ant(3'')* and *pmrB*_E123D. Notebly, topoisomerase genes mutation, *gyrA*_S83L and *parC*_S80I, were detected and they were restricted to children's faecal samples All the cereals and drinking water samples harboured *pmrB*_Y358N and *glpT*_E448K AMR genes, except one cereal sample (ET13-

cereal_Rural), characterised as EIEC, which additionally presented *aadA1*, *catA1*, *bla*$_{SHV-1}$, *sul1* and *tet(D).*

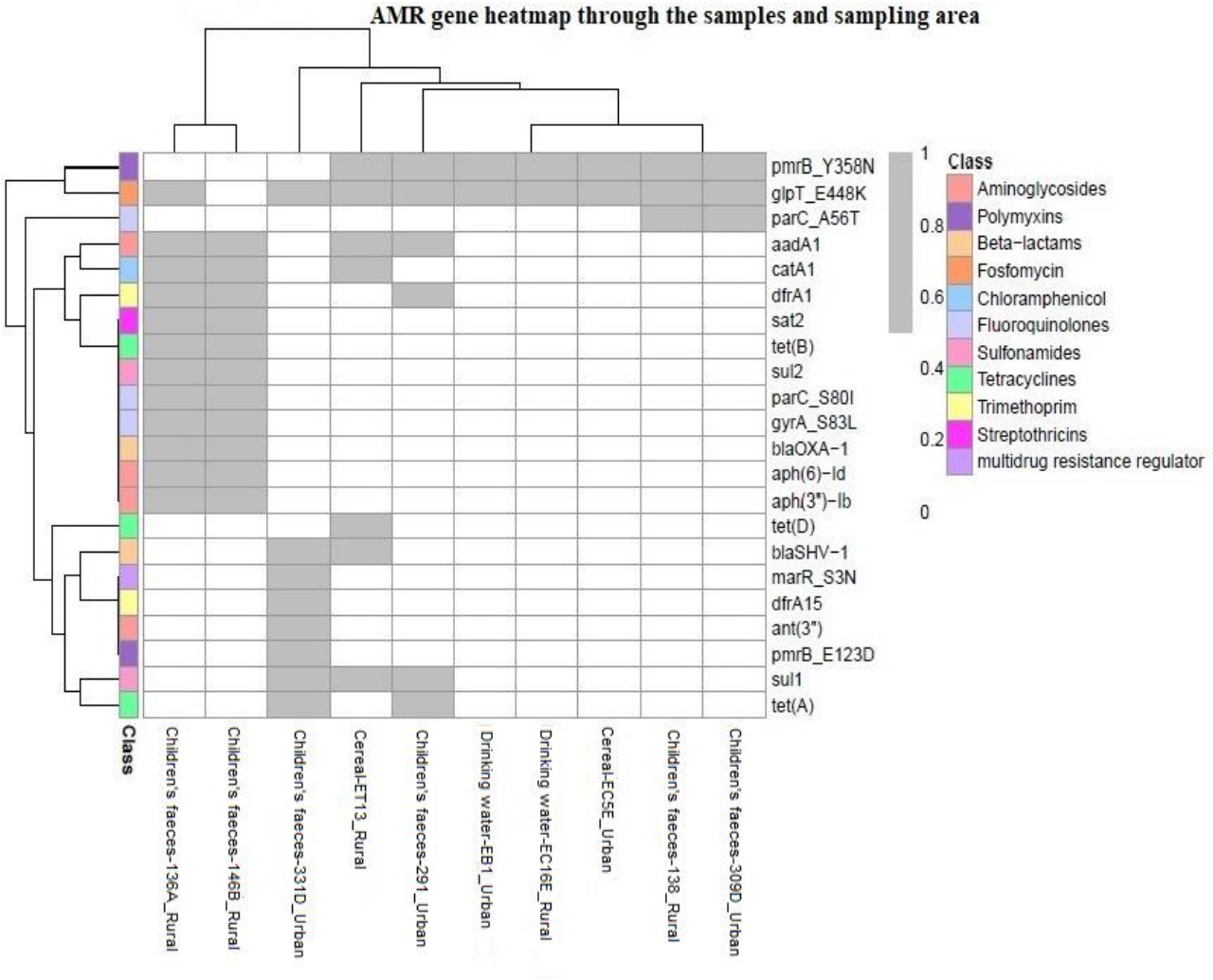

Figure 5.2. Heat map of antimicrobial resistance (AMR) genes in Diarrhoeagenic *E. coli* strains isolated from faecal samples of children under five with diarrhoea, and from food and drinking water consumed by these children, in rural and urban areas of Maputo.

5.4.4. Correlation between sample source, sampling area and AMR genes

Figure 5.3 represents the correlation heat map between sample source and AMR genes (A), and sampling area and AMR genes (B) detected in DEC strains isolated in cereal, drinking water and in faecal samples of children under five with diarrhoea, collected in rural and urban areas of Maputo. The results indicate that AMR genes such as *aph(6)-Id*, *aph(3'')-Ib*, *bla*$_{OXA-1}$, , *sul2*, *tet(A)*, *tet(B)*, *dfrA1* and *dfrA15*, and the topoisomerase genes

mutation, *gyrA*_S83L, *parC*_A56T and *parC*_S80I, were moderately correlated with children's faecal samples (r = 0.23 - 0.53), while only the *aadA1* gene presented a moderate correlation with drinking water. The AMR genes *catA1* and *sul1* presented a weak positive correlation with cereal and children's faecal samples dfrA15 (r = 0.10 - 0.11). Conversely, AMR genes like *pmr*B_Y358N and *tet(D)* presented a moderate positive correlation with cereal samples, while children's faecal samples demonstrated a moderate negative correlation with *dfrA15* (r = - 0.23 - (- 0.53).

From a sampling area perspective, an inverse correlation was observed between rural and urban areas with respect to the presence of AMR genes detected in DEC isolates. AMR genes that presented a moderate positive correlation in the rural area, namely, *aph(6)-Id*, *aph(3'')-Ib*, *bla*$_{OXA-1}$, *catA1*, *sul2*, *tet(B)*, *sat2* and *dfrA15*, and topoisomerase genes mutation, *gyrA*_S83L and *parC*_S80I, had moderate negative correlation in the urban area (r = 0.39 - 0.65).

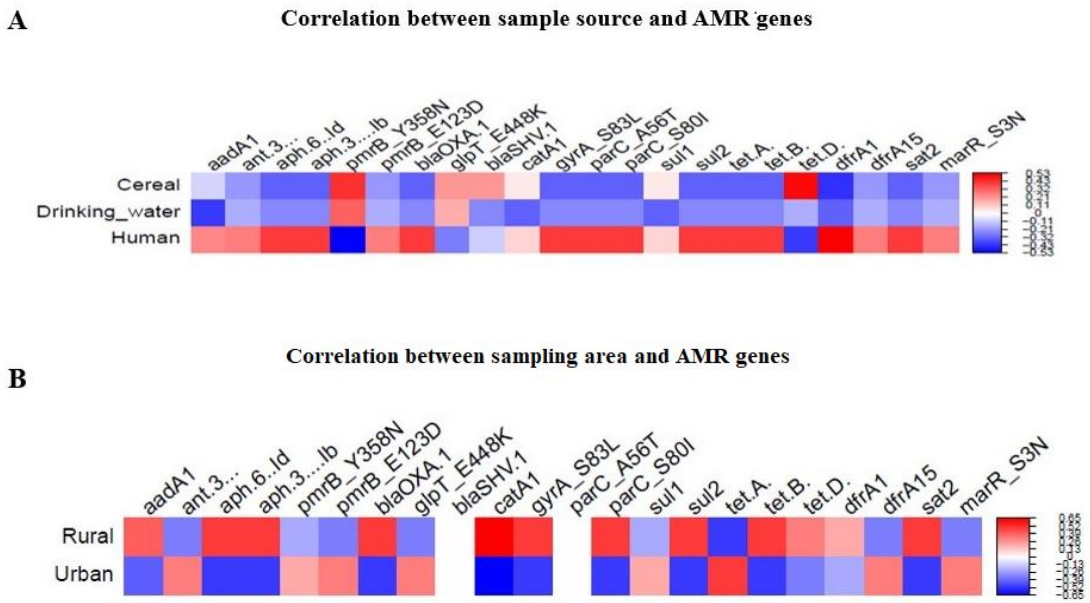

Figure 5.3. Correlation heat map between sample source (A), sampling are (B) and antimicrobial resistance (AMR) genes detected in Diarrhoeagenic *E. coli* strains isolated from faecal samples of children under five with diarrhoea, and from food and drinking water consumed by these children, in rural and urban areas of Maputo.

5.4.5. Network analysis between sequence type and AMR genes

Figure 5.4 represents network analysis between sequence type and AMR genes detected on DEC isolated from cereal, drinking water and faecal samples from children under five with diarrhoea, in rural and urban areas from Maputo. The results show that ST301

presented multiple connections with AMR genes, including *gyrA*_S83L, *aph(6)-Id*, *sat2*, *tet(B)*, *sul2*, *aph(3'')-Ib* and *bla*$_{OXA-1}$, and topoisomerase gene mutation, *parC*_S80I, which had strong correlation. Other sequence types, such as ST2356, ST5979 and ST206, were connected with AMR genes in less extension. AMR gene such as *tet(D)* (ST5979) and and the topoisomerase gene mutation like *parC*_A56T (ST2016), were linked to specific sequence types.

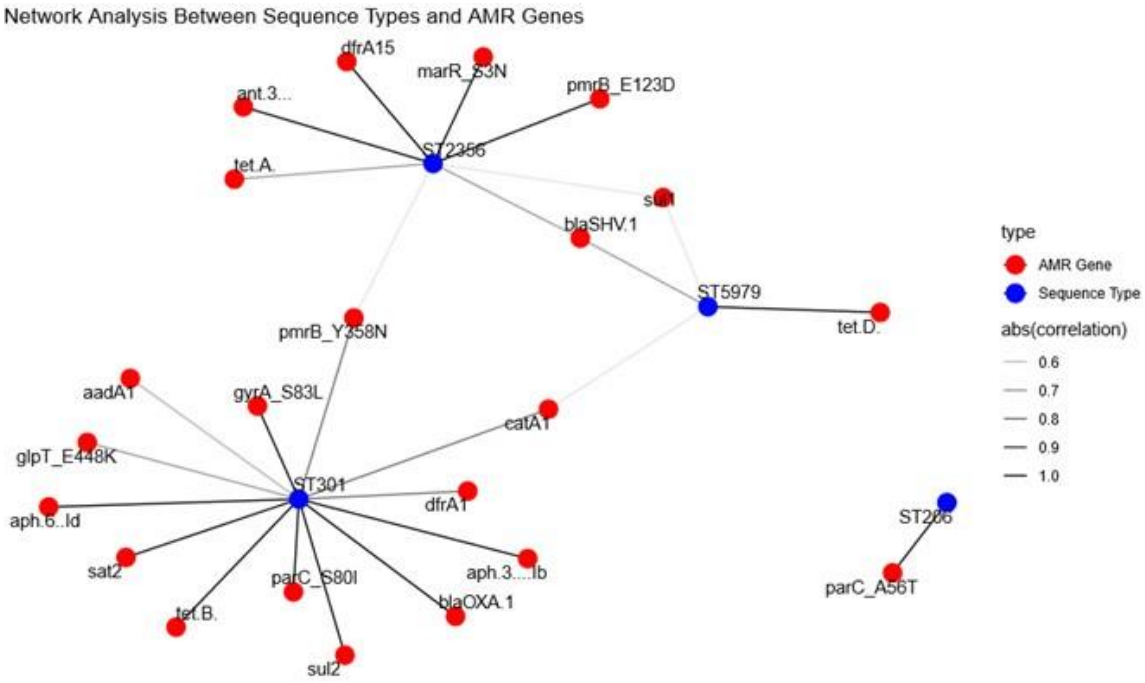

Figure 5.4. Network analysis between sequence type (ST) antimicrobial resistance (AMR) genes detected in Diarrhoeagenic *E. coli* strains isolated from faecal samples of children under five with diarrhoea, and from food and drinking water consumed by these children, in rural and urban areas of Maputo.

5.4.6. Phylogenetic analysis of Diarrhoeagenic *E. coli* isolates from children's faecal samples, food, and water

Figure 5.5 is the phylogenetic tree representing the genetic relatedness and hierarchical clustering of DEC strains isolated in food, drinking water and faecal samples of children under five with diarrhoea, in rural and urban areas of Maputo. The phylogenetic tree showed that the DEC strains were classified into three distinct clusters. Of the six strains isolated from children's faecal samples, four were in Cluster I, while the strains detected in drinking water samples were placed in Cluster II. The two strains from food samples were distributed across two clusters. One strain from a children's faecal sample collected

in the urban area was part of Cluster III. Among the five strains identified in the rural area, four were in Cluster I, while four out of five isolates from the urban area were in Cluster II. The EIEC strain (ET13-cereal_Rural) was part of Cluster I.

The study results attribute the DEC in children's faecal samples to food and drinking water based on the similarities of DEC isolates between food, drinking water and faecal samples of children under five with diarrhoea.

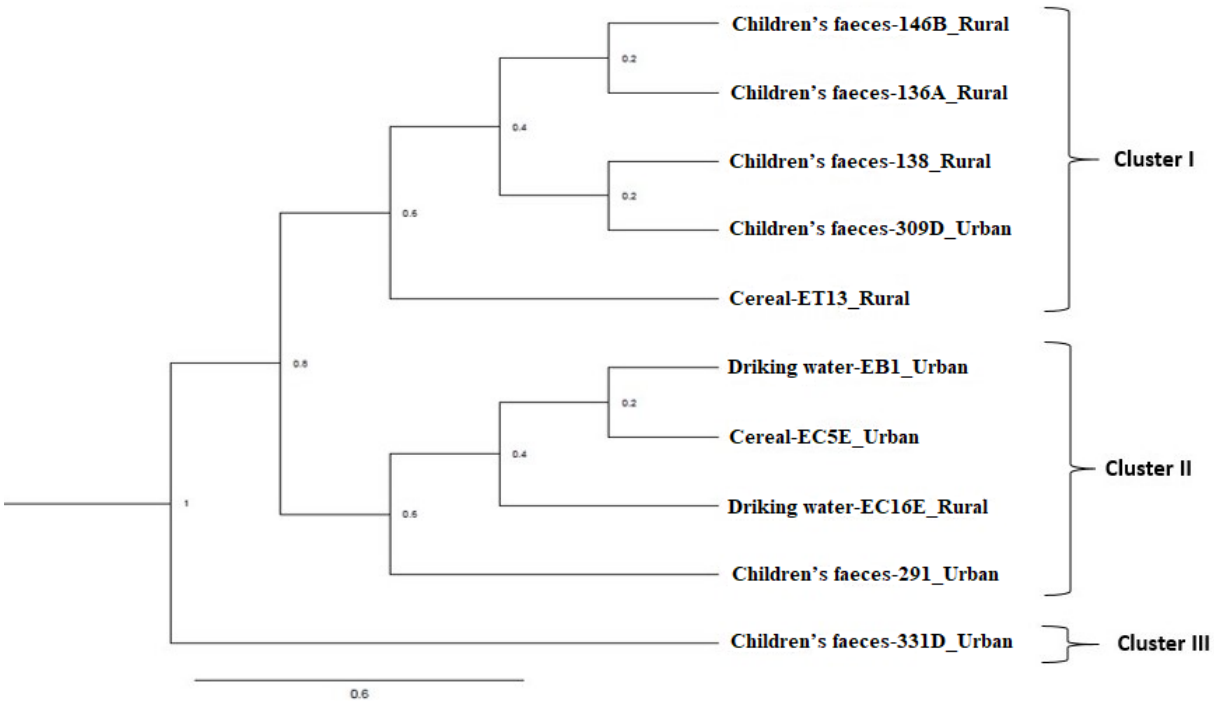

Figure 5.5. Phylogenetic tree representing the genetic relatedness and cluster analysis of Diarrhoeagenic *E. coli* strains, enteropathogenic *E. coli* (n = 9) and enteroinvasive *E. coli* (n = 1; Cereal-ET13_Rural) from faecal samples of children under five with diarrhoea, and from food and drinking water consumed by these children, in rural and urban areas of Maputo.

5.5. DISCUSSION

This study characterised ten DEC strains isolated from food, water and human faecal samples and analysed their relatedness. The identified strains belonged to A and B1 lineages. Similarly, these lineages were present in faecal and food samples, in Colombia, and are known as intestinal pathogens (Rúgeles *et al.*, 2010; Bakhtiari *et al.*, 2020). The EPEC O109:H21/ST40 strain was detected in both drinking water and children's faecal samples, across rural and urban areas, suggesting possible transmission from

contaminated water to children under five years old and indicating its potential for widespread environmental dissemination. Further, ST40 has been identified in previous studies and was shown to be a human diarrhoeagenic pathogen (Massella *et al.*, 2020; Saidenberg *et al.*, 2020). EPEC O80:H2/ST301 seems to be limited to the rural area. This strain has also been detected in previous studies in humans and calves infections (Ikeda *et al.*, 2023), pointing to possible zoonotic transmission. EPEC O88:H5/ST206 was identified in children's faecal samples from rural and urban areas, indicating its capacity to survive in different environments. ST206 was detected in faecal samples from healthy mink and broiler, indicating that animals can be reservoirs of this strain (Qiu *et al.*, 2019; Chisembe *et al.*, 2024). EIEC was characterised as ST11715 and O121:H30. The same sequence and serotypes were previously identified in South Africa in human samples (Hazen *et al.*, 2016).

The DEC from this study harboured antimicrobial resistance genes encoding resistance to multiple antimicrobial classes used in clinical practice, namely, aminoglycosides, fosfomycin, polymyxins, beta-lactam, fluoroquinolone, trimethoprim, tetracycline and sulphonamides. AMR genes such as *pmrB*_Y358N and *glpT*_E448K were present in cereal, drinking water and children's faecal samples from rural and urban areas, indicating a widespread distribution of these genes. Other genes were limited to children's faecal samples, including $bla_{\text{Oxa-1}}$, an ESBL gene, which constitutes a public health concern as its presence restricts the use of beta-lactam antimicrobials for infection treatment. Mutations in topoisomerase genes, *gyrA* and *parC*, were detected and play a key role in the development of high-level resistance to fluoroquinolones. These mutations are mainly observed in the quinolone resistance-determining region (QRDR), where they confer resistance to fluoroquinolones (Johnning *et al.*, 2015). A single mutation in *gyrA* can result in high-level resistance to nalidixic acid, while additional mutations in *gyrA* or in another topoisomerase gene such as *parC* contribute to high-level resistance to ciprofloxacin (Johnning *et al.*, 2015).

Differences in AMR profiles between rural and urban areas were observed, which may be related to human activities specific to each region, including urbanisation and self-administration of antimicrobials (Penders *et al.*, 2013; Hayward *et al.*, 2020; Krishna *et al.*, 2023).

In this study, genes conferring resistance to aminoglycosides, beta-lactams, fluoroquinolones, tetracycline, sulphonamide and trimethoprim were positively correlated with children's faecal samples, pointing to resistance against commonly used antimicrobials (Penders *et al.*, 2013). On the other hand, a negative correlation was observed between AMR genes and drinking water samples. This may be related to water treatment through chlorination of municipal water, which reduces the bacterial population and, consequently, limits the potential for horizontal gene transfer and the spread of antimicrobial resistance genes among bacteria.

Sequence type 301 was linked to multiple AMR genes and some AMR genes were correlated with specific sequence types, which can be related to horizontal genetic exchange by mobile genetic elements, facilitating the acquisition and spread of resistance genes (Partridge *et al.*, 2018).

The phylogenetic tree indicates genetic relatedness between the strains isolated from cereals, drinking water and faecal samples of children under five with diarrhoea. These results indicate that cereals and drinking water are potential sources of DEC causing diarrhoea in children. Although the DEC strains isolated from cereals and drinking water were not obtained from the same children whose faecal samples yielded the six DEC strains, their genetic relatedness highlights the potential public health significance of contaminated food and water as environmental reservoirs that may facilitate the spread of DEC among children in the same geographic setting.

5.6. CONCLUSIONS

This study focused on the molecular characterisation of the DEC pathotypes from faecal samples of under-five children with diarrhoea and food and water samples consumed by these children and also determining if these children's diarrhoea is caused by the food and water they consume. The most frequently detected DEC strains in this study were characterised as O109:H21/ST40, O88:H5/ST206, and O80:H2/ST301. The strains harboured AMR genes conferring resistance to important antimicrobial classes used in clinical practice, including aminoglycosides, fosfomycin, polymyxins, phenicol, beta-lactam, fluoroquinolone, trimethoprim, tetracycline and sulphonamides.

The DEC strains from food, water and faecal samples from children with diarrhoea were genetically related. These findings suggest that food and drinking water may be

contributing to the transmission of DEC among children residing in rural and urban areas of Maputo. The results of this study highlight the need for a One Health approach to effectively monitor, prevent, and control the spread of AMR across sectors. The detection of DEC in food and drinking water calls for education on good food preparation, conservation and water treatment practices.

CHAPTER 6: GENERAL DISCUSSION

6.1. Introduction

Children under five in Mozambique are at continuous risk of having diarrhoea due to factors such as lack of improved water, toilet facilities, and low education level of the caregivers, a scenario that characterises most countries in sub-Saharan Africa (He *et al*., 2023). These conditions facilitate the spread of diarrhoeagenic pathogens, mainly when good hygiene practices are not followed and drinking water is not treated, which leads to diarrhoeal diseases, mostly in children under five, who are at high risk. Diarrhoeagenic pathogens such as Rotavirus, Adenovirus, *Salmonella* spp., *Shigella* spp., *E. coli*, *V. cholerae*, and *Cryptosporidium* spp. have been involved in causing diarrhoea in children in Mozambique (Nhampossa *et al.*, 2015; Chissaque *et al.*, 2018; Dall, 2023).

Data from 2015 to 2019, indicate that in rural area of Maputo 1,373 cases of diarrhoea were reported in children under five years old, while three urban facilities in Maputo reported 7,668 cases (Machava *et al*., 2022a). Despite this continuous record of diarrhoea cases, studies have focused more on detecting diarrhoeagenic pathogens in stool samples from children. However, studies on risk factors that may contribute to the occurrence of diarrhoea and on microbial source attribution and transmission routes for foodborne pathogens are limited. The present study was carried out to feel this gap by (1) identifying DEC in children with diarrhoea and the factors associated with diarrhoea; (2) identifying diarrhoeagenic bacteria and factors associated with contamination of food and water consumed by children under five years with diarrhoea and (3) ascertain antimicrobial resistance presence, strain differences, microbial source attribution and transmission routes for foodborne pathogens.

The study was carried out in rural area (Marracuene), and urban area (KaMaxakeni), in Maputo having been characterised in field and laboratory work. Field work involved the collection of faecal samples from children under five with diarrhoea, sampling of food and drinking water consumed by these children, face-to-face household interviews with caregivers, and a one-week food recall.

The laboratory phase consisted of the identification of diarrhoeagenic bacteria in children's faeces and on food and water consumed by them, which consisted of *Salmonella* spp., *Shigella* spp. and DEC, the leading cause of foodborne diseases in the

world and particularly in Mozambique (WHO, 2024b; Nhampossa *et al*., 2015; Chissaque *et al*., 2018; Dall, 2023; Manhique-Coutinho *et al*., 2022).

The DEC identification was preceded by the isolation of *E. coli* from faecal, food and drinking water samples, as described in section 3.3.3.1 for human samples and section 4.3.4.1 for food and drinking water. The presumptive *E. coli* strains from food and drinking water were further confirmed using MALDI-TOF MS (section 4.3.4.2), which was not done for the human *E. coli* isolates. The confirmation of strains prior to molecular analysis is crucial to ensure that only those exhibiting characteristics similar to the target microorganism are analysed.

The identification of DEC in food and drinking water samples was performed using the SSI Diagnostics protocol, as described in section 4.3.5, while for human samples it was based on WGS detailed in section 4.3.5. Chapter 5 focused on the molecular characterisation and similarity evaluation between the DEC from human, food and drinking water based on the WGS, which made it necessary to perform the DEC identification and characterisation using the WGS.

The use of these molecular tests elucidated differences in the performance, as the isolates that were PCR may detect limited virulence genes, while WGS can identify several virulence genes. In addition, the differences may be related to the specificity of the PCR primers, which may be low. So, these molecular approaches may lead to different results in the identification of virulence genes and consequently, differences in the DEC strains identified.

6.2. General discussion

The WGS of the 300 faecal samples analyses showed that only 2.0% (3/300) harboured DEC strains, 1.0% (3/300) in rural and 1.0% (3/300) in urban areas, which consisted of EPEC. These findings support hypothesis 1, as presented in section 2.8.1. Based on the binomial logistic regression analysis, the EPEC diarrhoea was associated with foods such as fruits, vegetables, and juice, as well as animal contact with dogs and chickens. In this study, a small number of DEC (n = 6) was identified, which may have affected the reliability of the logistic regression model, having been identified the consumption of yoghurt as a protective factor and the consumption of fruits, vegetables and juice and contact with dogs and chickens as risky factors. However, more factors that may

constitute risk factors for diarrhoea in children were observed. For example, children from the rural area consumed more beef, pork, chicken and eggs a week before experiencing diarrhoea. These foods can harbour pathogenic bacteria, as the food animals serve as reservoir for foodborne pathogens (Heredia and García, 2018b).

Further, many caregivers from the rural area did not consider water as a source of diarrhoea, and children from both sampling areas were given untreated water for drinking, which is worrying as this is among the most important vehicles of foodborne pathogens (Bintsis, 2017). Caregivers of this area also attributed diarrhoea to lunar influences, as the communities believe the waning crescent moon phase increases vulnerability to illnesses, customs that constitute a barrier for disease prevention (Odo *et al.*, 2023).

Some households used pit latrines without covering slabs, and one household in a rural area did not have a toilet facility; lack of a toilet system and the use of uncovered pit latrines have been associated with diarrhoeal diseases (Belay *et al.*, 2022; Mabvouna *et al.*, 2023).

Since food and water are among the primary sources and transmission vehicles for foodborne pathogens, detecting these pathogens to assess safety is crucial, and this was the focus of the present study. In addition, the finding described above showed the relevance of food (fruits, vegetables and juice) in the transmission of EPEC strains, making it important to see if the food and water consumed by the children are contaminated.

The PCR analysis of *E. coli* from food and water consumed by children with diarrhoea allowed the identification of ETEC in cereals (n = 5), combined food (n = 3), yoghurt (n = 1) and drinking water (n = 6), EIEC only cereal (n = 1) and EPEC (n = 1) in drinking water. However, with WGS analysis, only EIEC and EPEC were identified. The strains identified as EIEC and EPEC by PCR were also confirmed by WGS. Other strains identified as DEC by the PCR were confirmed as negative by the WGS, and two isolates that were negative for PCR were identified as EPEC. The observed differences may indicate low primer specificity in the PCR assay.

The presence of DEC strains in food and drinking water consumed by children with diarrhoea was associated with fruit puree, infant formula, ready-to-eat meals and bottled water consumption. Coincidentally, fruits were identified as a risk factor for EPEC

diarrhoea and for food contamination by the DEC pathotypes, for this last is particularly the puree. This highlights the probability of inadequate fruit sanitisation before consumption, or even inappropriate conservation (Ekici, 2019).

Vegetables were among the risk factors for EPEC diarrhoea in children. However, no DEC strains were identified in the vegetable samples collected in the children's households, although some combined food and ready-to-eat meals, which harboured DEC strains, contained vegetables.

Drinking water was identified as a source of EPEC based on both PCR and WGS analyses, and regression analysis revealed a significant association between water and DEC contamination. ETEC was also detected in water samples by PCR. Interestingly, yoghurt consumption appeared to be a protective factor against EPEC-associated diarrhoea. However, one ETEC strain was detected by PCR in a yoghurt sample collected from a child's household, suggesting possible contamination during the preparation or storage of the yoghurt by caregivers. This finding highlights the need for further studies to explore food handling and hygiene practices at the household level.

Cereals (n = 2) and drinking water (n = 1) also harboured *Salmonella* spp., identified through WGS analysis. However, binomial logistic regression analysis was not conducted because the total number of isolates falls below the threshold for this kind of analysis. The presence of DEC strains and *Salmonella* spp. in food and drinking water consumed by children under five in Maputo confirms hypothesis 2, stated in section 2.8.2, except of *Shigella* spp., which was undetected.

The WGS analysis, besides the identification of the DEC strains in food, drinking water and humans, using bioinformatics tools, allowed the molecular characterisation of these strains. The molecular characterisation enabled the identification of the sequence types, serogroups, and AMR profiles of the DEC strains, as well as their genetic relatedness through phylogenetic analysis.

The obtained results demonstrated the applicability of WGS for both source attribution and AMR profiling, wich is not possible using routine methods (Adzitey *et al.*, 2013). Based on WGS analysis, ten strains were confirmed as DEC, two from cereals, two from drinking water and six from human samples. Most strains belonged to O109:H21/ST40, O88:H5/ST206 and O80:H2/ST301 serogroup and sequence types. The most frequent

AMR genes were *glpT*_E448K, *pmrB*_Y358N *aadA1*, *catA1*, *sul1*, and *dfrA1*, which confer resistance to fosfomycin, polymyxins, aminoglycosides, phenicol, sulphonamides and trimethoprim, antimicrobial classes used in clinical practices. ESBL genes, including $bla_{\text{OXA-1}}$ and $bla_{\text{SHV-1}}$, were also detected, constituting public health concerns by limiting the effectiveness of beta-lactam antimicrobials (Husna *et al.*, 2023). The WGS also enabled the identification of mutations in topoisomerase genes, such as *gyrA*_S83L, *parC*_A56T and *parC*_S80I. The coexistence of these mutations is associated with high-level resistance to the fluoroquinolone class of antimicrobials (Johnning *et al.*, 2015).

The identification of AMR genes and topoisomerase gene mutations WGS highlights the limitations of routine AMR testing. In LMICs the AMR testing often relies solely on phenotypic methods, which may fail to detect important resistance mechanisms. This limitation contributes to the spread of AMR, reduces treatment effectiveness, and can lead to increased morbidity and mortality.

In addition, phylogenetic analysis of the DEC isolated in food, drinking water, and faecal samples of children showed relatedness between the strains. The DEC strains were classified into three Clusters. Cluster I contained four DEC stains from children's faeces and one from cereal samples, highlighting their similarities. In contrast, Cluster II was heterogeneous, containing strains from food, water and children's faecal samples, and Cluster III comprised a single DEC strain isolated from children's faeces. The similarities observed in these strains underscore that food and drinking water are potential sources of DEC causing diarrhoea in children under five in Maputo, supporting hypothesis 3 presented in section 2.8.3.

CHAPTER 7: CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusions

EPEC pathotypes were recovered from 2.0% of faecal samples collected in children under five with diarrhoea in rural and urban areas of Maputo. EPEC-associated diarrhoea in these children was negatively associated with the consumption of yoghurt, which appeared to act as a protective factor. In contrast, diarrhoea was positively associated with the intake of fruits, vegetables, juice, and exposure to domestic animals such as dogs and chickens. However, this association may not be generalised to the broader population due to the low prevalence of EPEC in the study sample.

Food consumed by the children was contaminated with ETEC, EIEC and EPEC strains, with 13.0% prevalence, and *Salmonella* spp. with 2.2% prevalence. These strains were present in cereals, combined food, yoghurt and drinking water. DEC strains were associated with the consumption of infant formula, fruit puree, ready-to-eat meals, and bottled water.

WGS allowed the confirmation of 10 DEC strains isolated from food (n = 2), drinking water (n = 2) and faecal samples (n = 6), of which most belonged to O109:H21/ST40, O88:H5/ST206 and O80:H2/ST301 serogroup and sequence types. The strains harboured AMR genes conferring resistance to important antimicrobial classes in clinical practices, which include aminoglycosides, fosfomycin, polymyxins, beta-lactam, fluoroquinolone, trimethoprim, tetracycline and sulphonamides.

The DEC strains were genetically related, suggesting that food and drinking water were the potential sources of DEC causing diarrhoea in Maputo children under five years old. However, further epidemiological and molecular evidence based on robust data is needed to confirm food and water as definitive transmission routes of diarrhoea in children.

7.2. Recommendations

The presence of diarrhoeagenic pathogens in food and drinking water, along with the identification of these sources as potential reservoirs of DEC responsible for childhood diarrhoea, underscores the need for education on good practices in food preparation, storage, and water treatment. This education should be provided by health authorities, focusing on the Five Keys to Safer Food defined by the WHO. These include maintaining cleanliness, separating raw and cooked foods, using safe water and raw materials, and

keeping food at safe temperatures, thereby helping to prevent the spread of diarrhoeagenic pathogens. Studies on attitudes and practices during food preparation are also needed.

Further studies with more robust data are needed on the source attribution of diarrhoea in children. In this study, the association analyses were based on a small number of positive strains (DEC = 6), which limits the ability to draw reliable inferences for the broader population.

The presence of AMR genes conferring resistance to antimicrobial classes used in clinical practices, in food, drinking water, and children's faecal samples, underscores the importance of a One Health approach, for monitoring, preventing, and controlling the spread of AMR across different sectors.

CHAPTER 8: REFERENCES

Abakpa, G.O., Umoh, V.J., Ameh, J.B., Yakubu, S.E., Kwaga, J.K.P. and Kamaruzaman, S. (2015) 'Diversity and antimicrobial resistance of *Salmonella enterica* isolated from fresh produce and environmental samples', *Environmental Nanotechnology, Monitoring and Management*, 3, pp. 38–46. Available at: https://doi.org/10.1016/j.enmm.2014.11.004.

Abd-El-Malek, A.M. (2017) 'Cooked poultry meat and products as a potential source of some food poisoning bacteria', *IOSR Journal of Environmental Science, Toxicology and Food Technology*, 11(06), pp. 23–29. Available at: https://doi.org/10.9790/2402-1106032329.

Acácio, S., Mandomando, I., Nhampossa, T., Quintó, L., Vubil, D., Sacoor, C., Kotloff, K., Farag, T., Nasrin, D., Macete, E., Levine, M.M., Alonso, P. and Bassat, Q. (2019) 'Risk factors for death among children 0-59 months of age with moderate-to-severe diarrhea in Manhiça district, southern Mozambique', *BMC Infectious Diseases*, 19(1), pp. 1–14. Available at: https://doi.org/10.1186/s12879-019-3948-9.

Adam Mohamed, S., Nyerere, A., Sang, W.K. and Ngayo, M. (2020) 'Bottled water brands are contaminated with multidrug resistant bacteria which are associated with companies handling procedures in Nairobi, Kenya', *F1000Research*, 9, p. 1337. Available at: https://doi.org/10.12688/f1000research.24031.1.

Adu, G.A., Amegah, K.E., Addo, H.O., Andoh, T., Duvor, F., Antwi, G., Peprah, N.Y., Kenu, E., Bekoe, F.A. and Malm, K.L. (2024) 'Reduction in diarrhea cases following implementation of COVID-19 hand hygiene interventions in Ghana: A causal impact analysis', *PLoS ONE*, 19(8 August), pp. 1–13. Available at: https://doi.org/10.1371/journal.pone.0309202.

Adzitey, F., Huda, N. and Ali, G. (2013) 'Molecular techniques for detecting and typing of bacteria, advantages and application to foodborne pathogens isolated from ducks', *3 Biotech*, 3(2), pp. 97–107. Available at: https://doi.org/10.1007/s13205-012-0074-4.

AFRIKAIA (2022) *The impact of climate on sanitation in Maozambique*. Available at: https://www.wateraid.org/mz/sites/g/files/jkxoof341/files/the-impact-of-climate-change-on-sanitation-in-mozambique.pdf.

Akhondi, H., Goldin, J. and Simonsen, K.A. (2025) *Bacterial Diarrhea*. StatPearls [Internet]. Available at: https://www.ncbi.nlm.nih.gov/books/NBK551643/.

Akinlabi, O.C., Nwoko, E.S.Q., Dada, R.A., Ekpo, S., Omotuyi, A., Nwimo, C.C., Adepoju, A., Popoola, O., Dougan, G., Thomson, N.R. and Okeke, I.N. (2023) 'Epidemiology and risk factors for diarrheagenic *Escherichia coli* carriage among children in Northern Ibadan, Nigeria', *American Journal of Tropical Medicine and Hygiene*, 109(6), pp. 1223–1232. Available at: https://doi.org/10.4269/ajtmh.22-0618.

Akram, M., Hussain, S., Salim, M.W., Pervaiz, F., Karim, S.A. and Khan, H.I. (2023) 'Comparison of outcome of lactose-free formula milk with yogurt in children with acute diarrhea', *Pakistan Journal of Medical and Health Sciences*, 17(5), pp. 281–283. Available at: https://doi.org/10.53350/pjmhs2023175281.

Alemayehu, B., Mekonen, S. and Ambleu, A. (2024) 'Implications of COVID-19 prevention on the occurrence of childhood diarrhea in the Semen Bench district, Bench Sheko zone, southwestern Ethiopia', *Frontiers in Public Health*, 12. Available at: https://doi.org/10.3389/fpubh.2024.1379232.

Allard, M.W., Bell, R., Ferreira, C.M., Gonzalez-Escalona, N., Hoffmann, M., Muruvanda, T., Ottesen, A., Ramachandran, Padmini Reed, E., Sharma, S., Stevens, E., Timme, R., Zheng, J. and and Brown, E.W. (2018) 'Genomics of foodborne pathogens for microbial food safety', *Current Opinion in Biotechnology*, 49, p. :224–229.

Ameya, G., Tsalla, T., Getu, F. and Getu, E. (2018) 'Antimicrobial susceptibility pattern, and associated factors of *Salmonella* and *Shigella* infections among under five children in Arba Minch, South Ethiopia', *Annals of Clinical Microbiology and Antimicrobials*, 17(1), pp. 1–7. Available at: https://doi.org/10.1186/s12941-018-0253-1.

Ammari, S., Laglaoui, A., En-Nanei, L., Bertrand, S., Wildemauwe, C., Barrijal, S. and Abid, M. (2009) 'Isolation, drug resistance and molecular characterization of *Salmonella* isolates in northern Morocco', *Journal of Infection in Developing Countries*, 3(1), pp. 41–49. Available at: https://doi.org/10.3855/jidc.104.

Anderson, J.D., Bagamian, K.H., Muhib, F., Amaya, M.P., Laytner, L.A., Wierzba, T. and Rheingans, R. (2019) 'Burden of enterotoxigenic *Escherichia coli* and *Shigella* non-fatal diarrhoeal infections in 79 low-income and lower middle-income countries: a

modelling analysis', *The Lancet Global Health*, 7(3), pp. e321–e330. Available at: https://doi.org/10.1016/S2214-109X(18)30483-2.

Andreoletti, O., Budka, H., Buncic, S., Colin, P., Collins, J.D., De, A., Griffin, J., Havelaar, A., Hope, J., Klein, G., Kruse, H., Magnino, S., López, A.M., Mclauchlin, J., Nguyen-thé, C., Noeckler, K., Noerrung, B., Maradona, M.P., Roberts, T., Vågsholm, I. and Vanopdenbosch, E. (2008) 'Overview of methods for source attribution for human illness from food-borne microbiological hazards - Scientific Opinion of the Panel on Biological Hazards', *EFSA Journal*, 6(7), pp. 1–43. Available at: https://doi.org/10.2903/j.efsa.2008.764.

Andrews, W.H., Wang, H., Jacobson, A., Ge, B., Zhang, G. and Hammack, T. (2020) *BAM Chapter 5: Salmonella | FDA*. Available at: https://www.fda.gov/food/laboratory-methods-food/bam-chapter-5-salmonella (Accessed: 1 December 2020).

Askari, A., Ghanbarpour, R., Akhtardanesh, B., Aflatoonian, M.R., Sharifi, H., Jajarmi, M. and Molaei, R. (2020) 'Detection of zoonotic diarrheagenic pathotypes of escherichia coli in healthy household dogs', *Iranian Journal of Microbiology*, 12(6), pp. 522–530. Available at: https://doi.org/10.18502/ijm.v12i6.5026.

Assefa, A. and Girma, M. (2019) 'Prevalence and antimicrobial susceptibility patterns of *Salmonella* and *Shigella* isolates among children aged below five years with diarrhea attending Robe General Hospital and Goba Referral Hospital, South East Ethiopia', *Tropical Diseases, Travel Medicine and Vaccines*, 5(1), pp. 1–11. Available at: https://doi.org/10.1186/s40794-019-0096-6.

Bakare, A.A., Uchendu, O.C., Omotayo, O.E. and King, C. (2023) 'Feeding practices and nutritional status of under-five children in a peri-urban setting in Ibadan, Southwest Nigeria: a Comparative cross-sectional study', *Annals of Ibadan Postgraduate Medicine*, 21(1), p. 52. Available at: /pmc/articles/PMC10388425/%0A/pmc/articles/PMC10388425/?report=abstract%0Ahttps://www.ncbi.nlm.nih.gov/pmc/articles/PMC10388425/.

Bakhtiari, S., Mahmoudi, H., Seftjani, S.K., Amirzargar, M.A., Ghiasvand, S., Ghaffari, M.E. and Adabi, M. (2020) 'Antibiotic resistance pattern and phylogenetic groups of the uropathogenic *Escherichia coli* isolates from urinary tract infections in Hamedan, West

of Iran', *Iranian Journal of Microbiology*, 12(5), pp. 388–394. Available at: https://doi.org/10.18502/ijm.v12i5.4598.

Balew, M. and Kibret, M. (2023) 'Prevalence of enteric bacterial pathogens in diarrheic under-five children and their association with the nutritional status in Bahir Dar Zuria District, Northwest Ethiopia', *BMC Nutrition*, 9(1), pp. 1–9. Available at: https://doi.org/10.1186/s40795-023-00678-0.

Barnes, A.N., Anderson, J.D., Mumma, J., Mahmud, Z.H. and Cumming, O. (2018) 'The association between domestic animal presence and ownership and household drinking water contamination among peri-urban communities of Kisumu, Kenya', *PLoS ONE*, 13(6), pp. 1–17. Available at: https://doi.org/10.1371/journal.pone.0197587.

Basavaraju, M. and Gunashree, B.S. (2023) ' *Escherichia coli* : An overview of main characteristics ', *Escherichia coli - Old and New Insights* [Preprint], (November). Available at: https://doi.org/10.5772/intechopen.105508.

Bauleth, M.F., Mitonga, H.K. and Pinehas, L.N. (2020) 'Epidemiology and factors associated with diarrhoea amongst children under 5 years of age in Engela district in the Ohangwena region, Namibia', pp. 1–11.

Bauza, V., Ocharo, R.M., Nguyen, T.H. and Guest, J.S. (2017) 'Soil ingestion is associated with child diarrhea in an urban slum of Nairobi, Kenya', *American Journal of Tropical Medicine and Hygiene*, 96(3), pp. 569–575. Available at: https://doi.org/10.4269/ajtmh.16-0543.

Belay, D.G., Asratie, M.H., Aragaw, F.M., Tsega, N.T., Endalew, M. and Gashaw, M. (2022) 'Open defecation practice and its determinants among households in sub-Saharan Africa: pooled prevalence and multilevel analysis of 33 sub-Saharan Africa countries demographic and health survey', *Tropical Medicine and Health*, 50(1). Available at: https://doi.org/10.1186/s41182-022-00416-5.

Besser, J.M., Carleton, H.A., Trees, E., Stroika, S.G., Hise, K., Wise, M. and Gerner-Smidt, P. (2019) 'Interpretation of whole-genome sequencing for enteric disease surveillance and outbreak investigation', *Foodborne Pathogens and Disease*, 16(7), pp. 504–512. Available at: https://doi.org/10.1089/fpd.2019.2650.

Bhavnani, D., De Los Ángeles Bayas, R., Lopez, V.K., Zhang, L., Trueba, G., Foxman, B., Marrs, C., Cevallos, W. and Eisenberg, J.N.S. (2016) 'Distribution of enteroinvasive and enterotoxigenic *Escherichia coli* across space and time in northwestern Ecuador', *American Journal of Tropical Medicine and Hygiene*, 94(2), pp. 276–284. Available at: https://doi.org/10.4269/ajtmh.14-0337.

Bick, S., Perieres, L., D'Mello-Guyett, L., Baker, K.K., Brown, J., Muneme, B., Nala, R., Dreibelbis, R. and Cumming, O. (2020) 'Risk factors for child food contamination in low-income neighbourhoods of Maputo, Mozambique: An exploratory, cross-sectional study', *Maternal and Child Nutrition*, 16(4). Available at: https://doi.org/10.1111/mcn.12991.

Bintsis, T. (2017) 'Foodborne pathogens.', *AIMS microbiology*, 3(3), pp. 529–563. Available at: https://doi.org/10.3934/microbiol.2017.3.529.

Bortolaia, V., Kaas, R.S., Ruppe, E., Roberts, M.C., Schwarz, S., Cattoir, V., Philippon, A., Allesoe, R.L., Rebelo, A.R., Florensa, A.F., Fagelhauer, L., Chakraborty, T., Neumann, B., Werner, G., Bender, J.K., Stingl, K., Nguyen, M., Coppens, J., Xavier, B.B., Malhotra-Kumar, S., Westh, H., Pinholt, M., Anjum, M.F., Duggett, N.A., Kempf, I., Nykäsenoja, S., Olkkola, S., Wieczorek, K., Amaro, A., Clemente, L., Mossong, J., Losch, S., Ragimbeau, C., Lund, O. and Aarestrup, F.M. (2020) 'ResFinder 4.0 for predictions of phenotypes from genotypes', *Journal of Antimicrobial Chemotherapy*, 75(12), pp. 3491–3500. Available at: https://doi.org/10.1093/jac/dkaa345.

Boudraa, G., Touhami, M., Pochart, P., Soltana, R., Mary, J., Desjeuxaa, J., Touhami, M., Pochart, P., Soltana, R., Mary, J. and Desjeux, J. (1990) 'Effect of feeding yogurt versus milk in children with persistent diarrhea', *Journal of Pediatric Gastroenterology and Nutrition - JPGN*.

Brooks, G.F., Carroll, K.C., Butel, J.S., Morse, S.A. and Mietzner, T.A. (2013) *Medical Microbiology*. 26th edn. Edited by McGraw-Hill. New York.

Buermans, H.P.J. and den Dunnen, J.T. (2014) 'Next generation sequencing technology: Advances and applications', *Biochimica et Biophysica Acta - Molecular Basis of Disease*, 1842(10), pp. 1932–1941. Available at: https://doi.org/10.1016/j.bbadis.2014.06.015.

Butler, A.J., Thomas, M.K. and Pintar, K.D.M. (2015) 'Systematic review of expert elicitation methods as a tool for source attribution of enteric illness', *Foodborne*

Pathogens and Disease, 12(5), pp. 367–382. Available at: https://doi.org/10.1089/fpd.2014.1844.

Bywater, A., Dintwe, G., Alexander, K.A. and Ponder, M.A. (2024) 'Characterization of diarrheagenic *Escherichia coli* and *Salmonella enterica* from produce in the Chobe district of Botswana', *Journal of Food Protection*, 87(10), p. 100351. Available at: https://doi.org/10.1016/j.jfp.2024.100351.

Camacho, C., Coulouris, G., Avagyan, V., Ma, N., Papadopoulos, J., Bealer, K. and Madden, T.L. (2009) 'BLAST+: Architecture and applications', *BMC Bioinformatics*, 10, pp. 1–9. Available at: https://doi.org/10.1186/1471-2105-10-421.

Cantrell, M.E., Sylvestre, É., Wharton, H.C., Scheidegger, R., Curchod, L., Gute, D.M., Griffiths, J., Julian, T.R. and Pickering, A.J. (2023) 'Hands are frequently contaminated with fecal bacteria and enteric pathogens globally: A Systematic review and meta-analysis', *ACS Environmental Au*, 3(3), pp. 123–134. Available at: https://doi.org/10.1021/acsenvironau.2c00039.

Capone, D., Bivins, A., Knee, J., Cumming, O., Nalá, R. and Brown, J. (2021) 'Quantitative microbial risk assessment of pediatric infections attributable to ingestion of fecally contaminated domestic soils in low-income urban Maputo, Mozambique', *Environmental Science and Technology*, 55(3), pp. 1941–1952. Available at: https://doi.org/10.1021/acs.est.0c06972.

CDC (2018) *Attribution of Foodborne Illness: Overview*. Available at: https://www-cdc-gov.translate.goog/foodborneburden/attribution/overview.html?_x_tr_sl=en&_x_tr_tl=pt&_x_tr_hl=pt-PT&_x_tr_pto=sc.

CDC (2021) *Epi Info™ For Windows: version 7.2.5.0*. Available at: https://www.cdc.gov/epiinfo/support/downloads.html.

CDC (2024a) *E. coli Infection (Escherichia coli)*. Available at: https://www.cdc.gov/ecoli/about/kinds-of-ecoli.html.

CDC (2024b) *Shigellosis*. Available at: https://wwwnc.cdc.gov/travel/yellowbook/2024/infections-diseases/shigellosis.

Cervenka, L., Vytrasova, J. and Brozkova, I. (2006) 'Effects of the principal ingredients of biscuits upon water activity', 45, pp. 39–43.

Chakraborty, S., Johura, F.T., Sultana, M., Zhang, X., Sadique, A., George, C.M., Monira, S., Sack, D.A., Sack, R.B. and Alam, M. (2024) 'Epidemiology of enterotoxigenic *Escherichia coli* among children and adults seeking care at hospitals in two geographically distinct rural areas in Bangladesh', *Microorganisms*, 12(2). Available at: https://doi.org/10.3390/microorganisms12020359.

Chattaway, M.A., Langridge, G.C. and Wain, J. (2021) '*Salmonella* nomenclature in the genomic era: a time for change', *Scientific Reports*, 11(1), pp. 1–8. Available at: https://doi.org/10.1038/s41598-021-86243-w.

Chisembe, P., Suzuki, M., Dao, D.T., Njunga, G., Nkhoma, J., Mthilakuwili, L., Kinoshita-daitoku, R., Kuroda, E., Kimura, K. and Shibayama, K. (2024) 'JAC-Antimicrobial resistance: A nationwide survey of antimicrobial resistance of *Escherichia coli* isolated from broiler chickens in Malawi', *JAC-Antimicrobial Resistance*, 6(6), pp. 1–11. Available at: https://doi.org/10.1093/jacamr/dlae200.

Chissaque, A., de Deus, N., Vubil, D. and Mandomando, I. (2018) 'The Epidemiology of diarrhea in children under 5 years of age in Mozambique', *Current Tropical Medicine Reports*, 5(3), pp. 115–124. Available at: https://doi.org/10.1007/s40475-018-0146-6.

Chukwu, M.O., Abia, A.L.K., Ubomba-Jaswa, E., Obi, L.C. and Dewar, J.B. (2019) 'Antibiotic resistance profile and clonality of *E. coli* isolated from water and paediatric stool samples in the north-west, province South Africa', *Journal of Pure and Applied Microbiology*, 13(1), pp. 517–530. Available at: https://doi.org/10.22207/JPAM.13.1.58.

Chukwu, M.O., King Abia, A.L., Ubomba-Jaswa, E., Obi, L. and Dewar, J.B. (2019) 'Characterization and phylogenetic analysis of campylobacter species isolated from paediatric stool and water samples in the northwest province, south africa', *International Journal of Environmental Research and Public Health*, 16(12), pp. 1–22. Available at: https://doi.org/10.3390/ijerph16122205.

Cody, E.M. and Dixon, B.P. (2019) 'Hemolytic Uremic Syndrome', *Pediatric Clinics of North America*, 66(1), pp. 235–246. Available at: doi:10.1016/j.pcl.2018.09.011.

Cohen, A.L., Platts-Mills, J.A., Nakamura, T., Operario, D.J., Antoni, S., Mwenda, J.M., Weldegebriel, G., Rey-Benito, G., De Oliveira, L.H., Ortiz, C., Daniels, D.S., Videbaek, D., Singh, S., Njambe, E., Sharifuzzaman, M., Grabovac, V., Nyambat, B., Logronio, J., Armah, G., Dennis, F.E., Seheri, M.L., Magagula, N., Mphahlele, J., Fumian, T.M., Maciel, I.T.A., Gagliardi Leite, J.P., Esona, M.D., Bowen, M.D., Samoilovich, E., Semeiko, G., Abraham, D., Giri, S., Praharaj, I., Kang, G., Thomas, S., Bines, J., Liu, N., Kyu, H.H., Doxey, M., Rogawski Mcquade, E.T., Mcmurry, T.L., Liu, J., Houpt, E.R., Tate, J.E., Parashar, U.D. and Serhan, F. (2022) 'Aetiology and incidence of diarrhoea requiring hospitalisation in children under 5 years of age in 28 low-income and middle-income countries: findings from the global pediatric diarrhea surveillance network', *BMJ Global Health*, 7(9), pp. 1–12. Available at: https://doi.org/10.1136/bmjgh-2022-009548.

Collaborators, G.D.D. (2017) 'Estimates of global, regional, and national morbidity, mortality, and aetiologies of diarrhoeal diseases: a systematic analysis for the global burden of disease Study 2015', *The Lancet Infectious Diseases*, 17(9), pp. 909–948. Available at: https://doi.org/10.1016/S1473-3099(17)30276-1.

Conan, A., O'Reilly, C.E., Ogola, E., Ochieng, J.B., Blackstock, A.J., Omore, R., Ochieng, L., Moke, F., Parsons, M.B., Xiao, L., Roellig, D., Farag, T.H., Nataro, J.P., Kotloff, K.L., Levine, M.M., Mintz, E.D., Breiman, R.F., Cleaveland, S. and Knobel, D.L. (2017) 'Animal-related factors associated with moderate-to-severe diarrhea in children younger than five years in western Kenya: A matched case-control study', *PLoS Neglected Tropical Diseases*, 11(8). Available at: https://doi.org/10.1371/journal.pntd.0005795.

Croxen, M.A., Law, R.J., Scholz, R., Keeney, K.M., Wlodarska, M. and Finlay, B.B. (2013) 'Recent advances in understanding enteric pathogenic *Escherichia coli*', *Clinical Microbiology Reviews*, 26(4), pp. 822–880. Available at: https://doi.org/10.1128/CMR.00022-13.

Dade, Y., Kannaiyan, M., Dedecha, W., Daka, D., Husen, O., Gemechu, T., Aliyo, A. and Boru, K. (2025) 'Prevalence, antimicrobial susceptibility pattern and associated factors of Salmonella and Shigella among under five children with diarrhea attending Bule Hora University Teaching Hospital, Bule Hora, West Guji, Ethiopia', *BMC Infectious Diseases*, 25(1). Available at: https://doi.org/10.1186/s12879-025-10960-0.

Dall, C. (2023) *Study finds highly resistant diarrheal pathogens in Mozambique*. Available at: https://www.cidrap.umn.edu/antimicrobial-stewardship/study-finds-highly-resistant-diarrheal-pathogens-mozambique#:~:text=An analysis of stool samples,week in BMC Infectious Diseases.

Daniels, N.A. (2005) 'Enterotoxigenic *Escherichia coli* : Traveler ' s diarrhea comes home', 42(3), pp. 335–336.

Das, R., Palit, P., Haque, M.A., Ahmed, T. and Faruque, A.S.G. (2022) 'Association between pathogenic variants of diarrheagenic *Escherichia coli* and growth in children under 5 years of age in the global enteric multicenter study', *American Journal of Tropical Medicine and Hygiene*, 107(1), pp. 72–81. Available at: https://doi.org/10.4269/ajtmh.22-0096.

Das, R., Palit, P., Haque, M.A., Mahfuz, M., Faruque, A.S.G. and Ahmed, T. (2022) 'Site specific incidence rate of genomic subtypes of enteropathogenic *Escherichia coli* and association with enteric inflammation and child growth', *Scientific Reports*, 12(1), pp. 1–9. Available at: https://doi.org/10.1038/s41598-022-09730-8.

Delahoy, M.J., Wodnik, B., McAliley, L., Penakalapati, G., Swarthout, J., Freeman, M.C. and Levy, K. (2018) 'Pathogens transmitted in animal feces in low- and middle-income countries', *International Journal of Hygiene and Environmental Health*, 221(4), pp. 661–676. Available at: https://doi.org/10.1016/j.ijheh.2018.03.005.

Dessale, M., Mengistu, G. and Mengist, H.M. (2023) 'Prevalence, antimicrobial resistance pattern, and associated factors of *Salmonella* and *Shigella* among under five diarrheic children attending public health facilities in Debre Markos town, Northwest Ethiopia', *Frontiers in Public Health*, 11. Available at: https://doi.org/10.3389/fpubh.2023.1114223.

Desta, B.K., Assimamaw, N.T. and Ashenafi, T.D. (2017) 'Knowledge, practice, and associated factors of home-based management of diarrhea among caregivers of children attending under-five clinic in Fagita Lekoma District, Awi Zone, Amhara Regional State, Northwest Ethiopia, 2016', *Nursing Research and Practice*, 2017, pp. 1–8. Available at: https://doi.org/10.1155/2017/8084548.

van Dijk, E.L., Jaszczyszyn, Y., Naquin, D. and Thermes, C. (2018) 'The third revolution

in sequencing technology', *Trends in Genetics*, 34(9), pp. 666–681. Available at: https://doi.org/10.1016/j.tig.2018.05.008.

Doi, Y., Iovleva, A. and Bonomo, R.A. (2017) 'The ecology of extended-spectrum β-lactamases (ESBLs) in the developed world', *Journal of travel medicine*, 24(1), pp. S44–S51. Available at: https://doi.org/10.1093/jtm/taw102.

Dong, F. (2020) 'Urban-rural divide in foodborne illness in China: what can we learn from the socioeconomic factors?', *Journal of Global Health Reports*, 4. Available at: https://doi.org/10.29392/001c.14173.

Dow, M.A., Tóth, A., Malik, A., Herpay, M., Nógrády, N., Ghenghesh, K.S. and Nagy, B. (2006) 'Phenotypic and genetic characterization of enteropathogenic Escherichia coli (EPEC) and enteroaggregative *E. coli* (EAEC) from diarrhoeal and non-diarrhoeal children in Libya', *Comparative Immunology, Microbiology & Infectious Diseases*, pp. 100–113. Available at: https://doi.org/https://doi.org/10.1016/j.cimid.2006.01.005.

Doza, S., Rahman, M.J., Islam, M.A., Kwong, L.H., Unicomb, L., Ercumen, A., Pickering, A.J., Parvez, S.M., Naser, A.M., Ashraf, S., Das, K.K. and Luby, S.P. (2018) 'Prevalence and association of *Escherichia coli* and diarrheagenic *Escherichia coli* in stored foods for young children and flies caught in the same households in Rural Bangladesh', *American Journal of Tropical Medicine and Hygiene*, 98(4), pp. 1031–1038. Available at: https://doi.org/10.4269/ajtmh.17-0408.

EFSA (2015) *Public health risks associated with Enteroaggregative Escherichia coli (EAEC) as a food-borne pathogen*, *EFSA Journal*. Available at: https://doi.org/10.2903/j.efsa.2015.4330.

Ekici, G. and Dumen, E. (2019) '*Escherichia coli* and food safety', *Foodborne pathogens and disease* [Preprint]. Available at: https://doi.org/DOI: http://dx.doi.org/10.5772/intechopen.82375.

EPA (2011) 'Exposure factors handbook: 2011 Edition', *U.S. Environmental Protection Agency*, EPA/600/R-(September), pp. 1–1466. Available at: https://doi.org/EPA/600/R-090/052F.

Faife, S., Gichure, J. and Hald, T. (2024) 'Diarrhoeagenic *Escherichia coli* and

Salmonella spp. contamination of food and water consumed by children with diarrhoea in Maputo , Mozambique'.

Faife, S.L., Zimba, T., Sekyere, J.O., Govinden, U., Chenia, H.Y., Simonsen, G.S., Sundsfjord, A. and Essack, S.Y. (2020) 'β-lactam and fluoroquinolone resistance in Enterobacteriaceae from imported and locally-produced chicken in Mozambique', *Journal of infection in developing countries*, 14(5), pp. 471–478. Available at: https://doi.org/10.3855/jidc.10924.

Falcao, R.C., Edwards, M.R., Hurst, M., Fraser, E. and Otterstatter, M. (2024) 'A Review on microbiological source attribution methods of human salmonellosis: from subtyping to whole-genome sequencing', *Foodborne Pathogens and Disease*, 21(3), pp. 137–146. Available at: https://doi.org/10.1089/fpd.2023.0075.

Farag, T.H., Faruque, A.S., Wu, Y., Das, S.K., Hossain, A., Ahmed, S., Ahmed, D., Nasrin, D., Kotloff, K.L., Panchilangam, S., Nataro, J.P., Cohen, D., Blackwelder, W.C. and Levine, M.M. (2013) 'Housefly population density correlates with shigellosis among children in Mirzapur, Bangladesh: A time series analysis', *PLoS Neglected Tropical Diseases*, 7(6). Available at: https://doi.org/10.1371/journal.pntd.0002280.

FDA (2001) *Bacteriological Analytical Manual Online: Escherichia coli and the Coliform Bacteria*. Available at: http://higiene.unex.es/Bibliogr/Libros/BAMonlin/bam-4.htm.

Fellenz, D.F., Ruiz, J.M., Etcheverría, A.I., Colello, R., Velez, M. V., Sanz, M.E., Sparo, M.D., Lissarrague, S., Pereyra, J., Zanelli, G. and Padola, N.L. (2024) 'Detection of EPEC and STEC strains isolated from children with diarrhea in Argentina', *Revista Argentina de Microbiologia*, pp. 10–13. Available at: https://doi.org/10.1016/j.ram.2024.07.003.

Fiedoruk, K., Daniluk, T., Rozkiewicz, D., Oldak, E., Prasad, S. and Swiecicka, I. (2019) 'Whole-genome comparative analysis of *Campylobacter jejuni* strains isolated from patients with diarrhea in northeastern Poland', *Gut Pathogens*, 11(1), pp. 1–10. Available at: https://doi.org/10.1186/s13099-019-0313-x.

Franz, E., Gras, L.M. and Dallman, T. (2016) 'Significance of whole genome sequencing for surveillance, source attribution and microbial risk assessment of foodborne pathogens', *Current Opinion in Food Science*, 8, pp. 74–79. Available at:

https://doi.org/10.1016/j.cofs.2016.04.004.

Friedman, J., Hastie, T. and Tibshirani, R. (2010) 'Regularization paths for generalized linear models via coordinate descent', *Journal of Statistical Software*, 33(1), pp. 1–22.

Fufa, D.D., Abhram, A., Teshome, A., Teji, K., Abera, F., Tefera, M., Yeshitila, M., Mengistu, M., Gezahegn, M., Gure, T., Worku, T., Alemayehu, T. and Egata, G. (2020) 'Hygienic practice of complementary food preparation and associated factors among mothers with children aged from 6 to 24 months in rural kebeles of Harari region, Ethiopia', *Food Science and Technology (United States)*, 8(2), pp. 34–42. Available at: https://doi.org/10.13189/fst.2020.080203.

Garrine, M., Matambisso, G., Nobela, N., Vubil, D., Massora, S., Acácio, S., Nhampossa, T., Alonso, P. and Mandomando, I. (2020) 'Low frequency of enterohemorrhagic, enteroinvasive and diffusely adherent *Escherichia coli* in children under 5 years in rural Mozambique: A case-control study', *BMC Infectious Diseases*, 20(1), pp. 1–6. Available at: https://doi.org/10.1186/s12879-020-05380-1.

George, C.M., Birindwa, A., Beck, S., R Julian, T., Kuhl, J., Williams, C., Coglianese, N., D Thomas, E., Bauler, S., François, R., Ng, A., Presence, A.S., Claude, B.R.J., Tofail, F., Perin, J., Mirindi, P. and Cirhuza, L.B. (2022) 'Fecal contamination in child play spaces and on child hands are associated with subsequent adverse child developmental outcomes in rural Democratic Republic of the Congo: REDUCE Prospective Cohort Study', *The American journal of tropical medicine and hygiene*, 106(4), pp. 1141–1148. Available at: https://doi.org/10.4269/ajtmh.21-0706.

George, C.M., Perin, J., De Calani, K.J.N., Norman, W.R., Perry, H., Davis, T.P. and Lindquist, E.D. (2014) 'Risk factors for diarrhea in children under five years of age residing in peri-urban communities in Cochabamba, Bolivia', *American Journal of Tropical Medicine and Hygiene*, 91(6), pp. 1190–1196. Available at: https://doi.org/10.4269/ajtmh.14-0057.

Gerba, C.P. (2015) *Environmentally Transmitted Pathogens*, *Environmental Microbiology: Third Edition*. Available at: https://doi.org/10.1016/B978-0-12-394626-3.00022-3.

Getachew, A., Molla, M., Admasie, A. and Yenesew, M.A. (2024) 'Association between

domestic animal exposure and diarrhea prevalence in under- five children in low- and middle-income countries: a systematic review and meta-analysis', *BMC pediatrics*, 24(1), p. 601. Available at: https://doi.org/10.1186/s12887-024-05084-4.

Getaneh, D.K., Hordofa, L.O., Ayana, D.A., Tessema, T.S. and Regassa, L.D. (2021) 'Prevalence of Escherichia coli O157:H7 and associated factors in under-five children in Eastern Ethiopia', *PLoS ONE*, 16(1 January), pp. 1–15. Available at: https://doi.org/10.1371/journal.pone.0246024.

Gizaw, Z., Yalew, A.W., Bitew, B.D., Lee, J. and Bisesi, M. (2022) 'Fecal indicator bacteria along multiple environmental exposure pathways (water, food, and soil) and intestinal parasites among children in the rural northwest Ethiopia', *BMC Gastroenterology*, 22(1), pp. 1–17. Available at: https://doi.org/10.1186/s12876-022-02174-4.

Gobena, T., Belina, D., Kebede, A., Chimdessa, M., Jemal, A., Husen, C. and Hald, T. (2024) 'Foodborne bacterial pathogens in animal, food, and environmental samples collected from the physical exposure of children with diarrhea in Ethiopia: A One Health Approach', *Environmental Health Insights*, 18. Available at: https://doi.org/10.1177/11786302241288167.

Gomes, T.A.T., Elias, W.P., Scaletsky, I.C.A., Guth, B.E.C., Rodrigues, J.F., Piazza, R.M.F., Ferreira, L.C.S. and Martinez, M.B. (2016) 'Diarrheagenic *Escherichia coli*', *Brazilian Journal of Microbiology*, 47, pp. 3–30. Available at: https://doi.org/10.1016/j.bjm.2016.10.015.

Gonçalves-Tenório, A., Silva, B.N., Rodrigues, V., Cadavez, V. and Gonzales-Barron, U. (2018) 'Prevalence of pathogens in poultry meat: A Meta-analysis of European published surveys.', *Foods (Basel, Switzerland)*, 7(5). Available at: https://doi.org/10.3390/foods7050069.

Graaf, M. De, Beck, R., Caccio, S.M., Duim, B., Fraaij, P.L.A., Guyader, S. Le, Lecuit, M., Pendu, J. Le, Wit, E. De and Schultsz, C. (2020) 'Sustained fecal-oral human-to-human transmission following a zoonotic event', *Current Opinion in Virology*, 22, pp. 1–6. Available at: https://doi.org/https://doi.org/10.1016/j.coviro.2016.11.001.

Grace, D. (2015) 'Food safety in low and middle income countries', *International Journal*

of Environmental Research and Public Health, 12(9), pp. 10490–10507. Available at: https://doi.org/10.3390/ijerph120910490.

Grohmann, L., Barbante, A., Eriksson, R., Gatto, F., Georgieva, T., Huber, I., Hulin, J., Köppel, R., Marchesi, U., Marmin, L., Mazzara, M., Narendja, F., Owen, H., Perri, E., Scholtens, I., Sovová, T., Sowa, S., Stebih, D., Weidner, C. and Zdeňková, K. (2021) *Guidance document on multiplex real-time PCR methods*. Available at: https://doi.org/10.2760/243914.

Gu, Z., Eils, R. and Schlesner, M. (2016) 'Complex heatmaps reveal patterns and correlations in multidimensional genomic data', *Bioinformatics*, 32(18), pp. 2847–2849. Available at: https://doi.org/10.1093/bioinformatics/btw313.

Hadi, J., Rapp, D., Dhawan, S., Gupta, S.K., Gupta, T.B. and Brightwell, G. (2023) 'Molecular detection and characterization of foodborne bacteria: Recent progresses and remaining challenges', *Comprehensive Reviews in Food Science and Food Safety*, 22(3), pp. 2433–2464. Available at: https://doi.org/10.1111/1541-4337.13153.

Hadjimbei, E., Botsaris, G. and Chrysostomou, S. (2022) 'Beneficial Effects of Yoghurts and Probiotic Fermented Milks and Their Functional Food Potential', *Foods*, 11(2691).

Hamad, A.A., Sharaf, M., Hamza, M.A., Selim, S., Hetta, H.F. and El-Kazzaz, W. (2022) 'Investigation of the Bacterial Contamination and Antibiotic Susceptibility Profile of Bacteria Isolated from Bottled Drinking Water', *Microbiology Spectrum*, 10(1), pp. 1–7. Available at: https://doi.org/10.1128/spectrum.01516-21.

Hartman, R.M., Cohen, A.L., Antoni, S., Mwenda, J., Weldegebriel, G., Biey, J., Shaba, K., De Oliveira, L., Rey, G., Ortiz, C., Tereza, M., Fahmy, K., Ghoniem, A., Ashmony, H., Videbaek, D., Singh, S., Tondo, E., Sharifuzzaman, M., Liyanage, J., Batmunkh, N., Grabovac, V., Logronio, J., Serhan, F. and Nakamura, T. (2023) 'Risk Factors for Mortality Among Children Younger Than Age 5 Years With Severe Diarrhea in Low- and Middle-income Countries: Findings From the World Health Organization-coordinated Global Rotavirus and Pediatric Diarrhea Surveillance Networks', *Clinical Infectious Diseases*, 76(3), pp. E1047–E1053. Available at: https://doi.org/10.1093/cid/ciac561.

Havelaar, A.H., Kirk, M.D., Torgerson, P.R., Gibb, H.J., Hald, T., Lake, R.J., Praet, N.,

Bellinger, D.C., de Silva, N.R., Gargouri, N., Speybroeck, N., Cawthorne, A., Mathers, C., Stein, C., Angulo, F.J., Devleesschauwer, B., Adegoke, G.O., Afshari, R., Alasfoor, D., Baines, J., Balakrishnan, K., Hamza, W.M. Bin, Black, R.E., Bolger, P.M., Chaicumpa, W., Cravioto, A., Döpfer, D., Ehiri, J.E., Fazil, A., Ferreccio, C., Fèvre, E.M., Hall, G., Kasuga, F., Keddy, K.H., Lanata, C.F., Lei, H., Liu, X., Manyindo, B., Nasinyama, G., Ongolo-Zogo, P., Pitt, J.I., Rokni, M.B., Sripa, B., van Leeuwen, R., Verger, P., Willingham, A.L., Zhou, X.N., Aspinall, W., Buchanan, R., Budke, C., Caipo, M.L., Carabin, H., Cole, D., Cooke, R.M., Crump, J.A., El-Jardali, F., Fischer-Walker, C., Fürst, T., Haagsma, J.A., Hall, A.J., Henao, O., Hoffmann, S., Jensen, H., Jessani, N., Koopmans, M.P.G., Levine, M.M., de Noordhout, C.M., Majowicz, S., McDonald, S.A., Pires, S., Scallan, E., Sripa, B., Thomas, M.K., Verhoef, L., Wu, F. and Zeilmaker, M. (2015a) 'World Health Organization global estimates and regional comparisons of the burden of foodborne disease in 2010', *PLoS Medicine*, 12(12), pp. 1–23. Available at: https://doi.org/10.1371/journal.pmed.1001923.

Hayward, C., Ross, K.E., Brown, M.H. and Whiley, H. (2020) 'Water as a source of antimicrobial resistance and healthcare-associated infections', *Pathogens*, 9(8), pp. 1–21. Available at: https://doi.org/10.3390/pathogens9080667.

Hazen, T.H., Leonard, S.R., Lampel, K.A., Lacher, D.W., Maurelli, A.T. and Rasko, D.A. (2016) 'Investigating the Relatedness of Enteroinvasive *Escherichia coli* to Other E . coli and *Shigella* Isolates by Using comparative genomics', 84(8), pp. 2362–2371. Available at: https://doi.org/10.1128/IAI.00350-16.Editor.

He, Z., Ghose, B. and Cheng, Z. (2023) 'Diarrhea as a disease of poverty among under-five children in sub-saharan Africa: A Cross-Sectional Study', *Inquiry (United States)*, 60. Available at: https://doi.org/10.1177/00469580231202988.

Heather, J.M. and Chain, B. (2016) 'The sequence of sequencers: The history of sequencing DNA', *Genomics*, 107(1), pp. 1–8. Available at: https://doi.org/10.1016/j.ygeno.2015.11.003.

Heine, L., Alfinete, N.W., Potgieter, N. and Barnard, T.G. (2024) 'Multi- and extensively drug resistant *Escherichia coli* isolated from South African children under 5 years old with diarrhoea', *Diagnostic Microbiology and Infectious Disease*, 109(2), p. 116279. Available at: https://doi.org/10.1016/j.diagmicrobio.2024.116279.

Heredia, N. and García, S. (2018a) 'Animals as sources of food-borne pathogens: A review', *Animal Nutrition*, 4(3), pp. 250–255. Available at: https://doi.org/10.1016/j.aninu.2018.04.006.

Heredia, N. and García, S. (2018b) 'Animals as sources of food-borne pathogens: A review', *Animal Nutrition*. KeAi Communications Co., pp. 250–255. Available at: https://doi.org/10.1016/j.aninu.2018.04.006.

Hmar, E.B.L., Paul, S. and Sharma, H.K. (2024) 'The role of *Shigella* spp. in propagating bacillary dysentery in humans and the prominence of nanotechnology in disease prevention', *Future Journal of Pharmaceutical Sciences*, 10(1). Available at: https://doi.org/10.1186/s43094-024-00676-4.

Hoffmann, V., Simiyu, S., Sewell, D.K., Tsai, K., Cumming, O., Mumma, J. and Baker, K.K. (2022) 'Milk product safety and household food hygiene influence bacterial contamination of infant food in peri-urban Kenya', *Frontiers in Public Health*, 9. Available at: https://doi.org/10.3389/fpubh.2021.772892.

Hugho, E.A., Mmbaga, B.T., Lukambagire, A.H.S., Kinabo, G.D., Thomas, K.M., Kumburu, H.H. and Hald, T. (2024) 'Risk Factors for *Salmonella* infection in children under five years: a hospital-based study in Kilimanjaro region, Tanzania', *Pathogens*, 13(9). Available at: https://doi.org/10.3390/pathogens13090798.

Husna, A., Rahman, Masudur Badruzzaman, A.T.M., Sikder, Mahmudul Hasan Islam, Mohammad Rafiqul Rahman, T., Alam, J. and Ashou, H.M. (2023) 'Extended-spectrum β-lactamases (ESBL): Challenges and opportunities ', *BioMedicines*, 11(2937), pp. 1–22. Available at: https://pmc.ncbi.nlm.nih.gov/articles/PMC10669213/pdf/biomedicines-11-02937.pdf.

Ijaz, M.K. and Rubino, J.R. (2012) 'Impact of infectious diseases on cognitive development in childhood and beyond: Potential mitigational role of hygiene', *Open Infectious Diseases Journal*, 6(1), pp. 65–70. Available at: https://doi.org/10.2174/1874279301206010065.

Ikeda, R., Nakamura, K., Saulmont, M., Habets, A., Duprez, J., Korsak, N., Hayashi, T., Thiry, D. and Mainil, J.G. (2023) '*Escherichia coli* O80 in healthy cattle: absence of shigatoxigenic and enteropathogenic *E. coli* O80:H2 and (Phylo) Genomics of Non-

Clonal Complex 165 *E. coli* O80', 11, pp. 1–13.

INE (2013a) 'Estatísticas do distrito de Ka Maxaqueni', p. 32.

INE (2013b) 'Estatísticas do Distrito de Marracuene', p. 29. Available at: www.ine.gov.mz.

INE (2024) *Inquérito Demográfico e de Saúde 2022–23*. Maputo.

Jagadeesan, B., Baert, L., Wiedmann, M. and Orsi, R.H. (2019) 'Comparative analysis of tools and approaches for source tracking *Listeria monocytogenes* in a food facility using whole-genome sequence data.', *Frontiers in microbiology*, 10, p. 947. Available at: https://doi.org/10.3389/fmicb.2019.00947.

Jarman, A.F., Long, S.E., Robertson, S.E., Nasrin, S., Alam, N.H., McGregor, A.J. and Levine, A.C. (2019) 'Sex and gender differences in acute pediatric Diarrhea: A secondary analysis of the DHAKA study', *International Journal of Computational Intelligence Systems*, 8(2), pp. 54–58. Available at: https://doi.org/ 10.2991/j.jegh.2018.08.102

Jay, J., Loessner, M. and Golden, D. (2005) *Modern Food Microbiology text book 7th Ed Jay, Loessner, Golden IMP*. 7th edn. Edited by F.S.T. Series. New York.

Jay, J.M., Loessner, M.J. and Golden, D.A. (2005) *Modern Food Microbiology*. 7th edn. Edited by Spinger. New York.

Jepleting, M., Mutai, W., Musyoki, V.M., Oduor, B., Ayodo, C., Mugoh, R., Kariuki, S. and Omulo, S. (2023) '*Escherichia coli* pathotypes in children with acute diarrhea in an informal settlement in Nairobi, Kenya', *Advances in Microbiology*, 13(04), pp. 181–192. Available at: https://doi.org/10.4236/aim.2023.134011.

Johnning, A., Kristiansson, E., Fick, J., Weijdegård, B. and Larsson, D.G.J. (2015) 'Resistance mutations in *gyrA* and *parC* are common in *Escherichia* communities of both fluoroquinolone-polluted and uncontaminated aquatic environments.', *Frontiers in microbiology*, 6, p. 1355. Available at: https://doi.org/10.3389/fmicb.2015.01355.

Kagambèga, A., Martikainen, O., Siitonen, A., Traoré, A.S., Barro, N. and Haukka, K. (2012) 'Prevalence of diarrheagenic *Escherichia coli* virulence genes in the feces of slaughtered cattle, chickens, and pigs in Burkina Faso', *MicrobiologyOpen*, 1(3), pp.

276–284. Available at: https://doi.org/10.1002/mbo3.30.

Kasumba, I.N., Powell, H., Omore, R., Hossain, M.J., Sow, S.O., Ochieng, J.B., Badji, H., Verani, J.R., Widdowson, M.A., Sen, S., Nasrin, S., Permala-Booth, J., Jones, J.A., Roose, A., Nasrin, D., Sugerman, C.E., Juma, J., Awuor, A., Jones, J.C.M., Doh, S., Okoi, C., Zaman, S.M.A., Antonio, M., Hunsperger, E., Onyango, C., Platts-Mills, J., Liu, J., Houpt, E., Neuzil, K.M., Kotloff, K.L. and Tennant, S.M. (2023) 'Prevalence of *Salmonella* in stool during the vaccine impact on diarrhea in Africa (VIDA) Study, 2015-2018', *Clinical Infectious Diseases*, 76, pp. S87–S96. Available at: https://doi.org/10.1093/cid/ciac985.

Kchouk, M., Gibrat, J.F. and Elloumi, M. (2017) 'Generations of sequencing technologies: From first to next generation', *Biology and Medicine*, 09(03). Available at: https://doi.org/10.4172/0974-8369.1000395.

Khabo-Mmekoa, C.M., Genthe, B. and Momba, M.N.B. (2022) 'Enteric Pathogens Risk factors associated with household drinking water: A case study in Ugu District Kwa-Zulu Natal Province, South Africa', *International Journal of Environmental Research and Public Health*, 19(8). Available at: https://doi.org/10.3390/ijerph19084431.

Kim, M., Rodriguez-R, L.M., Hatt, J.K., Kayali, O., Nalá, R., Dunlop, A.L., Brennan, P.A., Corwin, E., Smith, A.K., Brown, J. and Konstantinidis, K.T. (2022) 'Higher pathogen load in children from Mozambique vs. USA revealed by comparative fecal microbiome profiling', *ISME Communications*, 2(1). Available at: https://doi.org/10.1038/s43705-022-00154-z.

Knee, J., Sumner, T., Adriano, Z., Berendes, D., de Bruijn, E., Schmidt, W.P., Nalá, R., Cumming, O. and Brown, J. (2018) 'Risk factors for childhood enteric infection in urban Maputo, Mozambique: A cross-sectional study', *PLoS Neglected Tropical Diseases*, 12(11), pp. 1–19. Available at: https://doi.org/10.1371/journal.pntd.0006956.

Komarulzaman, A., Smits, J. and De Jong, E. (2017) 'Clean water, sanitation and diarrhoea in Indonesia: Effects of household and community factors', *Global Public Health*, 12(9), pp. 1141–1155. Available at: https://doi.org/10.1080/17441692.2015.1127985.

Kombat, M.Y., Kushitor, S.B., Sutherland, E.K., Boateng, M.O. and Manortey, S. (2024)

'Prevalence and predictors of diarrhea among children under five in Ghana', *BMC Public Health*, 24(1), pp. 1–11. Available at: https://doi.org/10.1186/s12889-023-17575-7.

Koutsoumanis, K., Allende, A., Alvarez-Ordóñez, A., Bolton, D., Bover-Cid, S., Chemaly, M., Davies, R., De Cesare, A., Hilbert, F., Lindqvist, R., Nauta, M., Peixe, L., Ru, G., Simmons, M., Skandamis, P., Suffredini, E., Jenkins, C., Malorny, B., Ribeiro Duarte, A.S., Torpdahl, M., da Silva Felício, M.T., Guerra, B., Rossi, M. and Herman, L. (2019) 'Whole genome sequencing and metagenomics for outbreak investigation, source attribution and risk assessment of food-borne microorganisms', *EFSA Journal*, 17(12). Available at: https://doi.org/10.2903/j.efsa.2019.5898.

Kralicek, S.E., Sitaraman, L.M., Kuprys, P. V., Harrington, A.T., Ramakrishna, B., Osman, M. and Hecht, G.A. (2022) 'Clinical manifestations and stool load of atypical enteropathogenic *Escherichia coli* infections in united states children and adults', *Gastroenterology*, 163(5), pp. 1321–1333. Available at: https://doi.org/10.1053/j.gastro.2022.07.064.

Krishna, M., Makwana, N., Kakde, G.S., Puri, S. and Kharat, A.S. (2023) 'Knowledge and attitude toward antibiotic use and identification of financially feasible options to curb the spread of antibiotics in environment', 2023.

Kuria, J.K.N. (2024) 'Salmonellosis in food and companion animals and its public health importance', *Salmonella - perspectives for low-cost prevention, control and treatment* [Preprint]. Available at: https://doi.org/10.5772/intechopen.109324.

Lake, I.R. and Barker, G.C. (2018) 'Climate Change, foodborne pathogens and illness in higher-income countries', *Current environmental health reports*, 5(1), pp. 187–196. Available at: https://doi.org/10.1007/s40572-018-0189-9.

Lamichhane, B., Mawad, A.M.M., Saleh, M., Kelley, W.G., Harrington, P.J., Lovestad, C.W., Amezcua, J., Sarhan, M.M., El Zowalaty, M.E., Ramadan, H., Morgan, M. and Helmy, Y.A. (2024) 'Salmonellosis: An overview of epidemiology, pathogenesis, and innovative approaches to mitigate the antimicrobial resistant infections', *Antibiotics*, 13(1). Available at: https://doi.org/10.3390/antibiotics13010076.

Lennox, J.A., John, G.E. and Echa, C. (2020) 'Isolation of *Escherichia coli* 0157:H7 from selected food samples sold in local markets in Nigeria', *African Journal of Food Science*,

14(2), pp. 32–37. Available at: https://doi.org/10.5897/ajfs2019.1866.

Liu, Y., Chen, X., Luifu, J., Zhao, J., He, X. and Xie, T. (2023) 'Extended-spectrum β-lactamase-producing Enterobacteriaceae from ready-to-eat foods: Genetic diversity and antibiotic susceptibility', *Food Science and Nutrition*, 11(9), pp. 5565–5572. Available at: https://doi.org/10.1002/fsn3.3513.

López-Mendoza, M.C., Lozano, M., García-Romero, E., Ruiz-García, P., Gormaz, M., Jordano, R., Medina, L.M., Ramón-Beltrán, A. and Silvestre, D. (2023) 'Growth of *Escherichia coli* in human milk and powdered infant formula under various treatments and feeding conditions in neonatal units', *Applied Sciences (Switzerland)*, 13(15). Available at: https://doi.org/10.3390/app13158978.

Mabvouna, R.B., Béchir, A.S. Ben, Nkamedjie, M.P.P., Colizzi, V. and Sanou, M.S. (2023) 'Influence of latrine coverage and usage on diarrhoea incidence among children under 5 living in slum areas of Douala 5th sub-division, Cameroon', *Pan African Medical Journal*, 44. Available at: https://doi.org/10.11604/pamj.2023.44.82.11996.

Machava, N.E., Mulaudzi, F.M. and Salvador, E.M. (2022) 'Household factors of foodborne diarrhea in children under five in two Districts of Maputo, Mozambique', *International Journal of Environmental Research and Public Health*, 19(23). Available at: https://doi.org/10.3390/ijerph192315600.

Machava, N.E., Salvador, E.M. and Mulaudzi, F. (2022) 'Assessment of diagnosis and treatment practices of diarrhoea in children under five in Maputo-Mozambique', *International Journal of Africa Nursing Sciences*, 17(March). Available at: https://doi.org/10.1016/j.ijans.2022.100507.

Macicame, I., Kante, A.M., Wilson, E., Gilbert, B., Koffi, A., Nhachungue, S., Monjane, C., Duce, P., Adriano, A., Chicumbe, S., Jani, I., Kalter, H.D., Datta, A., Zeger, S., Black, R.E., Gudo, E.S. and Amouzou, A. (2023) 'Countrywide mortality surveillance for action in Mozambique: Results from a national sample-based vital statistics system for mortality and cause of death', *American Journal of Tropical Medicine and Hygiene*, 108(Suppl 5), pp. 5–16. Available at: https://doi.org/10.4269/ajtmh.22-0367.

Mahamat, S., Founou, R.C., Founou, L.L., Tchouangueu, T.F., Dimani, B.D., Nkengkana, O.A., Mafo, L.D., Chelo, D., Fonkoua, M.C., Boum-Ii, Y., Gonsu, H., Noubom, M.,

Olivier Koki Ndombo, P. and Gatsing, D. (2024) 'Occurrence of Extended-spectrum β-lactamase (ESBL) and Carbapenemase-producing *Escherichia coli* isolated from childhood diarrhoea in Yaoundé, Cameroon', *BMC microbiology*, 24(1), p. 401. Available at: https://doi.org/10.1186/s12866-024-03559-z.

Mahmud, I., Das, S., Khan, S.H., Faruque, A.S.G. and Ahmed, T. (2020) 'Gender disparity in care-seeking behaviours and treatment outcomes for dehydrating diarrhoea among under-5 children admitted to a diarrhoeal disease hospital in Bangladesh: An analysis of hospital-based surveillance data', *BMJ Open*, 10(9), pp. 1–9. Available at: https://doi.org/10.1136/bmjopen-2020-038730.

Mahmud, Z.H., Kabir, M.H., Ali, S., Moniruzzaman, M., Imran, K.M., Nafiz, T.N., Islam, M.S., Hussain, A., Hakim, S.A.I., Worth, M., Ahmed, D., Johnston, D. and Ahmed, N. (2020) 'Extended-spectrum beta-lactamase-producing *Escherichia coli* in drinking water samples from a forcibly displaced, densely populated community setting in Bangladesh', *Frontiers in Public Health*, 8(June), pp. 1–14. Available at: https://doi.org/10.3389/fpubh.2020.00228.

Makarova, M.A. (2023) 'A modern view of diarrheagenic *Escherichia coli*— a causative agent of acute intestinal infections', *Zhurnal Mikrobiologii Epidemiologii i Immunobiologii*, 100(4), pp. 333–344. Available at: https://doi.org/10.36233/0372-9311-410.

Makinde, O.M., Ayeni, K.I., Sulyok, M., Krska, R., Adeleke, R.A. and Ezekiel, C.N. (2020) 'Microbiological safety of ready-to-eat foods in low- and middle-income countries: A comprehensive 10-year (2009 to 2018) review', *Comprehensive Reviews in Food Science and Food Safety*, 19(2), pp. 703–732. Available at: https://doi.org/10.1111/1541-4337.12533.

Manhique-Coutinho, L., Chiani, P., Michelacci, V., Taviani, E., Bauhofer, A.F.L., Chissaque, A., Cossa-Moiane, I., Sambo, J., Chilaúle, J., Guimarães, E.L., Salência, J., Cassocera, M., Bero, D.M., Langa, J.P. and de Deus, N. (2022) 'Molecular characterization of diarrheagenic *Escherichia coli* isolates from children with diarrhea: A cross-sectional study in four provinces of Mozambique: Diarrheagenic *Escherichia coli* in Mozambique', *International Journal of Infectious Diseases*, 121, pp. 190–194. Available at: https://doi.org/10.1016/j.ijid.2022.04.054.

Marege, A., Regassa, B., Seid, M., Tadesse, D., Siraj, M. and Manilal, A. (2023) 'Bacteriological quality and safety of bottle food and associated factors among bottle-fed babies attending pediatric outpatient clinics of Government Health Institutions in Arba Minch, southern Ethiopia', *Journal of Health, Population and Nutrition*, 42(1), pp. 1–18. Available at: https://doi.org/10.1186/s41043-023-00387-1.

Massella, E., Reid, C.J., Cummins, M.L., Anantanawat, K., Zingali, T., Serraino, A., Piva, S., Giacometti, F. and Djordjevic, S.P. (2020) 'Snapshot study of whole genome sequences *of Escherichia coli* from healthy companion animals, Livestock, Wildlife, Humans and Food in Italy', pp. 1–22.

Mbae, C., Mwangi, M., Gitau, N., Irungu, T., Muendo, F., Wakio, Z., Wambui, R., Kavai, S., Onsare, R., Wairimu, C., Ngetich, R., Njeru, F., Van Puyvelde, S., Clemens, J., Dougan, G. and Kariuki, S. (2020) 'Factors associated with occurrence of salmonellosis among children living in Mukuru slum, an urban informal settlement in Kenya', *BMC Infectious Diseases*, 20(1), pp. 1–12. Available at: https://doi.org/10.1186/s12879-020-05134-z.

Mensah, P., Mwamakamba, L., Mahomed, C. and Nsue-Milang, D. (2012) *Public health and food safety in the who African region*.

Meza-Segura, M., Zaidi, M.B., Vera-Ponce de León, A., Moran-Garcia, N., Martinez-Romero, E., Nataro, J.P. and Estrada-Garcia, T. (2020) 'New Insights Into DAEC and EAEC pathogenesis and phylogeny', *Frontiers in Cellular and Infection Microbiology*, 10(October). Available at: https://doi.org/10.3389/fcimb.2020.572951.

Miri-Aliabad, G., Khajeh, A. and Teimouri, A. (2021) 'Infants teething problems and mothers' beliefs in south east of iran', *Journal of Comprehensive Pediatrics*, 12(1), pp. 0–4. Available at: https://doi.org/10.5812/COMPREPED.62531.

MISAU (1997) *Manual de Microbiologia Alimentar*. Maputo.

Mohapi, D.A., Ramatla, T., Thekisoe, O., Khetsha, Z.P. and Nkhebenyane, J. (2025) 'Antimicrobial resistance profiles of bacteria isolated from fresh vegetables in Free State Province, South Africa', *Foods*, 14(12), p. 2139. Available at: https://doi.org/10.3390/foods14122139.

Mokhtari, W., Nsaibia, S., Majouri, D., Ben Hassen, A., Gharbi, A. and Aouni, M. (2012) 'Detection and characterization of *Shigella* species isolated from food and human stool samples in Nabeul, Tunisia, by molecular methods and culture techniques', *Journal of Applied Microbiology*, 113(1), pp. 209–222. Available at: https://doi.org/10.1111/j.1365-2672.2012.05324.x.

Momtaz, H. and Yadollahi, S. (2013) 'Molecular characterization of *Listeria monocytogenes* isolated from fresh seafood samples in Iran.', *Diagnostic pathology*, 8, p. 149. Available at: https://doi.org/10.1186/1746-1596-8-149.

Moran-Gilad, J. (2017) 'Whole genome sequencing (WGS) for food-borne pathogen surveillance and control-taking the pulse', p. 3. Available at: https://doi.org/10.2807/1560-7917.ES.2017.22.23.30547.

Mulu, B.M., Belete, M.A., Demlie, T.B., Tassew, H. and Sisay Tessema, T. (2024) 'Characteristics of pathogenic *Escherichia coli* associated with diarrhea in children under five years in Northwestern Ethiopia', *Tropical Medicine and Infectious Disease*, 9(3). Available at: https://doi.org/10.3390/tropicalmed9030065.

Mwape, K., Bosomprah, S., Chibesa, K., Silwamba, S., Luchen, C.C., Sukwa, N., Mubanga, C., Phiri, B., Chibuye, M., Liswaniso, F., Somwe, P., Chilyabanyama, O., Chisenga, C.C., Muyoyeta, M., Simuyandi, M., Barnard, T.G. and Chilengi, R. (2023) 'Prevalence of Diarrhoeagenic *Escherichia coli* among children aged between 0–36 months in peri-urban areas of Lusaka', *Microorganisms*, 11(11), pp. 1–13. Available at: https://doi.org/10.3390/microorganisms11112790.

Mwisha Kinkese, D., Bernard Hang, M., Toure, O., Kinkese, T. and Kangwa, E. (2018) 'Contamination of complementary weaning foods for children with *Escherichia coli* and *Salmonella* species in Lusaka District', *Zambia. Journal of Preventive and Rehabilitative Medicine*, 1(1), pp. 19–31. Available at: https://doi.org/10.21617/jprm.2018.0101.3.

Nakamura, M., Hamazaki, K., Matsumura, K., Kasamatsu, H., Tsuchida, A., Inadera, H. and Group, on behalf of the J.E. and C.S. (2022) 'Dietary intake of yogurt and cheese in children at age 1 year and sleep duration at age 1 and 3 years: the Japan Environment and Children's Study', *BMC Pediatrics*, 22(1), pp. 1–10. Available at: https://doi.org/10.1186/s12887-022-03633-3.

Nemeth, V. and Pfleghaar, N. (2022) 'Diarrhea', in. Treasure Island (FL): StatPearls Publishing: StatPearls [Internet]. Available at: https://pubmed.ncbi.nlm.nih.gov/28846339/.

Ngaywa, C., Aboge, G.O., Obiero, G., Omwenga, I., Ngwili, N., Wamwere, G., Wainaina, M. and Bett, B. (2019) 'Antimicrobial resistant *Escherichia coli* isolates detected in raw milk of livestock in pastoral areas of northern Kenya', *Food Control*, 102(December 2018), pp. 173–178. Available at: https://doi.org/10.1016/j.foodcont.2019.03.008.

Nguyen, T.Y.C., Fagbayigbo, B.O., Cissé, G., Redi, N., Fuhrimann, S., Okedi, J., Schindler, C., Röösli, M., Armitage, N.P., Carden, K. and Dalvie, M.A. (2021) 'Diarrhoea among children aged under five years and risk factors in informal settlements: A cross-sectional study in Cape Town, South Africa', *International Journal of Environmental Research and Public Health*, 18(11). Available at: https://doi.org/10.3390/ijerph18116043.

Nhampossa, T., Mandomando, I., Acacio, S., Quintó, L., Vubil, D., Ruiz, J., Nhalungo, D., Sacoor, C., Nhabanga, A., Nhacolo, A., Aide, P., Machevo, S., Sigaúque, B., Nhama, A., Kotloff, K., Farag, T., Nasrin, D., Bassat, Q., Macete, E., Levine, M.M. and Alonso, P. (2015) 'Diarrheal disease in rural Mozambique: Burden, risk factors and etiology of diarrheal disease among children aged 0-59 months seeking care at health facilities', *PLoS ONE*, 10(5), pp. 12–23. Available at: https://doi.org/10.1371/journal.pone.0119824.

Nisa, I., Qasim, M., Driessen, A., Nijland, J., Rafiullah, Ali, A., Mirza, M.R., Khan, M.A., Khan, T.A., Jalal, A. and Rahman, H. (2021) 'Prevalence and associated risk factors of *Shigella flexneri* isolated from drinking water and retail raw foods in Peshawar, Pakistan', *Journal of Food Science*, 86(6), pp. 2579–2589. Available at: https://doi.org/10.1111/1750-3841.15777.

Nouws, S., Bogaerts, B., Verhaegen, B., Denayer, S., Cromb, F., De Rauw, K., Pi, D., Marchal, K., Vanneste, K., Roosens, N.H.C. and De Keersmaecker, S.C.J. (2020) 'The benefits of whole genome sequencing for foodborne outbreak investigation from the perspective of a national reference laboratory in a smaller country', *Foods*, 9(1030).

O'Reilly, C.E., Jaron, P., Ochieng, B., Nyaguara, A., Tate, J.E., Parsons, M.B., Bopp,

C.A., Williams, K.A., Vinjé, J., Blanton, E., Wannemuehler, K.A., Vulule, J., Laserson, K.F., Breiman, R.F., Feikin, D.R., Widdowson, M.A. and Mintz, E. (2012) 'Risk factors for death among children less than 5 years old hospitalized with diarrhea in rural Western Kenya, 2005-2007: A cohort study', *PLoS Medicine*, 9(7), pp. 2005–2007. Available at: https://doi.org/10.1371/journal.pmed.1001256.

Ocampo, K., Riveros, M., Pinedo-Bardales, M., Ruiz, J. and Ochoa, T.J. (2021) 'Frequency of serine protease autotransporters of enterobacteriaceae (Spate) encoding genes in diffusely adherent escherichia coli (daec) isolates from children with and without diarrhea', *Revista Peruana de Medicina Experimental y Salud Publica*, 38(1), pp. 124–129. Available at: https://doi.org/10.17843/rpmesp.2021.381.5750.

Odo, C., Onalu, C., Nwatu, U., Nwafor, N. and Ebimgbo, S. (2023) 'Factors associated with the prevalence of diarrhoea among children in rural areas of Enugu State, Nigeria: Practice considerations for social workers', *International Social Work*, 66(2), pp. 518–533. Available at: https://doi.org/10.1177/00208728211034229.

Ogunbiyi, T.S., Fayemi, O.E., Akanni, G.B., Ayolabi, C.I. and Hald, T. (2023) 'Molecular characterization of hetero-pathogenic and diarrheagenic *Escherichia coli* pathotypes in diarrheic children under five years and exposure environment in Ogun State, South-West Nigeria', *Pathogens*, 12(11), pp. 1–17. Available at: https://doi.org/10.3390/pathogens12111358.

Okeke, I.N. (2009) 'Diarrheagenic *Escherichia coli* in sub-Saharan Africa: Status, uncertainties and necessities', *Journal of Infection in Developing Countries*, 3(11), pp. 817–842. Available at: https://doi.org/10.3855/jidc.586.

Okumu, N.O., Muloi, D.M., Moodley, A., Ochien'g, L., Watson, J., Kiarie, A., Ngeranwa, J.J.N., Cumming, O. and Cook, E.A.J. (2025) 'Epidemiology of Antimicrobial-resistant diarrheagenic *Escherichia coli* pathotypes from children, livestock and food in Dagoretti South, Nairobi Kenya', *International Journal of Antimicrobial Agents*, 65(3), p. 107419. Available at: https://doi.org/10.1016/j.ijantimicag.2024.107419.

Okumu, N.O., Ngeranwa, J.J.N., Muloi, D.M., Ochien, L., Moodley, A., Mutisya, C., Kiarie, A., Wasonga, J.O., Watson, J., Amon-Tanoh, M.A., Cumming, O. and Cook,

E.A.J. (2023) 'Risk factors for diarrheagenic *Escherichia coli* infection in children aged 6–24 months in peri-urban community, Nairobi, Kenya', *PLOS Global Public Health*, 3(11), pp. 1–16. Available at: https://doi.org/10.1371/journal.pgph.0002594.

Olayanju, A., Mellor, D., Khatri, Y. and Pickles, N. (2023) 'The efficacy of fermented foods in the treatment and management of diarrhoeal diseases: A systematic review and meta-analysis', *Nutrition and Health*, 29(1), pp. 71–83. Available at: https://doi.org/10.1177/02601060221095678.

ONU (2019) *Em Moçambique, mais de 500 mil pessoas tiveram doenças causadas por consumo de alimentos inseguros | ONU News*. Available at: https://news.un.org/pt/story/2019/06/1675251 (Accessed: 27 April 2020).

Pakbin, B., Brück, W.M. and Brück, T.B. (2023) 'Molecular mechanisms of *Shigella* pathogenesis; Recent advances', *International Journal of Molecular Sciences*, 24(3). Available at: https://doi.org/10.3390/ijms24032448.

Partridge, S.R., Kwong, S.M., Firth, N. and Jensen, S.O. (2018) 'Mobile genetic elements associated with antimicrobial resistance', 31(4), pp. 1–61.

Parvez, S.M., Azad, R., Pickering, A.J., Kwong, L.H., Arnold, B.F., Rahman, M.J., Rahman, M.Z., Alam, M., Sen, D., Islam, S., Rahman, M., Colford, J.M., Luby, S.P., Unicomb, L. and Ercumen, A. (2019) 'Microbiological contamination of young children's hands in rural Bangladesh: Associations with child age and observed hand cleanliness as proxy', *PLoS ONE*, 14(9), pp. 1–15. Available at: https://doi.org/10.1371/journal.pone.0222355.

Parvez, S.M., Kwong, L., Rahman, M.J., Ercumen, A., Pickering, A.J., Ghosh, P.K., Rahman, M.Z., Das, K.K., Luby, S.P. and Unicomb, L. (2017) '*Escherichia coli* contamination of child complementary foods and association with domestic hygiene in rural Bangladesh', *Tropical Medicine and International Health*, 22(5), pp. 547–557. Available at: https://doi.org/10.1111/tmi.12849.

Pashapour, N. and Iou, S.G. (2006) 'Evaluation of yogurt effect on acute diarrhea in 6-24-month-old hospitalized infants', *Turkish Journal of Pediatrics*, 48(2), pp. 115–118.

Patro-Gołab, B., Shamir, R. and Szajewska, H. (2015) 'Yogurt for treating acute

gastroenteritis in children: Systematic review and meta-analysis', *Clinical Nutrition*, 34(5), pp. 818–824. Available at: https://doi.org/10.1016/j.clnu.2014.09.004.

Penakalapati, G., Swarthout, J., Delahoy, M.J., McAliley, L., Wodnik, B., Levy, K. and Freeman, M.C. (2017) 'Exposure to animal feces and human health: a systematic review and proposed research priorities', *Environmental Science and Technology*, 51(20), pp. 11537–11552. Available at: https://doi.org/10.1021/acs.est.7b02811.

Penders, J., Stobberingh, E.E., Savelkoul, P.H.M. and Wolffs, P.F.G. (2013) 'The human microbiome as a reservoir of antimicrobial resistance', 4(April), pp. 1–7. Available at: https://doi.org/10.3389/fmicb.2013.00087.

Pickering, A.J., Ercumen, A., Arnold, B.F., Kwong, L.H., Parvez, S.M., Alam, M., Sen, D., Islam, S., Kullmann, C., Chase, C., Ahmed, R., Unicomb, L., Colford, J.M. and Luby, S.P. (2018) 'Fecal indicator bacteria along multiple environmental transmission pathways (water, hands, food, soil, flies) and subsequent child diarrhea in rural Bangladesh', *Environmental Science and Technology*, 52(14), pp. 7928–7936. Available at: https://doi.org/10.1021/acs.est.8b00928.

Pickering, A.J., Julian, T.R., Marks, S.J., Mattioli, M.C., Boehm, A.B., Schwab, K.J. and Davis, J. (2012) 'Fecal contamination and diarrheal pathogens on surfaces and in soils among Tanzanian households with and without improved sanitation', *Environmental Science and Technology*, 46(11), pp. 5736–5743. Available at: https://doi.org/10.1021/es300022c.

Pinto, A. (1996) 'Doenças de origem microbiana transmitidas pelos alimentos', *Millenium*, (February), pp. 91–100.

Pires, S.M., Majowicz, S., Gill, A. and Devleesschauwer, B. (2019) 'Global and regional source attribution of shiga toxin-producing *Escherichia coli* infections using analysis of outbreak surveillance data', *Epidemiology and Infection*, 147. Available at: https://doi.org/10.1017/S095026881900116X.

Pires, S.M., Vieira, A.R., Hald, T. and Cole, D. (2014) 'Source attribution of human salmonellosis: An overview of methods and estimates', *Foodborne Pathogens and Disease*, 11(9), pp. 667–676. Available at: https://doi.org/10.1089/fpd.2014.1744.

Podolak, R., Enache, E., Stone, W., Black, D.G. and Elliott, P.H. (2010) 'Sources and risk factors for contamination, survival, persistence, and heat resistance of Salmonella in low-moisture foods', *Journal of Food Protection*, 73(10), pp. 1919–1936. Available at: https://doi.org/10.4315/0362-028X-73.10.1919.

Poirel, L., Madec, J., Lupo, A., Schink, A., Kieffer, N., Nordmann, P. and Schwarz, S. (2018) 'Antimicrobial resistance in *Escherichia coli* Sepsis', *Microbiol Spectrum*, 6(4), pp. 1–27. Available at: https://doi.org/10.1128/microbiolspec.ARBA-0026-2017.

Pornsukarom, S., Van Vliet, A.H.M. and Thakur, S. (2018) 'Whole genome sequencing analysis of multiple *Salmonella* serovars provides insights into phylogenetic relatedness, antimicrobial resistance, and virulence markers across humans, food animals and agriculture environmental sources', *BMC Genomics*, 19(1), p. 801. Available at: https://doi.org/10.1186/s12864-018-5137-4.

Potgieter, N., Obi, C.L., Bessong, P.O., Igumbor, E.O., Samie, A. and Nengobela, R. (2005) 'Bacterial contamination of Vhuswa - A local weaning food and stored drinking-water in impoverished households in the Venda region of South Africa', *Journal of Health, Population and Nutrition*, 23(2), pp. 150–155.

Qiu, J., Jiang, Z., Ju, Z., Zhao, X., Yang, J., Guo, H. and Sun, S. (2019) 'molecular and phenotypic characteristics of *Escherichia coli* isolates from farmed minks in Zhucheng , China', 2019. Available at: https://doi.org/10.1155/2019/3917841.

Rambaut, A. (2018) *Molecular evolution, phylogenitics and epidemiology*. Available at: http://tree.bio.ed.ac.uk/software/figtree/.

Rappelli, P., Folgosa, E., Solinas, M.L., DaCosta, J.L., Pisanu, C., Sidat, M., Melo, J., Cappuccinelli, P. and Colombo, M.M. (2005) 'Pathogenic enteric *Escherichia coli* in children with and without diarrhea in Maputo, Mozambique', *FEMS Immunology and Medical Microbiology*, 43(1), pp. 67–72. Available at: https://doi.org/10.1016/j.femsim.2004.07.006.

Raya, S., Malla, B., Thakali, O., Angga, M.S., Segawa, T., Sherchand, J.B. and Haramoto, E. (2024) 'Validation and application of high-throughput quantitative PCR for the simultaneous detection of microbial source tracking markers in environmental water', *Science of the Total Environment*, 940(May), p. 173604. Available at:

https://doi.org/10.1016/j.scitotenv.2024.173604.

Rich, K.M., Schaefer, K.A., Thapa, B., Hagerman, A.D. and Shear, H.E. (2022) 'An overview of meat processing in Africa', in. resakss.org, pp. 1–11.

Ripley, B., Venables, B., Bates, D.M., Hornik, K., Gebhardt, A. and Firth, D. (2024) *Package ' MASS '*.

Rohmah, H., Hafsah, T. and Rakhmilla, L.E. (2015) 'Role of exclusive breastfeeding in preventing diarrhea', *Althea Medical Journal*, 2(1), pp. 78–81. Available at: https://doi.org/10.15850/amj.v2n1.436.

Roy, B., Das, T. and Bhattacharyya, S. (2023) 'Overview on old and new biochemical test for bacterial identification scholastic microbiology overview on old and new biochemical test for bacterial identification', (October). Available at: https://www.researchgate.net/publication/374755011.

Rúgeles, L.C., Bai, J., Martínez, A.J., Vanegas, M.C. and Gómez-Duarte, O.G. (2010) 'Molecular characterization of diarrheagenic *Escherichia coli* strains from stools samples and food products in Colombia', *International Journal of Food Microbiology*, 138(3), pp. 282–286. Available at: https://doi.org/10.1016/j.ijfoodmicro.2010.01.034.

Rychert, J. (2019) 'Benefits and limitations of MALDI-TOF Mass Spectrometry for the identification of microorganisms', *Journal of Infectiology*, 2(4), pp. 1–5. Available at: https://doi.org/10.29245/2689-9981/2019/4.1142.

Saha, J., Mondal, S., Chouhan, P., Hussain, M., Yang, J. and Bibi, A. (2022) 'Occurrence of diarrheal disease among under-five children and associated sociodemographic and household environmental factors: an investigation based on national family health survey-4 in Rural India', *Children*, 9(5). Available at: https://doi.org/10.3390/children9050658.

Saidenberg, A.B.S., Vliet, A.H.M. Van, Eduardo, P., Rose, L., Sá, M. De, Paulo, M., Cunha, V., Roberto, M., Rose, L., Sá, M. De, Paulo, M., Cunha, V., Ragione, R.M. La and S, L.R.M. De (2020) 'Genomic characterization of enteropathogenic *Escherichia coli* (EPEC) of avian origin and rabbit ileal loop response ; a pet macaw (Ara chloropterus) as a possible zoonotic reservoir', *Veterinary Quarterly*, 40(1), pp. 331–341. Available at: https://doi.org/10.1080/01652176.2020.1845916.

Saka, H.K., Dabo, N.T., Muhammad, B., García-Soto, S., Ugarte-Ruiz, M. and Alvarez, J. (2019) 'Diarrheagenic *Escherichia coli* pathotypes from children younger than 5 years in Kano State, Nigeria', *Frontiers in Public Health*, 7(November), pp. 1–8. Available at: https://doi.org/10.3389/fpubh.2019.00348.

Salamandane, A., Alves, S., Chambel, L., Malfeito-Ferreira, M. and Brito, L. (2022) 'Characterization of *Escherichia coli* from water and food sold on the streets of Maputo: Molecular typing, virulence genes, and antibiotic resistance', *Applied Microbiology*, 2(1), pp. 133–147. Available at: https://doi.org/10.3390/applmicrobiol2010008.

Salamandane, A., Vila-Boa, F., Malfeito-Ferreira, M. and Brito, L. (2021) 'High fecal contamination and high levels of antibiotic-resistant enterobacteriaceae in water consumed in the city of Maputo, Mozambique', *Biology*, 10(6). Available at: https://doi.org/10.3390/biology10060558.

Samad, A., Abbas, F., Yousaf, M., Hassan, Y., Naeem, M., Zahid, M., Pokryshko, O. and Diaconescu, S. (2018) 'Isolation & identification of *Shigella* species from food and water samples of Quetta, Pakistan', *Pure and Applied Biology*, 7(1), pp. 227–235. Available at: https://doi.org/10.19045/bspab.2018.70027.

Sambe-Ba, B., Espié, E., Faye, M.E., Timbiné, L.G., Sembene, M. and Gassama-Sow, A. (2013) 'Community-acquired diarrhea among children and adults in urban settings in Senegal: Clinical, epidemiological and microbiological aspects', *BMC Infectious Diseases*, 13(1). Available at: https://doi.org/10.1186/1471-2334-13-580.

Santos, F.S. tabno., Santos, F.C. ésa. S., Santos, L.H. unald. dos, Leite, A.M. orae. and Mello, D.F. de (2015) 'Breastfeeding and protection against diarrhea: an integrative review of literature', *Einstein (São Paulo, Brazil)*, pp. 435–440. Available at: https://doi.org/10.1590/S1679-45082015RW3107.

Scarano, C., Veneruso, I., De Simone, R.R., Di Bonito, G., Secondino, A. and D'Argenio, V. (2024) 'The Third-generation sequencing challenge: novel insights for the omic sciences', *Biomolecules*, 14(5), pp. 1–20. Available at: https://doi.org/10.3390/biom14050568.

Schadron, T., van den Beld, M., Mughini-Gras, L. and Franz, E. (2024) 'Use of whole genome sequencing for surveillance and control of foodborne diseases: status quo and

quo vadis', *Frontiers in Microbiology*, 15(September), pp. 1–13. Available at: https://doi.org/10.3389/fmicb.2024.1460335.

Schneider, K.R., Ahn, S., Goodrich-schneider, R.M., Shigella, W. and Gram-negative, S. (2012) 'Preventing Foodborne Illness : Shigellosis', pp. 1–5.

Sinigalliano, C., Kim, K., Gidley, M., Yuknavage, K., Knee, K., Palacios, D., Bautista, C., Bonacolta, A., Lee, H.W. and Maurin, L. (2021) 'Microbial source tracking of fecal indicating bacteria in coral reef waters, recreational waters, and groundwater of saipan by real-time quantitative PCR', *Frontiers in Microbiology*, 11(January), pp. 1–20. Available at: https://doi.org/10.3389/fmicb.2020.596650.

Siregar, U.R. and Susanna, D. (2020) '*Escherichia Coli* in Musca Domestica Flies toward the incidence of diarrhoea in children under five years in around the location of traditional fish processing in the North Jakarta', *Indian Journal of Public Health Research & Development*, 11(03), pp. 1196–1200. Available at: https://doi.org/10.37506/ijphrd.v11i3.1576.

Soubeiga, A.P., Kpoda, D.S., Compaoré, M.K.A., Somda-Belemlougri, A., Kaseko, N., Rouamba, S.S., Ouedraogo, S., Traoré, R., Karfo, P., Nezien, D., Nikiéma, F., Kabre, E., Zongo, C. and Savadogo, A. (2022) 'Molecular characterization and the antimicrobial resistance profile of *Salmonella* spp. isolated from ready-to-eat foods in Ouagadougou, Burkina Faso', *International Journal of Microbiology*, 2022. Available at: https://doi.org/10.1155/2022/9640828.

Staley, Z.R., Boyd, R.J., Shum, P. and Edge, T.A. (2018) 'Microbial source tracking using quantitative and digital PCR to identify sources of fecal contamination in stormwater, river water, and beach water in a Great Lakes area of concern', *Applied and Environmental Microbiology*, 84(20). Available at: https://doi.org/10.1128/AEM.01634-18.

Suge, T., Magu, D. and Wanzala, P. (2023) 'Correlation between *Escherichia coli* resistant gene isolated from stool samples of children under five years and that from consumed water', *Scientific African*, 22(September), p. e01937. Available at: https://doi.org/10.1016/j.sciaf.2023.e01937.

Taşkale Karatuğ, N., Yüksel, F.N., Akçelik, N. and Akçelik, M. (2018) 'Genetic diversity

of food originated *Salmonella* isolates', *Biotechnology and Biotechnological Equipment*, 32(3), pp. 638–645. Available at: https://doi.org/10.1080/13102818.2018.1451779.

Tenea, G.N., Reyes, P., Molina, D. and Ortega, C. (2023) 'Pathogenic Microorganisms linked to fresh fruits and juices purchased at low-cost markets in Ecuador, Potential Carriers of Antibiotic Resistance', *Antibiotics*, 12(2). Available at: https://doi.org/10.3390/antibiotics12020236.

Tenny, S., Kerndt, C.C. and Hoffman, M.R. (2023) 'Case Control Studies', *Textbook of Clinical Epidemiology: for Medical Students*, pp. 83–104. Available at: https://doi.org/10.1007/978-981-99-3622-9_5.

Todar, K. (2012) *Bacterial Resistance to Antibiotics*. Available at: http://textbookofbacteriology.net/resantimicrobial.html (Accessed: 12 February 2020).

Tokponnon, T.F., Agassounon, B.B.E., Kougblenou, D.S., Osse, R., Dougnon, V., Daga, C.N., Kossou, J., Gounou Yerima, I.J., Houessinon, F., Zoulkifilou, S.D. and Akogbeto, M. (2023) 'Potential Risk of Transmission of *Escherichia coli* and *Salmonella* spp. Infections by the Musca domestica Fly and the Periplaneta americana Cockroach in the City of Cotonou (South Benin)', *Advances in Microbiology*, 13(09), pp. 448–461. Available at: https://doi.org/10.4236/aim.2023.139029.

Tosisa, W., Mihret, A., Ararsa, A., Eguale, T. and Abebe, T. (2020) 'Prevalence and antimicrobial susceptibility of *Salmonell*a and *Shigella* species isolated from diarrheic children in Ambo town', *BMC Pediatrics*, 20(1), pp. 1–8. Available at: https://doi.org/10.1186/s12887-020-1970-0.

Trabulsi, L.R., Keller, R. and Tardelli Gomes, T.A. (2002) 'Typical and atypical enteropathogenic Escherichia coli', *Emerging Infectious Diseases*, 8(5), pp. 508–513. Available at: https://doi.org/10.3201/eid0805.010385.

Tsai, K., Hoffmann, V., Simiyu, S., Cumming, O., Borsay, G. and Baker, K.K. (2022) 'Bacteroides Microbial Source Tracking Markers Perform Poorly in Predicting Enterobacteriaceae and Enteric Pathogen Contamination of Cow Milk Products and Milk-Containing Infant Food', *Frontiers in Microbiology*, 12(January). Available at: https://doi.org/10.3389/fmicb.2021.778921.

Tsai, K., Simiyu, S., Mumma, J., Aseyo, R.E., Cumming, O., Dreibelbis, R. and Baker, K.K. (2019) 'Enteric pathogen diversity in infant foods in low-income neighborhoods of kisumu, kenya', *International Journal of Environmental Research and Public Health*, 16(3). Available at: https://doi.org/10.3390/ijerph16030506.

Ugbogu, O.C., Nwachukwu, N.C. and Ogbuagu, U.N. (2006) 'Isolation of Salmonella and Shigella species from house flies (Musca domestica l.) in Uturu, Nigeria', *African Journal of Biotechnology*, 5(11), pp. 1090–1091. Available at: https://doi.org/10.4314/ajb.v5i11.42976.

UNICEF (2021) *The situation of children in Mozambique*. Available at: https://www.unicef.org/mozambique/media/4976/file/The Situation of Children in Mozambique 2021.pdf.

Urio, E.M., Collison, E.K., Gashe, B.A., Sebunya, T.K. and Mpuchane, S. (2001) '*Shigella* and *Salmonella* strains isolated from children under 5 years in Gaborone, Botswana, and their antibiotic susceptibility patterns', *Tropical Medicine and International Health*, 6(1), pp. 55–59. Available at: https://doi.org/10.1046/j.1365-3156.2001.00668.x.

Varela, G., Batthyány, L., Bianco, M.N., Pérez, W., Pardo, L., Algorta, G., Robino, L., Suárez, R., Navarro, A., Pírez, M.C. and Schelotto, F. (2015) 'Enteropathogens associated with acute diarrhea in children from households with high socioeconomic level in uruguay', *International Journal of Microbiology*, 2015. Available at: https://doi.org/10.1155/2015/592953.

Vincent, A.T., Derome, N., Boyle, B., Culley, A.I. and Charette, S.J. (2017) 'Next-generation sequencing (NGS) in the microbiological world: How to make the most of your money', *Journal of Microbiological Methods*, 138, pp. 60–71. Available at: https://doi.org/10.1016/j.mimet.2016.02.016.

Vu Nguyen, T., Le Van, P., Le Huy, C., Nguyen Gia, K. and Weintraub, A. (2006) 'Etiology and epidemiology of diarrhea in children in Hanoi, Vietnam', *International Journal of Infectious Diseases*, 10(4), pp. 298–308. Available at: https://doi.org/10.1016/j.ijid.2005.05.009.

Vubil, D., Acácio, S., Quintò, L., Balleste-Delpierre, C., Nhampossa, T., Kotloff, K.,

Levine, M.M., Alonso, P., Nataro, J.P., Farag, T.H., Vila, J. and Mandomando, I. (2018) 'Clinical features, risk factors, and impact of antibiotic treatment of diarrhea caused by Shigella in children less than 5 years in Manhiça district, rural Mozambique', *Infection and Drug Resistance*, 11, pp. 2095–2106. Available at: https://doi.org/10.2147/IDR.S177579.

Walker-Daniels, J. (2012) 'Current PCR Methods', *Materials and Methods*, 2. Available at: https://doi.org/10.13070/mm.en.2.119.

Wells, J., Abugo, D.G., Angong, J., Lamwaka, N.G., Gallandat, K., Hassan, J.L., Deng, L., Save, D., Braun, L., Gose, M., Amanya, J., Ayoub, K., King, S., Stobaugh, H., Cumming, O. and D'Mello-Guyett, L. (2023) 'Risk factors for food contamination among children discharged from community management of acute malnutrition programmes in South Sudan: A cross-sectional study and hazard analysis critical control point approach', *Maternal and Child Nutrition* [Preprint], (April). Available at: https://doi.org/10.1111/mcn.13612.

WHO (2015) *WHO estimates of the global burden of foodborne diseases*. Available at: https://apps.who.int/iris/bitstream/handle/10665/199350/9789241565165_eng.pdf.

WHO (2018a) *A child under 15 dies every 5 seconds around the world*. Available at: https://www.who.int/news/item/18-09-2018-a-child-under-15-dies-every-5-seconds-around-the-world-.

WHO (2018b) *E. coli*. Available at: https://www.who.int/news-room/fact-sheets/detail/e-coli.

WHO (2019) *Food safety*. Available at: https://www.who.int/news-room/fact-sheets/detail/food-safety (Accessed: 2 December 2019).

WHO (2020) 'Children: improving survival and well-being'. Available at: https://www.who.int/news-room/fact-sheets/detail/children-reducing-mortality (Accessed: 12 February 2020).

WHO (2022) *Enterotoxigenic Escherichia coli (ETEC)*. Available at: https://www.who.int/teams/immunization-vaccines-and-biologicals/diseases/enterotoxigenic-escherichia-coli-(etec).

WHO (2023) *Antimicrobial resistance*. Available at: https://www.who.int/news-room/fact-sheets/detail/antimicrobial-resistance.

WHO (2024a) *Diarrhoeal disease*. Available at: https://www.who.int/news-room/fact-sheets/detail/diarrhoeal-disease.

WHO (2024b) *Food safety*. Available at: https://www.who.int/news-room/fact-sheets/detail/food-safety (Accessed: 4 February 2025).

WHO (2025) *Estimating the global burden of foodborne diseases*. Available at: https://www.who.int/activities/estimating-the-burden-of-foodborne-diseases.

Winfield, M.D. and Groisman, E.A. (2003) 'Role of nonhost environments in the lifestyles of *Salmonella* and *Escherichia coli*', *Applied and Environmental Microbiology*, 69(7), pp. 3687–3694. Available at: https://doi.org/10.1128/AEM.69.7.3687-3694.2003.

Winn, W., Alen, S., Koneman, E. and Procop, G. Schreckenberger, P. Woods, G. (2010) *Diagnóstico Microbiológico: Texto e Atlas Colorido*. 6th edn. Edited by G. Koogan. Rio de Janeiro.

Woldu, W., Bitew, B.D. and Gizaw, Z. (2016) 'Socioeconomic factors associated with diarrheal diseases among under-five children of the nomadic population in northeast Ethiopia', *Tropical Medicine and Health*, 44(1), pp. 7–14. Available at: https://doi.org/10.1186/s41182-016-0040-7.

World Bank (2018) *Food-borne Illnesses Cost US$ 110 Billion Per Year in Low- and Middle-Income Countries*. Available at: ww.worldbank.org/en/news/press-release/2018/10/23/food-borne-illnesses-cost-us-110-billion-per-year-in-low-and-middle-income-countries.

Worrall, G. (2011) 'Diagnosing ARIs Series Acute cough in children', 57, pp. 315–318.

Yeda, R., Makalliwa, G., Apondi, E., Sati, B., Riziki, L., Ouma, C., Anguko, E., Opot, B., Okoth, R., Koech, E.J., Ochieng, B., Gachohi, J. and Kikuvi, G. (2024) 'Comparative prevalence of diarrheagenic *Escherichia coli* between children below five years with close contact to food animals in Kisumu County, Kenya', *Pan African Medical Journal*, 47. Available at: https://doi.org/10.11604/pamj.2024.47.25.41197.

Yihunie, F.B., Belete, M.A., Fentahun, G. and Dubie, T. (2024) 'Molecular detection and antibiogram of Shiga toxin-producing *Escherichia coli* (STEC) from raw milk in and around Bahir Dar town dairy farms, Ethiopia', *Heliyon*, 10(7), pp. 0–7. Available at: https://doi.org/10.1016/j.heliyon.2024.e28839.

Zahra, A.M., Niaj Murshidi, G., Das Moon, U., Sultana, S. and Haque, F.K.M. (2025) 'Seasonal Analysis of Pathogenic *Escherichia coli* Contamination in Vegetables, Washing Water, and Vendor Hygiene: Virulence Group Classification and Antibiotic Resistance', *Food Science and Nutrition*, 13(1), pp. 1–19. Available at: https://doi.org/10.1002/fsn3.4723.

Zelelie, T.Z., Eguale, T., Yitayew, B., Abeje, D., Alemu, A., Seman, A., Jass, J., Mihret, A. and Abebe, T. (2023) 'Molecular epidemiology and antimicrobial susceptibility of diarrheagenic *Escherichia coli* isolated from children under age five with and without diarrhea in Central Ethiopia', *PLoS ONE*, 18(7 July), pp. 1–19. Available at: https://doi.org/10.1371/journal.pone.0288517.

Zhang, H.N., Hou, P. Bin, Chen, Y.Z., Ma, Y., Li, X.P., Lv, H., Wang, M., Tan, H.L. and Bi, Z.W. (2016) 'Prevalence of foodborne pathogens in cooked meat and seafood from 2010 to 2013 in Shandong Province, China', *Iranian Journal of Public Health*, 45(12), pp. 1577–1585.

Zhao, Y. (2005) 'Pathogens in fruit', in W. Jongen (ed.) *Improving the Safety of Fresh Fruit and Vegetables*. Woodhead Publishing, pp. 44–88. Available at: https://doi.org/https://doi.org/10.1533/9781845690243.1.44.

Zhou, Z., Alikhan, N.F., Mohamed, K., Fan, Y. and Achtman, M. (2020) 'The EnteroBase user's guide, with case studies on *Salmonella* transmissions, *Yersinia pestis* phylogeny, and *Escherichia* core genomic diversity', *Genome Research*, 30(1), pp. 138–152. Available at: https://doi.org/10.1101/gr.251678.119.

APPENDIX 1: Questionnaire

This questionnaire aims to obtain information related to demographics of the children with diarrhoea and their caregivers, source of diarrhoea as perceived by the caregivers, house conditions, animals in the house, water supply, and food consumed by the children a week before experiencing diarrhoeal episodes.

Important note: these questions are part of an extensive questionnaire of the focal project focused on Foodborne Disease Epidemiology, Surveillance and Control in African LMIC (Mozambique, Tanzania, Nigeria and Ethiopia).

1. Questions to be pre-filled

1.1.Data collector/interviewer:

1.2.Health care institution/facility:

1.3 Child case number/ID:

1.4 Parent/caretaker number/ID:

1.5 Laboratory ID:

1.6 Date of interview: __ /__ / __ (dd/mm/year)

Start of the interview

Interviewer: Thank you for agreeing to participate in the study. We are going to start by asking you some questions about your child, your household, your activities, and livestock you may have. Please let me know if you don't understand any of the questions, and feel free to ask any question you may have during the course of the interview, which will take approximately 30 min.

2. Demographics

2.1. Name of the child: _____

2.2. Gender child:

□ Female

□ Male

2.3. Age of child: *(Consult baby card, if available)*

_____ years _____ months

Birthday ___/___/___ *(dd/mm/year)*

□ Don't have exact date

2.4. Name of parent/caretaker: _____

2.5.Gender of parent/caretaker:

□ Female
□ Male

2.6.What is your marital status

□ Married
□ Divorced
□ Widowed
□ Single, but living with someone
□ Single, living alone

2.7.Age of parent/caretaker:

_____ years

Birthday ___/___/___ *(dd/mm/year)*

□ Don't have exact date

2.8.Relationship with child:

□ Mother
□ Father
□ Relative, specify:
_____
□ Other, specify: _____

2.9.Address/household identifiers:

Street name:	
Village name and ID:	
House number/ID:	
Location ID:	
Region:	
Latitude:	
Longitude:	

3. Possible source/vehicle of infection

3.1. According to you, what do you think caused your child's illness?

_____

3.2. Did your child consume food or drinks that in your opinion smelled or tasted like they were spoiled?

□ Yes
□ No

If yes, what food/drink was it:
_____
when was it:_____
where was it:
_____

□ Don't remember

4. Food eaten in the household

4.1. Which of the following types of food did your child eat during the week before the illness? *Click all that applies and specify, from where the food was bought/obtained*

Meat and eggs		Cooked	Semi-cooked	Raw	Food bought/obtained from
Beef	☐	☐	☐	☐	
Pork	☐	☐	☐	☐	
Lamb/sheep meat	☐	☐	☐	☐	
Goat meat	☐	☐	☐	☐	
Chicken meat	☐	☐	☐	☐	
Eggs	☐	☐	☐	☐	
Other poultry, specify_____	☐	☐	☐	☐	
Fish or shellfish, specify	☐	☐	☐	☐	
Other meats, specify	☐		☐		
Don't remember	☐				

Dairy		Pasteurised	Fermented	Raw	Food bought/obtained from
Milk	☐	☐	☐	☐	
Yogurts	☐	☐	☐	☐	
Cheese	☐	☐	☐	☐	
Other dairy, specify	☐	☐	☐	☐	

Vegetables and fruit		Food bought/obtained from
Cooked vegetables, specify	☐	
Raw vegetables (e.g. leafy green salads), specify_____	☐	
Berries, specify	☐	
Other fruits, specify_____	☐	

5. Food products for very young children (< 2 years)

5.1. What did your child eat and drink during the week before the illness?

		Where was it bought (Name and location of the shop/place)	How was the nature of it (e.g. powder, liquid)	How was it prepared/eaten?
Food product		Not applicable	Not applicable	Not applicable
Milk (breastfeeding)	☐	☐	☐	☐
Infant formula	☐	☐	☐	☐
Follow-on formula	☐	☐	☐	☐
Fermented cereals	☐	☐	☐	☐
Ready-to-eat meal	☐	☐	☐	☐
Fruit puree	☐	☐	☐	☐
Biscuits, rusks, cookies	☐	☐	☐	☐
Fruit or vegetable juices	☐	☐	☐	☐
Other food, specify___	☐	☐	☐	☐
Specify, _____				☐
				☐

5.2. Does your child drink any of the above products from baby bottles?

☐ Yes, specify which products

_____

☐ No
☐ Don't know

5.3. What kind of water is used for preparing the above mentioned products?

☐ Bottled water, specify the brand _____
☐ Boiled water from the household's usual water supply* (reaching boiling point)
☐ Water (not boiled) from the household's water supply
☐☐ Other, specify

6. Water supply

6.1. What kind of water do you use in your household for drinking?

Drinking water source	Primary source (choose only one)	Secondary source (choose only one)	Other sources (tick all that apply)
Piped water into home	☐	☐	☐
Public/communal well/ or standpipe	☐	☐	☐
Lake, name: _____	☐	☐	☐
River, name: _____	☐	☐	☐
Creek	☐	☐	☐
Pond or dam (standing water) directly	☐	☐	☐
Private well or pump, protected	☐	☐	☐
Private well or pump, unprotected	☐	☐	☐
Spring, protected	☐	☐	☐
Spring, unprotected	☐	☐	☐
Rainwater	☐	☐	☐
Tanker truck	☐	☐	☐
Cart or wheelbarrow with small tank or drum	☐	☐	☐
Bottled water	☐	☐	☐
Other, specify_____	☐	☐	☐

6.1. Is the drinking water treated before consumption (e.g. disinfected with chlorine or UV, filtered, etc.)?

☐ Yes
☐ No
☐ Don't know/Don't remember

If yes,
☐ Boiling
☐ Strain it through a cloth
☐ Adding disinfectant, sich as chloringe or bleach
☐ Sedimentation and decant
☐ Filtering
☐ Solar disinfection
☐ Other, specify

7. Toilet system

7.1. What type of toilet sytem do members of your household use when at home? *(chose only one)*

Bathing water source	Primary source (choose only one)	Secondary source (choose only one)	Other sources (tick all that apply)
Flush or pour toilet with septic tank, including squat toilet	☐	☐	☐
Flush or pour toilet connected to sewer pipe	☐	☐	☐
Pit latrine with covering slab	☐	☐	☐
Pit latrine without covering slab	☐	☐	☐
Ventilated improved pit latrine (VIP)	☐	☐	☐
Bucket or plastic bags	☐	☐	☐
No facilities or field or bush	☐	☐	☐
Other, specify_____	☐	☐	☐

8. Contact with animals

8.1. Does anyone in your household own any of these animals?

	Yes	How many	Location of animals		
			Inside your house	Around your house	Distant location
Cattle	☐		☐	☐	☐
Goats	☐		☐	☐	☐
Sheep	☐		☐	☐	☐
Pigs	☐		☐	☐	☐
Chicken	☐		☐	☐	☐
Ducks	☐		☐	☐	☐
Dogs	☐		☐	☐	☐
Cats	☐		☐	☐	☐
Horses	☐		☐	☐	☐
Donkeys	☐		☐	☐	☐
Rabbits	☐		☐	☐	☐
Other	☐		☐	☐	☐

- Specify

8.2. Did your child have contact with the following animals? By contact we mean feeding or touching the animal, or contact with droppings or manure from the animals.

	Contact frequency				
	Yes	Daily	4-6 times per week	1-3 times per week	Less than 1 times per week
Cattle	☐	☐	☐	☐	☐
Goats	☐	☐	☐	☐	☐
Sheep	☐	☐	☐	☐	☐
Pigs	☐	☐	☐	☐	☐
Chicken	☐	☐	☐	☐	☐
Ducks	☐	☐	☐	☐	☐
Dogs	☐	☐	☐	☐	☐
Cats	☐	☐	☐	☐	☐
Horses	☐	☐	☐	☐	☐
Donkeys	☐	☐	☐	☐	☐
Rabbits	☐	☐	☐	☐	☐
Other	☐	☐	☐	☐	☐
- Specify					

8.3.Did your child have the possibility to be in contact with any of the following wild or free roaming animals? By contact we mean feeding or touching the animal or contact with droppings or manure from the animals.

	Contact frequency				
	Yes	Daily	4-6 times per week	1-3 times per week	Less than 1 times per week
Caged wild animals, specify	☐	☐	☐	☐	☐
Wild birds, specify	☐	☐	☐	☐	☐
Bats	☐	☐	☐	☐	☐
Wild boars	☐	☐	☐	☐	☐
Deer	☐	☐	☐	☐	☐
Foxes	☐	☐	☐	☐	☐
Rats	☐	☐	☐	☐	☐
Mice	☐	☐	☐	☐	☐
Rabbits	☐	☐	☐	☐	☐
…	☐	☐	☐	☐	☐
…	☐	☐	☐	☐	☐
Other, specify	☐	☐	☐	☐	☐

9. Occupation

9.1. Who in the household produces income for the family?

- □ Adult male
- □ Adult female
- □ Male child (13-18 years old)
- □ Female child (13-18 years old)
- □ Male child (<13 years old)
- □ Female child (<13 years old)
- □ Other, specify_____

9.2. What is the average monthly income of the main earner in the household?

Enter amount: _____
Enter currency: _____

- □ Don't know/not sure
- □ Prefer not to respond

10. Closure questions

10.1. May we contact you again if we have more questions?

- □ No
- □ Yes, contact info (how can we contact you):

_____

10.2. Is there any additional information you would like to share?

_____

_____

Interviewer: On behalf of the project team, thank you for taking the time to respond to this questionnaire. If you have questions about the investigation, please contact […..]